

## Lowering Hyperhomocysteinemia to Diminish the Risk of Cardiovascular, Cerebrovascular and Peripheral Artery Disease: A Review of Evidence on Nutritional Preventive Interventions

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### Abstract

Hyperhomocysteinemia is an independent risk factor for atherothrombotic cardiovascular disease, lacunar infarction, cerebrovascular disease and peripheral artery disease. However, early hallmark clinical studies on the benefits of nutrients supplementation for decreasing homocysteine (Hcy) levels and vascular events showed contradictory results. In the present review, recent meta-analyses and selected clinical studies on the use of micronutrients as diet complements to prevent hyperhomocysteinemia and related vascular disease are summarised. Its objective is to identify subgroups of patients that could benefit the most from closer nutritional surveillance and eventual dietary complementation. Results on preventive nutritional interventions were more consistent for stroke than for myocardial infarction. The reviewed publications showed that oral dietary complementation with the combination of folic acid, B<sub>6</sub>-, and B<sub>12</sub>-vitamins substantially lowers circulating Hcy levels compared to vitamins given alone. Patients younger than 69 years of age, with intracranial small vessel disease, from regions without folic acid food fortification or with poor dietary intake, with higher baseline cholesterol and Hcy levels and those not receiving antiplatelet or lipid-lowering drugs may obtain the larger benefits from nutritional interventions. Better results may be observed with a duration of interventions of > 36 months. Given the frequent underdiagnosis of B<sub>12</sub> vitamin deficiency, serum B<sub>12</sub> and total Hcy should be checked routinely in cardiovascular and cerebrovascular disease patients and elevated total Hcy should be treated.

**Keywords:** *Hyperhomocysteinemia; Cardiovascular Disease; Cerebrovascular Disease; Nutrition; Review*

### Background

Homocysteine (Hcy) is a non-protein forming alpha-amino acid derived from the essential animal protein animal acid methionine through the methylation process. Hcy is characterized by containing sulfur and being an intermediary in the metabolism of methionine and cysteine. It is metabolized through two main routes: methylation and transsulfuration, and mainly eliminated through renal catabolism [1].

Human plasma contains reduced and oxidized Hcy and total Hcy is the sum of all Hcy forms that exist in plasma or serum [1]. Normal plasma Hcy levels for healthy adults has been established in 5 - 15 μmol/L [2]. Hcy circulating levels can be varied by a diversity of factors. They can be increased by hereditary defects in enzymes of the metabolism of methionine, deficiencies of vitamins B<sub>6</sub>, B<sub>12</sub> and folate or by feeding methionine enriched diets, also with raising age, male sex, smoking, coffee consumption, high blood pressure, unfavourable lipid

profile, high creatinine, and the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism [3]. Some pharmaceutical products frequently used in clinical practice, such as lipid-lowering, diuretics and other anti-hypertensive [4,5] and anti-Parkinsonian drugs [6] modify Hcy levels. Additionally, several disease states, pregnancy, and lactation contribute to variations in Hcy levels [7]. Physical activity, moderate alcohol consumption, and good folate or vitamin B-12 status are associated with lower total Hcy levels [3].

In 1969, McCully, *et al.* [8] described the vascular pathology in homocystinuria pediatric patients, which is characterized by smooth muscle proliferation, progressive arterial stenosis, and procoagulant hemostatic changes, revealing the importance of severe hyperhomocysteinemia in the early development of arteriosclerosis and thromboembolism [1]. Since then, the potential relationship between high Hcy in plasma and disease of the vessels of the heart, brain and the periphery has been extensively studied [9]. Hyperhomocysteinemia, or increased circulating levels of total Hcy, has been suggested as a probably independent risk factor for developing cardiovascular, cerebrovascular and peripheral artery disease [10-12].

Some evidence suggest that the risk of coronary disease exists at levels of 10  $\mu\text{mol/L}$ , and therefore this should be considered the upper normal limit [1]. Although three arbitrary levels of hyperhomocysteinemia have been determined as mild (15 - 30  $\mu\text{mol/L}$ ), moderate (30 - 100  $\mu\text{mol/L}$ ) and severe (>100  $\mu\text{mol/L}$ ) [1] a continuous concentration-response relation with no apparent threshold concentration has been reported [3].

Prospective studies on the causality between Hcy levels and cardiovascular disease have shown that the odds ratios (OR) for a 5  $\mu\text{mol/L}$  increase in serum Hcy were, for ischaemic heart disease, 1.32 (95% confidence interval (CI): 1.19 to 1.45); and, for stroke, 1.59 (95% CI: 1.29 to 1.96) [13]. An early meta-analysis that included 27 retrospective and prospective studies showed an incremental increase in risk of coronary artery disease per 5  $\mu\text{mol/L}$  increase in total Hcy concentration (men, OR = 1.6, CI 95% 1.4 - 1.7; women OR = 1.8, CI 95% 1.3 - 1.9) [14]. From this result, the authors extrapolated that 10% of the populations' coronary artery disease risk was attributable to hyperhomocysteinemia, and that up to 50,000 coronary artery disease deaths could be prevented annually by Hcy level reduction [15]. These initial conclusions were supported by subsequent findings from the Homocysteine Studies' Collaboration meta-analysis that showed a risk reduction for ischemic heart disease by 11% and for stroke by 19% per 3  $\mu\text{mol/L}$  reduction in Hcy concentration [16]. Likewise, in type 2 diabetes mellitus patients, Hcy levels were associated with the angiographic severity of peripheral artery disease (PAD) with an odds ratio, 2.77 (95% CI: 1.14, 6.72) for patients with Hcy levels above the median vs. those under the median [17].

However, previous hallmark clinical studies on the benefits of nutrients supplementation for decreasing Hcy levels and, in consequence, cardiovascular, cerebrovascular or peripheral artery disease showed contradictory results [18,19]. In the present review, recent meta-analyses and selected clinical studies on the use of micronutrients as diet complements to prevent hyperhomocysteinemia and related cardiovascular, cerebrovascular and peripheral artery disease are summarised. It aims to identify subgroups of patients that could benefit the most from a closer nutritional surveillance and eventual dietary complementation.

### **Vitamin prevention of hyperhomocysteinemia: overview**

Hyperhomocysteinemia can be reduced significantly or reversed by vitamin treatment of affected patients, except those bearing severe functional defects of methionine synthase or a thermostable mutation of MTHFR who would benefit from a combination of betaine and/or methionine supplement with folinic acid or 5-methyl-tetrahydrofolate (metylTHF) [20,21]. A number of observations suggest that except in vitamin B<sub>12</sub> deficiency, folate treatment should be the most adequate therapy to reverse hyperhomocysteinemia. Folate supplement also improves endothelial function in hyperhomocysteinemic subjects while folic acid plus pyridoxine (vitamin B<sub>6</sub>) treatment may effectively contribute to the reduction of the atherothrombotic risk [20].

The treatment with folic acid should be combined with pyridoxine in vitamin B<sub>6</sub>-deficient patients. Additionally, it is important to correct cobalamin (vitamin B<sub>12</sub>) deficiency before starting the folic acid treatment or provide vitamin B<sub>12</sub> together with folic acid. A folic acid

supplement, particularly at high dosage (> 5 mg per day) could mask the hematologic manifestations of megaloblastic anemia induced by cobalamin deficiency and could thus exacerbate the associated neurologic disorders [15,20].

The optimal doses of therapeutic vitamins remain to be clearly defined. To date, folic acid doses of 5 to 10 mg per day are currently used. It is noteworthy that an excessive dietary intake of methionine-rich animal protein is an additional cause of hyperhomocysteinemia. In this case, a dietary reduction of methionine in conjunction with pyridoxine treatment should be instituted to correct hyperhomocysteinemia [15].

### **Prevention of cardiovascular and cerebrovascular disease**

#### **Folic acid supplementation is associated with a reduced risk of overall cardiovascular and cerebrovascular disease**

The early French trial conducted to investigate whether dietary supplementation with B vitamins (5-methyltetrahydrofolate, 560 µg; B<sub>6</sub> vitamin, 3 mg; B<sub>12</sub> vitamin, 20 µg) or omega 3 fatty acids (600 mg of eicosapentaenoic acid and docosahexaenoic acid at a ratio of 2:1), or both, found no significant effects on major cardiovascular events, defined as a composite of non-fatal myocardial infarction, stroke or death from cardiovascular disease [22].

In another meta-analysis that examined the effect vitamins therapy in the primary prevention of stroke, it was found that folic acid supplementation decreased the relative risk (RR) for stroke by 18% (RR = 0.82, 95% CI, 0.68 - 1.00), and a greater benefit was observed with treatment duration of > 36 months [23]. Although in populations with established or increasing intake of folate and vitamin B<sub>12</sub>, lowering total plasma Hcy with folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> prevention of any stroke (0.91, 0.82-1.00, I<sup>2</sup> = 11%) or of recurrent stroke (RR 0.92, 95% CI 0.81 - 1.06) is similar to placebo [24], a reduction in total plasma Hcy might effectively prevent stroke in people with a low intake of folate or vitamin B<sub>12</sub> [25], intracranial small vessel disease [26] and those not taking or who are unable to take anti-platelet therapies, such as aspirin [27].

More recently Li, *et al.* [28] conducted a meta-analysis of 30 randomized controlled trials (RCTs) involving 82,334 participants to establish the efficacy of lowering Hcy with folic acid supplementation for decreasing cardiovascular disease risk. The pooled relative risks of folic acid supplementation compared with controls were 0.90 (95% CI 0.84 - 0.96; p = 0.002) for stroke, 1.04 (95% CI 0.99 - 1.09; p = 0.16) for coronary heart disease, and 0.96 (95% CI 0.92-0.99; p = 0.02) for overall cardiovascular disease. The intervention effects for both stroke and combined cardiovascular disease were more pronounced among participants with lower plasma folate levels at baseline (both p < 0.02 for interaction) while in stratified analyses, a greater beneficial effect for overall cardiovascular disease was seen among participants without pre-existing cardiovascular disease (p = 0.006 for interaction) or in trials with larger reduction in Hcy levels (p = 0.009 for interaction). Based on these results, the authors concluded that a 10% lower risk of stroke and a 4% lower risk of overall cardiovascular disease with folic acid supplementation could be expected, but no significant effect on the risk of coronary heart disease can be anticipated.

#### **Greater risk reduction of vascular events can be obtained after combining folic acid and vitamins B<sub>6</sub> and B<sub>12</sub>**

Lee, *et al.* [29] reported the findings of a meta-analysis of 13 RCTs (n = 39,005 participants) of folic acid therapy to reduce Hcy and stroke as an outcome measure. Across all trials, folic acid supplementation was associated with a trend towards a mild benefit that did not reach statistical significance in reducing the risk of primary stroke (RR 0.93, 95% CI 0.85 - 1.03; p = 0.16). In stratified analyses, a greater beneficial effect was seen in the trials testing combination therapy of folic acid plus vitamins B<sub>6</sub> and B<sub>12</sub> (RR 0.83, 0.71 - 0.97; p = 0.02) and in the trials that disproportionately enrolled male patients (men/women > 2, RR 0.84, 0.74 - 0.94; p = 0.003).

#### **Nutritional interventions reduce the risk of vascular events, but not events severity or related disability**

Saposnik, *et al.* [30] sought to determine whether vitamin therapy reduces the risk of ischaemic and hemorrhagic stroke, as well as stroke-related disability by analyzing stroke outcomes among participants of the Heart Outcomes Prevention Evaluation 2 (HOPE 2) trial.

A total of 5,522 adults with known cardiovascular disease were randomized to a daily combination of 2.5 mg of folic acid, 50 mg of vitamin B<sub>6</sub>, and 1 mg of vitamin B<sub>12</sub>, or matching placebo, for 5 years. Findings showed that geometric mean Hcy concentration decreased by 2.2 μmol/L in the vitamin therapy group and increased by 0.80 μmol/L in the placebo group. The incidence rate of stroke was 0.88 per 100 person-years in the vitamin therapy group and 1.15 per 100 person-years in the placebo group (hazard ratio [HR], 0.75; 95% CI: 0.59, 0.97). Vitamins therapy also reduced the risk of nonfatal stroke (HR, 0.72; 95% CI: 0.54, 0.95) but did not impact on neurological deficit at 24 hours (p = 0.45) or functional dependence at discharge or at 7 days (OR, 0.95; 95% CI: 0.57, 1.56). In subgroup analysis, patients aged younger than 69 years, from regions without folic acid food fortification, with higher baseline cholesterol and Hcy levels, and those not receiving antiplatelet or lipid-lowering drugs at study enrolment had a larger treatment benefit. Therefore, lowering of Hcy with folic acid and vitamins B<sub>6</sub> and B<sub>12</sub> did reduce the risk of overall stroke, but not stroke severity or disability.

A meta-analysis of 14 RCTs was carried out by Zen., *et al.* [31] to check how different folate fortification status might affect the effects of folic acid supplementation in lowering Hcy and reducing stroke risk. A total of 39,420 patients were studied. A significant difference was observed in Hcy reduction between the subgroups with folate fortification and without folate fortification (p = 0.05). The relative risk of stroke was 0.88 (95% CI 0.77, 1.00, p = 0.05) in the subgroup without folate fortification, 0.94 (95% CI 0.58, 1.54, p = 0.82) in the subgroup with folate fortification and 0.91 (95% CI 0.82, 1.01, p = 0.09) in the subgroup with partial folate fortification. The authors found that folic acid supplementation alone might have a modest benefit on stroke prevention in regions without folate fortification.

### **Renal function and older age determine the response to nutritional interventions for lowering hyperhomocysteinemia and the associated risk of vascular events**

The earlier double-blinded RCT (the Vitamin Intervention for Stroke Prevention, VISP) from Toole., *et al.* [32] conducted in 3,680 adults with nondisabling cerebral infarction to determine whether high doses of folic acid, pyridoxine (vitamin B<sub>6</sub>) and cobalamin (vitamin B<sub>12</sub>), given to lower total Hcy levels, reduced the risk of recurrent stroke over a 2-year period compared with low doses of these vitamins. The mean reduction of total Hcy was 2 μmol/L greater in the high dose group than in the low-dose group. The unadjusted relative risk for any stroke, coronary heart disease event, or death was 1.0 (95% CI: 0.8 - 1.1), with chances of an event within 2 years of 18.0% in the high-dose group and 18.6% in the low-dose group. There was a persistent and graded association between baseline total Hcy level and outcomes. The authors concluded that although a moderate reduction of total Hcy after nondisabling cerebral infarction had no effect on vascular outcomes during the 2 years of follow-up, there were consistent findings of an association of total Hcy with vascular risk suggesting that the hypothesis warrants further exploration.

However, in a post hoc analysis of the VISP trial reported by Towfighi., *et al.* [33] the effects of high-dose vitamin replacement on primary (stroke, myocardial infarction, or death) and secondary (stroke) outcomes were assessed after stratifying by age (< vs. ≥ median age, 67 years) and adjusting for demographic and clinical factors. The authors found that among individuals older than 67 years, high-dose vitamin therapy was associated with reduced risk of stroke, myocardial infarction or death (adjusted HR 0.76, 95% CI 0.58 - 0.99) and a trend towards reduced likelihood of stroke (adjusted HR 0.86, 95% CI 0.59 - 1.25). Noteworthy, high-dose vitamin therapy did not impact outcomes among individuals younger than 67 years demonstrating that age modified the association between B vitamin therapy and recurrent vascular risk among stroke survivors with elevated serum total Hcy levels. Older individuals with stroke were more likely to benefit from B vitamin therapy than younger individuals.

More recently it has also been pointed out that cyanocobalamin (a form of vitamin B<sub>12</sub>) can accelerate decline in renal function and increase the risk of cardiovascular events in patients with impaired renal function [34]. Although the early trial by Toole., *et al.* [32] did not show benefit in reduction of stroke, these results might have been due to harm in participants with impaired renal function. In patients with diabetic nephropathy, cyanocobalamin is harmful, whereas B vitamins appear to reduce cardiovascular events in study participants with normal renal function [34]. A recent meta-analysis of individual patient data from the two large trials of B vitamin therapy (VISP

and VITATOPS) [32,35] indicates that patients with impaired renal function who are exposed to high-dose cyanocobalamin do not benefit from therapy with B vitamins for the prevention of stroke (RR: 1.04, 95% CI: 0.84, 1.27), however, patients with normal renal function who are not exposed to high-dose cyanocobalamin benefit significantly from this treatment (RR: 0.78, 95% CI: 0.67, 0.90; interaction  $p = 0.03$ ) [36]. These observations show that the potential benefits of B vitamin therapy with folic acid and methylcobalamin or hydroxycobalamin, instead of cyanocobalamin, to lower Hcy concentrations in people at high risk of stroke requires further investigation.

#### **Folic acid complementation enhances the preventive effects of antihypertensives in patients at risk of vascular events**

Huo, *et al.* [37] tested the hypothesis that therapy with enalapril and folic acid was more effective in reducing first stroke than enalapril alone among Chinese adults with hypertension using data from the CSPPT (China Stroke Primary Prevention Trial). The authors found that during a median treatment duration of 4.5 years, the enalapril-folic acid group had a significant risk reduction in first stroke (HR: 0.79; 95% CI: 0.68, 0.93), first ischemic stroke (HR: 0.76; 95% CI: 0.64, 0.91), and composite cardiovascular events consisting of cardiovascular death, myocardial infarction and stroke (3.1% with enalapril-folic acid vs 3.9% with enalapril alone; HR: 0.80; 95% CI: 0.69, 0.92) compared with the enalapril alone group. These findings showed that the combined use of enalapril and folic acid, compared with enalapril alone, significantly reduced the risk of first stroke among adults with hypertension in China without a history of stroke or myocardial infarction.

Likewise, Qin, *et al.* [38] sought to determine whether folic acid supplementation can independently reduce the risk of the first stroke associated with elevated total cholesterol levels in a subanalysis of data from the CSPPT. After a median treatment of 4.5 years, folic acid supplementation reduced the risk of the first stroke associated with elevated total cholesterol ( $\geq 200$  mg/dL) by 31% among hypertensive adults without a history of major cardiovascular diseases.

#### **Betaine and choline may add anti-inflammatory benefits in the prevention of vascular events**

In a review of epidemiological studies on the association between dietary intakes of choline and betaine and traditional and novel cardiovascular disease risk factors it was found that higher dietary intakes of choline and betaine were associated with metabolic syndrome components in diverging directions, with lower serum concentrations of serum inflammatory markers, such as reactive protein-C, IL-6 and TNF- $\alpha$ , and to reduction of total Hcy levels which may eventually lead to cardiovascular disease prevention [39].

Prevention of peripheral artery disease.

#### **Findings in peripheral artery disease prevention remain inconclusive**

Andras, *et al.* [40] reported the findings of a systematic review conducted to assess the effects of plasma Hcy lowering therapy on the clinical progression of disease in people with PAD and hyperhomocysteinemia including, as a subset, those who had undergone surgical or radiological intervention. In one randomised trial with a total of 133 participants there was a significant improvement in ankle brachial index in participants who received folic acid compared with placebo (mean difference 0.07, 95% CI: 0.04 to 0.11,  $p < 0.001$ ) and in participants who received 5-methyltetrahydrofolate (5-MTHF) versus placebo (MD 0.05, 95% CI 0.01 to 0.10,  $p = 0.009$ ). In a second trial with a total of 18 participants there was no difference ( $p$  non-significant) in ABI in participants who received a multivitamin B supplement (mean  $\pm$  SEM:  $0.7 \pm 0.1$ ) compared with placebo (mean  $\pm$  SEM:  $0.8 \pm 0.1$ ). No major events were reported in neither of both trials. Given these contradictory results, the authors concluded that well-constructed trials are required before making recommendations for Hcy lowering therapy.

#### **Interpretation of findings**

Epidemiologic reports have established that elevated levels of Hcy are an independent risk factor for atherothrombotic cardiovascular disease, lacunar infarction and stroke [3]. Hyperhomocysteinemia also adds a higher risk for cognitive impairment (elderly), depres-

sion and osteoporosis-induced bone fracture [41]. The reviewed publications have shown that oral dietary complementation with the combination of folic acid, B<sub>6</sub>, and B<sub>12</sub>-vitamins substantially lowers circulating Hcy levels. However, it has been harder to demonstrate the association between lowering Hcy levels and improvement of outcomes in the primary and secondary prevention of cardiovascular, cerebrovascular or peripheral artery disease. Overall, the results of vitamins B<sub>6</sub>, B<sub>9</sub> or B<sub>12</sub> given alone or in combination as Hcy lowering interventions were more consistent for stroke than for myocardial infarction [9].

Reasons for some major clinical studies failing to demonstrate significant reductions in cardiovascular or cerebrovascular events may be the studies being conducted in populations with high folate consumption, with folate fortification or in patients with only mildly elevated homocysteine levels. Among folate-replete subjects, the main nutritional determinant of high total Hcy is B<sub>12</sub> deficiency [34]. Metabolic B<sub>12</sub> deficiency is very common among stroke patients particularly amongst those of older age [42] and frequently missed and not diagnosed constituting an additional potential explanation for inconsistencies in clinical studies. Throughout the review, it also became apparent that in the early clinical studies, impairment from cyanocobalamin among study participants with renal dysfunction hidden the benefit of B vitamins among participants with normal renal function [36]. Future clinical studies of Hcy-lowering interventions to prevent cardiovascular, cerebrovascular and peripheral artery disease should consider demographic and clinical features and test combinations of folic acid and methylcobalamin or hydroxocobalamin.

## Conclusion

Research into the role of nutritional interventions to lowering hyperhomocysteinemia as a measure to decrease the risk of cardiovascular, cerebrovascular and peripheral artery disease is evolving. So far, the evidence suggests that, compared to the single-use, a greater beneficial effect can be expected from combining folic acid plus vitamins B<sub>6</sub> and B<sub>12</sub> [23]. Adding betaine to the combination may affix anti-inflammatory advantages [39]. Patients younger than 69 years of age, with intracranial small vessel disease, from regions without folic acid food fortification or with poor dietary intake, with higher baseline cholesterol and Hcy levels, and those not receiving antiplatelet or lipid-lowering drugs may obtain the larger benefits from nutritional interventions [24,27-29]. Patients on enalapril or with high total cholesterol may get extra benefits from adding folic acid to their therapeutic regimen. Better results may be observed with a duration of interventions of > 36 months [25]. Given the frequent underdiagnosis of B<sub>12</sub> vitamin deficiency, serum B<sub>12</sub> and total Hcy should be checked routinely in cardiovascular and cerebrovascular disease patients and elevated total Hcy should be treated.

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