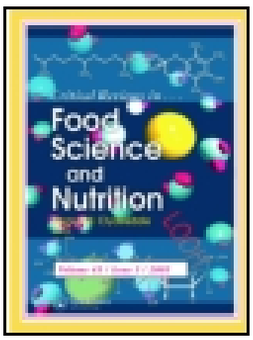

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

The role of B vitamins in stroke prevention

Geir Bjørklund, Massimiliano Peana, Maryam Dadar, Iryna Lozynska, Salvatore Chirumbolo, Roman Lysiuk, Larysa Lenchyk, Taras Upyr & Beatrice Severin

To cite this article: Geir Bjørklund, Massimiliano Peana, Maryam Dadar, Iryna Lozynska, Salvatore Chirumbolo, Roman Lysiuk, Larysa Lenchyk, Taras Upyr & Beatrice Severin (2021): The role of B vitamins in stroke prevention, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.1885341

To link to this article: https://doi.org/10.1080/10408398.2021.1885341

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1885341

Taylor & Francis
Taylor & Francis Group

REVIEW

The role of B vitamins in stroke prevention

Geir Bjørklund[a], Massimiliano Peana[b], Maryam Dadar[c], Iryna Lozynska[d,e], Salvatore Chirumbolo[f,g], Roman Lysiuk[e,h], Larysa Lenchyk[i,j], Taras Upyr[j,k], and Beatrice Severin[l]

[a]Council for Nutritional and Environmental Medicine (CONEM), Mo i Rana, Norway; [b]Department of Chemistry and Pharmacy, University of Sassari, Italy; [c]Education and Extension Organization (AREEO), Razi Vaccine and Serum Research Institute, Agricultural Research, Karaj, Iran; [d]Department of Biochemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine; [e]CONEM Ukraine Life Science Research Group, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine; [f]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy; [g]CONEM Scientific Secretary, Verona, Italy; [h]Department of Pharmacognosy and Botany, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine [i]Department of Quality, Standardization and Certification of Medicines of IATPS, National University of Pharmacy, Kharkiv, Ukraine; [j]CONEM Ukraine Pharmacognosy and Natural Product Chemistry Research Group National University of Pharmacy, Kharkiv, Ukraine; [k]Department of Pharmacognosy, National University of Pharmacy, Kharkiv, Ukraine [l]Faculty of Medicine, Ovidius University of Constanta, Constanta, Romania

ABSTRACT

Elevated plasma levels of homocysteine (Hcy) are a recognized risk factor for stroke. This relationship represents one aspect of the debated `Hcy hypothesis'. Elevated Hcy may be an independent and treatable cause of atherosclerosis and thrombotic vascular diseases. Further observations indicate that proper dietary supplementation with B-vitamins decreases total plasma Hcy concentrations and may be an effective intervention for stroke prevention. Metabolic vitamin B_{12} deficiency is a nutritional determinant of total Hcy and stroke risk. Genetic factors may link B vitamins with stroke severity due to the impact on Hcy metabolism of polymorphism in the genes coding for methylenetetrahydrofolate reductase, methionine-synthase, methionine synthase reductase, and cystathionine β-synthase. Several meta-analyses of large randomized controlled trials exist. However, they are not completely in agreement about B vitamins' role, particularly folic acid levels, vitamin B_{12}, and B_6, in lowering the homocysteine concentrations in people at high stroke risk. A very complex relationship exists between Hcy and B vitamins, and several factors appear to modify the preventive effects of B vitamins in stroke. This review highlights the regulating factors of the active role of B vitamins active in stroke prevention. Also, inputs for further large, well-designed studies, for specific, particularly sensitive subgroups are given.

KEYWORDS

B vitamins; folic acid; cobalamin; riboflavin; pyridoxine; nutrients; sources; stroke; homocysteine; hyperhomocysteinemia

Introduction

B vitamins are strictly connected to homocysteine (Hcy) through their role in methionine (Met) metabolism, regeneration, and Hcy breakdown. The vitamins involved in Hcy metabolism and turn over encompass vitamin B_9 (folic acid), vitamin B_{12} (cobalamin), and, to a lesser extent, vitamin B_6 (pyridoxal 5′-phosphate) and vitamin B_2 (riboflavin) (Spence, Yi, and Hankey 2017; McNulty et al. 2008; Sanchez-Moreno, Jimenez-Escrig, and Martin 2009) (Figures 1 and 2). There are numerous studies of Hcy as a risk factor for stroke, most of which show a positive association (Li et al. 2003; Bos et al. 2005; Ashjazadeh, Fathi, and Shariat 2013; Devasia, Joy, and Tarey 2016; Narang et al. 2009; Dhamija et al. 2009). In some cases, Hcy levels in stroke were about 20% and 30% higher than in controls (Ashjazadeh, Fathi, and Shariat 2013; Narang et al. 2009), with a report showing Hcy levels two times higher (Dhamija et al. 2009). Some studies evidenced a most reliable association relatively with small vessel strokes (Piao et al. 2018; Larsson, Traylor, and Markus 2019; Khan et al. 2008), while other reports showed elevated Hcy for brain atrophy in post-stroke patients (Yang et al. 2007; Shi et al. 2018; Pascoe et al. 2012).

Three meta-analyses confirmed the relationship mentioned above. One (Homocysteine Studies Collaboration 2002) included 30 observational studies involving a total of 5073 ischemic heart disease events and 1113 stroke events and established that elevated Hcy is an independent predictor but less strongly related risk factor of ischemic heart disease and stroke in healthy peoples than suggested before (Boushey et al. 1995). Among prospective studies in individuals with no history of cardiovascular disease (CVD) and after the proper corrections and adjustments, the stroke risk was reduced by 19% for every 25% decrease in Hcy level (Homocysteine Studies Collaboration 2002). The second meta-analysis collected 20 sets of prospective data (3820 participants), resulting in an OR = 1.59, which means that a future stroke patient would have a 5 µmol/l increase in serum Hcy from the baseline level (Wald, Law, and Morris 2002). The genetic and prospective studies evidenced a clear causal association between Hcy and CVD. The authors

CONTACT Geir Bjørklund bjorklund@conem.org Council for Nutritional and Environmental Medicine, Toften 24, 8610 Mo i Rana, Norway.

concluded that lowering Hcy concentrations by 3 μmol/l from current levels by increasing folic acid intake (about 0.8 mg/day) would reduce the stroke risk by 24%. In the third meta-analysis, among 14 studies involving 1769 stroke cases, the pooled OR estimate of ischemic stroke associated with hyperhomocysteinemia (HHcy) was 1.79 (Kelly et al. 2002).

The relationship between Hcy level and reduced stroke risk with supplementation of B vitamins has been analyzed in several epidemiological studies. There are many reports, although not completely in agreement, about stroke being associated with folic acid levels (Weng et al. 2008; Robinson et al. 1998; Huo et al. 2015; He et al. 2004; Yang et al. 2007; Larsson et al. 2008; Zeng et al. 2015), vitamin B_{12} levels (Spence 2016; Ahmed et al. 2019; He et al. 2004) and vitamin B_6 (Robinson et al. 1998; He et al. 2004; Kelly et al. 2004). A single study found lower vitamin B_2 levels in stroke patients than controls (Ullegaddi, Powers, and Gariballa 2005), revising previous data (Ullegaddi, Powers, and Gariballa 2004). Fundamental factors that affect folate's ability to prevent stroke are platelet disorders and cigarette smoking (Kong et al. 2018; Zhou et al. 2018; Larsson et al. 2008). A recent study reported that Hcy levels have a strong interaction with antiplatelet therapy in recurrent stroke in female subjects ($p = 0.010$) rather than in male individuals ($p = 0.595$), after correcting statistics for confounders (Li et al. 2020). This evidence suggests that an outstanding investigation on the role of B vitamins supplementation and Hcy marker in stroke might take into account age and sex distribution. But not only that. The polymorphism C677T in the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR), has been shown to induce a predisposition to HHcy and to have an influence in determining susceptibility to ischemic stroke (Kelly et al. 2002). The promising relationship between reduction of total plasma Hcy in hypertension subjects and vitamin B supplementation is dependent on various parameters, including disease states, polymorphism of MTHFR and other genes, total Hcy, baseline folate, age, sex, race, educational level, and lifestyle choice as chronic alcohol intake and smoking (Zhou et al. 2018). Therefore, a very complex relationship can be outlined between Hcy and B vitamins in stroke, which deserves great attention. This narrative review aimed to highlight the associated factors that may regulate B vitamins' active role in stroke prevention and provide input for further large, well-designed studies, particularly for specific sensitive subgroups.

A brief insight into the characteristics and sources of B vitamins

Vitamin B_9

Vitamin B_9, or folic acid, is a synthetically produced water-soluble vitamin, formed by a para-aminobenzoic acid with a pteridine jointed to its amino end and with a carboxyl group bound to the α-amino group of glutamic acid forming an amide bond (Figure 1). Folate is the naturally occurring form of folic acid that is converted into folate in the human body (Blancquaert et al. 2010). Since folate is an essential nutrient and cannot be biosynthesized *de novo* by human beings, it must be necessarily provided through the diet or as a supplement to prevent nutritional deficiency. The active form of folic acid is known as tetrahydrofolate (THF). Folate plays an important role in DNA synthesis, DNA and RNA modifications, in the metabolism of amino acids required for cell division, and in a series of cellular reactions known as folate-mediated one-carbon metabolism, such as the synthesis of amino acids and nucleic acids (Lan, Field, and Stover 2018). Deficiency of folate can lead to several outcomes including CVDs, macrocytic and megaloblastic anemia, depression, dementia, and cancer (especially in the colon). During early pregnancy, adequate folate intake prevents unborn babies' risk of having a neural tube defect by 40–70% (Barua, Kuizon, and Junaid 2014). A probable mechanism regarding folate deficiency causing neurological function involves HHcy associated with CVDs that chronically limit the brain's blood flow. This condition can turn into an acute status such as stroke or heart attack. The main natural source of folate for humans comes from plants and animals like chicken and calf liver and cheese, eggs, milk, fruits, and nuts. Since folate is highly susceptible to degradation, its bioavailability depends on cooking methods, preparation, and storage (Arcot and Shrestha 2005).

The average daily recommended dietary allowances (RDA) of folate depend on age, pregnancy, and lactation status. The RDA for an adult is 400 μg/d of dietary folate equivalent (DFE). DFE is used because of the higher bioavailability of folic acid than that of food folate (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate other B Vitamins and Choline 1998)

Folate deficiency is still a treat affecting millions of people worldwide, although strategies involving food fortification (cereals, cornmeal, rice, grain, and wheat products) are mandatory practice in nearly 80 countries together with folic acid supplementation. Often this policy remains inaccessible, particularly for poor rural populations in developing countries (Blancquaert et al. 2010).

Vitamin B_{12}

Vitamin B_{12} or cobalamin is a tetrapyrrolic cofactor in which the central cobalt atom is coordinated by four equatorial nitrogen ligands donated by pyrroles A-D of the corrin ring (Takahashi-Iñiguez et al. 2012) (Figure 1). It was described in 1925 by Whipple and Minot and Murphy as an antipernicious anemia factor (Robscheit-Robbins and Whipple 1925; Minot and Murphy 1926). Cobalamin is involved in many cells' metabolism in fatty acid and amino acid metabolism (Yamada 2013; Banerjee and Ragsdale 2003). Vitamin B_{12} plays a role in the synthesis of myelin, a lipid-rich sheath that surrounds nerve cell axons, and thus is necessary for the normal functioning of the nervous system and also for the maturation of developing red blood cells in the bone marrow and for DNA synthesis (Green 2013; Combs, Gerald and McClung 2016). Vitamin B_{12} is essential

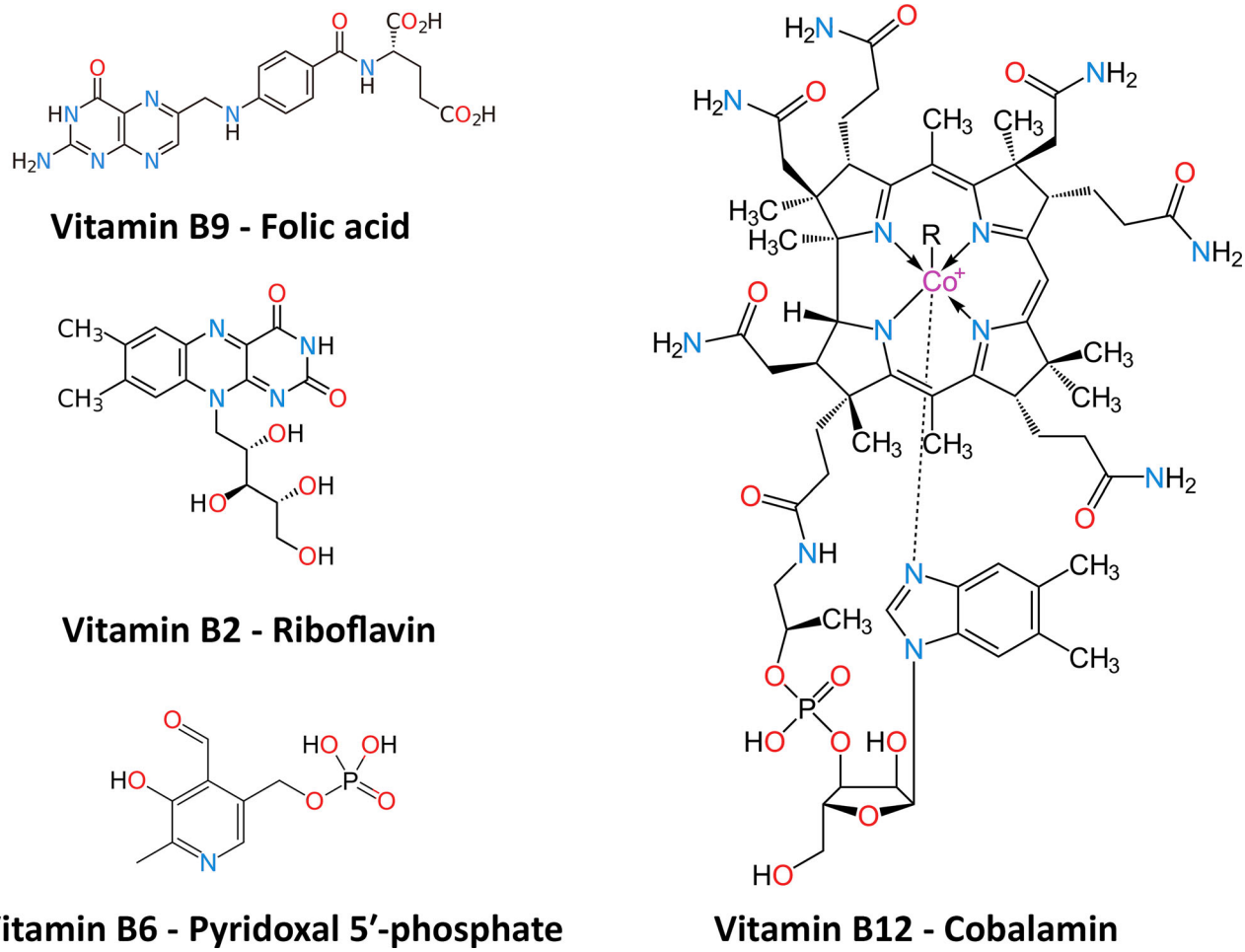

Figure 1. The chemical structures of B vitamins.

for the production of methionine (Met), the precursor for S-adenosylmethionine (SAM) required for methylation, which is essential for myelin preservation and nerve function. Vitamin B_{12} deficiency leads to demyelination, a process in which myelin sheath is damaged, causing disruption of nerve impulse transmission and several neurological problems (Miller et al. 2005; Calderon-Ospina and Nava-Mesa 2020; Acıpayam et al. 2020). In recent decades, the understanding of the biochemistry of vitamin B12 and the reaction mechanisms of its enzymes and their functions have been greatly improved (Banerjee and Ragsdale 2003; Smith, Warren, and Refsum 2018). Selective absorption of cobalamin requires interaction between the three transporting proteins – haptocorrin, intrinsic factor, transcobalamin, and several receptors. A complex transport system guarantees a very effective absorption of the vitamin, but failure in any link causes vitamin B_{12} deficiency (Fedosov 2012). Cobalamin deficiency is associated with megaloblastic anemia, weakness, fatigue, constipation, weight loss, and neurological disturbance as depression (Combs, Gerald and McClung 2016; Obeid et al. 2019). Cobalamin is a critical vitamin for the elderly (Wolters, Strohle, and Hahn 2004). It is considered that approximately 40% of the elderly are affected. Poor cobalamin status, often caused by atrophic gastritis, results in declining gastric acid and a decreasing intestinal absorption of the cobalamin protein complexes from food. Also, a reduction in acid secretion leads to alkalization of the small intestine, causing a further decrease in the cobalamin's bioavailability. Vitamin absorption is also affected by certain drugs, such as proton pump inhibitors or H2 receptor antagonists, which inhibit the intestinal absorption of vitamin B_{12}. Even a moderately reduced level of vitamin B_{12} is associated with vascular diseases and neurocognitive disorders. This is especially noticeable if folate deficiency is also present (Wolters, Strohle, and Hahn 2004).

The RDA for vitamin B_{12} in adults is 2.4 µg/d (2.6–2.8 µg/d in pregnant or lactating women) (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate other B Vitamins and Choline 1998). Dietary sources of vitamin B_{12} are animal products such as fish and seafood, clams, mussels, mackerel, crab, salmon, trout, as well as meat, milk, and eggs, whereas it is generally not present in significant amount in plant foods. Thus, to prevent vitamin B_{12} deficiency in vegetarians, specific plant foods particularly rich in vitamin B_{12} are required, such as fermented beans and vegetables, edible mushrooms, black trumpet

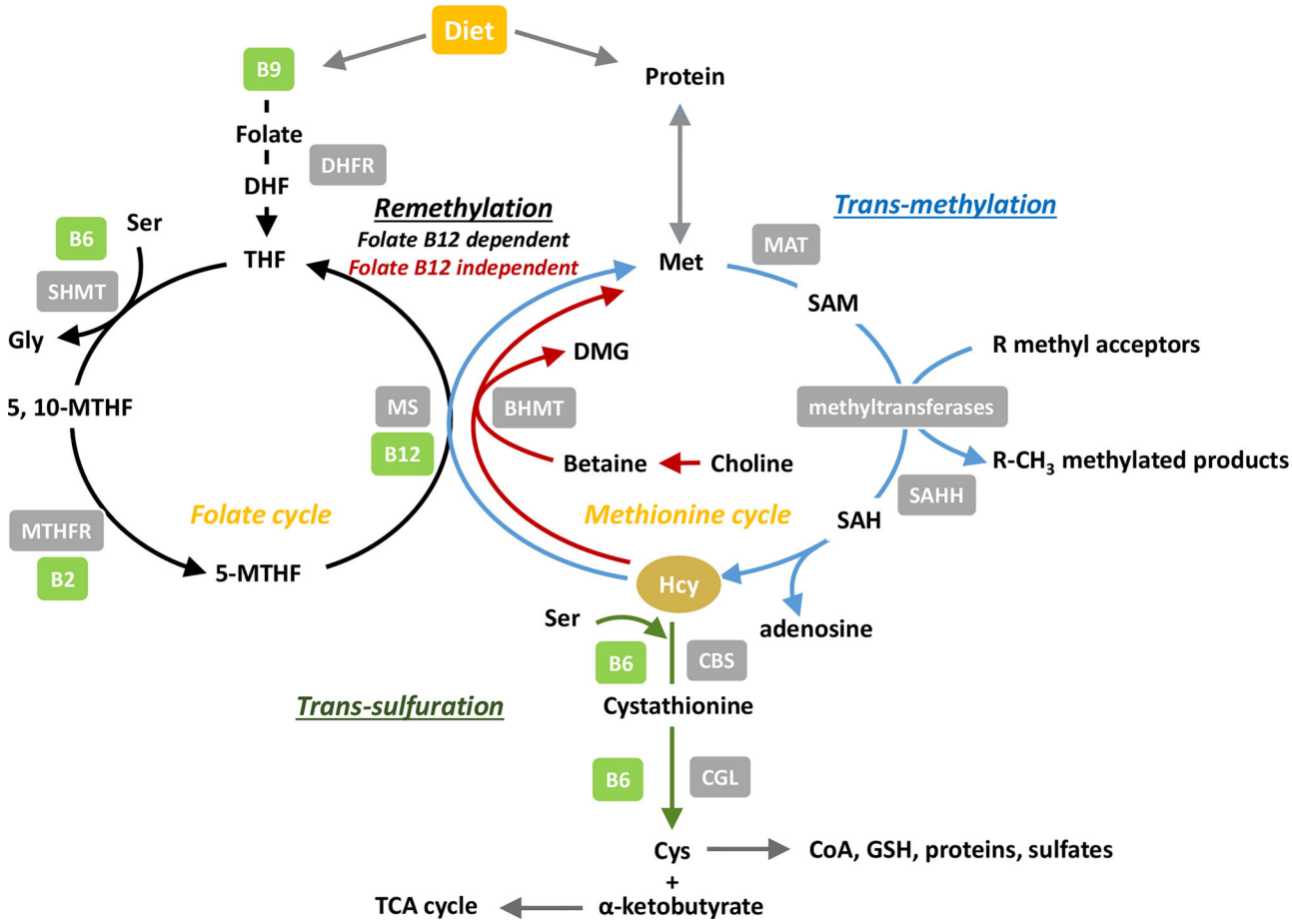

Figure 2. The pathways of homocysteine metabolism and the role of B vitamins.
Dietary vitamin B9 enters the "folate cycle" after its conversion to dihydrofolate (DHF) and then to tetrahydrofolate (THF). The vitamin B_6 dependent enzyme serine hydroxymethyltransferase (SHMT) catalyzes serine's reversible conversions to glycine and THF in 5,10-methylenetetrahydrofolate (5,10-MTHF). The enzyme 5,10-methyltetrahydrofolate reductase (MTHFR) converts 5,10-MTHF to 5-MTHF in response to vitamin B2. Vitamin B_{12} is required for the conversion of homocysteine (Hcy) to methionine (Met) by methionine synthase (MS), which transfers a one-carbon methyl group from 5-MTHF to Hcy, thereby generating Met and THF in a folate/vitamin B_{12} dependent remethylation. Hcy conversion to Met can also occur through the folate/vitamin B_{12} independent remethylation reaction of betaine, which is synthesized from choline by betaine-homocysteine S-methyltransferase (BHMT). In the "methionine cycle," dietary Met is converted to Hcy through S-adenosyl methionine (SAM), by methionine adenosyltransferase (MAT), and S-adenosyl homocysteine (SAH), by S-Adenosylhomocysteine hydrolase (SAHH), and then back to Met. SAM serves as methyl-donor in various cellular methylations, including biosynthesis of different compounds and epigenetic modulations. In the trans-sulfuration pathway, Hcy reacts with serine (Ser) in vitamin B_6 dependent reactions to generate cysteine (Cys) in the presence of rate-limiting enzymes cystathionine-β synthase (CBS) and cystathionine-γ lyase (CGL). In the first step, cystathionine is formed, which is further hydrolyzed to Cys and α-ketobutyrate. Cys is further incorporated/converted to glutathione (GSH), Coenzyme A (CoA), proteins, and sulfates, whereas α-ketobutyrate is a precursor of succinyl-CoA, which enters the citric acid cycle (TCA cycle).

(*Craterellus cornucopioides*), and golden chanterelle (*Cantharellus cibarius*). Edible algae as dried green laver (*Enteromorpha* sp.) and purple laver, or nori (*Porphyra* sp.), common in Japan, contain a significant amount of vitamin B_{12} (Watanabe et al. 2014; Rizzo et al. 2016). For instance, 4 g of dried purple laver, served at breakfast, or in some sushi receipts, supplies the RDA of vitamin B_{12} (Watanabe et al. 2014).

Vitamin B_6

Vitamin B_6 is the generic name for a group of six compounds, "vitamers", with the same activity. The active form of vitamin B_6 is pyridoxal 5′-phosphate (PLP) (Figure 1) that is a cofactor/regulator of more than 140 enzymes, particularly Hcy, involved in many aspects of amino acid metabolism, lipid and glucose metabolism, gene expression, neurotransmitters (i.e., serotonin) and hemoglobin synthesis and function (Combs, Gerald and McClung 2016). The main natural food sources of vitamin B_6 included fish, liver and other organ meats, bananas, potatoes, pistachios, nuts, and vegetables. However, its bioavailability is largely variable depending on cooking, storage, and processing methods. The RDA of vitamin B_6 for an adult is 1.3 mg/d (1.9–2.0 mg/d in pregnant or lactating women stage). Adults over 50 years old should increase the intake of vitamin B_6 (1.7–1.5 mg/d for men and women respectively) (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate other B Vitamins and Choline 1998). Vitamin B_6 deficiency has been correlated with CVD, diabetes, cancer, and stroke. Severe deficiency evolves in sideroblastic microcytic anemia, depression, confusion, convulsions, and peripheral neuropathy (Stover and Field 2015; Brown, Ameer, and Beier 2020). However, a syndrome of vitamin B_6

deficiency is somewhat a rare condition. It occurs in association with other B vitamins deficiency or adverse health condition such as liver disease, systemic inflammation, type 1 diabetes, rheumatoid arthritis, osteoporosis, Crohn's disease, celiac disease, ulcerative colitis (Younes-Mhenni et al. 2004; Wang, Chen, et al. 2019; Mascolo and Verni 2020). In addition, wrong style life choices such as alcohol abuse are connected with vitamin B_6 deficiency due to the inability of chronic alcoholics with liver disease to use food as a source of vitamin B (Cravo and Camilo 2000). Vitamin B_6 deficiency is also observed in conditions such as obesity or pregnancy. Conversely, excess vitamin B_6, reached with improper supplementation, has been correlated with toxic effects such as neurological, gastrointestinal disturbances, and dermatological lesions (Bacharach, Lowden, and Ahmed 2017; Scott, Zeris, and Kothari 2008).

Vitamin B_2

Vitamin B_2 or riboflavin, the least hydrosoluble of the B vitamins, is the precursor of two coenzymes known as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) that are crucial for normal tissue respiration, tryptophan to niacin (vitamin B_3) conversion, pyridoxine activation, fat, carbohydrate, and protein metabolism, and glutathione reductase mediated detoxification (Suwannasom et al. 2020). Riboflavin is composed of flavin moiety (7,8-dimethyl-benzo[g]pteridine-2,4-dione) with an isoalloxazine tricyclic ring (Figure 1). It is an essential nutrient required by the diet since humans are unable to synthesize it. However, riboflavin is easily found in various food, especially grain products, cereals, bread, organ meats, poultry, eggs, fish, nuts, certain fruits, legumes, and dairy products such as milk and cheese. Dark green leafy vegetables, mushrooms, and turnips are also moderately rich in riboflavin (Pinto and Zempleni 2016). These foods are also typically sources of other B vitamins and flavoenzymes. Therefore, a riboflavin deficiency is often accompanied by a deficiency of other B vitamins. The bioavailability of riboflavin can vary widely since fermenting, blanching, prolonged storage of milk products in clear bottles (ultraviolet and visible light inactivate riboflavin) and food processing, as well as sun-drying of fruits and vegetables, can result in physical removal, degradation, or vitamin photo-oxidation (Pinto and Zempleni 2016). Riboflavin has been indicated as a direct or indirect player in preventing several health conditions, including cancer, hypertension, hyperglycemia, diabetes mellitus, and oxidative stress (Thakur et al. 2017; Suwannasom et al. 2020). The RDA for riboflavin for adult men and women is 1.1 and 1.3 mg/d, respectively (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate other B Vitamins and Choline 1998). Riboflavin deficiency is a rare condition associated with several outcomes. These include endocrine abnormalities, skin disorders, hyperemia, mitochondria dysfunction, brain dysfunction, degeneration of the liver system, and others (Coates et al. 2015; Thakur et al. 2017).

Homocysteine metabolism and role of vitamin B-dependent enzymes

Methionine serves as a precursor for the synthesis of homocysteine, which is a non-essential sulfur-containing amino acid. The active form of Met, SAM, is a major donor of the methyl group used by many enzymes for their substrates' methylation. The transfer of methyl group from SAM to an acceptor in transmethylation reaction leads to S-adenosylhomocysteine (SAH) production, which is further hydrolyzed to Hcy and adenosine (Figure 2) (Mato, Martinez-Chantar, and Lu 2008).

Hcy metabolism includes remethylation and transsulfuration pathways. In the remethylation pathway, 5-methyl tetrahydrofolate (5-MTHF) serves as a donor of the methyl group and leads to methionine regeneration from homocysteine. This reaction is catalyzed by the B_{12}-dependent enzyme methionine synthase (MS). It should be noted that folate-dependent remethylation of Hcy is also essential for releasing THF and its reuse in one-carbon metabolism. In the folate/vitamin B_{12}-independent remethylation pathway, which occurs mainly in the liver, betaine is used as a methyl group donor (Esse et al. 2019).

In the transsulfuration pathways, B_6-dependent enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CTH) catalyze homocysteine condensation with serine to cystathionine and its cleavage to cysteine and α-ketobutyrate, respectively. In cysteine synthesis, the carbon skeleton is transferred from serine, while Hcy provides the sulfur atom. Cysteine is a precursor for synthesizing important compounds, namely coenzyme A, glutathione, and proteins (Škovierová et al. 2016). Thus, the failure to regenerate methionine from Hcy in folate/B_{12}-dependent pathway or transsulfuration to cysteine in a vitamin B_6-dependent pathway results in elevated plasma levels of Hcy (Zaric et al. 2019).

Causes and mechanism underlying hyperhomocysteinemia-related pathologies

Hyperhomocysteinemia, an elevated level of Hcy in the blood (greater than 15 µmol/L), arises from several causes. These include diseases condition (chronic renal failure, hypothyroidism, diabetes mellitus, and malignant tumors), the lack of cofactors involved in the metabolism of Hcy (diet poor in B vitamins), enzyme defect associated with homocysteine metabolism (polymorphisms), excessive methionine intake, and certain drugs (cholestyramine, metformin, methotrexate, niacin, fibric acid derivatives, oral contraceptive pills) (Kim et al. 2018). Other causes of HHcy are dependent on physiological factors such as age, sex, and lifestyle factors: smoking, coffee and alcohol abuse, and physical inactivity (Obeid et al. 2019).

High plasma concentrations of total homocysteine (tHcy) induce vascular endothelial dysfunction, cerebral vascular dysfunction, oxidative stress, promote inflammatory monocyte generation, and accelerates atherosclerosis (Faraci and Lentz 2004; Zhang et al. 2009; Azzini, Ruggeri, and Polito 2020).

HHcy leads to posttranslational modification of proteins, followed by significant restriction of their functions. N-homocysteinylation of proteins is based on the formation of amide linkage between lysine residues of proteins and homocysteine-thiolactone, and such proteins become prone to aggregation (Jakubowski 2019). It has been reported that low-density lipoproteins (LDL), which play a key role in the elevation of plasma cholesterol, are susceptible to N-homocysteinylation, leading to an increase in LDL atherogenicity. It was revealed in other studies that LDL modification is due to interactions between thiol groups of apolipoprotein B-100 and homocysteine by *S*-linkage (Zinellu et al. 2009). Modified LDL leads to the oxidative damage of vascular endothelial cells and could play an important role in developing atherogenesis and stroke (Pirillo et al. 2000).

Meta-analyses have shown an association between homocysteine level and CVDs involved via several different mechanisms, such as the negative impact on vascular endothelium and smooth muscle cells with resultant vessel damage (Kuo et al. 2005). These mechanisms are based on increasing arterial stiffness, muscle cell proliferation, deposition of sulfated glycosaminoglycans, and further loss of vasodilatation, especially in the elderly (Ganguly and Alam 2015). Recent studies demonstrated that Hcy might initiate an inflammatory response by inducing C-reactive protein expression in vascular smooth muscle cells (VSMCs) via the NMDAr-ROS-ERK1/2/p38-NF-kB signal pathway. The strong connection between Hcy level and C-reactive protein in VSMCs is mediated through reactive oxygen species (ROS), N-methyl-D-aspartate receptor (NMDA), and mitogen-activated protein kinase (MAPK) signal pathway (Curro et al. 2014; Pang et al. 2014; Ridker, Stampfer, and Rifai 2001).

The oxidation process is a key mechanism of increasing Hcy level and further developing atherosclerosis (McCully 2015; Moretti and Caruso 2019; Ganguly and Alam 2015; Lentz 2005). The plausible mechanism of the increased risk of atherosclerosis and stroke progression includes endothelial dysfunction, which is believed to occur primarily from oxidative stress. (Esse et al. 2019; Wu et al. 2019; Incalza et al. 2018). Thus, Hcy can trigger the generation of free radical species via auto-oxidation, inhibition of glutathione peroxidase, or oxidation of LDL. Also, Hcy, due to an increase in the levels of asymmetric dimethylarginine (ADMA) strong inhibitor of nitric oxide synthase (eNOS), suppresses the vasodilator nitric oxide (Lehotský et al. 2016; Stuhlinger et al. 2001; Incalza et al. 2018).

Genetic variability beyond the issue of the Hcy level

Defects in enzymes associated with Hcy metabolism are the most common cause of HHcy. Extensively documented proofs showed that a gene mutation C677T (cytosine 677 replaced by thymidine), which decodes for MTHFR enzyme involved in folate metabolism, reduces its activity, leading to impaired Hcy metabolism. A meta-analysis including 19 case-control studies involving 5159 individuals confirmed the susceptibility to ischemic stroke in association with the particular MTHFR genotype (TT) in which Hcy recycling is compromised (Li and Qin 2014). One of the first meta-analyses performed in 2002 reported the OR = 1.23 for ischemic stroke in the TT genotype (Kelly et al. 2002), this association was later confirmed (Li et al. 2003; Alluri et al. 2005; Cui 2016; Li et al. 2017). Analysis of the data of 20424 hypertensive adults who participated in the China Stroke Primary Prevention Trial, with a median follow-up period of 4,5 years, demonstrated that in patients with hypertension, the impact of Hcy on the first stroke significantly depended on the MTHFR C677T genotype and vitamin B9 supplementation (Li et al. 2017).

Moreover, other polymorphisms, such as the genes coding for methionine-synthase (MS), methionine synthase reductase (MTRR), and cystathionine β-synthase (CBS), has been highlighted as a cause of their low or lack of activity in the Hcy clearance mechanism, which results in the dysregulation of Hcy circulating level and increases HHcy risk (Li et al. 2017; Kim et al. 2018). In particular, the combination of Hcy metabolism gene polymorphisms and folate deficiency leads to an increased risk of HHcy. Excess Hcy could inhibit the expected migration of progenitor cells from bone marrow to repair vascular endothelium damaged in stroke patients (Dong et al. 2016; Zhu et al. 2006; Alam et al. 2009; Jakubowski 2006; Sato et al. 2020). Genetic variability profoundly affects this issue, relating to Hcy, stroke, and arterial physiology. For example, arterial cranial stenosis, the most common cause of ischemic stroke worldwide, has been associated with genetic RNF213 single nucleotide variant c.14429G > A (p.Arg4810Lys, rs112735431) (Hongo et al. 2020). RNF213 polymorphism has also been positively associated with Moyamoya Disease, a cerebrovascular occlusive disorder (Bang et al. 2016; Kim et al. 2016).

Mechanisms involving the Hcy-vitamins relationship

B vitamins influence the metabolism of Hcy, and their role in stroke prevention emerged due to the association between HHcy and CVDs (Wald, Law, and Morris 2002; Homocysteine Studies Collaboration 2002) and the ability of vitamin B supplementation to lowering tHcy level (Homocysteine Lowering Trialists' Collaboration 1998). Since then, a series of studies have been carried out regarding vitamin B supplementation in the treatment of HHcy. Therapy with B vitamins, able to lower homocysteine, appeared to help prevent stroke, if not other vascular outcomes (Spence 2007a). The next section will discuss key studies devoted to the role of vitamin B in stroke prevention.

Epidemiological data and evidence of the role of B vitamins in stroke prevention

A study based on a detailed food frequency questionnaire conducted in 1980 on approximately 80000 women in the United States over 14 years confirmed that consumption of folic acid and vitamin B_6, higher than currently

recommended, is an important factor for the primary prevention of coronary heart disease (CHD) among women (Rimm et al. 1998). Specifically, a folic acid consumption at a dose of 400 µg/d, either from food sources or from dietary supplements, was recommended. A recent dose–response meta-analysis of prospective cohort studies demonstrated that a higher intake of folate and vitamin B_6 is connected with a lower risk of CHD in the general population (Jayedi and Zargar 2019). A similar study involving about 44000 men, free of CVDs and diabetes at baseline, followed over four years, showed that the intake of vitamin B_{12} and folate increase, but not B_6, was inversely associated with the risk of ischemic stroke (He et al. 2004).

The HOPE-2 study (Heart Outcomes Prevention Evaluation-2 Trial) that involved 5000 participants started in December 1999 and was completed in October 2005. The goal of the research was to determine if long-term supplementation with folic acid, vitamin B_6, and vitamin B_{12}, aimed at decreasing Hcy, was able to reduce the rates of major fatal and nonfatal cardiovascular events in patients with established CVDs and/or diabetes mellitus (Lonn, Held, et al. 2006). Although tHcy levels and stroke rate were reduced (25% protection against stroke incidence), long-term supplementation with folic acid, vitamin B_6, and vitamin B_{12} did not reduce mortality or the incidence of myocardial infarction in high-risk patients with vascular disease (Lonn, Yusuf, et al. 2006). Consequently, lowering Hcy levels through vitamin B supplementation was not recommended in patients with established vascular disease (Kullo 2006).

HOPE-2 earned a major impact in the RCTs that have looked at this question (Ebbing et al. 2008; Albert et al. 2008; Bonaa et al. 2006; Zoungas et al. 2006; Lonn, Yusuf, et al. 2006; Righetti et al. 2006; Spence et al. 2005; Toole et al. 2004; Liem et al. 2005; Liem et al. 2004; Wrone et al. 2004; Mark et al. 1996) and formed the basis of separate meta-analyses (Marti-Carvajal et al. 2009; Bazzano et al. 2006; Wang et al. 2007). These meta-analyses, not considering the same data and conditions, did not completely agree with each other. Therefore, it was hard to find strong support for the idea that giving vitamins to reduce Hcy prevents stroke. Although 7/11 RCTs only showed a risk reduction, in one of them we can earn a clear significance. (Lonn, Yusuf, et al. 2006) concluded that supplements combining folic acid and vitamins B_6 and B_{12} did not reduce the risk of major cardiovascular events in patients with vascular disease.

Results from double-blind randomized controlled trial "SEARCH" (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine) involving 12064 survivors of myocardial infarction concluded that substantial long-term Hcy-lowering with folic acid and vitamin B_{12} supplementation does not have beneficial effects on CVDs, including stroke (Armitage et al. 2010). Conversely, from the meta-analysis of Wang, a positive association has been found between folic acid supplementation with effective reduction of Hcy level (more than 20%) and reduced the risk of stroke in primary prevention by 18% (Wang et al. 2007). Two next RCTs study were published in 2008 do not support the use of vitamin B in lowering the risk of mortality, stroke, and other cardiovascular events, although both were affected by some limitations. The first involved 5442 women treated for 7.3 years with a supplement of folic acid, vitamin B_6, and vitamin B_{12}. The result did not reduce cardiovascular events among high-risk women; even significant homocysteine lowering was observed (Albert et al. 2008). A second RTC, stopped prematurely and involving 3096 adult participants undergoing coronary angiography, did not show a positive effect after treatment with folic acid, vitamin B_6, and B_{12} on total cardiovascular mortality (Ebbing et al. 2008). The limitations of such trials have been related to the use of low-dose supplements to treat patients affected by advanced atherosclerotic disease (Marcus, Sarnak, and Menon 2007). Potential confounder factors need to be also considered. For instance, in a WAFACS (Women's Antioxidant and Folic Acid Cardiovascular Study) (Kang et al. 2008), the control group would take multivitamin supplements up to the RDA level (Bassuk et al. 2004).

Other potential limitations and potential confounders can arise from concurrent treatment (statin therapy, antacids), Hcy metabolic dysregulated state, renal failure, hypertension, diabetes, physical activity, smoking, alcohol consumption, high cyanocobalamin dose in patients with impaired renal function, and concurrent antiplatelet therapy and a high baseline level of vitamin or, on the contrary, vitamin malabsorption due to a series of diseases such as autoimmune gastritis (Wang et al. 2007; Debreceni and Debreceni 2014; Spence 2007b; Hankey 2018). It appeared that a meaningful association between vitamin B and stroke is dependent on a well-defined population.

The other two large RCTs involving Hcy-vitamins and stroke prevention were SU.FOL.OM3 study, on around 2500 patients who had CHD or stroke within the previous year, and VITATOPS (A Study of B VITAmins To Prevent Stroke), conducted in Australia with a target of 8000 patients who had a recent transient ischemic attack (Bazzano 2009; Galan et al. 2008). The SU.FOL.OM3 study, started in 2003 in France, was a secondary prevention trial testing the impact of supplementation with folate and B-vitamins and/or Omega-3 PUFA. The results of SU.FOL.OM3 trial does not support the routine use of dietary supplements containing n-3 PUFA and advises against supplementation with B vitamins in coronary patients in secondary cardiovascular prevention (Blacher et al. 2013).

A meta-analysis of data concerning individuals who took part in the RCT studies VITATOPS (VITATOPS Trial Study Group 2010) and VISP (Vitamins Intervention for Stroke Prevention) (Spence et al. 2005; Toole et al. 2004) did not show any benefits for stroke prevention in persons with impaired renal function, compared with patients with normal renal function, exposed to high intake of vitamin B_{12} (Spence, Yi, and Hankey 2017; Hankey 2018). The toxic effects of cyanocobalamin among participants with renal failure (diabetic nephropathy) obscured the treatment's benefit. Consequently, it has been suggesting using more safe methylcobalamin or oxocobalamin, instead of cyanocobalamin, combined with folic acid to lower Hcy

concentrations in people at high risk of stroke with the impaired renal condition (Spence, Yi, and Hankey 2017).

In a meta-analysis of Lee et al., supplementation of folic acid alone did not demonstrate a major effect in averting stroke (Lee et al. 2010). However, the authors concluded that potential moderate benefits in primary stroke prevention can be seen when a folic acid combined with other B vitamins is administered.

The RCT FAVORIT (Folic Acid for Vascular Outcome Reduction in Transplantation Trial) investigated if lowering Hcy levels in renal transplant recipients with a multivitamin (folic acid, vitamin B_6, and vitamin B_{12}) reduced the occurrence of cardiovascular disease outcome (Bostom et al. 2006). Despite a significant reduction in Hcy level, the treatment did not reduce a composite cardiovascular disease outcome, all-cause mortality, or dialysis-dependent kidney failure (Bostom et al. 2011). The RCT HOST (Homocysteine Study) tested the hypothesis that folate administration, pyridoxine, and cyanocobalamin in high doses to patients with advanced chronic renal failure and abnormally HHcy would lower the Hcy levels and the death rate compared to patients who received placebo. Moreover, HOST tested the hypothesis that the intake of the vitamins compared to placebo decreases the incidence of myocardial infarction, disabling stroke, amputation of a lower extremity, and vascular access site thrombosis in patients receiving hemodialysis (Jamison et al. 2004). It was concluded that the treatment with high doses of folic acid and B vitamins did not improve survival or reduce the incidence of vascular disease in patients with advanced chronic kidney disease or end-stage renal disease (Jamison et al. 2007).

Not a general consensus has been found between stroke risk reduction and Hcy-lowering through vitamin treatments. However, some positive proofs come from a series of studies in which it has been demonstrated a significant regression of carotid intima-media thickness (CIMT), which is a measure of atherosclerosis and stroke risk (Sun et al. 2017; Jung et al. 2013; Zhang et al. 2020), by a reduction of Hcy level through vitamin supplementation (Ntaios et al. 2010; Qin et al. 2012; Kwok et al. 2012). Conversely, some other studies have reported that long-term homocysteine-lowering did not significantly improve flow-mediated dilation (FMD) or CIMT in people with a stroke history (Potter et al. 2008).

The efficacy of folic acid therapy in the primary prevention of stroke among adults with hypertension has been verified in China's RTC study. The CSPPT (China Stroke Primary Prevention Trial) RCT study, involved 20702 adults with hypertension without a history of stroke or myocardial infarction, demonstrated that the combined therapy by enalapril and folic acid, compared with enalapril alone, significantly decreased the risk of first stroke (Huo et al. 2015). Moreover, meta-analysis research on 17 studies involving 86393 patients, seven therapy strategies, and a placebo group revealed that supplementation of folic acid and vitamin B_6 reduced stroke risk (Dong et al. 2015). The study highlighted folic acid's role in combination with vitamin B_6 for an ideal therapy for stroke prevention, respect the monotherapy based on folic acid alone.

The examination of 14 RCT with a total of 39420 adult patients, considering a different folate fortification status and the effects of folic acid supplementation in lowering Hcy and reducing stroke risk, give the conclusion that a modest benefit in the prevention of stroke can be seen in the region without folate fortification (Zeng et al. 2015).

Whereas the study of Miller et al. concluded that folic acid had no impact on the prevention of CVD or stroke (Miller et al. 2010), a more recent meta-analysis of 30 RCTs, involving 82334 patients, resulted in the demonstration of evidence of a 10% lower risk of stroke and a 4% lower risk of overall CVDs due to folic acid supplementation (Li et al. 2016). The best results were observed for people with lower plasma folate levels and without preexisting CVD and in studies with larger decreases in Hcy levels. Folic acid supplementation versus control caused a lower risk of any future stroke (RR = 0.85; 95% CI = 0.77–0.95) in patients from countries without mandatory vitamin food fortification (Hsu et al. 2018), confirming previous results (Huo et al. 2012). The greater benefit has been seen in patients receiving folic acid alone or with a minimal cyanocobalamin dose. Analysis of 7 RCTs with a total number of 9846 stroke patients demonstrated that supplementation with vitamin B (folic acid, vitamin B_{12}, and vitamin B_6) might avert the recurrence of stroke (RR = 0.64, 95% CI = 0.47–0.87) and decrease plasma Hcy levels in stroke patients (Dai et al. 2017). On the contrary, niacin (nicotinic acid, vitamin B_3) did not show evidence in preventing cardiovascular events, considering outcomes of 23 RCTs, which included 39195 participants in total (Schandelmaier et al. 2017).

The third update of the Cochrane review concludes that supplementation with folic acid, vitamin B_{12}, and vitamin B_6, alone or in combination, decreases tHcy level in blood by about 25%, but found a small difference in reducing the risk of stroke compared with placebo (risk ratio RR = 0.90, 95% CI = 0.82–0.99), and no differences compared with placebo on myocardial infarction and death (Marti-Carvajal et al. 2017). A meta-analysis of Tian et al., considering eleven studies with a total of 65790 participants, concluded that folic acid supplementation is effective in stroke prevention in patients with CVD (RR = 0.90; 95% CI: 0.84–0.97) (Tian et al. 2017). A similar result in stroke reduction (RR = 0.85, 95% CI = 0.77–0.94) has been achieved in a more recent meta-analysis that considered a total of 12 randomized controlled trials involving 47523 patients with CVD who received folic acid therapy (Wang, Jin, et al. 2019). A dose–response meta-analysis based on 11 prospective studies involving 389938 participants and 10749 cases of stroke showed a clear correlation between dietary intake of B vitamins and stroke risk (Chen et al. 2020). In particular, a linear inverse association between folate and vitamin B_6 intake and the risk of stroke were found: (RR = 0.94, 95% CI: 0.90–0.98) for 100 µg/d increments in folate intake and (RR = 0.94, 95% CI: 0.89–0.99) for 0.5 mg/d increments in vitamin B_6 intake. Conversely, the stroke risk does not increase significantly in association with 3 µg/d increments in vitamin B_{12} intake (RR= 1.01, 95% CI: 0.97–1.06). These data support the theory that increasing habitual folate and

vitamin B_6 intake might be beneficial, though having a modest stroke prevention effect.

Conclusion

The association between elevated Hcy, levels of B vitamins, and the incidence and severity of stroke appear strong. The present review highlights the evidence that applied B vitamins in the lowering concentrations of Hcy can decrease stroke risk. This benefit has often been obscured in past trial data due to masking effect or confounding factors. The metabolic vitamin B_{12} deficiency seems like the main nutritional determinant of tHcy and the risk of stroke. The relationship between tHcy level, adequate vitamin B supplementation, and stroke prevention is dependent on various parameters, including age, sex, genetic polymorphism, baseline folate, race, educational level, health condition, and lifestyle choice. A personalized nutritional approach to stroke prevention could be an effective solution in the next future. The individual variability and the interactions between the multiple factors should be highlighted in novel experimental designs to reach this goal. For example, patients with MTHFR C677T polymorphism likely need higher folic acid doses than those obtained in current fortified food. Further large, well-designed trials with specific B vitamin supplementation (alone or in combination, with dose and time of treatment defined) are urgently needed for specific subgroup as in people with a kind of advanced atherosclerotic disease, with metabolic B_{12} deficiency, with genetic polymorphism, or for people residing in regions without folic acid fortification. These studies should attempt to clarify the association between dietary B-vitamins intake and the risk of specific stroke subtypes in the perspective of personalized preventive treatment.

Abbreviations

Met	methionine
CBS	cystathionine β-synthase
CHD	coronary heart disease
CTH	cystathionine γ-lyase
CVD	cardiovascular disease
Hcy	homocysteine
HHcy	hyperhomocysteinemia
LDL	low-density lipoproteins
tHcy	total homocysteine
MS	methionine synthase
5-MTHF	5-methyl tetrahydrofolate
MTHFR	5,10-methylenetetrahydrofolate reductase
MTRR	methionine synthase reductase
PUFA	Polyunsaturated fatty acids
SAM	S-adenosylmethionine
SAH	S-adenosylhomocysteine
THF	tetrahydrofolate

Disclosure statement

The authors reported no potential conflict of interest.

Funding

The authors received no financial support for this article.

ORCID

Geir Bjørklund http://orcid.org/0000-0003-2632-3935
Massimiliano Peana http://orcid.org/0000-0002-3306-0419
Iryna Lozynska http://orcid.org/0000-0002-4120-032X
Salvatore Chirumbolo http://orcid.org/0000-0003-1789-8307
Roman Lysiuk http://orcid.org/0000-0003-0961-2970
Larysa Lenchyk http://orcid.org/0000-0002-6551-9036
Taras Upyr http://orcid.org/0000-0003-2374-5497
Beatrice Severin http://orcid.org/0000-0001-6499-1577

References

Acı payam, C., H. Güneş, O. Güngör, S. İpek, N. Sarı şı k, and N. Ş. Demir. 2020. Cerebral atrophy in 21 hypotonic infants with severe vitamin B12 deficiency. *Journal of Paediatrics and Child Health* 56 (5):751–6. doi: 10.1111/jpc.14733.

Ahmed, S., C. Bogiatzi, D. G. Hackam, A. C. Rutledge, L. A. Sposato, A. Khaw, J. Mandzia, M. R. Azarpazhoo, V. Hachinski, and J. David Spence. 2019. Vitamin B ((12)) deficiency and hyperhomocysteinaemia in outpatients with stroke or transient ischaemic attack: A cohort study at an academic medical centre. *BMJ Open* 9 (1): e026564-e. doi: 10.1136/bmjopen-2018-026564.

Alam, M. M., A. A. Mohammad, U. Shuaib, C. Wang, U. Ghani, B. Schwindt, K. G. Todd, and A. Shuaib. 2009. Homocysteine reduces endothelial progenitor cells in stroke patients through apoptosis. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism* 29 (1):157–65. doi: 10.1038/jcbfm.2008.99.

Albert, C. M., N. R. Cook, J. M. Gaziano, E. Zaharris, J. MacFadyen, E. Danielson, J. E. Buring, and J. E. Manson. 2008. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: A randomized trial. *JAMA* 299 (17):2027–36. doi: 10.1001/jama.299.17.2027.

Alluri, R. V., V. Mohan, S. Komandur, K. Chawda, J. R. Chaudhuri, and Q. Hasan. 2005. MTHFR C677T gene mutation as a risk factor for arterial stroke: A hospital based study. *European Journal of Neurology* 12 (1):40–4. doi: 10.1111/j.1468-1331.2004.00938.x.

Arcot, J., and A. Shrestha. 2005. Folate: Methods of analysis. *Trends in Food Science and Technology* 16 (6-7):253–66. doi: 10.1016/j.tifs.2005.03.013.

Armitage, J. M., L. Bowman, R. J. Clarke, K. Wallendszus, R. Bulbulia, K. Rahimi, R. Haynes, S. Parish, P. Sleight, R. Peto, et al. 2010. Effects of homocysteine-lowering with folic acid plus vitamin B12 vs placebo on mortality and major morbidity in myocardial infarction survivors: A randomized trial. *JAMA* 303 (24):2486–94. doi: 10.1001/jama.2010.840.

Ashjazadeh, N., M. Fathi, and A. Shariat. 2013. Evaluation of homocysteine level as a risk factor among patients with ischemic stroke and its subtypes. *Iranian Journal of Medical Sciences* 38 (3):233–9.

Azzini, E., S. Ruggeri, and A. Polito. 2020. Homocysteine: Its possible emerging role in at-risk population groups. *International Journal of Molecular Sciences* 21 (4)1421. doi: 10.3390/ijms2104:.

Bacharach, R., M. Lowden, and A. Ahmed. 2017. Pyridoxine toxicity small fiber neuropathy with dysautonomia: A case report. *Journal of Clinical Neuromuscular Disease* 19 (1):43–6. doi: 10.1097/CND.0000000000000172.

Banerjee, R., and S. W. Ragsdale. 2003. The many faces of vitamin B12: Catalysis by cobalamin-dependent enzymes. *Annual Review of Biochemistry* 72:209–47. doi: 10.1146/annurev.biochem.72.121801.161828.

Bang, O. Y., J.-W. Chung, J. Cha, M. J. Lee, J. Y. Yeon, C.-S. Ki, P. Jeon, J.-S. Kim, and S. C. Hong. 2016. A polymorphism in RNF213

Is a susceptibility gene for intracranial atherosclerosis. *PloS One* 11 (6):e0156607. doi: 10.1371/journal.pone.0156607.

Barua, S., S. Kuizon, and M. A. Junaid. 2014. Folic acid supplementation in pregnancy and implications in health and disease. *Journal of Biomedical Science* 21:77. 77. doi: 10.1186/s12929-014-z.

Bassuk, S. S., C. M. Albert, N. R. Cook, E. Zaharris, J. G. MacFadyen, E. Danielson, M. Van Denburgh, J. E. Buring, and J. E. Manson. 2004. The Women's antioxidant cardiovascular study: design and baseline characteristics of participants. *Journal of women's health (2002)* 13 (1):99–117. doi: 10.1089/154099904322836519.

Bazzano, L. A. 2009. Folic acid supplementation and cardiovascular disease: The state of the art. *The American Journal of the Medical Sciences* 338 (1):48–9. doi: 10.1097/MAJ.0b013e3181aaefd6.

Bazzano, L. A., K. Reynolds, K. N. Holder, and J. He. 2006. Effect of Folic acid supplementation on risk of cardiovascular diseases: A meta-analysis of randomized controlled trials . *JAMA* 296 (22): 2720–6. doi: 10.1001/jama.296.22.2720.

Blacher, J., S. Czernichow, F. Paillard, P. Ducimetiere, S. Hercberg, and P. Galan. 2013. Cardiovascular effects of B-vitamins and/or N-3 fatty acids: The SU.FOL.OM3 trial. *International Journal of Cardiology* 167 (2):508–13. doi: 10.1016/j.ijcard.2012.01.060.

Blancquaert, D., S. Storozhenko, K. Loizeau, H. De Steur, V. De Brouwer, J. Viaene, S. Ravanel, F. Rébeillé, W. Lambert, and D. Van Der Straeten. 2010. Folates and folic acid: From fundamental research toward sustainable health. *critical reviews in plant sciences Critical Reviews in Plant Sciences* 29 (1):14–35. doi: 10.1080/07352680903436283.

Bonaa, K. H., I. Njolstad, P. M. Ueland, H. Schirmer, A. Tverdal, T. Steigen, H. Wang, J. E. Nordrehaug, E. Arnesen, and K. Rasmussen. 2006. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *The New England Journal of Medicine* 354 (15):1578–88. doi: 10.1056/NEJMoa055227.

Bos, M. J., M. L. van Goor, P. J. Koudstaal, and D. W. Dippel. 2005. Plasma homocysteine is a risk factor for recurrent vascular events in young patients with an ischaemic stroke or TIA. *Journal of Neurology* 252 (3):332–7. doi: 10.1007/s00415-005-0647-9.

Bostom, A. G., M. A. Carpenter, J. W. Kusek, L. G. Hunsicker, M. A. Pfeffer, A. S. Levey, P. F. Jacques, and J. McKenney. 2006. Rationale and design of the folic acid for vascular outcome reduction in transplantation (FAVORIT) trial. *American Heart Journal* 152 (3):448 e1-7–448.e7. doi: 10.1016/j.ahj.2006.03.004.

Bostom, A. G., M. A. Carpenter, J. W. Kusek, A. S. Levey, L. Hunsicker, M. A. Pfeffer, J. Selhub, P. F. Jacques, E. Cole, L. Gravens-Mueller, et al. 2011. Homocysteine-lowering and cardiovascular disease outcomes in kidney transplant recipients: Primary results from the folic acid for vascular outcome reduction in transplantation trial. *Circulation* 123 (16):1763–70. doi: 10.1161/CIRCULATIONAHA.110.000588.

Boushey, C. J., S. A. Beresford, G. S. Omenn, and A. G. Motulsky. 1995. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 274 (13):1049–57. doi: 10.1001/jama.1995.03530130055028.

Brown, M. J., M. A. Ameer, and K. Beier. 2020. Vitamin B6 deficiency (pyridoxine). In *StatPearls*. Treasure Island (FL). https://www.ncbi.nlm.nih.gov/books/NBK470579/ .

Calderon-Ospina, C. A., and M. O. Nava-Mesa. 2020. B Vitamins in the nervous system: Current knowledge of the biochemical modes of action and synergies of thiamine, pyridoxine, and cobalamin. *CNS Neuroscience & Therapeutics* 26 (1):5–13. doi: 10.1111/cns.13207.

Chen, L., Q. Li, X. Fang, X. Wang, J. Min, and F. Wang. 2020. Dietary intake of homocysteine metabolism-related b-vitamins and the risk of stroke: A dose-response meta-analysis of prospective studies. *Advances in Nutrition*. doi: 10.1093/advances/nmaa061.

Coates, P. M., J. M. Betz, M. R. Blackman, G. M. Cragg, M. Levine, J. Moss, and J. D. White. 2015. *Encyclopedia of dietary supplements*. Boca Raton: CRC Press.. doi: 10.1201/b14669.

Combs, J., F. Gerald, and J. P. McClung. 2016. *The vitamins: fundamental aspects in nutrition and health*. Cambridge, MA: Academic Press.

Cravo, M. L., and M. E. Camilo. 2000. Hyperhomocysteinemia in chronic alcoholism: Relations to folic acid and vitamins b6 and b12 status. *Nutrition* 16 (4):296–302. doi: 10.1016/S0899-9007(99)00297-X.

Cui, T. 2016. MTHFR C677T mutation increased the risk of Ischemic Stroke, especially in large-artery atherosclerosis in adults: An updated meta-analysis from 38 researches. *International Journal of Neuroscience* 126 (1):10–9. doi: 10.3109/00207454.2014.990559.

Curro, M., A. Gugliandolo, C. Gangemi, R. Risitano, R. Ientile, and D. Caccamo. 2014. Toxic effects of mildly elevated homocysteine concentrations in neuronal-like cells. *Neurochemical Research* 39 (8): 1485–95. doi: 10.1007/s11064-014-1338-7.

Dai, G., H. Du, H. Wang, Y. Wei, Y. Xie, and Z. Li. 2017. Preventive effect of Vitamin B supplementation on recurrent stroke: A meta-analysis. *Zhonghua Wei Zhong Bing ji Jiu yi Xue* 29 (5):419–24. doi: 10.3760/cma.j.issn.2095-4352.2017.05.007.

Debreceni, B., and L. Debreceni. 2014. The Role of Homocysteine-Lowering B-Vitamins in the Primary Prevention of Cardiovascular Disease. *Cardiovascular Therapeutics* 32 (3):130–8. doi: 10.1111/1755-5922.12064.

Devasia, A. J., B. Joy, and S. D. Tarey. 2016. Serum homocysteine as a risk factor for carotid intimal thickening in acute stroke: A cross sectional observational study. *Annals of Indian Academy of Neurology* 19 (1):48–51. doi: 10.4103/0972-2327.168633.

Dhamija, R. K., P. Gaba, S. Arora, A. Kaintura, M. Kumar, and J. Bhattacharjee. 2009. Homocysteine and lipoprotein (a) correlation in ischemic stroke patients. *Journal of the Neurological Sciences* 281 (1-2):64–8. doi: 10.1016/j.jns.2009.02.341.

Dong, H., F. Pi, Z. Ding, W. Chen, S. Pang, W. Dong, and Q. Zhang. 2015. Efficacy of supplementation with b vitamins for stroke prevention: A network meta-analysis of randomized controlled trials. *PloS One* 10 (9):e0137533. doi: 10.1371/journal.pone.0137533.

Dong, Y., Q. Sun, T. Liu, H. Wang, K. Jiao, J. Xu, X. Liu, H. Liu, and W. Wang. 2016. Nitrative stress participates in endothelial progenitor cell injury in hyperhomocysteinemia. *PloS One* 11 (7):e0158672. doi: 10.1371/journal.pone.0158672.

Ebbing, M., O. Bleie, P. M. Ueland, J. E. Nordrehaug, D. W. Nilsen, S. E. Vollset, H. Refsum, E. K. Pedersen, and O. Nygard. 2008. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: A randomized controlled trial. *Jama* 300 (7):795–804. doi: 10.1001/jama.300.7.795.

Esse, R., M. Barroso, I. Tavares de Almeida, and R. Castro. 2019. The contribution of homocysteine metabolism disruption to endothelial dysfunction: State-of-the-art. *International Journal of Molecular Sciences* 20 (4)867. doi: 10.3390/ijms20040:.

Faraci, F. M., and S. R. Lentz. 2004. Hyperhomocysteinemia, oxidative stress, and cerebral vascular dysfunction. *Stroke* 35 (2):345–7. doi: 10.1161/01.STR.0000115161.10646.67.

Fedosov, S. N. 2012. Physiological and molecular aspects of cobalamin transport. *Sub-Cellular Biochemistry* 56:347–67. doi: 10.1007/978-94-007-2199-9_18.

Galan, P., S. Briancon, J. Blacher, S. Czernichow, and S. Hercberg. 2008. The SU.FOL.OM3 Study: A secondary prevention trial testing the impact of supplementation with folate and B-vitamins and/or Omega-3 PUFA on fatal and non fatal cardiovascular events, design, methods and participants characteristics. *Trials* 9:35 doi: 10.1186/1745-6215-9-35.

Ganguly, P., and S. F. Alam. 2015. Role of homocysteine in the development of cardiovascular disease. *Nutrition Journal* 14:6. doi: 10.1186/1475-2891-14-6.

Green, R. 2013. Vitamin B12: Physiology, dietary sources, and requirements. In *Encyclopedia of human nutrition* (3rd Ed.), ed. Benjamin Caballero, 351–6. Waltham: Academic Press.

Hankey, G. J. 2018. B vitamins for stroke prevention. *Stroke and Vascular Neurology* 3 (2):51–8. doi: 10.1136/svn-2018-000156.

He, K., A. Merchant, E. B. Rimm, B. A. Rosner, M. J. Stampfer, W. C. Willett, and A. Ascherio. 2004. Folate, vitamin B6, and B12 intakes in relation to risk of stroke among men. *Stroke* 35 (1):169–74. doi: 10.1161/01.STR.0000106762.55994.86.

Homocysteine Lowering Trialists' Collaboration. 1998. Lowering blood homocysteine with folic acid based supplements: Meta-analysis of randomised trials. *BMJ* 316 (7135):894–8. doi: 10.1136/bmj.316.7135.894.

Homocysteine Studies Collaboration. 2002. Homocysteine and risk of ischemic heart disease and stroke: A meta-analysis. *JAMA* 288 (16):2015–22. doi: 10.1001/jama.288.16.2015.

Hongo, H., S. Miyawaki, H. Imai, M. Shimizu, S. Yagi, J. Mitsui, H. Ishiura, J. Yoshimura, K. Doi, and W. Qu. 2020. Comprehensive investigation of RNF213 nonsynonymous variants associated with intracranial artery stenosis. *Scientific Reports* 10 (1):11942. doi: 10.1038/s41598-020-68888-1.

Hsu, C. Y., S. W. Chiu, K. S. Hong, J. L. Saver, Y. L. Wu, J. D. Lee, M. Lee, and B. Ovbiagele. 2018. Folic acid in stroke prevention in countries without mandatory folic acid food fortification: A meta-analysis of randomized controlled trials. *Journal of Stroke* 20 (1):99–109. doi: 10.5853/jos.2017.01522.

Huo, Y., J. Li, X. Qin, Y. Huang, X. Wang, R. F. Gottesman, G. Tang, B. Wang, D. Chen, M. He, et al. 2015. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: The CSPPT randomized clinical trial. *Jama* 313 (13):1325–35. doi: 10.1001/jama.2015.2274.

Huo, Y., X. Qin, J. Wang, N. Sun, Q. Zeng, X. Xu, L. Liu, X. Xu, and X. Wang. 2012. Efficacy of folic acid supplementation in stroke prevention: New insight from a meta-analysis. *International Journal of Clinical Practice* 66 (6):544–51. doi: 10.1111/j.1742-1241.2012.02929.x.

Incalza, M. A., R. D'Oria, A. Natalicchio, S. Perrini, L. Laviola, and F. Giorgino. 2018. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascular Pharmacology* 100:1–19. doi: 10.1016/j.vph.2017.05.005.

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate other B Vitamins and Choline. 1998. *Dietary Reference Intakes for Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, Pantothenic acid, biotin and choline*, Washington (DC): National Academies Press (US): National Academies Press (US).

Jakubowski, H. 2006. Pathophysiological consequences of homocysteine excess. *The Journal of Nutrition* 136 (6 Suppl):1741S–9S. doi: 10.1093/jn/136.6.1741S.

Jakubowski, H. 2019. Homocysteine modification in protein structure/function and human disease. *Physiological Reviews* 99 (1):555–604. doi: 10.1152/physrev.00003.2018.

Jamison, R. L., P. Hartigan, J. M. Gaziano, S. P. Fortmann, D. S. Goldfarb, J. A. Haroldson, J. Kaufman, P. Lavori, K. S. McCully, and K. Robinson. 2004. Design and statistical issues in the homocysteinemia in kidney and end stage renal disease (HOST) study. *Clinical Trials (London, England)* 1 (5):451–60. doi: 10.1191/1740774504cn038oa.

Jamison, R. L., P. Hartigan, J. S. Kaufman, D. S. Goldfarb, S. R. Warren, P. D. Guarino, J. M. Gaziano, and F. t Veterans Affairs Site Investigators. 2007. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: A randomized controlled trial. *JAMA* 298 (10):1163–70. doi: 10.1001/jama.298.10.1163.

Jayedi, A., and M. S. Zargar. 2019. Intake of vitamin B6, folate, and vitamin B12 and risk of coronary heart disease: A systematic review and dose-response meta-analysis of prospective cohort studies. *Critical Reviews in Food Science and Nutrition* 59 (16):2697–707. doi: 10.1080/10408398.2018.1511967.

Jung, J. M., D. Y. Kwon, C. Han, I. Jo, S. A. Jo, and M. H. Park. 2013. Increased carotid intima-media thickness and plasma homocysteine levels predict cardiovascular and all-cause death: A population-based cohort study. *European Neurology* 70 (1-2):1–5. doi: 10.1159/000343807.

Kang, J. H., N. Cook, J. Manson, J. E. Buring, C. M. Albert, and F. Grodstein. 2008. A trial of B vitamins and cognitive function among women at high risk of cardiovascular disease. *The American Journal of Clinical Nutrition* 88 (6):1602–10. doi: 10.3945/ajcn.2008.26404.

Kelly, P. J., J. P. Kistler, V. E. Shih, R. Mandell, N. Atassi, M. Barron, H. Lee, S. Silveira, and K. L. Furie. 2004. Inflammation, homocysteine, and vitamin B6 status after ischemic stroke. *Stroke* 35 (1):12–5. doi: 10.1161/01.STR.0000106481.59944.2F.

Kelly, P. J., J. Rosand, J. P. Kistler, V. E. Shih, S. Silveira, A. Plomaritoglou, and K. L. Furie. 2002. Homocysteine, MTHFR 677C–>T polymorphism, and risk of ischemic stroke: Results of a meta-analysis. *Neurology* 59 (4):529–36. doi: 10.1212/WNL.59.4.529.

Khan, U., C. Crossley, L. Kalra, A. Rudd, C. D. Wolfe, P. Collinson, and H. S. Markus. 2008. Homocysteine and its relationship to stroke subtypes in a UK black population: The south London ethnicity and stroke study. *Stroke* 39 (11):2943–9. doi: 10.1161/STROKEAHA.107.513416.

Kim, E. H., M. S. Yum, Y. S. Ra, J. B. Park, J. S. Ahn, G. H. Kim, H. W. Goo, T. S. Ko, and H. W. Yoo. 2016. Importance of RNF213 polymorphism on clinical features and long-term outcome in moyamoya disease. *Journal of Neurosurgery* 124 (5):1221–7. doi: 10.3171/2015.4.JNS142900.

Kim, J., H. Kim, H. Roh, and Y. Kwon. 2018. Causes of hyperhomocysteinemia and its pathological significance. *Archives of Pharmacal Research* 41 (4):372–83. doi: 10.1007/s12272-018-1016-4.

Kong, X., X. Huang, M. Zhao, B. Xu, R. Xu, Y. Song, Y. Yu, W. Yang, J. Zhang, L. Liu, et al. 2018. Platelet count affects efficacy of folic acid in preventing first stroke. *Journal of the American College of Cardiology* 71 (19):2136–46. doi: 10.1016/j.jacc.2018.02.072.

Kullo, I. J. 2006. HOPE 2: Can supplementation with folic acid and B vitamins reduce cardiovascular risk? *Nature Clinical Practice. Cardiovascular Medicine* 3 (8):414–5. doi: 10.1038/ncpcardio0611.

Kuo, H. K., F. A. Sorond, J. H. Chen, A. Hashmi, W. P. Milberg, and L. A. Lipsitz. 2005. The role of homocysteine in multisystem age-related problems: A systematic review. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 60 (9):1190–201. doi: 10.1093/gerona/60.9.1190.

Kwok, T., P. Chook, M. Qiao, L. Tam, Y. K. Poon, A. T. Ahuja, J. Woo, D. S. Celermajer, and K. S. Woo. 2012. Vitamin B-12 supplementation improves arterial function in vegetarians with subnormal vitamin B-12 status. *The Journal of Nutrition, Health & Aging* 16 (6):569–73. doi: 10.1007/s12603-012-0036-x.

Lan, X., M. S. Field, and P. J. Stover. 2018. Cell cycle regulation of folate-mediated one-carbon metabolism. *Wiley Interdisciplinary Reviews. Systems Biology and Medicine* 10 (6):e1426. doi: 10.1002/wsbm.1426.

Larsson, S. C., S. Mannisto, M. J. Virtanen, J. Kontto, D. Albanes, and J. Virtamo. 2008. Folate, vitamin B6, vitamin B12, and methionine intakes and risk of stroke subtypes in male smokers. *American Journal of Epidemiology* 167 (8):954–61. doi: 10.1093/aje/kwm395.

Larsson, S. C., M. Traylor, and H. S. Markus. 2019. Homocysteine and small vessel stroke: A Mendelian randomization analysis. *Annals of Neurology* 85 (4):495–501. doi: 10.1002/ana.25440.

Lee, M., K. S. Hong, S. C. Chang, and J. L. Saver. 2010. Efficacy of homocysteine-lowering therapy with folic acid in stroke prevention: A meta-analysis. *Stroke* 41 (6):1205–12. doi: 10.1161/STROKEAHA.109.573410.

Lehotský, J., B. Tothová, M. Kovalská, D. Dobrota, A. Beňová, D. Kalenská, and P. Kaplán. 2016. Role of homocysteine in the ischemic stroke and development of ischemic tolerance. *Frontiers in Neuroscience* 10:538 doi: 10.3389/fnins.2016.00538.

Lentz, S. R. 2005. Mechanisms of homocysteine-induced atherothrombosis. *Journal of Thrombosis and Haemostasis: JTH* 3 (8):1646–54. doi: 10.1111/j.1538-7836.2005.01364.x.

Li, A., Y. Shi, L. Xu, Y. Zhang, H. Zhao, Q. Li, X. Zhao, X. Cao, H. Zheng, and Y. He. 2017. A possible synergistic effect of MTHFR C677T polymorphism on homocysteine level variations increased risk for ischemic stroke. *Medicine* 96 (51):e9300. doi: 10.1097/MD.0000000000009300.

Li, J., Y. Wang, H. Li, Z. Zuo, J. Lin, A. Wang, X. Zhao, L. Liu, and Y. Wang. 2020. Homocysteine level predicts response to dual antiplatelet in women with minor stroke or transient ischemic attack: subanalysis of the CHANCE trial. *Arteriosclerosis, Thrombosis, and Vascular Biology* 40 (3):839–46. doi: 10.1161/ATVBAHA.119.313741.

Li, P., and C. Qin. 2014. Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and susceptibility to ischemic stroke: A meta-analysis. *Gene* 535 (2):359–64. doi: 10.1016/j.gene.2013.09.066.

Li, Y., T. Huang, Y. Zheng, T. Muka, J. Troup, and F. B. Hu. 2016. Folic acid supplementation and the risk of cardiovascular diseases: A meta-analysis of randomized controlled trials. *Journal of the American Heart Association* 5 (8). doi: 10.1161/JAHA.116.003768.

Li, Z., L. Sun, H. Zhang, Y. Liao, D. Wang, B. Zhao, Z. Zhu, J. Zhao, A. Ma, Y. Han, et al. 2003. Elevated plasma homocysteine was associated with hemorrhagic and ischemic stroke, but methylenetetrahydrofolate reductase gene C677T polymorphism was a risk factor for thrombotic stroke: A multicenter case-control study in China. *Stroke* 34 (9):2085–90. doi: 10.1161/01.STR.0000086753.00555.0D.

Liem, A. H., A. J. van Boven, N. J. G. M. Veeger, A. J. Withagen, R. M. Robles de Medina, J. G. P. Tijssen, and D. J. van Veldhuisen. 2004. Efficacy of folic acid when added to statin therapy in patients with hypercholesterolemia following acute myocardial infarction: A randomised pilot trial. *International Journal of Cardiology* 93 (2-3): 175–9. doi: 10.1016/j.ijcard.2003.02.001.

Liem, A., G. H. Reynierse-Buitenwerf, A. H. Zwinderman, J. W. Jukema, and D. J. van Veldhuisen. 2005. Secondary prevention with folic acid: Results of the Goes extension study. *Heart (British Cardiac Society)* 91 (9):1213–4. doi: 10.1136/hrt.2004.035030.

Lonn, E., C. Held, J. M. Arnold, J. Probstfield, M. McQueen, M. Micks, and J. Pogue. 2006. Rationale, design and baseline characteristics of a large, simple, randomized trial of combined folic acid and vitamins B6 and B12 in high-risk patients: The Heart Outcomes Prevention Evaluation (HOPE)-2 trial. *The Canadian Journal of Cardiology* 22 (1):47–53. doi: 10.1016/S0828-282X(06)70238-0.

Lonn, E., S. Yusuf, M. J. Arnold, P. Sheridan, J. Pogue, M. Micks, M. J. McQueen, J. Probstfield, G. Fodor, C. Held, et al. 2006. Homocysteine lowering with folic acid and B vitamins in vascular disease. *The New England Journal of Medicine* 354 (15):1567–77. doi: 10.1056/NEJMoa060900.

Marcus, J., M. J. Sarnak, and V. Menon. 2007. Homocysteine lowering and cardiovascular disease risk: Lost in translation. *The Canadian Journal of Cardiology* 23 (9):707–10. doi: 10.1016/S0828-282X(07)70814-0.

Mark, S. D., W. Wang, J. F. Fraumeni, J. Y. Li, P. R. Taylor, G. Q. Wang, W. Guo, S. M. Dawsey, B. Li, and W. J. Blot. 1996. Lowered risks of hypertension and cerebrovascular disease after vitamin/mineral supplementation: The Linxian Nutrition Intervention Trial. *American Journal of Epidemiology* 143 (7):658–64. doi: 10.1093/oxfordjournals.aje.a008798.

Marti-Carvajal, A. J., I. Sola, D. Lathyris, and M. Dayer. 2017. Homocysteine-lowering interventions for preventing cardiovascular events. *The Cochrane Database of Systematic Reviews* 8:CD006612. doi: 10.1002/14651858.CD006612.pub5.

Marti-Carvajal, A. J., I. Sola, D. Lathyris, and G. Salanti. 2009. Homocysteine lowering interventions for preventing cardiovascular events. *The Cochrane Database of Systematic Reviews* (4):CD006612. doi: 10.1002/14651858.CD006612.pub2.

Mascolo, E., and F. Verni. 2020. Vitamin B6 and Diabetes: Relationship and Molecular Mechanisms. *International Journal of Molecular Sciences* 21 (10):3669. doi: 10.3390/ijms21103669.

Mato, J. M., M. L. Martinez-Chantar, and S. C. Lu. 2008. Methionine metabolism and liver disease. *Annual Review of Nutrition* 28:273–93. doi: 10.1146/annurev.nutr.28.061807.155438.

McCully, K. S. 2015. Homocysteine and the pathogenesis of atherosclerosis. *Expert Review of Clinical Pharmacology* 8 (2):211–9. doi: 10.1586/17512433.2015.1010516.

McNulty, H., K. Pentieva, L. Hoey, and M. Ward. 2008. Homocysteine, B-vitamins and CVD. *The Proceedings of the Nutrition Society* 67 (2):232–7. doi: 10.1017/S0029665108007076.

Miller, A., M. Korem, R. Almog, and Y. Galboiz. 2005. Vitamin B12, demyelination, remyelination and repair in multiple sclerosis. *Journal of the Neurological Sciences* 233 (1-2):93–7. doi: 10.1016/j.jns.2005.03.009.

Miller, E. R., 3rd, S. Juraschek, R. Pastor-Barriuso, L. A. Bazzano, L. J. Appel, and E. Guallar. 2010. Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. *The American Journal of Cardiology* 106 (4):517–27. doi: 10.1016/j.amjcard.2010.03.064.

Minot, G. R., and W. P. Murphy. 1926. Treatment of pernicious anemia by a special diet. *JAMA: The Journal of the American Medical Association* 87 (7):470–6. doi: 10.1001/jama1926.02680070016005. .

Moretti, R., and P. Caruso. 2019. The controversial role of homocysteine in neurology: from labs to clinical practice. *International Journal of Molecular Sciences* 20 (1):231. doi: 10.3390/ijms20010231.

Narang, A. P., I. Verma, S. Kaur, A. Narang, S. Gupta, and G. Avasthi. 2009. Homocysteine–risk factor for ischemic stroke? *Indian Journal of Physiology and Pharmacology* 53 (1):34–8.

Ntaios, G., C. Savopoulos, D. Karamitsos, I. Economou, E. Destanis, I. Chryssogonidis, I. Pidonia, P. Zebekakis, C. Polatides, M. Sion, et al. 2010. The effect of folic acid supplementation on carotid intima-media thickness in patients with cardiovascular risk: A randomized, placebo-controlled trial. *International Journal of Cardiology* 143 (1): 16–9. doi: 10.1016/j.ijcard.2009.01.023.

Obeid, R., S. G. Heil, M. M. A. Verhoeven, E. G. H. M. van den Heuvel, L. C. P. G. M. de Groot, and S. J. P. M. Eussen. 2019. Eghm van den Heuvel, Lcpgm de Groot, and Sjpm Eussen. 2019. Vitamin B12 Intake From Animal Foods, Biomarkers, and Health Aspects. *Frontiers in Nutrition* 6:93 doi: 10.3389/fnut.2019.00093.

Pang, X., J. Liu, J. Zhao, J. Mao, X. Zhang, L. Feng, C. Han, M. Li, S. Wang, and D. Wu. 2014. Homocysteine induces the expression of C-reactive protein via NMDAr-ROS-MAPK-NF-κB signal pathway in rat vascular smooth muscle cells. *Atherosclerosis* 236 (1):73–81. doi: 10.1016/j.atherosclerosis.2014.06.021.

Pascoe, M. C., S. G. Crewther, L. M. Carey, K. Noonan, D. P. Crewther, and T. Linden. 2012. Homocysteine as a potential biochemical marker for depression in elderly stroke survivors. *Food & Nutrition Research* 56 (1):14973. doi: 10.3402/fnr.v56i0.14973.

Piao, X., G. Wu, P. Yang, J. Shen, A. De, J. Wu, and Q. Qu. 2018. Association between homocysteine and cerebral small vessel disease: A meta-analysis. *Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association* 27 (9):2423–30. doi: 10.1016/j.jstrokecerebrovasdis.2018.04.035.

Pinto, J. T., and J. Zempleni. 2016. Riboflavin. *Advances in Nutrition (Bethesda, Md.)* 7 (5):973–5. doi: 10.3945/an.116.012716.

Pirillo, A., W. Zhu, G. D. Norata, T. Zanelli, L. Barberi, P. Roma, and A. L. Catapano. 2000. Oxidized lipoproteins and endothelium. *Clinical Chemistry and Laboratory Medicine* 38 (2):155–60. doi: 10.1515/CCLM.2000.023.

Potter, K., G. J. Hankey, D. J. Green, J. Eikelboom, K. Jamrozik, and L. F. Arnolda. 2008. The effect of long-term homocysteine-lowering on carotid intima-media thickness and flow-mediated vasodilation in stroke patients: A randomized controlled trial and meta-analysis. *BMC Cardiovascular Disorders* 8 (1):24. doi: 10.1186/1471-2261-8-24.

Qin, X., M. Xu, Y. Zhang, J. Li, X. Xu, X. Wang, X. Xu, and Y. Huo. 2012. Effect of folic acid supplementation on the progression of carotid intima-media thickness: A meta-analysis of randomized controlled trials. *Atherosclerosis* 222 (2):307–13. doi: 10.1016/j.atherosclerosis.2011.12.007.

Ridker, P. M., M. J. Stampfer, and N. Rifai. 2001. Novel risk factors for systemic atherosclerosis: A comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA* 285 (19):2481–5. doi: 10.1001/jama.285.19.2481.

Righetti, M., P. Serbelloni, S. Milani, and G. Ferrario. 2006. Homocysteine-Lowering Vitamin B Treatment Decreases Cardiovascular Events in Hemodialysis Patients. *Blood Purification* 24 (4):379–86. doi: 10.1159/000093680.

Rimm, E. B., W. C. Willett, F. B. Hu, L. Sampson, G. A. Colditz, J. E. Manson, C. Hennekens, and M. J. Stampfer. 1998. Folate and vitamin B6 from diet and supplements in relation to risk of coronary

heart disease among women. *JAMA* 279 (5):359–64. doi: 10.1001/jama.279.5.359.

Rizzo, G., A. Laganà, A. Rapisarda, G. La Ferrera, M. Buscema, P. Rossetti, A. Nigro, V. Muscia, G. Valenti, F. Sapia, et al. 2016. Vitamin B12 among vegetarians: Status, assessment and supplementation. *Nutrients* 8 (12):767. doi: 10.3390/nu8120767.

Robinson, K., K. Arheart, H. Refsum, L. BrattströM, G. Boers, P. Ueland, P. Rubba, R. Palma-Reis, R. Meleady, L. Daly, et al. 1998. Low circulating folate and vitamin B6 concentrations: Risk factors for stroke, peripheral vascular disease, and coronary artery disease. *Circulation* 97 (5):437–43. doi: 10.1161/01.CIR.97.5.437.

Robscheit-Robbins, F. S., and G. Whipple. 1925. Favorable influence of liver, heart and skeletal muscle in diet. *American Journal of Physiology-Legacy Content* 72 (3):408–18. doi: 10.1152/ajplegacy.1925.72.3.408.

Sanchez-Moreno, C., A. Jimenez-Escrig, and A. Martin. 2009. Stroke: Roles of B vitamins, homocysteine and antioxidants. *Nutrition Research Reviews* 22 (1):49–67. doi: 10.1017/S0954422409990023.

Sato, K., Y. Morofuji, N. Horie, T. Izumo, T. Anda, and T. Matsuo. 2020. Hyperhomocysteinemia causes severe intraoperative thrombotic tendency in superficial temporal artery-middle cerebral artery bypass. *Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association* 29 (5):104633. doi: 10.1016/j.jstrokecerebrovasdis.2019.104633.

Schandelmaier, S., M. Briel, R. Saccilotto, K. K. Olu, A. Arpagaus, L. G. Hemkens, and A. J. Nordmann. 2017. Niacin for primary and secondary prevention of cardiovascular events. *The Cochrane Database of Systematic Reviews* 6:CD009744. doi: 10.1002/14651858.CD009744.pub2.

Scott, K., S. Zeris, and M. J. Kothari. 2008. Elevated B6 levels and peripheral neuropathies. *Electromyography and Clinical Neurophysiology* 48 (5):219–23.

Shi, Z., S. Liu, Y. Guan, M. Zhang, H. Lu, W. Yue, B. Zhang, M. Li, J. Xue, and Y. Ji. 2018. Changes in total homocysteine levels after acute stroke and recurrence of stroke. *Scientific Reports* 8 (1):6993. doi: 10.1038/s41598-018-25398-5.

Škovierová, H., E. Vidomanová, S. Mahmood, J. Sopková, A. Drgová, T. Červeňová, E. Halašová, and J. Lehotský. 2016. The Molecular and Cellular Effect of Homocysteine Metabolism Imbalance on Human Health. *International Journal of Molecular Sciences* 17 (10)1733. doi: 10.3390/ijms1710:.

Smith, A. D., M. J. Warren, and H. Refsum. 2018. Chapter Six - Vitamin B12. In *Advances in food and nutrition research*, ed. N. A. Michael Eskin, vol. 83, 215–79. Academic Press.

Spence, J. D. 2007a. Homocysteine-lowering therapy: A role in stroke prevention? *The Lancet. Neurology* 6 (9):830–8. doi: 10.1016/S1474-4422(07)70219-3.

Spence, J. D. 2007b. Perspective on the efficacy analysis of the Vitamin Intervention for Stroke Prevention trial. *Clinical Chemistry and Laboratory Medicine* 45 (12):1582–5. doi: 10.1515/CCLM.2007.325.

Spence, J. D. 2016. Metabolic vitamin B12 deficiency: A missed opportunity to prevent dementia and stroke. *Nutrition Research* 36 (2):109–16. doi: 10.1016/j.nutres.2015.10.003.

Spence, J. D., H. Bang, L. E. Chambless, and M. J. Stampfer. 2005. Vitamin intervention for stroke prevention trial: An efficacy analysis. *Stroke* 36 (11):2404–9. doi: 10.1161/01.STR.0000185929.38534.f3.

Spence, J. D., Q. Yi, and G. J. Hankey. 2017. B vitamins in stroke prevention: Time to reconsider. *The Lancet Neurology* 16 (9):750–60. doi: 10.1016/S1474-4422(17)30180-1.

Stover, P. J., and M. S. Field. 2015. Vitamin B-6. *Advances in Nutrition (Bethesda, Md.)* 6 (1):132–3. doi: 10.3945/an.113.005207.

Stuhlinger, M. C., P. S. Tsao, J. H. Her, M. Kimoto, R. F. Balint, and J. P. Cooke. 2001. Homocysteine impairs the nitric oxide synthase pathway: Role of asymmetric dimethylarginine. *Circulation* 104 (21):2569–75. doi: 10.1161/hc4601.098514.

Sun, K., J. Song, K. Liu, K. Fang, L. Wang, X. Wang, J. Li, X. Tang, Y. Wu, X. Qin, et al. 2017. Associations between homocysteine metabolism related SNPs and carotid intima-media thickness: A Chinese sib pair study. *Journal of Thrombosis and Thrombolysis* 43 (3):401–10. doi: 10.1007/s11239-016-1449-x.

Suwannasom, N., I. Kao, A. Pruß, R. Georgieva, and H. Bäumler. 2020. Riboflavin: The Health benefits of a forgotten natural vitamin. *International Journal of Molecular Sciences* 21 (3):950. doi: 10.3390/ijms21030950.

Takahashi-Iñiguez, T., E. García-Hernandez, R. Arreguín-Espinosa, and M. E. Flores. 2012. Role of vitamin B12 on methylmalonyl-CoA mutase activity. *Journal of Zhejiang University. Science. B* 13 (6):423–37. doi: 10.1631/jzus.B1100329.

Thakur, K., S. K. Tomar, A. K. Singh, S. Mandal, and S. Arora. 2017. Riboflavin and health: A review of recent human research. *Critical Reviews in Food Science and Nutrition* 57 (17):3650–60. doi: 10.1080/10408398.2016.1145104.

Tian, T., K. Q. Yang, J. G. Cui, L. L. Zhou, and X. L. Zhou. 2017. Folic acid supplementation for stroke prevention in patients with cardiovascular disease. *The American Journal of the Medical Sciences* 354 (4):379–87. doi: 10.1016/j.amjms.2017.05.020.

Toole, J. F., M. R. Malinow, L. E. Chambless, J. D. Spence, L. C. Pettigrew, V. J. Howard, E. G. Sides, C. H. Wang, and M. Stampfer. 2004. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: The Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *Jama* 291 (5):565–75. doi: 10.1001/jama.291.5.565.

Ullegaddi, R., H. J. Powers, and S. E. Gariballa. 2005. Antioxidant supplementation enhances antioxidant capacity and mitigates oxidative damage following acute ischaemic stroke. *European Journal of Clinical Nutrition* 59 (12):1367–73. doi: 10.1038/sj.ejcn.1602248.

Ullegaddi, R., H. J. Powers, and S. E. Gariballa. 2004. B-group vitamin supplementation mitigates oxidative damage after acute ischaemic stroke. *Clinical Science* 107 (5):477–84. doi: 10.1042/CS20040134.

VITATOPS Trial Study Group. 2010. B vitamins in patients with recent transient ischaemic attack or stroke in the VITAmins TO Prevent Stroke (VITATOPS) trial: A randomised, double-blind, parallel, placebo-controlled trial. *The Lancet Neurology* 9 (9):855–65. doi: 10.1016/S1474-4422(10)70187-3.

Wald, D. S., M. Law, and J. K. Morris. 2002. Homocysteine and cardiovascular disease: Evidence on causality from a meta-analysis. *BMJ (Clinical Research ed.)* 325 (7374):1202. doi: 10.1136/bmj.325.7374.1202.

Wang, J., L. Chen, Y. Zhang, C-g Li, H. Zhang, Q. Wang, X. Qi, L. Qiao, W-w Da, X-j Cui, et al. 2019. Association between serum vitamin B6 concentration and risk of osteoporosis in the middle-aged and older people in China: A cross-sectional study. *BMJ Open* 9 (7):e028129. doi: 10.1136/bmjopen-2018-028129.

Wang, X., X. Qin, H. Demirtas, J. Li, G. Mao, Y. Huo, N. Sun, L. Liu, and X. Xu. 2007. Efficacy of folic acid supplementation in stroke prevention: A meta-analysis. *Lancet (London, England)* 369 (9576):1876–82. doi: 10.1016/S0140-6736(07)60854-X.

Wang, Y., Y. Jin, Y. Wang, L. Li, Y. Liao, Y. Zhang, and D. Yu. 2019. The effect of folic acid in patients with cardiovascular disease: A systematic review and meta-analysis. *Medicine* 98 (37):e17095. doi: 10.1097/MD.0000000000017095.

Watanabe, F., Y. Yabuta, T. Bito, and F. Teng. 2014. Vitamin B_{12}-containing plant food sources for vegetarians . *Nutrients* 6 (5):1861–73. doi: 10.3390/nu6051861.

Weng, L.-C., W.-T. Yeh, C.-H. Bai, H.-J. Chen, S.-Y. Chuang, H.-Y. Chang, B.-F. Lin, K.-J. Chen, and W.-H. Pan. 2008. Is ischemic stroke risk related to folate status or other nutrients correlated with folate intake? *Stroke* 39 (12):3152–8. doi: 10.1161/STROKEAHA.108.524934.

Wolters, M., A. Strohle, and A. Hahn. 2004. Cobalamin: A critical vitamin in the elderly. *Preventive Medicine* 39 (6):1256–66. doi: 10.1016/j.ypmed.2004.04.047.

Wrone, E. M., J. M. Hornberger, J. L. Zehnder, L. M. McCann, N. S. Coplon, and S. P. Fortmann. 2004. Randomized trial of folic acid for prevention of cardiovascular events in end-stage renal disease. *Journal of the American Society of Nephrology: JASN* 15 (2):420–6. doi: 10.1097/01.asn.0000110181.64655.6c.

Wu, X., L. Zhang, Y. Miao, J. Yang, X. Wang, C. C. Wang, J. Feng, and L. Wang. 2019. Homocysteine causes vascular endothelial dysfunction by disrupting endoplasmic reticulum redox homeostasis. *Redox Biology* 20:46–59. doi: 10.1016/j.redox.2018.09.021.

Yamada, K. 2013. Cobalt: Its role in health and disease. *Metal Ions in Life Sciences* 13:295–320. doi: 10.1007/978-94-007-7500-8_9.

Yang, L. K., K. C. Wong, M. Y. Wu, S. L. Liao, C. S. Kuo, and R. F. S. Huang. 2007. Correlations between folate, B12, homocysteine levels, and radiological markers of neuropathology in elderly post-stroke patients. *Journal of the American College of Nutrition* 26 (3):272–8. doi: 10.1080/07315724.2007.10719611.

Younes-Mhenni, S., L. Derex, M. Berruyer, N. Nighoghossian, F. Philippeau, M. Salzmann, and P. Trouillas. 2004. Large-artery stroke in a young patient with Crohn's disease. Role of vitamin B6 deficiency-induced hyperhomocysteinemia. *Journal of the Neurological Sciences* 221 (1-2):113–5. doi: 10.1016/j.jns.2004.03.016.

Zaric, B. L., M. Obradovic, V. Bajic, M. A. Haidara, M. Jovanovic, and E. R. Isenovic. 2019. Homocysteine and hyperhomocysteinaemia. *Current Medicinal Chemistry* 26 (16):2948–61. doi: 10.2174/0929867325666180313105949.

Zeng, R., C.-H. Xu, Y.-N. Xu, Y.-L. Wang, and M. Wang. 2015. The effect of folate fortification on folic acid-based homocysteine-lowering intervention and stroke risk: A meta-analysis. *Public Health Nutrition* 18 (8):1514–21. doi: 10.1017/s1368980014002134.

Zhang, D., X. Jiang, P. Fang, Y. Yan, J. Song, S. Gupta, A. I. Schafer, W. Durante, W. D. Kruger, X. Yang, et al. 2009. Hyperhomocysteinemia promotes inflammatory monocyte generation and accelerates atherosclerosis in transgenic cystathionine beta-synthase-deficient mice. *Circulation* 120 (19):1893–902. doi: 10.1161/CIRCULATIONAHA.109.866889.

Zhang, T., Y. Jiang, S. Zhang, T. Tie, Y. Cheng, X. Su, Z. Man, J. Hou, L. Sun, M. Tian, et al. 2020. The association between homocysteine and ischemic stroke subtypes in Chinese: A meta-analysis. *Medicine* 99 (12):e19467. doi: 10.1097/MD.0000000000019467.

Zhou, Z., J. Li, Y. Yu, Y. Li, Y. Zhang, L. Liu, Y. Song, M. Zhao, Y. Wang, G. Tang, et al. 2018. Effect of smoking and folate levels on the efficacy of folic acid therapy in prevention of stroke in hypertensive men. *Stroke* 49 (1):114–20. doi: 10.1161/STROKEAHA.117.018273.

Zhu, J. H., J. Z. Chen, X. X. Wang, X. D. Xie, J. Sun, and F. R. Zhang. 2006. Homocysteine accelerates senescence and reduces proliferation of endothelial progenitor cells. *Journal of Molecular and Cellular Cardiology* 40 (5):648–52. doi: 10.1016/j.yjmcc.2006.01.011.

Zinellu, A., S. Sotgia, B. Scanu, G. Pintus, A. M. Posadino, A. Cossu, L. Deiana, S. Sengupta, and C. Carru. 2009. S-homocysteinylated LDL apolipoprotein B adversely affects human endothelial cells in vitro. *Atherosclerosis* 206 (1):40–6. doi: 10.1016/j.atherosclerosis.2009.01.035.

Zoungas, S. B. P., McGrath, P. Branley, P. G. Kerr, C. Muske, R. Wolfe, R. C. Atkins, K. Nicholls, M. Fraenkel, B. G. Hutchison, et al. 2006. Cardiovascular morbidity and mortality in the Atherosclerosis and Folic Acid Supplementation Trial (ASFAST) in chronic renal failure: A multicenter, randomized, controlled trial. *Journal of the American College of Cardiology* 47 (6):1108–16. doi: 10.1016/j.jacc.2005.10.064.