

High Homocysteine Serum Levels as A Cause of Early and Massive Atherosclerosis: Vitamins B-6-9-12 Supplementation: More Shadows Than Lights

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ABSTRACT

Folic acid and/or Vitamins B₆₋₁₂ deficiency induces high-homocysteine (H-Hcy) serum levels for reduced activity of methylene-tetrahydrofolate reductase (MTHFR). This metabolic derangement can be responsible for early and massive atherosclerosis, that could favour ischemic acute events. It can be assumed that vitamins' supplementation, reducing the elevated Hcy serum concentration, could reduce atherosclerotic risk. In this review, we evaluated if, the reduction of the high Hcy values by the B-vitamins' supplementation, is able to reduce the incidence of atherosclerotic events. Retrospective trials performed in patients already suffered of acute ischemic episodes, demonstrated that vitamins B₆₋₉₋₁₂ supplementation was unable to reduce the incidence of new ischemic events, even if it lowers the high Hcy levels. On the contrary, perspective studies carried out in patients not previously suffered of cardiovascular acute events, evidenced that the vitamins' supplementation significantly reduced both Hcy serum concentration and atherosclerotic risk. These conflicting results demonstrate that folic acid and vitamins B_{6/12} supplementation is effective in to reduce high Hcy serum concentration in patients with signs of previous atherosclerosis, but is unable to reduce the atherosclerotic risk. On the contrary, the supplementation is useful in to lower both high Hcy serum levels and atherosclerotic risk in patients without atherosclerotic marks. In

addition, some experiences performed in this field demonstrated that these nutrients could favor some negative effects, as the growth of an unknown neoplastic mass, especially the cells of prostate cancer. Therefore, the supplementation with folates and other vitamins of B group can be performed cautiously in patients with increased Hcy serum concentration.

Key words: Homocysteine; Ischemic risk; Folate and B₆₋₁₂ vitamins supplementation

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INTRODUCTION

Apart from traditional risk factors for cardiovascular disease, a powerful cause for raised cardiovascular morbidity and mortality can be the inherited or acquired hyper-homocysteinemia (H-Hcy). In this field, several reports referred that H-Hcy can be responsible for early and massive atherosclerosis, inducing some vascular diseases life threatening, such as myocardial infarction (MI), ischemic acute stroke (IS), and peripheral vascular disease (PVD)^[1-5]. Concerning this, several studies and trials demonstrated that elevated plasma total Hcy may promote atherothrombosis with several mechanisms^[6-8]. Among these, increased Hcy and S-adenosyl-homocysteine (SAH) serum values, inhibiting the methyltransferases, result in abnormal smooth muscle cell proliferation and reduction of endothelial cells' growth. A critical role in the atherosclerotic process is played by DNA hypomethylation^[9,10]. But, H-Hcy also causes down regulation of cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), resulting in depletion of H₂S, an angiogenic agent with antioxidant and vasorelaxing properties. Therefore, its depletion results in

vascular damage and vascular disease^[11]. Another mechanism HHcy-related, that favours the atherosclerotic lesions consists in its pro-oxidant action, responsible for the formation of reactive oxygen species (ROS). These can lead to lipid peroxidation (that initiates an inflammatory response) and are involved in establishing foam cells, a key atherosclerotic lesion component^[12]. In addition, Hcy has been shown to inhibit nitric oxide synthase (NOS) activity, further leading to endothelial dysfunction^[13]. Direct endothelial damage by the Hcy accumulation in endothelial cells, and increased platelets' aggregability is also involved^[14]. Further, Hcy affects the hemostatic process, for hypercoagulable state and a higher risk of thrombosis characterizing HHcy-patients. Therefore, increased plasma Hcy levels may increase cardiovascular risk by increasing thrombotic tendency^[15,16].

HOMOCYSTEINE METABOLISM

There are three pathways that maintain low serum levels concentration of Hcy. The first involves the methylation of Hcy and is catalyzed by methionine synthase (MS). In this, Hcy is converted into Methionine by the enzyme^[5,10] methylene-tetra-hydrofolatereductase (MTHFR) requiring folic acid and vitamin B₁₂ as cofactors. In turn, Methionine (reacting with ATP) forms S-adenosyl-methionine (SAM), the principal cellular methyl donor. Giving its methyl-group (C-H3), SAM is converted in S-adenosyl-homocysteine (SAH). Inside cells, SAH is turned in Hcy by the enzyme SAH-hydrolase. But, when intracellular Hcy concentration increased, the SAH-hydrolase reaction proceeds in favor of SAH formation. In this connection, Yi and others provided evidence that moderate Hcy increases were highly correlated with elevations in plasma SAH. That, in turn, was associated with hypomethylation involving DNA, phospholipids, proteins and catecholamine neurotransmitters^[17].

In the trans-sulfuration pathway (second metabolization pathway), Hcy can be irreversibly converted in cystathionine by the enzyme cystathionine-β-synthase (CBS), requiring vitamin B₆ as co-enzyme. Subsequently, cystathionine is metabolized into cysteine, a precursor for glutathione. This same is a powerful antioxidant, acting against oxidative damage.

A third route of Hcy metabolism happens in kidney and liver alone. In that, Hcy is remethylated by the enzyme betaine homocysteine methyl-transferase (BHMT), requiring vitamin B₆ as cofactor. BHMT transfers a methyl group from betaine to Hcy, producing dimethylglycine (DMG) and Methionine respectively (Figure 1).

Conclusively, Hcy metabolism requires three co-enzymes (co-factors) as folic acid (vitamin B₉), vitamin B₁₂ (cobalamin), and vitamin B₆ (pyridoxal phosphate). Folic acid is a substrate for production of 5-methyltetrahydrofolate (MTHF). Vitamin B₁₂ is required, as cofactor, for MS activity. Vitamin B₆ is a cofactor for CBS and BHMT.

Multiple experiences evidenced that the reduced presence/action of these cofactors causes an increase of Hcy levels, responsible for increased trend to early and massive atherosclerotic lesions. In this connection, it was hypothesized that folate and B₆₋₁₂ vitamins supplementation is useful in to lower both Hcy elevated serum levels and atherosclerotic trend^[18]. But, contrary to the expected results, large controlled trials have found that this supplementation, even through lowers Hcy levels, does not significantly reduce the incidence of cardiac, peripheral and cerebro-vascular atherosclerotic events. Concerning that, the Vitamin for Stroke Prevention (VISP) study, a multicenter randomized non-placebo trial performed in patients with a history of previous ischemic stroke, demonstrated that both low (2.0 mg. of folate, 20 mg. of B₆, 6 mcg. of B₁₂) or high (2.5 mg. of folate, 25 mg. of B₆, 400 mcg. of B₁₂) doses of folate and B₆₋₁₂ vitamins are unable to prevent ischemic acute events, even if lowers high Hcy levels^[19]. In the Heart Outcomes Prevention Evaluation (HOPE) 2 study, 5522 patients with pre-existing cardiovascular disease (CVD) were randomly assigned to Hcy-lowering therapy and treated with folate and B vitamins (2.5 mg of folate, 50 mg. of B₆, 1 mg of B₁₂) or placebo. After a five-year follow-up, in this study also no differences in cardiovascular disease or cardiovascular death were detected among any of the four groups. Referring to these results, the authors concluded that the active treatment with folates and B-vitamins is not recommended^[21]. Previously, the VITATOPS (VITamins TO Prevent Stroke) study, a multicenter, randomized, double blind, placebo controlled secondary stroke prevention trial, was performed to determine whether the addition of vitamins B₆₋₁₂ is able to reduce the combined incidence of vascular events (stroke, MI) and vascular death in patients with recent stroke or TIA. In this trial too, the results obtained showed that vitamins B₆₋₉₋₁₂ supplementation (2 mg of folate, 25 mg of B₆, 0.5 mg of B₁₂) is safe but not statistically significantly in reducing the risk of major vascular events^[22]. In accordance, a meta-analysis of several randomized, controlled trials performed by Bazzano *et al* concluded that folic acid supplementation did not reduce the risk of CVD and all cause mortality among participants with prior history of CVD^[23].

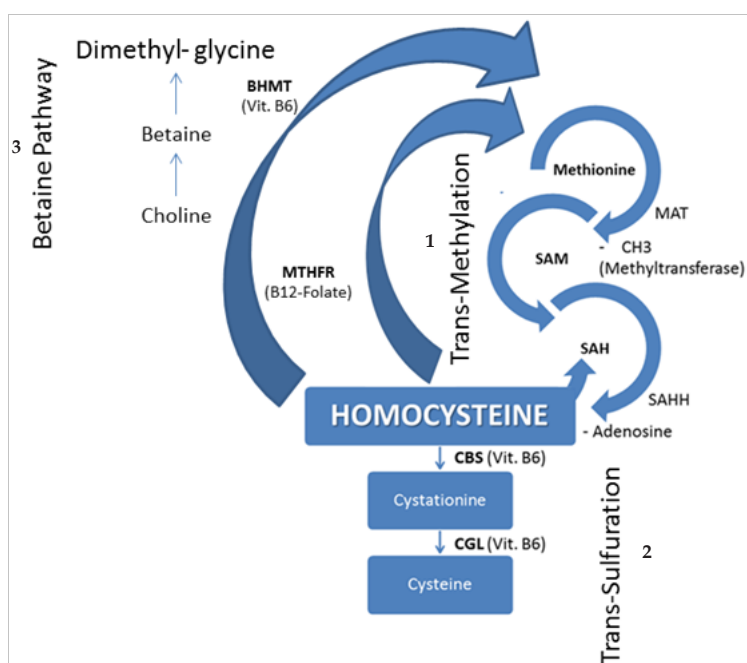


Figure 1 Homocysteine metabolism: three pathways. MTHFR: Methylene-Tetra-Hydro-Folate-Reductase; MAT: Methionine-Adenosyl-Transferase; SAM: S-Adenosyl-Methionine; SAH: S-Adenosyl-Homocysteine; SAH-H: SAH-Hydrolase; CBS: Cystathionine-Beta-Synthase; CGL: Cystathionine-Gamma-Lyase; BHMT: Betaine-Homocysteine-Methyl-Transferase.

Contrarily to these retrospective trials, a meta-analysis of prospective observational studies showed that 25% lower Hcy levels was associated with an 11% lower ischemic heart disease risk and a 19% lower stroke risk^[2]. In addition, Wald *et al* have evaluated 20 prospective studies and concluded that lowering Hcy concentration by 3 $\mu\text{mol/L}$ decreased the risk of ischemic heart disease by 16% and stroke by 24%^[24]. Other studies have reported that folic acid supplementation has been found to improve endothelial function, a surrogate end point for cardiovascular risk, both in healthy and in patients with H-Hcy^[25-29].

The conflicting results obtained in these studies are summarized in Table 1. The exact mechanisms underlying the ameliorative effects on atherosclerotic risk obtained in the last studies are still unknown. Perhaps, this effect may be reported to an independent lowering-Hcy mechanism and could referred to direct pharmacological actions of Vitamins B₆₋₉₋₁₂ on atherosclerosis, rather than to indirect consequence of the reduction in Hcy serum levels. Likely, 5-methyl tetrahydrofolate (a derived compound of folic acid) improves nitric oxide production and prevents superoxide generation, via uncoupling of nitric oxide synthase by stabilizing tetrahydrobiopterin or regenerating this same from its inactive form^[30,31]. In confirmation of that, a study of Doshi *et al* provides evidence indicating that an improvement in endothelial function (assessed by flow-mediated dilatation) was observed after the first dose of folic acid and was present before any significant reduction in Hcy serum levels. This observation demonstrated that the improvement in endothelial function is due to direct pharmacologic actions of folic acid rather than reductions in Hcy concentrations^[32]. Another folate study performed more recently confirmed that daily supplementation of oral folic acid improves arterial function in patients with peripheral arterial disease^[33]. Concerning this, a randomized study conducted in 103 patients at increased risk of stroke, investigated the effect of folic acid supplementation on carotid intima-media thickness (IMT). After 18 months of folic acid (vitamin B₉) supplementation, a significant regression of carotid IMT in the treated group compared to IMT progression recorded in the placebo group was found^[34]. Similar results were reported in a study examining the influence of folic acid on carotid artery atherosclerosis. Another perspective study showed an inverse association between plasma folate and plaque calcification score^[35]. In addition, a recent analysis of multiple studies suggests that folic acid supplement can reduce the risk of stroke in people with H-Hcy who have not already suffered a stroke, but did not reduce the risk of second stroke in people who have already had one. This observation raises the possibility that Hcy lowering is beneficial at early stages of vascular disease elaboration, but is less effective in the face of established advanced disease^[36]. An improvement of flow-mediated dilation was also found in hyperhomocysteinemic patients with unstable angina^[37]. Finally, Kolling *et al* described that folic acid supplementation is useful in to prevent Hcy-induced peroxidation, ROS production, antioxidant and NO reduction in rats^[38]. On the contrary, the negative results on cardiovascular risk reported in studies previously performed by vitamins B supplementation in patients with HHcy serum levels must be referred to numerous design and methodological flaws including limited statistical power, and relatively short duration of follow-up in pts. evaluated. It must be also added that these trials were addressed to secondary prevention of atherosclerotic acute events. On the contrary, the Hcy-lowering with folate and vitamins B₁₂₋₆ supplementation could play a more important role in to reduce atherosclerotic risk in the primary prevention. It must be also added that the reversion of an atherosclerotic process in patients already suffered of ischemic acute events is more

Table 1 Results of B₆₋₉₋₁₂ supplementation in several trials and meta-analyses.

	Stroke	AMI	CVD	Sudden Death
VISP 2004 ^[19]	-	-	-	-
NORVIT 2006 ^[21]	-	-	-	-
HOPE-2 2006 ^[20]	-	-	-	-
VITATOPS 2010 ^[22]	-	-	-	-
Bazzano 2006 ^[23]	-	-	-	-
Wald 2002 ^[24]	+	+	-	-
H.C.S. 2002 ^[2]	+	+	-	-
Verhaar 2002 ^[25]	-	-	+	-
Moat 2006 ^[26]	-	+	-	-
Moens 2007 ^[27]	-	+	+	-
Shirodaria 2007 ^[28]	-	-	+	-
Tian 2008 ^[29]	-	+	-	-

AMI: Acute Myocardial Infarction; CVD: Cardio-Vascular Disease; (-): negative results; (+): positive results.

difficult. It must also be added that, although folate and vitamins B₁₂₋₆ supplementation lowers Hcy levels, they may simultaneously increase atherosclerotic risk through other Hcy-independent mechanisms. Concerning this, an editorial by Loscalzo postulated that folic acid and B vitamins supplementation may even promote atherosclerosis, by increasing cell proliferation in atherosclerotic plaques enhancing methylation of DNA, and augmenting levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide (NO) synthesis^[39,40]. Another possibility to explain the negative results is that H-Hcy is a clinically important risk factor only when plasma Hcy is elevated to extremely high levels, but it is less important for light or mild H-Hcy, as happens in the greatest number of patients with H-Hcy^[41-43]. In confirmation of that, in contrast to the evident clinical benefits of Hcy-lowering therapy obtained in patients with severe H-Hcy, in those with mild-moderate Hcy (values ranged between 15-30 $\mu\text{mol/L}$), the reduction of vascular risk is negligible by the B vitamins supplementation, although the Hcy concentration lowers^[44].

CONCLUSIVE REMARKS

Referring to these conflicting results, we may affirm that the folic acid and B₆₋₁₂-vitamins supplementation is useless when an acute ischemic event is already happened. On the contrary, this supplementation could efficaciously be carried out early, when the atherosclerotic lesions didn't show yet. A further caution concerning the vitamins-B₆₋₉₋₁₂ supply referred to some, frequent side effects, as black stools, diarrhea, incomplete or infrequent bowel movements, intense abdominal pain, depression or bronchospasm. It was also added that the supplementation must be given cautiously because of some contradictory published data suggesting that folic acid could induce the growth of neoplastic mass once constituted^[45] or could favour its beginning. That can be caused by folate-mediated one-carbon metabolism (which involves B-vitamins), by inducing epigenomic changes and influencing the DNA synthesis^[46]. Concerning this topic, it must be added that high folate concentrations were especially associated with increased prostate cell proliferation^[47]. These limitations justify the uncertain about the folates and Vitamins B supplementation in to prevent the atherosclerotic marks in patients with increased Hcy serum levels.

In conclusion, the evidences of several studies published over two decades suggest that vitamins-B supplementation in patients suffering from H-Hcy could be a nontoxic and easy method in the primary prevention of CVD. On the contrary, its employment in the secondary prevention of atherosclerotic disease seems not to be indicated or heedless, even if the supplementation significantly reduces the high Hcy serum levels.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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