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Review Article

Optimizing Vitamin Status to Sustain Vascular and Pulmonary Health

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Abstract

Vitamins, particularly folate (vitamin B9), vitamins B6 and B12, and vitamins C, D, and E, are indispensable regulators of endothelial function, and their deficiency or insufficiency has been implicated in a broad range of vascular and degenerative disorders, including those affecting the pulmonary circulation. This narrative review synthesizes current evidence on the mechanisms by which vitamin status influences vascular health, with emphasis on folate metabolism, homocysteine regulation, and the clinical consequences of deficiency across systemic, retinal, cerebral, and pulmonary vascular beds.

Folate is a central cofactor in one-carbon metabolism, supporting the remethylation of homocysteine to methionine. Elevated homocysteine impairs endothelial function through oxidative stress, reduced nitric oxide bioavailability, and accumulation of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase. These mechanisms are directly relevant to the pulmonary microvasculature: asymmetric dimethylarginine has been shown to predict the development of pulmonary arterial hypertension under hypoxic conditions, and severe vitamin B12 deficiency has been reported to produce pulmonary vascular congestion, edema, and hypoxemia. Cross-sectional data in elderly and chronic obstructive pulmonary disease populations further link lower serum folate and higher homocysteine to impaired spirometric parameters.

Beyond homocysteine lowering, folate, particularly in its bioactive form, L-methylfolate, enhances nitric oxide production through regeneration of tetrahydrobiopterin, a cofactor for nitric oxide synthase. Clinical trials in patients with coronary artery disease demonstrate that supplementation improves flow-mediated dilation, a validated measure of endothelial function, at doses of 5 to 10 milligrams per day of folic acid. Evidence from retinal disease studies using medical-food doses of L-methylfolate with vitamins B12 and B6 shows reduction in homocysteine, restoration of capillary perfusion, and improved clinical outcomes in diabetic retinopathy, glaucoma, and age-related macular degeneration.

However, excessive supplementation with synthetic folic acid raises concerns: it generates unmetabolized folic acid in the bloodstream, which may reduce cellular folate availability and is associated with increased cardiovascular mortality at high intake levels. Genetic polymorphisms in folate-metabolizing enzymes further affect individual responses to supplementation. Plasma homocysteine serves as a practical, low-cost biomarker for identifying patients likely to benefit from correction of folate and vitamin B12 status, with preference for bioactive reduced forms over synthetic folic acid. Prospective trials measuring pulmonary capillary perfusion and gas exchange before and after correction of vitamin insufficiency are warranted.

Keywords: *Folate; Homocysteine; Vitamin B12; Endothelial Function; Nitric Oxide; Edema; Pulmonary Vascular Disease; L-Methylfolate*

Abbreviations

L-5-MTHF: 5-Methyl-(6S)-Tetrahydrofolate, L-Methylfolate; MTHFR: MTHF Reductase; DHFR: Dihydrofolate Reductase; CVD: Cardiovascular Disease; FMD: Flow Mediated Diffusion; CAD: Coronary Artery Disease; VEGF: Vascular Endothelial Growth Factor; UMFA: Unmetabolized Folic Acid; DFE: Dietary Folate Equivalent; CFD: Cerebral Folate Deficiency; AMD: Age-related Macular Degeneration; MCI: Mild Cognitive Impairment; ASD: Autism Spectrum Disorder

Vitamins in vascular health

The endothelium, a continuous monolayer of cells lining the inner surface of blood and lymphatic vessels, is central to vascular homeostasis. Its integrity governs vascular tone, blood fluidity, and barrier function. When vitamin absorption is compromised, as frequently occurs in conditions such as age-related macular degeneration, lymphedema, or pulmonary vascular disease, endothelial function deteriorates and disease progression is accelerated [1,2]. Among the micronutrients that sustain this system, vitamins of the B group hold particular importance, as do vitamins C, D, and E. These nutrients collectively support endothelial processes including one-carbon metabolism, DNA synthesis and repair, gene methylation, and the attenuation of oxidative and inflammatory signaling. This chapter surveys the vitamins most consequential for vascular endothelial health, with particular attention to vitamin B9 (folate), its chemical forms, relative bioavailability, and the vascular consequences of its deficiency.

Vitamins essential for endothelial health: Folate

Folate (vitamin B9) occupies a pivotal position in one-carbon metabolism, a network of biochemical reactions underpinning nucleotide biosynthesis, DNA and RNA replication, and cell division and repair [3-5]. Within this network, folate additionally participates in methylation reactions that control gene expression and govern amino acid interconversions [4].

Together with vitamin B12, folate drives the remethylation of homocysteine to methionine. This conversion is clinically significant: when folate or B12 status is inadequate, homocysteine accumulates to concentrations that are directly damaging to vascular endothelium [3,6] (See figure 1).

Dietary folate exists in two broad forms. The naturally occurring form, abundant in leafy green vegetables, legumes, and liver, is a reduced compound that must undergo enzymatic transformation within the body before reaching its biologically active state [3,5]. The synthetic counterpart, folic acid, is an oxidized and chemically stable molecule used in dietary supplements and mandatory food fortification programs. Folic acid is absorbed with greater efficiency than food folate (approximately 85% vs. 50% bioavailability), yet it must first be reduced in the intestinal tract and liver before ultimately being converted to the active metabolite 5-methyl-(6S)-tetrahydrofolate (L-5-MTHF, commonly termed L-methylfolate) [3,5]. Figure 1 illustrates these parallel conversion pathways and the central role of L-methylfolate as the biologically active end-product [6].

A practically relevant distinction among commercially available supplements concerns stereochemistry. Certain products contain a racemic mixture of L- and D-methylfolate in equal proportions. Because only the L-isomer is metabolically active, achieving a given therapeutic dose requires twice the total amount of the racemic preparation compared with a pure L-methylfolate product. The same principle applies to folinic acid (leucovorin): the active enantiomer, levoleucovorin, delivers equivalent clinical effect at half the dose of the racemate. Plants biosynthesize exclusively the L-form; this stereoselectivity is not replicated in all synthetic preparations. Throughout this paper, "methylfolate" refers specifically to the active L-methylfolate (L-5-MTHF), and we note that the full chemical designation L-5-methyl-(6S)-tetrahydrofolate and the abbreviated L-methylfolate denote the identical molecule. While L-methylfolate is generally available without a prescription, leucovorin and levoleucovorin are regulated pharmaceutical products. In clinical practice, formulations containing exclusively the L-isomer of either methylfolate or folinic acid are preferred, as they avoid the diluting effect of the inactive D-isomer on therapeutic potency.

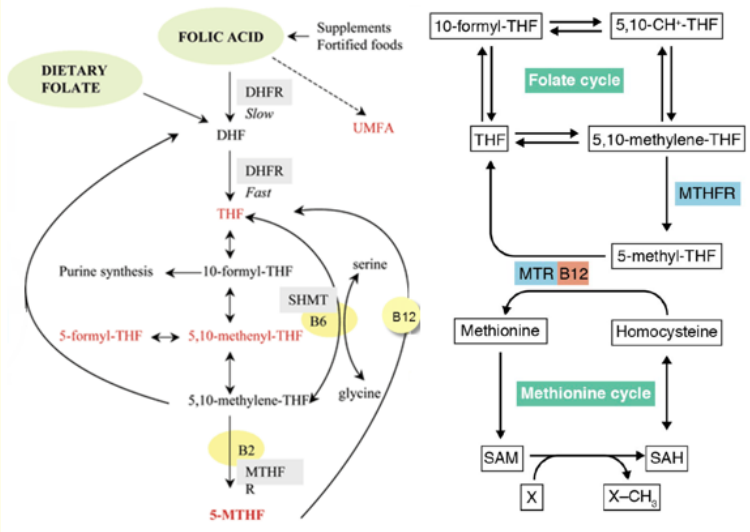

Figure 1: *Metabolism of vitamin B9. The left panel depicts conversion of dietary folate or supplemental folic acid to the biologically active form, L-methylfolate (5-MTHF), via sequential reduction steps in the gut and liver. The right panel illustrates how folate, in concert with vitamins B6 and B12, supports the conversion of homocysteine to methionine, thereby preventing homocysteine-mediated endothelial injury [7,8].*

Steps in the folate pathway

Ingested folates are predominantly in polyglutamated form; they are cleaved to monoglutamates within the small intestinal brush border and then absorbed through dedicated transport proteins, principally the proton-coupled folate transporter. Following entry into the circulation, folate is distributed to target tissues via reduced folate carriers and folate receptors expressed on cell surfaces [5,9].

Inside cells, sequential reduction by the enzyme dihydrofolate reductase (DHFR) converts folate first to dihydrofolate (DHF) and subsequently to tetrahydrofolate (THF). THF serves as the core carrier of one-carbon units across a range of oxidation states, giving rise to functionally distinct derivatives including 5,10-methylene-THF, 5-methyl-THF, and 10-formyl-THF [5,10].

These THF derivatives feed three major metabolic outputs: synthesis of purines and thymidylate (dTMP) for DNA and RNA assembly; regeneration of methionine from homocysteine (through 5-methyl-THF and methionine synthase); and provision of methyl groups for S-adenosylmethionine (SAM)-dependent methylation reactions [5].

Folate metabolism proceeds concurrently in the cytosol and mitochondria, with each compartment housing distinct enzymatic machinery and folate derivatives. Tight regulatory controls and dedicated repair mechanisms help maintain folate homeostasis within cells [10,11].

Folate deficiency/insufficiency and vascular dysfunction

Inadequate folate, whether frank deficiency or subclinical insufficiency, disrupts nucleotide synthesis, produces megaloblastic anemia, and elevates circulating homocysteine, each of which independently heightens risk for vascular disease including atherosclerosis, stroke, and related degenerative conditions [3,4]. The elevation of homocysteine arising from poor folate status impairs endothelial-dependent signaling and amplifies oxidative stress, a recognized driver of vascular pathology [3,6].

Repleting folate status through folic acid or L-methylfolate supplementation improves endothelial function, particularly among individuals with cardiovascular risk factors or established disease, by augmenting nitric oxide (NO) bioavailability and attenuating oxidative burden [3,6,12]. While mandatory folic acid fortification in the United States has meaningfully lowered the prevalence of neural tube defects and raised population-level folate status, concerns persist regarding the accumulation of unmetabolized folic acid (UMFA) at high supplementation doses, which may competitively impair cellular uptake or utilization of L-methylfolate [4,5].

Vitamins essential for endothelial health: Pyridoxine and cobalamin

Both vitamin B6 (pyridoxine) and vitamin B12 (cobalamin) function as cofactors in homocysteine metabolism, acting alongside folate to complete the conversion of homocysteine to methionine [3,5]. Vitamin B6 enables the transsulfuration pathway, in which homocysteine is sequentially converted to cystathionine and then cysteine. Adequate B6 thereby prevents hyperhomocysteinemia and its downstream endothelial toxicity [3]. Vitamin B6 additionally dampens the expression of endothelial adhesion molecules and pro-inflammatory cytokines, providing a separate anti-inflammatory benefit within the vasculature [13].

Vitamin B12, acting synergistically with folate, is required for the remethylation branch of homocysteine disposal; deficiency in either nutrient potentiates homocysteine elevation and the attendant vascular risk [14]. Beyond the vasculature, B12 and folate together contribute to myelin formation and maintenance, suggesting a potential therapeutic role for B12 in neuropathic conditions involving demyelination and impaired nerve regeneration [15].

Both vitamins are efficiently absorbed from dietary and supplemental sources under normal physiological conditions, and both support DNA synthesis and genomic stability [4]. Absorption capacity, however, declines with advancing age and is compromised by various gastrointestinal disorders [5]. Deficiency in either nutrient can precipitate elevated homocysteine and an increased burden of cardiovascular risk [16].

Vitamins essential for endothelial health: Ascorbic acid

Vitamin C (ascorbic acid) is a potent hydrophilic antioxidant that scavenges reactive oxygen species, thereby limiting oxidative damage to endothelial cells and reducing oxidative stress-driven dysfunction. It also participates in the regeneration of bioactive nitric oxide, a vasodilatory signaling molecule, thus supporting healthy vascular tone and countering hypertension. Beyond its antioxidant functions, vitamin C provides a structural contribution to vascular integrity through its requirement for collagen biosynthesis, which underpins the mechanical stability of the vessel wall [3,17].

Vitamin C is absorbed rapidly and with high efficiency from fruits and vegetables, reflecting its water-soluble nature and the widespread expression of intestinal ascorbate transporters [3].

Vitamins essential for endothelial health: Vitamin D

Endothelial cells express vitamin D receptors, and receptor activation shifts the transcriptional balance toward suppression of pro-inflammatory genes while upregulating anti-inflammatory pathways. Through this mechanism, vitamin D modulates endothelial immune responsiveness and helps maintain vascular resilience [17-21]. At the hemodynamic level, vitamin D participates in regulation of the renin-angiotensin-aldosterone axis, a principal determinant of blood pressure [19]. Insufficient vitamin D is associated with reduced endothelial conductance and increased arterial stiffness [22], and a recent meta-analysis has linked low serum vitamin D with elevated cardiovascular disease risk, with supplementation conferring measurable protective effects [20].

Vitamin D is obtained through cutaneous synthesis upon ultraviolet light exposure, as well as from fortified foods and supplements [3,17].

Vitamins essential for endothelial health: Tocopherol and vitamin K2

Vitamin E, and alpha-tocopherol in particular, is a lipid-soluble antioxidant that integrates into cell membranes and protects endothelial phospholipids from peroxidation. By reducing LDL oxidation, it may slow atheromatous plaque formation, and there is evidence that it modulates platelet aggregation and vascular inflammation as well [3,18]. Dietary vitamin E is absorbed in conjunction with fat and is found principally in nuts, seeds, and plant-derived oils [3].

Vitamin K2 activates matrix Gla-protein, an inhibitor of soft tissue calcification, and through this mechanism reduces arterial stiffness. Given the high prevalence of vitamin K deficiency in the United States population, inadequate K2 status may represent a meaningful, underrecognized contributor to cardiovascular disease risk [23].

Public health aspects

Nutritional sufficiency

Epidemiological data consistently reveal that suboptimal intake of key vitamins is widespread, including in high-income nations, and is associated with progression of atherosclerotic disease and adverse cardiovascular outcomes. Vitamins B9, B12, D, and C are among those most frequently found to be below optimal levels in population surveys [18,24,25]. Addressing these gaps is ideally accomplished through adoption of cardiovascular-protective dietary patterns [26]; however, supplementation remains an appropriate strategy in individuals with impaired absorption, or in those with genetic or immune conditions that limit bioavailability of specific nutrients.

Genetic polymorphisms

Variants in genes encoding enzymes of folate and homocysteine metabolism, most notably MTHFR polymorphisms, reduce the efficiency of conversion from folic acid to metabolically active forms, thereby amplifying the clinical consequences of suboptimal folate intake and increasing vascular risk. In these individuals, direct provision of L-methylfolate, or of the pharmaceutical-grade drug product levoleucovorin, circumvents the impaired enzymatic step and restores adequate active folate [4,27].

Concerns about whether genetic heterogeneity might alter the efficacy or safety profile of such interventions are addressed by decades of clinical experience: leucovorin and levoleucovorin have been administered in cancer treatment protocols, including in pediatric patients at doses far exceeding those relevant to vascular supplementation, without evidence of harm, and with consistent benefit across diverse ethnic groups [28].

Supplementation and disease prevention

Mandatory folic acid fortification has generated clear public health gains, most notably in reducing the incidence of neural tube defects. This benefit must nonetheless be weighed against emerging evidence that individuals who cannot efficiently convert folic acid to its active form, including those who are otherwise replete and those carrying relevant gene variants, may experience adverse effects from high-dose folic acid supplementation [12,18].

Vitamins are indispensable for vascular health through their complementary roles in endothelial cell physiology, modulation of oxidative and inflammatory signaling, and regulation of homocysteine metabolism. Early identification and correction of vitamin deficiencies or insufficiencies, targeted supplementation in at-risk individuals, and consistent adherence to nutrient-rich dietary patterns together represent a compelling evidence-based framework for reducing vascular disease burden and supporting healthy aging.

Folate sources, absorption, and bioavailability

Vitamin B9, or folate, is a water-soluble essential nutrient whose functions span nucleotide biosynthesis, amino acid interconversion, and the methylation reactions that regulate gene expression. It occurs in nature as a family of reduced compounds found across a wide range of foods, and also as the synthetic oxidized derivative folic acid, which is deployed in supplements and fortification programs owing

to its chemical stability. A working understanding of these distinct sources, their comparative absorption efficiencies, and the genetic factors that shape individual folate metabolism is fundamental to preventing deficiency-related disease and optimizing therapeutic strategies [4,25,29,30].

Folate from foods

Folate is distributed across many food categories, with the richest concentrations found in dark leafy greens, organ meats, and certain vegetables; spinach, liver, asparagus, and Brussels sprouts are among the most concentrated sources [27,30]. Representative food categories and examples are compiled in table 1. The predominant folate species in these foods are polyglutamated tetrahydrofolate derivatives, including L-methylfolate, that serve essential metabolic roles. Before absorption can occur, the glutamate chains must be enzymatically cleaved in the intestinal lumen to yield the monoglutamate form that enters the circulation and becomes available to cells [31].

Food Category	Example Foods
Dark green leafy vegetables	Spinach, kale, collard greens, romaine lettuce
Legumes	Beans, lentils, peas
Fruits	Oranges, avocados, bananas, melons
Nuts and seeds	Peanuts, sunflower seeds
Other vegetables	Asparagus, Brussels sprouts, broccoli, beets
Animal products	Liver, eggs (liver especially high)
Whole grains	Fortified and unfortified whole grain products

Table 1: *Key food sources of folate.*

Sources: [4,25,27,30].

Folic acid, by contrast, is a monoglutamate compound in its fully oxidized state. This chemical structure confers long-term stability and eliminates the need for deconjugation from a food matrix prior to absorption. Folic acid is incorporated into fortified grain products and dietary supplements, where it provides consistent and high-bioavailability folate activity, compensating for the variability and processing losses inherent to food-derived folate and contributing to the reduction of population-level deficiency [30,32,33].

A further category of folate-containing products encompasses medical foods and pharmaceutical agents. Medical foods formulated with L-methylfolate (L-5-MTHF) provide the biologically active form directly, bypassing the conversion steps required for folic acid. Leucovorin and its active enantiomer levoleucovorin are classified as drug products; they are used clinically to replenish folate depleted by cytotoxic chemotherapy and have also been investigated as a treatment for social communication impairments in autism [34]. Both agents are available in pure L-isomer formulations, though historically racemic mixtures of the L- and D-isomers were common. In racemic preparations only 50% of the compound is metabolically active, necessitating doses twice as large as those required when the pure L-isomer is administered.

Bioavailability and absorption

The bioavailability of naturally occurring food folate is both inherently variable and substantially lower than that of synthetic folic acid. Conventional estimates place the bioavailability of food folate at roughly 50 - 60% relative to folic acid consumed with a meal [5,29,35].

Three principal factors account for this reduction. First, folate molecules may be tightly sequestered within plant cell structures, limiting their physical accessibility during digestion. Second, the polyglutamated nature of food folate necessitates enzymatic deconjugation before

intestinal absorption can proceed. Third, folate is susceptible to degradation by heat, light, and oxidation, so a meaningful proportion is lost during food preparation and storage [5,29,35].

More granular analyses suggest that bioavailability across different foods and preparation methods may span approximately 44% to 80%, with a central estimate near 65% relative to folic acid [29,35,36]. Food labeling employs the dietary folate equivalent (DFE) to standardize these comparisons, with 1 DFE defined as 0.6 µg of folic acid or L-methylfolate. This variability in absorption efficiency, combined with matrix-dependent folate release and processing sensitivity, is the primary rationale for public health policies centered on folic acid fortification, policies that have demonstrably elevated population folate status and reduced neural tube defect incidence [5,30].

Genetic influences on folate metabolism

A number of relatively common gene variants alter folate absorption, intracellular metabolism, and overall methylation capacity, with meaningful clinical implications.

MTHFR (methylenetetrahydrofolate reductase) C677T and A1298C polymorphisms impair the enzymatic conversion of folic acid and dietary folates to the active L-methylfolate species, resulting in elevated circulating homocysteine and heightened susceptibility to neural tube defects and, in certain populations, to autism spectrum disorder (ASD) [37-40]. Carriers of these variants may not adequately benefit from folic acid supplementation and are often better served by direct provision of L-methylfolate [1,7,41].

MTR, MTRR, FOLH1, RFC1, and SHMT gene variants each affect distinct steps in folate absorption, tissue distribution, or metabolic utilization, with downstream consequences for DNA methylation patterns and associated health outcomes [37-40].

DHFR (dihydrofolate reductase) variability influences the rate at which ingested folic acid is reduced to tetrahydrofolate, the obligatory precursor for active folate derivatives. Individuals with diminished DHFR activity may accumulate unmetabolized folic acid, a form with uncertain biological effects [42].

Folate transport into the brain

Neurological function depends on adequate folate delivery across the blood-brain barrier (BBB), a process mediated by at least two specialized transport systems.

Folate receptor alpha (FRα) executes high-affinity, active transport of folate across the choroid plexus into the cerebrospinal fluid and brain parenchyma. Loss-of-function mutations in the FOLR1 gene encoding FRα cause cerebral folate deficiency (CFD), a condition marked by neurological deterioration despite potentially normal blood folate levels.

The reduced folate carrier (RFC, SLC19A1) facilitates folate uptake into diverse tissues including the brain, but operates by passive facilitated diffusion rather than active transport, meaning it can only equilibrate brain folate concentrations to the prevailing plasma level rather than concentrate folate above it. Under normal circumstances, brain folate concentrations run two- to fivefold higher than those in blood, with the ratio being highest, approximately fivefold, in early childhood and declining progressively through adolescence toward the adult twofold differential. Genetic variation in the RFC gene may therefore constrain folate delivery to the central nervous system. Emerging evidence additionally implicates the vitamin D receptor (VDR) in regulating folate transport across the BBB, particularly in specific neurological disease contexts [44,45].

When any of these transport mechanisms is compromised, whether by inherited mutations, circulating autoantibodies targeting FRα (FRAAs), or intercurrent disease, the result is cerebral folate deficiency: insufficient brain folate even when systemic folate status appears

adequate. Administration of leucovorin or levoleucovorin can in some cases circumvent the transport defect and normalize brain folate concentrations [45].

Taken together, these genetic determinants explain much of the interindividual variation in folate requirements, the heterogeneity of responses to supplementation, and differential susceptibility to conditions associated with folate insufficiency at the plasma and cerebral levels, including congenital anomalies, cardiovascular disease, and autism spectrum disorder [37-40].

Ongoing research continues to delineate the interplay among genetic variation, environmental exposures, and folate-dependent metabolic pathways, with the longer-term goal of enabling genuinely personalized folate intake recommendations [46].

Cardiovascular health

Folate supports endothelial function through both homocysteine-dependent and homocysteine-independent mechanisms. Human clinical data demonstrate that folate can enhance vascular reactivity and reduce cardiovascular disease (CVD) event rates, particularly in populations with low baseline folate or elevated homocysteine. Cardiovascular benefit is dose-dependent, and excessive supplementation, especially with folic acid, carries potential risks. Other vitamins, notably B6, B12, C, D, E, and K, contribute complementary vascular protection through antioxidative, anti-inflammatory, and structural mechanisms.

Folate and endothelial function

As described in the preceding chapter, folate is indispensable for one-carbon metabolism and for the remethylation of homocysteine to methionine. Elevated circulating homocysteine is an established independent risk factor for CVD. At the mechanistic level, excess homocysteine promotes endothelial dysfunction by amplifying oxidative stress, curtailing nitric oxide (NO) bioavailability, and directly injuring endothelial cells. By accelerating homocysteine clearance through remethylation, folate supplementation reduces plasma homocysteine and may thereby attenuate these adverse vascular consequences [6,12,47,48].

Beyond this homocysteine-lowering effect, folate exerts direct endothelial benefits through independent pathways. Experimental work has shown that both folic acid and its bioactive metabolite L-methylfolate can improve endothelial function by reducing intracellular superoxide and augmenting NO production within endothelial cells [6,12]. This mechanism accounts for the observation that endothelial-dependent vasodilation often improves with folate even when reductions in homocysteine are modest or absent.

Endothelial dysfunction is an early and central event in atherogenesis, preceding plaque formation and influencing disease progression. Improvements in endothelial function, typically quantified by flow-mediated dilation (FMD), correlate with reduced cardiovascular risk [12,49]. Both hyperhomocysteinemia and suboptimal folate status have been associated with elevated cardiovascular risk in epidemiological and mechanistic studies. One notable exception, published over a decade ago, did not identify a connection between large vessel disease and homocysteine elevation [50]. The body of work cited in this paper nonetheless demonstrates that elevated homocysteine is linked to small vessel disease and that L-methylfolate supplementation can lower homocysteine and restore capillary diffusion capacity.

Evidence for improved blood flow

A convergence of animal and human data supports the view that folate, together with adequate B6 and B12, improves vascular reactivity and tissue perfusion. In animal models, folate supplementation has been shown to reverse hyperhomocysteinemia-induced endothelial dysfunction [3].

In human studies, a series of randomized controlled trials and observational investigations have demonstrated that high-dose folic acid (5 - 10 mg/day) improves FMD and overall endothelial function in patients with and without hyperhomocysteinemia [6,12,49,51].

Critically, vascular improvements frequently precede detectable changes in homocysteine, implicating additional protective mechanisms including enhanced antioxidant capacity and direct effects on NO generation.

Vitamins C, E, D, and K contribute further to circulatory health through their antioxidant properties, their roles in blood pressure regulation, and their structural support of the vessel wall, collectively reducing risk for atherosclerosis and peripheral vascular disease [3,17].

Clinical evidence

Trials in patients with coronary artery disease (CAD) have shown that folic acid supplementation at 5 mg for six weeks raises plasma folate and improves FMD even when homocysteine falls only modestly [6,12,49]. Meta-analyses indicate that folic acid supplementation is associated with modest reductions in overall cardiovascular event rates and stroke risk, with the most pronounced benefits observed in primary prevention and in individuals who begin with low folate status or substantial homocysteine elevation [53].

While moderate folate intake correlates with reduced cardiovascular mortality, data suggest that escalating folic acid doses beyond a certain threshold yield no further incremental benefit and may introduce risk [54-56]. A plausible explanation lies in the limited daily capacity for folic acid conversion: an estimated ceiling of approximately 400 μg of folic acid can be processed per day [57]. Intake beyond this level results in accumulation of unmetabolized folic acid (UMFA) in the circulation. This constraint appears to be particularly pronounced in humans relative to rodents, where conversion capacity is roughly 80-fold greater [57]. Accumulating evidence indicates that UMFA impairs cellular access to active folate [58,59], rendering high-dose folic acid supplementation counterproductive in some circumstances [60].

Large randomized trials in specific high-risk populations, such as those with chronic kidney disease, have not consistently demonstrated cardiovascular event reduction with prolonged folate supplementation, underscoring the importance of individual baseline folate status, homocysteine levels, and comorbid factors in predicting treatment response [61].

Key trial outcomes are summarized in table 2.

Outcome	Dose/Duration/Ref	Population	Notes
Improved FMD†, lowered homocysteine	5 mg folic acid, 6 wks [6,12]	CAD† patients	Benefits independent of homocysteine
Improved FMD†	5-10 mg, 6-8 wks [49]	Hyperhomocysteinemia	
Reduced plaque progression	2.5-5 mg, 4y [49]	Premature atherosclerosis	With B6, B12
J-shaped risk curve; benefit at modest folate intake	Various (food/suppl) [54]	US adults at CVD† risk	High intake possibly adverse
↓ CVD† events (RR ~0.96), ↓ stroke (RR ~0.90)	Various [53]	General and high-risk adults	Greater benefit with low baseline
No effect on CVD† risk	Folic acid, 2 y [61]	CVD† patients	

Table 2: *Evidence for folate in cardiovascular conditions.*

† FMD: Flow-Mediated Dilation; CAD: Coronary Artery Disease; CVD: Cardiovascular Disease.

The therapeutic implications for CVD management are as follows. When homocysteine is elevated, folate supplementation at the doses listed in table 2 improves outcomes. In the absence of homocysteine elevation, however, benefit appears minimal, establishing plasma homocysteine as an essential biomarker for guiding treatment decisions. Folate toxicity is not generally a concern [26], with one important exception: patients presenting with both elevated homocysteine and elevated plasma folic acid should not receive additional folic acid, as the presence of UMFA may worsen outcomes. In such cases, a reduced-form folate, either folinic acid or L-methylfolate, is the preferred treatment vehicle. As a general clinical principle, patients with elevated homocysteine should have plasma folate measured and should be screened for vitamin B12 deficiency, which elevated homocysteine may mask. Where supplementation is indicated, L-methylfolate is advisable, with concurrent monitoring and treatment of any B12 insufficiency. Folic acid remains an option in this setting but carries the risk of UMFA accumulation and its downstream consequences [60].

Peripheral circulation, lymphedema, and glymphatic function

Folate exerts a wide-ranging influence on circulatory physiology, encompassing improvements in endothelial function, augmentation of nitric oxide signaling, and potential enhancement of both blood and lymphatic flow. Clinical data point to meaningful benefits in peripheral artery disease and lymphedema, with emerging implications for lymphatic vessel biology. The question of whether folate influences glymphatic function in the brain remains at the speculative frontier, but its well-characterized vascular effects provide a compelling rationale for investigating its potential role in cerebral waste clearance and the prevention of neurodegenerative disease [62,63].

Folate and peripheral circulation

The endothelial benefits of folate extend into the peripheral vasculature and are not fully explained by homocysteine reduction alone [62]. Acute administration of high-dose folic acid has been shown to lower blood pressure and augment vasodilator-stimulated blood flow in patients with coronary artery disease, an effect attributable primarily to increased nitric oxide bioavailability [3,63]. The underlying mechanism involves the enzymatic restoration of tetrahydrobiopterin, an obligatory cofactor for nitric oxide synthase, thereby sustaining NO production and supporting arterial dilation [62,63].

Folic acid supplementation has additionally been shown to prevent the endothelial dysfunction and nitrate tolerance that develop with continuous nitroglycerin therapy, further establishing its role in preserving nitric oxide synthase activity and vascular homeostasis [3,62]. Importantly, these protective effects are evident in both coronary and peripheral arterial beds, indicating that folate's hemodynamic influence operates across the systemic circulation rather than being confined to a single vascular territory [62-64].

Folate and lymphedema

Folate's circulatory effects appear to extend to the lymphatic system, where it may stimulate lymphangiogenesis, the sprouting and remodeling of lymphatic vessels, and facilitate lymphatic drainage. Human clinical evidence supports this possibility: a reported case study documented successful management of lymphatic congestion and limb swelling in a patient with primary lymphedema through L-methylfolate supplementation [2]. This observation raises the possibility that folate could serve as a useful adjunct in lymphedema management, potentially acting by improving lymphatic vessel contractility and reducing pathological accumulation of interstitial fluid [2,67]. Such an approach would carry a more favorable adverse-effect profile than pharmacological strategies involving exogenous endothelial growth factors, which have been proposed for the same indication [67].

Glymphatic system

The glymphatic system is a perivascular waste-clearance network in the brain, functionally analogous to the peripheral lymphatic system, that removes metabolic byproducts from neural tissue and is increasingly implicated in the pathogenesis of neurodegenerative conditions including dementia [68-70]. Direct evidence linking folate to glymphatic function is currently limited; however, by analogy with the mechanisms documented in peripheral and lymphatic vessels, it has been proposed that folate may support glymphatic flow

through its capacity to enhance nitric oxide production and improve vascular health more broadly [2]. Specifically, if folate sustains perivascular nitric oxide signaling, it may help maintain the patency of perivascular spaces and the function of astroglial aquaporin-4 water channels, the two structural elements that drive convective glymphatic fluid movement [71].

Impaired glymphatic clearance has been hypothesized to permit the accumulation of neurotoxic proteins and metabolic waste in the brain, potentially accelerating dementia risk [72,73]. Were folate to enhance glymphatic efficiency [74], it might confer neuroprotective benefits in aging populations, an effect that has been demonstrated in the retina [1] but that remains to be established across the blood-brain barrier in controlled trials. This hypothesis, while not yet confirmed, carries sufficient therapeutic significance to merit dedicated investigation. Efforts to test this conjecture are underway, including the prospective evaluation of L-methylfolate as an adjunct intervention within an ongoing study of cognitive decline.

Folate, vitamin B12, vascular perfusion, edema, and pulmonary oxygenation

Folate and vitamin B12 are indispensable cofactors in the enzymatic remethylation of homocysteine to methionine, and inadequate status of either nutrient produces hyperhomocysteinemia, a condition with well-characterized consequences for systemic microvascular perfusion and endothelial nitric oxide (NO) signaling [3,75]. Given that pulmonary gas exchange depends critically on adequate perfusion of the alveolar capillary bed and on the integrity of the pulmonary microvasculature, the same pathophysiological mechanisms that compromise systemic and retinal perfusion in hyperhomocysteinemic states carry plausible relevance to pulmonary vascular tone, alveolar-capillary fluid balance, and arterial oxygenation. This chapter reviews the evidence connecting folate and B12 nutritional status to microvascular perfusion, edema formation, and pulmonary oxygenation, and considers the therapeutic implications for pulmonary disease management.

Homocysteine, nitric oxide, and microvascular perfusion

Excess homocysteine disrupts endothelium-dependent, NO-mediated vasodilation through several converging mechanisms: uncoupling of endothelial nitric oxide synthase (eNOS), accumulation of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA), heightened superoxide generation, and the establishment of a chronic low-grade inflammatory state [3,52]. These effects have been directly demonstrated in microvascular preparations: in morbidly obese individuals, the combination of low folate, low B12, and elevated homocysteine was associated with diminished flow-induced dilation of isolated adipose arterioles and reduced responsiveness to exogenous nitric oxide donors [75]. Coronary microvascular endothelial dysfunction has similarly been linked to elevated serum homocysteine in patients presenting with angina and non-obstructive coronary artery disease, an association that persisted independently of conventional cardiovascular risk factors [76].

Because ADMA competitively inhibits nitric oxide synthase across vascular territories, including the pulmonary circulation, the rise in ADMA driven by folate/B12-dependent hyperhomocysteinemia constitutes a mechanistic bridge between B-vitamin nutritional status and pulmonary vascular tone. In a prospective study of military personnel subjected to chronic intermittent hypobaric hypoxia, elevated baseline ADMA predicted subsequent development of high-altitude pulmonary arterial hypertension, implicating impaired NO-dependent pulmonary vasodilation in hypoxic vascular remodeling [77]. Since folate and B12 repletion lower both homocysteine and ADMA concentrations, correction of these deficiencies may help preserve NO-dependent pulmonary vasodilatory reserve under conditions of hypoxic stress [78].

B12 deficiency, pulmonary edema, and hypoxemia

Severe vitamin B12 deficiency can manifest with overt pulmonary pathology. One documented case of severe megaloblastic anemia attributable to B12 deficiency presented with cardiomegaly and pulmonary vascular congestion on chest imaging; the treating clinicians noted that transfusion to correct the anemia risked precipitating or worsening pulmonary edema [79]. In a separate case, a patient

with profound B12 deficiency, markedly elevated homocysteine, and elevated methylmalonic acid developed acute bilateral pulmonary embolism accompanied by hypoxemia (oxygen saturation 94%) and bilateral lower-extremity pitting edema, illustrating the combined thrombotic and fluid-regulatory consequences of B12-deficiency-driven hyperhomocysteinemia [80].

Although these cases represent advanced deficiency rather than subclinical insufficiency, they establish that the vascular and hematologic sequelae of B12 depletion are not restricted to the systemic circulation but can directly perturb pulmonary vascular congestion, interstitial fluid homeostasis, and gas exchange efficiency. They highlight the clinical relevance of assessing folate and B12 status in patients presenting with unexplained dyspnea, pulmonary edema, or pulmonary vascular congestion, particularly when macrocytic anemia or other hematologic indicators of B-vitamin deficiency are present.

Folate, homocysteine, and pulmonary function in chronic respiratory disease

A cross-sectional investigation of elderly Korean adults identified positive associations between serum folate concentration and multiple spirometric measures, including forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and peak expiratory flow; these relationships were observed in men but not in women [24]. Among patients with chronic obstructive pulmonary disease (COPD), folate status has been found to be lower and plasma homocysteine higher than in healthy controls; a pilot intervention found that six weeks of folic acid supplementation significantly reduced homocysteine concentrations in COPD patients, though FEV1 did not change significantly over this abbreviated treatment window [80]. A larger multicenter investigation spanning six pulmonology departments examined serum B12, folate, vitamin D, and homocysteine in stable COPD patients and assessed their relationships to pulmonary function parameters and respiratory symptom burden [81].

Hyperhomocysteinemia has additionally been proposed as a contributor to pulmonary vascular remodeling in pulmonary hypertension, including pulmonary hypertension arising in the context of COVID-19 pneumonia, operating through the oxidative endothelial injury pathways described in section homocysteine, nitric oxide, and microvascular perfusion. Because folate and B12 concentrations are inversely correlated with homocysteine, and because combined supplementation with folic acid, B12, and B6 reliably lowers homocysteine at moderate doses with a favorable safety profile, correction of B-vitamin insufficiency has been proposed as a low-risk adjunctive strategy in pulmonary hypertension patients with elevated homocysteine, complementing dietary optimization and avoidance of homocysteine-raising exposures such as smoking [82].

Considered together, the evidence reviewed in this chapter indicates that folate and B12 insufficiency, acting through hyperhomocysteinemia, ADMA accumulation, and impaired NO-dependent vasodilation, constitute a modifiable contributor to microvascular perfusion deficits, pathological fluid accumulation, and potentially compromised pulmonary oxygenation in patients with chronic respiratory and pulmonary vascular disease. As in the retinal and systemic vascular territories discussed elsewhere in this review, plasma homocysteine may prove a practical, low-cost biomarker for identifying pulmonary patients most likely to benefit from B-vitamin repletion, with preference given to bioactive forms such as L-methylfolate in individuals whose conversion of folic acid is limited by genetic variants or absorptive dysfunction. Prospective trials directly measuring pulmonary capillary perfusion, alveolar-capillary diffusion capacity (DLCO), and arterial oxygenation before and after targeted correction of folate and B12 status are needed to firmly establish the clinical significance of these associations.

Retinal vascularization and retinal disease

Folate deficiency produces hyperhomocysteinemia, which inflicts damage on retinal vascular endothelial cells through oxidative stress, inflammatory signaling, and breakdown of barrier integrity. Adequate folate status and efficient folate transport are therefore prerequisites for homocysteine detoxification, endothelial protection, and preservation of retinal vascular structure in conditions such as diabetic retinopathy, retinal vascular occlusions, glaucoma, and age-related macular degeneration (AMD) [83-86]. Across all four of these

disorders, the retinal capillaries show particular vulnerability to hyperhomocysteinemia, manifesting as reduced vascular perfusion and elevated retinal venous pressure [1,41,87].

Retinal hyperhomocysteinemia and vascular dysfunction

As outlined in earlier chapters, folate is a principal dietary regulator of plasma homocysteine. When folate is sufficient, remethylation of homocysteine to methionine proceeds efficiently, keeping circulating homocysteine within safe limits. Folate deficiency is the most prevalent cause of hyperhomocysteinemia [83,84,86], and the resulting excess homocysteine exerts direct toxic effects on retinal vascular endothelial cells, contributing to the development of retinal vascular occlusions, diabetic retinopathy, glaucoma, and AMD. Mechanistically, homocysteine impairs endothelial cell integrity by triggering oxidative stress, downregulating tight junction proteins, increasing vascular permeability, and activating inflammatory cascades, effects that collectively erode the blood-retinal barrier and compromise the structural stability of the retinal vasculature [84,85,88].

Animal and cellular models demonstrate that sustained elevation of homocysteine causes retinal ganglion cell loss and thinning of inner retinal layers, changes that are partially reversible with folate supplementation [86]. This restorative capacity suggests that folate's protective role in the retina extends beyond homocysteine normalization and may include direct support of endothelial cell viability and antioxidant defense.

The degree to which folate protects the retinal vasculature depends on its successful delivery into retinal cells, predominantly as L-methylfolate. Efficient intracellular folate transport is essential for sustaining inner blood-retinal barrier integrity and ischemic resistance. Critically, transport impairment or localized retinal folate insufficiency can arise even when serum folate concentrations appear normal, resulting in elevated local homocysteine and regional vascular dysfunction [83,89]. L-methylfolate additionally supports nitric oxide (NO) synthesis in retinal endothelial cells; when folate availability is compromised, the resulting reduction in NO production promotes vasoconstriction and amplifies ischemic injury within the retina [83].

Retinal oxidative stress and antioxidants

Oxidative stress is the central mechanism through which hyperhomocysteinemia drives retinal injury. Elevated homocysteine stimulates reactive oxygen species (ROS) generation in retinal endothelial and glial cells, overwhelming the eye's intrinsic antioxidant defenses and precipitating cellular dysfunction and tissue damage [85,90,91].

The oxidative burden imposed by excess homocysteine reduces tight junction protein expression in retinal endothelial cells, increasing vascular permeability and driving blood-retinal barrier (BRB) breakdown, a pathological hallmark shared by diabetic retinopathy and AMD [85,92]. Accumulated ROS further activates inflammatory signaling and induces apoptosis in retinal neurons, including retinal ganglion cells, progressively degrading retinal architecture and visual function [90,92,93]. Compounding this damage, homocysteine simultaneously depletes antioxidant capacity: protective enzymes including superoxide dismutase and glutathione peroxidase, as well as total antioxidant capacity, are reduced in both affected patients and experimental models [91].

The oxidative microenvironment generated by hyperhomocysteinemia also promotes endothelial toxicity and hypercoagulability, raising the risk of thrombotic events such as central retinal vein occlusion [91]. Experimental intervention with antioxidant agents can attenuate these processes, reducing ROS burden and partially restoring barrier function in homocysteine-exposed retinal cell preparations [85]. Oxidative stress thus operates as a master mediator of homocysteine-driven retinal pathology, coordinating vascular dysfunction, barrier failure, neuroinflammation, and neuronal attrition across the spectrum of retinal disease [85,90,93].

Clinical cases

Recent clinical investigations have established the therapeutic value of prescription-grade B-vitamin supplementation, specifically using the L-methylfolate form of vitamin B9, in lowering homocysteine and improving retinal outcomes in patients with ocular disease. The treatment protocol in each study combined L-methylfolate with vitamins B12 and B6, together with the antioxidant formulation validated by the AREDS2 trial, in a product known as Ocufolin. This combination constitutes a medical food that integrates the established AREDS2 antioxidant platform with L-methylfolate and complementary B vitamins to support endothelial cell health, an expanded formulation that some investigators have proposed designating AREDS3. Across all studies, treatment consistently reduced homocysteine and improved retinal perfusion, translating into better clinical outcomes within each retinal disease category.

In diabetic retinopathy, L-methylfolate supplementation lowered homocysteine and improved retinal perfusion while concurrently reducing stroke risk, leading investigators to recommend that these patients undergo retinal imaging as a window into central nervous system microangiopathy [94].

In a small case series, a similar vitamin regimen produced benefit in glaucomatous disease. Retinal venous pressure, measured directly in both high-tension and normal-tension glaucoma patients, was found to be elevated in both groups, and Ocufolin therapy reduced this pressure, improving retinal perfusion and patient outcomes [7,41]. This study carries particular significance for normal-tension glaucoma, a condition notoriously difficult to manage, by demonstrating that elevated retinal venous pressure is present even in the absence of elevated intraocular pressure, and that targeted vitamin therapy can address this treatable component. Retinal venous pressure measurement offers a more direct assessment of the intraocular hemodynamic conditions that reduce vascular perfusion and underlie disorders such as glaucoma [87].

AMD patients evaluated under the same framework showed analogous findings: Ocufolin therapy reduced both retinal venous pressure and homocysteine levels. A further observation was that this oral vitamin regimen may reduce the frequency of anti-VEGF intravitreal injections, which represent the current standard of care for neovascular AMD, with outcomes appearing stronger when vitamin therapy was used alongside anti-VEGF treatment than with anti-VEGF alone [1]. This finding suggests that simultaneous reduction of homocysteine and retinal venous pressure may enhance the efficacy of anti-VEGF therapy, and raises the prospect of extending the interval between injections under appropriate monitoring. Given the absence of adverse effects associated with the vitamin regimen, and pending confirmation in larger trials, such an approach warrants consideration as a standard adjunct to anti-VEGF treatment for neovascular AMD.

Neurodegenerative disorders: Dementia and cognitive decline

Folate deficiency and insufficiency, together with the resultant elevation in plasma homocysteine, have emerged as significant modifiable risk factors for neurodegenerative conditions, particularly dementia and cognitive decline [95]. This chapter synthesizes epidemiological, clinical, and mechanistic evidence to characterize folate's contributions to brain health and its role in slowing neurodegeneration.

The cumulative evidence supports folate's neuroprotective capacity through several converging pathways: homocysteine regulation, endothelial maintenance, genomic integrity, and suppression of neuroinflammation. Although population-level fortification has largely eliminated severe deficiency, suboptimal folate status continues to represent a meaningful risk factor for cognitive deterioration. Targeted supplementation offers benefit in deficient individuals, but broad preventive recommendations require refinement according to age, genetic background, and baseline nutritional status.

Neurodegenerative disease and folate

As elaborated in preceding chapters, folate deficiency elevates homocysteine, a neurotoxic amino acid whose accumulation is associated with vascular injury and neuronal apoptosis. Hyperhomocysteinemia disrupts endothelial function, amplifies oxidative stress, and has been mechanistically linked to Alzheimer's pathology and cerebrovascular disease [96-98].

Folate is equally critical for nucleotide biosynthesis and epigenetic methylation. Experimental evidence from mice lacking the uracil repair enzyme uracil-DNA glycosylase demonstrates that folate deficiency worsens uracil misincorporation into neuronal DNA, precipitating hippocampal neuron death and diminished expression of brain-derived neurotrophic factor (BDNF) [96]. Such impairment of DNA repair capacity may accelerate the trajectory of age-related cognitive deterioration.

Low folate status also correlates with raised concentrations of pro-inflammatory cytokines, including IL-6 and TNF-α, and with depletion of glutathione, a primary cellular antioxidant. These perturbations act in concert to damage neurons and suppress hippocampal neurogenesis [96,99].

Folate deficiency linked to cognitive decline

Clinical evidence for a causal relationship between folate insufficiency and cognitive decline is extensive. A longitudinal study enrolling 3,140 older Irish adults found that serum folate below 5 ng/mL predicted accelerated global cognitive deterioration, while levels below 9 ng/mL were specifically associated with impaired episodic memory [100]. For reference, serum folate in the 4 - 9 ng/mL range is considered borderline, and values below 4 ng/mL are regarded as deficient [30]. An earlier investigation, the Sacramento Area Latino Study, demonstrated a direct relationship between folate status and cognitive performance and an inverse relationship between folate and dementia risk [101].

A Korean cohort study found that low-normal serum folate (1.5 - 5.9 ng/mL) significantly elevated the risk of cognitive impairment, with dementia incidence over a four-year follow-up being markedly higher among individuals in this folate range [102]. An Israeli study reported that serum folate below 4.4 ng/mL in an older population was associated with a 68% increase in dementia risk and a threefold increase in all-cause mortality [97]. Taken alongside the Irish and Latino findings, these data suggest that even post-fortification, a persistent association between low-normal folate and cognitive impairment exists, implying that the thresholds defining adequacy may need to be revised upward [100,101].

Consistently across studies, reduced serum and red blood cell folate concentrations are associated with heightened risk of cognitive decline, Alzheimer's disease, and vascular dementia in elderly populations. Prospective data indicate that deficiency in either folate or vitamin B12 can approximately double the probability of developing Alzheimer's disease [103]. Large cohort analyses further indicate that higher dietary folate intake is associated with lower odds of cognitive impairment in a linear dose-response fashion.

Folate supplementation slows cognitive decline

The potential of folate supplementation to preserve or restore cognitive function in elderly populations at risk has been examined across numerous trials, with converging evidence of benefit in those with mild cognitive impairment (MCI) or depleted baseline folate.

A 12-month randomized trial in 180 Chinese patients with MCI showed that daily folic acid supplementation at 400 μg improved global cognition across multiple standardized assessments, meaningfully raised serum folate, lowered homocysteine, and reduced circulating IL-6 and TNF-α by approximately 30% [99]. This dose aligns with current US recommended intake for folic acid supplementation [30].

A high-dose regimen of 5 mg/day in Japanese older adults with cognitive impairment or Alzheimer's disease produced significant improvements in Mini-Mental State Examination (MMSE) scores, with the degree of improvement correlating with the magnitude of

homocysteine reduction [105]. A subsequent meta-analysis confirmed that nutritional intervention with folate plays a meaningful role in slowing cognitive deterioration, while also identifying the need for clearer guidance on optimal dosing and underscoring plasma homocysteine as a potentially valuable therapeutic biomarker [106]. Among the studies included, all seven that incorporated folate, whether alone or combined with B12, demonstrated cognitive benefit and reductions in neurodegeneration biomarkers, whereas the ten trials evaluating B6 and B12 without folate showed no cognitive improvement [106].

A 2024 meta-analysis of seven randomized controlled trials involving more than 1,100 older adults (mean age 65 - 80) with MCI found that folic acid supplementation produced significant gains across multiple cognitive domains, including Full Scale IQ, arithmetic, information processing, digit span, and block design performance, while simultaneously reducing inflammatory cytokines and homocysteine [107].

A comprehensive review encompassing 51 studies, 23 of which were meta-analyzed, concluded that folate-based B-vitamin supplementation exerts a broadly positive effect on cognitive function in older adults, with the most pronounced benefits occurring in populations not covered by mandatory folic acid fortification. In countries with established fortification policies, additional supplementation appeared to confer no significant cognitive advantage, likely because baseline folate status in those populations is already adequate [108].

A six-month trial in China, a country without folic acid fortification, found that elderly individuals with MCI who received 400 µg/day of folic acid showed significant cognitive gains, including improvements in Full Scale IQ, Digit Span, and Block Design scores, relative to controls, accompanied by rises in serum folate and B12 and reductions in homocysteine [109].

Historical open-label studies have additionally reported that folic acid supplementation, often at 5 mg/day, produced improvements in mood, initiative, alertness, and cognitive function in folate-deficient elderly patients, with some individuals demonstrating substantial functional recovery [103].

The published supplementation literature to date has predominantly used folic acid [98,110]. Particularly compelling are the findings of Smith and Refsum, who demonstrated in a blinded trial that among individuals with elevated homocysteine, those receiving B-vitamin therapy exhibited virtually no brain atrophy over the study period, while placebo recipients showed substantial atrophy [110]. MRI quantification revealed a grey matter atrophy rate of 5.2% over two years in the placebo group compared with just 0.6% in the B-vitamin group [110].

Given that a subset of individuals may derive limited benefit from folic acid due to metabolic conversion limitations, studies using L-methylfolate directly would be informative, as this bioactive form should theoretically produce equivalent benefit across all individuals regardless of conversion capacity. Such a study is in development to evaluate whether L-methylfolate may exert a more universally protective effect against cognitive decline.

Efficacy of folate in slowing cognitive decline

The cognitive benefits of folate supplementation are most reliably observed in populations with low baseline folate status or residing in countries without mandatory fortification. Where widespread folic acid fortification has already normalized population folate levels, incremental supplementation is unlikely to yield further cognitive protection in most older adults [108].

Those with MCI or confirmed folate deficiency stand to benefit most from supplementation, while cognitively intact, folate-replete individuals show less consistent responses [107,109]. High-dose supplementation warrants caution in specific risk groups, including individuals with cardiovascular disease, and must be used carefully in the presence of vitamin B12 deficiency or epilepsy, given potential for harm in these contexts [103,104,111].

Because folate absorption and metabolic utilization require adequate B12, a coexisting B12 deficiency can undermine supplementation efficacy and may independently contribute to disease progression [112]. Further high-quality trials are needed to establish optimal dosing regimens and identify the subgroups most likely to derive benefit [107-109]. Machine learning applied to large epidemiological repositories such as the UK Biobank may offer a promising avenue for identifying which nutrients most robustly modify dementia risk at a population level [113].

Risks of excessive folate in disease

While adequate folate is protective for cardiovascular and neurological health, excessive supplementation, particularly with synthetic folic acid, carries risks that are especially relevant in older adults with established CVD. These include masking of vitamin B12 deficiency, potential increases in mortality, and attenuation of cognitive benefit. Supplementation must therefore be individually calibrated, with high-dose folic acid avoided in at-risk groups. These risks appear to be largely specific to the folic acid form, suggesting that routine use of L-methylfolate may confer the cognitive benefits of folate repletion while avoiding folic acid's adverse effects. Table 3 summarizes key risks associated with excessive folic acid intake.

Excess folic acid and increased mortality risk

Several studies have identified an association between excess folic acid and elevated CVD mortality. Whereas modest folate intake is associated with improved long-term survival, high-dose folic acid in CVD patients has been linked to increased mortality [54,115]. Specifically, red blood cell folate concentrations exceeding 1,080 nmol/L are associated with a 32% higher risk of CVD mortality and a 25% increase in all-cause mortality in older adults with pre-existing CVD [104,114].

These findings reveal a U-shaped dose-response relationship: both low red blood cell folate (below 476 nmol/L) and high levels are associated with elevated CVD mortality in patients with type 2 diabetes [104]. Across studies, moderate dietary folate intake in the range of 400 - 600 μg/day is associated with reduced mortality risk, whereas supplemental folic acid exceeding 1,000 μg/day raises mortality risk by an estimated 18 - 24%.

Excess folic acid and attenuated cognitive benefits

As described above, folate supplementation improves cognition in deficient individuals. However, intake beyond adequate levels may not only fail to provide incremental cognitive protection but could actively diminish the protective effect, particularly in individuals with CVD or diabetes. The normally observed association between red blood cell folate and protection against cognitive impairment appears to disappear entirely in CVD patients, possibly because CVD-related insulin resistance and cerebral microcirculation damage override folate's neuroprotective mechanisms.

Pathways of harm from excess folic acid

Several mechanisms have been proposed to explain why excess folic acid is problematic, particularly in CVD patients:

- **Folate oversaturation in fortified populations:** More than half of US CVD patients already meet recommended folate intake through diet alone, yet approximately 25% additionally use supplements, creating a risk of unintended excess.
- **Interaction with CVD pathophysiology:** High folate concentrations may accelerate atherosclerosis progression in individuals with established CVD through mechanisms that remain incompletely characterized [114].
- **The homocysteine paradox:** Despite folic acid's established capacity to lower homocysteine, excessive supplementation in CVD patients has not translated into mortality benefit [116].
- **Masking of vitamin B12 deficiency:** Elevated folate intake can normalize red blood cell morphology while concealing the hematological signs of B12 deficiency, which is common in older adults, allowing neurological damage from B12 depletion to advance undetected and potentially become irreversible [59].

- **Unmetabolized folic acid (UMFA) accumulation:** As discussed in chapter 3, synthetic folic acid intake beyond the daily conversion ceiling of approximately 400 µg leads to UMFA accumulation in the circulation. UMFA has been associated with impaired immune function and a possible increase in cancer risk [58,117]. During pregnancy and lactation, circulating UMFA has additionally been linked to elevated risk of neurodevelopmental disorders including autism [118,119] and a threefold increase in gestational diabetes incidence [120]. Given that excessive folic acid supplementation is the root cause of UMFA accumulation, the current supplementation paradigm warrants reconsideration. Average supplemental folic acid intake in the US is approximately 200 µg, though individual intake varies widely, with some consuming two- to threefold more than the 400 µg that can be metabolically processed each day. Transitioning to L-methylfolate supplementation would substantially reduce this risk.

Risk Factor	Evidence
Masking B12 deficiency	Well-established, especially in elderly
Increased CVD/all-cause mortality	U-shaped association in epidemiological studies
Attenuated cognitive benefit	Observed in CVD/diabetes populations with high folate intake
Unmetabolized folic acid	Potential immune/cancer risk; further research needed

Table 3. *Excessive folate risks.*

Note: The risks listed refer specifically to folate in the folic acid form. With the exception of B12 masking, use of a reduced folate form such as L-methylfolate is expected to mitigate these risks.

Guidelines for folate supplementation in CVD patients

The available evidence supports avoiding high-dose folic acid supplementation, defined as intake exceeding 400 µg/day, in older adults with a history of CVD. A red blood cell folate concentration above 900 nmol/L in this population warrants clinical caution [104].

Practically, this means prioritizing dietary folate from whole food sources such as leafy greens and legumes over synthetic folic acid, and monitoring red blood cell folate levels in CVD patients who use B-complex vitamin products. Where dietary folate is insufficient and supplementation is necessary, a reduced-form folate, L-methylfolate, is preferable to folic acid. Supporting this preference, high-dose folinic acid (leucovorin), another reduced folate form used as a prescription pharmaceutical for vitamin B9 replacement in the context of chemotherapy, has been administered for over 75 years without evidence of cardiotoxicity. The one noted exception, a 3% cardiotoxicity rate with leucovorin combined with 5-fluorouracil, was equivalent to the 3% rate observed with 5-fluorouracil alone, indicating that the toxicity was attributable to the chemotherapy agent rather than leucovorin [121].

Clinical guidelines for folate supplementation in CVD patients can be summarized as follows:

- Avoid folic acid supplements exceeding 400 µg/day in older adults with CVD unless a specific deficiency warrants it under medical supervision.
- Routinely assess vitamin B12 status in older adults receiving folic acid, particularly those at elevated risk of deficiency.
- Favor dietary folate sources over high-dose synthetic supplements unless clinically indicated.
- When supplementation is necessary, use a reduced folate form, L-methylfolate or leucovorin, in preference to folic acid.
- Individualize supplementation decisions based on personal risk factors, comorbidities, and baseline folate and B12 status.
- In cases where specific diagnostic findings warrant it, such as genetic testing revealing MTHFR variants or a positive folate receptor autoantibody (FRAA) test, consider high-dose treatment with a pharmaceutical-grade leucovorin or levoleucovorin drug product.

Impact of vitamin deficiencies and insufficiencies

Accumulated evidence establishes that deficiencies or insufficiencies in key vitamins, folate (B9), vitamins B6, B12, C, D, and E, occupy a central position in the pathogenesis of endothelial dysfunction, arterial stiffness, and the downstream development of a broad spectrum of vascular and degenerative disorders, including those affecting the pulmonary circulation. Restoring adequate vitamin status, particularly through bioactive forms and targeted supplementation protocols, improves endothelial performance and microvascular health across vascular territories, including the pulmonary vasculature, underscoring the genuine therapeutic potential of correcting these nutritional gaps. Table 4 maps the disorders reviewed throughout this paper to the specific vitamin deficiencies or excesses implicated in each.

Disorder	Folate/B9	B12	B6	C	D
Cardiovascular Disease	Deficiency, Excess†	Deficiency	Deficiency	Deficiency	Deficiency
Pulmonary Vascular Disease/COPD	Deficiency	Deficiency			
Cerebrovascular Disease	Deficiency	Deficiency	Deficiency	Deficiency	Deficiency
Peripheral Artery Disease	Deficiency	Deficiency	Deficiency	Deficiency [122]	Deficiency
Lymphedema	Deficiency	Deficiency	Deficiency	Deficiency	Deficiency [123]
Age-related Macular Degeneration	Deficiency	Deficiency	Deficiency	Deficiency [123]	
Diabetic Retinopathy	Deficiency	Deficiency	Deficiency		
Glaucoma	Deficiency	Deficiency	Deficiency		[123,124]
Dementia/Cognitive Decline	Deficiency, Excess†	Deficiency	Deficiency		[123,124]

Table 4: *Vitamin deficiencies or excesses implicated in disorders discussed.*

Excess† (B9/Folate) indicates that disease risk or adverse outcomes can be aggravated by excessive folic acid supplementation, as documented in CVD, neurodegeneration, and autism risk contexts. Blank cells indicate that evidence for vitamin involvement in that condition is limited or absent.

At the clinical level, compromised vascular health arising from impaired vitamin metabolism or absorption can present across a wide range of phenotypes: cerebrovascular disease, lymphedema, cognitive deterioration, age-related macular degeneration, glaucoma, and diabetic microvascular complications. The mechanistic pathways unifying these associations, hyperhomocysteinemia, oxidative stress, disrupted nitric oxide signaling, defective collagen synthesis, and chronic inflammation, are each amenable to nutritional intervention and targeted supplementation, as illustrated in figure 2.

Notwithstanding the strength of observational and mechanistic data linking vitamin deficiency to vascular risk, interventional trials have produced heterogeneous results, particularly with respect to hard cardiovascular endpoints. Nevertheless, normalization of vitamin levels, especially vitamin D and the homocysteine-lowering B vitamins administered in their most bioavailable forms, consistently improves surrogate measures of vascular function and attenuates established risk factors. These findings collectively argue for clinical strategies that prioritize adequate vitamin intake, individualized supplementation, and the preferential use of bioavailable forms, with the aim of improving patient outcomes and arresting the progression of disorders rooted in poor vascular health. Notably, neurodevelopmental disorders such as autism spectrum disorder are also shaped by cerebral folate deficiency and demonstrate measurable benefit from supplementation with natural folate in the form of leucovorin.

A unifying theme throughout this review has been the distinction between folate sources, natural forms present in food and in L-methylfolate supplements versus synthetic folic acid used in grain fortification and most commercially available oral supplements. In

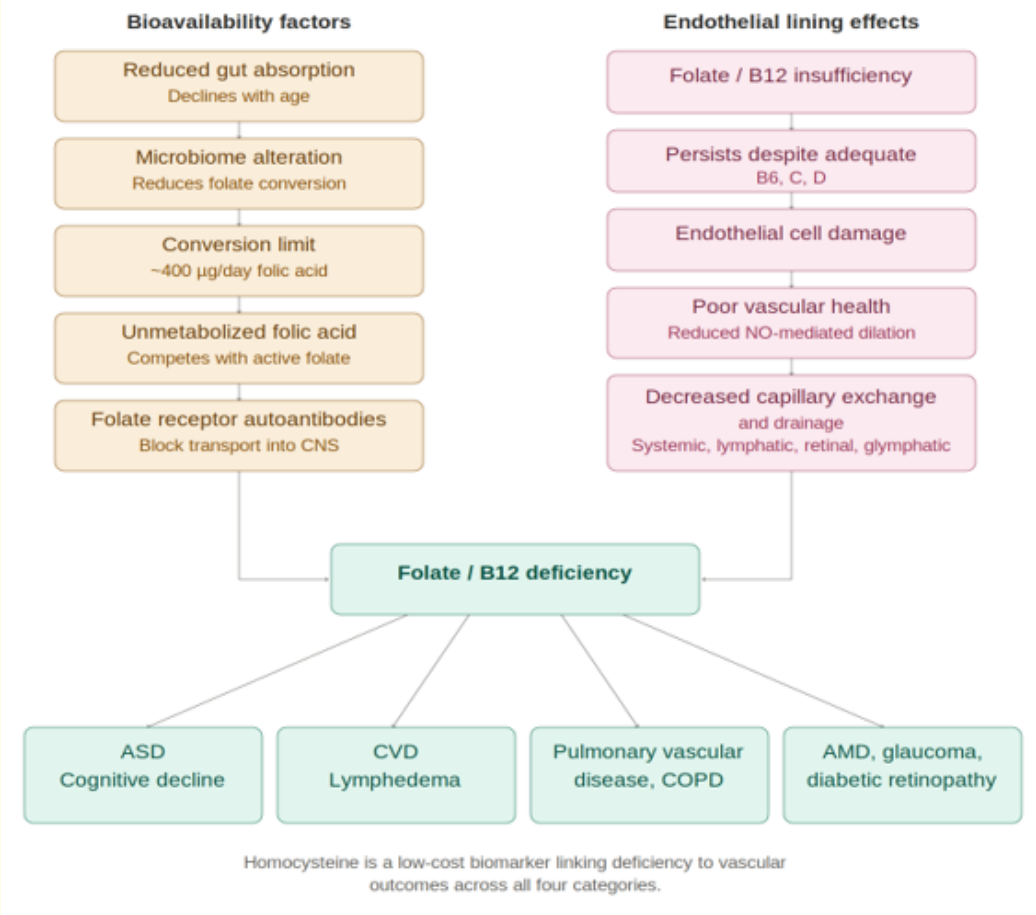

Figure 2: *Factors involved in disorders linked to folate deficiency. Disorders are grouped into three categories according to whether the folate deficiency is systemic, retinal, or cerebral. Systemic disorders include cardiovascular disease and lymphedema. Retinal disorders encompass AMD, glaucoma, and diabetic retinopathy. Cerebral disorders include neurodevelopmental conditions such as autism (ASD) and neurodegenerative conditions such as dementia and cognitive decline. The common underlying driver across categories is a reduction in endothelial health, which diminishes capillary exchange and impairs drainage from perfused tissue, whether in systemic blood or lymphatic capillaries, retinal capillaries, or cerebral glymphatic capillaries. Endothelial health is governed by the availability of bioactive folate and vitamin B12. Bioactive folate availability can be compromised through five distinct mechanisms: age-related decline in intestinal folate absorption; gut microbiome alterations that reduce the biochemical conversion of ingested folate; consumption of excess folic acid, whose unmetabolized form (UMFA) competes with active folate for cellular uptake; and, in a subset of the population, the presence of folate receptor autoantibodies (FRAA) that block folate entry into the cerebral compartment. Homocysteine serves as a reliable biomarker across most of these scenarios, with even modest elevations signaling declining vascular health. Treatment typically involves reducing folic acid supplementation to below 400 µg/day, confirming adequate vitamin B12 availability, increasing natural folate through dietary sources and/or L-methylfolate or leucovorin supplementation, and, in more severe cases, using a pharmaceutical drug product containing leucovorin or levoleucovorin, with consideration of strategies to restore microbiome diversity.*

countries such as the United States, where mandatory grain fortification with folic acid is in place, estimated average daily vitamin B9 intake in adults ranges from approximately 450 µg in women to 600 µg in men, with roughly half derived from folic acid [125]. Given that the recommended daily requirement for folates is 400 µg, many individuals find themselves in a state of net excess. However, the distribution of intake is highly skewed: some individuals consume twice the average while others take in as little as 20% of it. When this distributional reality is overlaid on the fraction of the population carrying metabolic polymorphisms or immune responses that reduce folic acid utilization, and on the demonstrated capacity of UMFA to compete with active folate for cellular and cerebral uptake, a picture emerges of harm arising at both extremes, excess folic acid in one group and frank deficiency in another, with consequences spanning the disorders catalogued in table 4.

This pattern raises the question of whether healthy adults might be best served by obtaining their B vitamins from whole food sources, reserving supplementation for those at elevated disease risk due to genetic factors, immune conditions, age-related absorption decline, or pregnancy planning. The last point carries an important caveat: excess folic acid during pregnancy has been identified as a potential risk factor for gestational diabetes [120] and for autism spectrum disorder in genetically susceptible offspring [126]. Those who do require supplementation may be better served by natural reduced-form folate to eliminate the risk of UMFA accumulation. This is an area demanding substantially more research, including cross-national comparisons of disorder incidence stratified by natural versus synthetic folate exposure. Recognizing that many of the conditions reviewed here share folate deficiency or folic acid excess as an underlying contributor, that B12 insufficiency can be concealed by UMFA, and that homocysteine measurement, supplemented by UMFA quantification where indicated, represents the most practical biomarker for disease risk, offers a clinically actionable framework with broad potential benefit.

On a policy level, we suggest that existing folic acid fortification and supplementation guidelines warrant careful reconsideration, given that the current approach may be maladaptive for a substantial fraction of the population, particularly those with MTHFR polymorphisms or immune-mediated production of folate receptor autoantibodies (FRAA). A large cohort of susceptible individuals may be inadvertently accumulating excess folic acid through routine consumption of fortified processed foods, and a downward revision of fortification levels merits serious evaluation. In clinical practice, we recommend routine screening for hyperhomocysteinemia; when elevated homocysteine is identified, follow-up testing should include vitamin B12 status and plasma folic acid measurement, with treatment directed at lowering homocysteine through L-methylfolate supplementation at doses sufficient to achieve normalization. In cases of severe cerebral folate insufficiency, such as that seen in autism spectrum disorder, pharmaceutical drug products containing leucovorin or levoleucovorin are warranted, typically guided by diagnostic testing for cerebral folate deficiency, including the FRAA assay. Several published studies have documented successful management through daily provision of several milligrams of L-methylfolate together with vitamins B12, B6, and D, without adverse effects.

Achieving optimal vascular health ultimately requires a multidimensional strategy: identifying and correcting vitamin deficiencies, characterizing their molecular consequences for endothelial and vascular smooth muscle cell biology, and embedding nutritional therapy within standard clinical care pathways. Further well-designed intervention trials are needed to establish optimal protocols and clinically meaningful endpoints for vitamin-based approaches to the prevention and management of degenerative vascular disease.

Conclusion

Vitamins, most prominently folate (B9), B6, B12, C, D, and E, function as indispensable regulators of endothelial biology, and their depletion or insufficiency is mechanistically implicated in a broad array of vascular and degenerative conditions, extending to disorders of the pulmonary circulation. The pathophysiological pathways through which these deficiencies operate are notably convergent: hyperhomocysteinemia, heightened oxidative burden, compromised nitric oxide signaling, and structural weakening of collagen scaffolding each represent targetable processes amenable to correction through nutritional and supplementation strategies.

Four principal conclusions can be drawn from the body of evidence reviewed in this paper.

1. **Homocysteine is the central clinical biomarker:** Across the cardiovascular, pulmonary, retinal, and neurological disease domains reviewed here, elevated plasma homocysteine consistently reflects insufficient folate and vitamin B12 status and prospectively identifies individuals who are most likely to respond to nutritional correction. Incorporating routine hyperhomocysteinemia screening, with reflex testing for vitamin B12 deficiency and plasma folic acid where elevation is confirmed, into the clinical assessment of patients presenting with unexplained vascular dysfunction, deteriorating pulmonary function, or cognitive impairment represents a low-cost, high-yield diagnostic step.
2. **Bioactive folate forms outperform synthetic folic acid in vulnerable individuals:** When synthetic folic acid is consumed at levels exceeding approximately 400 µg per day, the surplus that cannot be metabolically processed accumulates in the circulation as unmetabolized folic acid (UMFA), which interferes with cellular folate uptake and has been associated with elevated cardiovascular mortality at high intake levels. Individuals carrying folate-metabolizing gene variants, those with circulating folate receptor autoantibodies, and those with impaired intestinal absorption cannot reliably convert folic acid to its active form and derive specific benefit from direct supplementation with L-methylfolate or, in cases of severe deficiency, from pharmaceutical preparations containing leucovorin or levoleucovorin. When supplementation is clinically indicated, reduced-form folates are the preferred vehicle over synthetic folic acid.
3. **The therapeutic window is critically important:** The relationship between folate intake and clinical outcomes follows a non-linear trajectory. Moderate intake of dietary folate is associated with reduced vascular risk, whereas escalating synthetic supplementation in older adults with established cardiovascular disease is linked to diminished cognitive protection and higher mortality. Rather than applying uniform high-dose recommendations, supplementation decisions should be anchored in individualized assessment of baseline folate and B12 status, existing comorbidities, and genetic risk profile.
4. **Interventional evidence in pulmonary populations remains an important gap:** The mechanistic and observational case for a role of folate and B12 insufficiency in pulmonary vascular tone, microvascular perfusion, and gas exchange efficiency is compelling, but direct interventional data in pulmonary disease populations are currently limited. Prospective trials designed to measure diffusing capacity, alveolar-capillary perfusion, and arterial oxygenation before and after targeted correction of vitamin insufficiency, with preference for bioactive folate forms, would substantially strengthen the evidence base and clarify the clinical relevance of these associations.

Collectively, the evidence reviewed throughout this paper supports the integration of vitamin status assessment into routine clinical management of vascular and pulmonary disease. The emphasis should fall on correcting deficiencies using the most bioavailable forms available, actively avoiding excess synthetic folic acid exposure, and escalating to appropriate pharmacological or medical food formulations when nutritional insufficiency persists despite first-line measures.

Conflict of Interest

The author declares no conflicts of interest.

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