

Review Article

Homocysteinemia, cardiovascular risk and long-term enteral nutrition

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ABSTRACT: *The problem of micronutrients and vitamin supplementation in artificial nutrition is widely debated as it involves a large number of clinically heterogeneous patients. Homocysteine is a sulfur-containing amino acid produced in the metabolism of the essential amino acid methionine. In the metabolic pathways involved in homocysteine turnover, folic acid (FA) and vitamins B6 and B12 are important regulators. Experimental and clinical evidence has long suggested a causal relationship between hyperhomocysteinemia and cardiovascular disease. Reducing plasma homocysteine by multivitamin therapy consisting of FA and vitamins B6 and B12 has been proposed in clinical practice as a strategy to prevent cardiovascular disease in high-risk patients. Recently, however, the results of clinical controlled trials failed to show a conclusive relationship between homocysteine and cardiovascular disease. Thus, while multivitamin supplementation is indicated to prevent malnutrition and the clinical consequences of vitamin deficiency, its potential implications for the prevention of atherosclerosis need to be reassessed. (Nutritional Therapy & Metabolism 2007; 25: 1-7)*

KEY WORDS: *Homocysteine, Atherosclerosis, Artificial nutrition, Outcome*

INTRODUCTION

Adequate vitamin intake is essential to avoid functional deficiencies. In addition, some vitamins, especially those belonging to the B group, are involved in the metabolism of intermediate products and their deficiency may have detrimental effects. Homocysteine, a sulfur-containing amino acid produced in the metabolism of the essential amino acid methionine, depends for its turnover on the availability of folic acid and the vitamins B6 and B12 (1). Increased plasma levels of homocysteine have long been associated with cardiovascular disease, following the observation of premature extended atherosclerosis in patients with homocystinuria, an inborn error of methionine metabolism (2). After this seminal observation many experimental (3-5) and clinical studies (6-8) have suggested a link between atherosclerosis and cardiovascular disease.

The pathophysiologic mechanisms by which homocysteine relates to atherosclerosis may involve a direct toxic effect on the vessel wall and on the thrombogenic pathways. Homocysteine promotes inflammation (9) and oxidative stress (10), endothelial dysfunction (11) and smooth muscle cell proliferation (12); it enhances

platelet aggregation (13), tissue factor activity (14) and binding of monocytes to endothelial cells (15). In addition, it is associated with increased PAI-1 and TPA levels, resulting in impaired fibrinolytic potential (16).

In the light of these observations, controlled clinical trials have been performed to confirm the findings in large study populations and to validate the use of multivitamin supplementation to lower increased plasma homocysteine in high-risk patients. The results, however, have been disappointing because they failed to demonstrate any causal relationship between plasma homocysteine and vascular disease. This has nutritional implications since adequate vitamin B and folic acid supplementation is required to prevent nutritional deficiency but not hyperhomocysteinemia and vascular disease.

HOMOCYSTEINE METABOLISM AND MODULATION OF PLASMA LEVELS

Plasma homocysteine levels are the product of a number of intersecting metabolic pathways involved in its production and removal. Homocysteine is produced as a result of methylation reactions and represents an in-

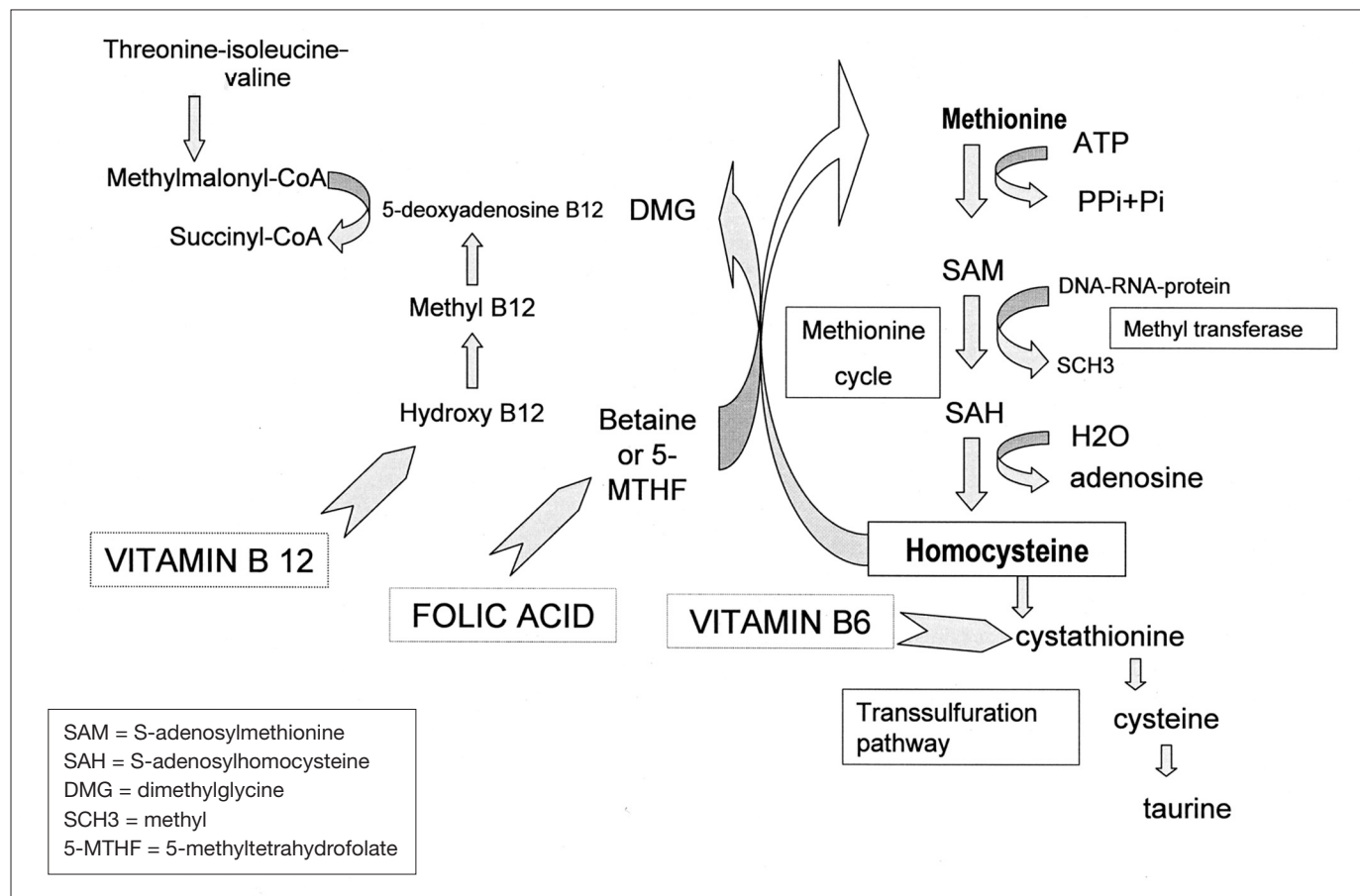


Fig. 1 - Homocysteine metabolism.

intermediate product of the metabolism of the essential amino acid methionine. The importance of methionine in cellular function relies on the fact that, together with cysteine, it is a sulfur donor in the cellular metabolism. In addition, methionine is used to synthesize S-adenosyl methionine (SAM), an important methyl donor.

Once produced, homocysteine is metabolized via remethylation, resulting in the formation of methionine, or via transsulfuration, resulting in the formation of cysteine and finally taurine (17) (Fig. 1). The remethylation pathway of homocysteine is strongly dependent on the availability of folic acid in the active form of 5-methyltetrahydrofolate and vitamin B12. The latter is essential for optimal activity of the enzyme methionine synthase, which is responsible for methylation of homocysteine to methionine (18). In addition to being important cofactors in the metabolism of homocysteine, both folates and vitamin B12 play a pivotal role in the synthesis of purine and pyrimidine bases, which are essential for the synthesis of nucleic acids (19).

In the transsulfuration pathway of homocysteine, which results in the formation of cysteine and taurine, an essential cofactor is the active form of vitamin B6, pyridoxal-5'-phosphate (P5P). Adequate levels of this vitamin are provided by a balanced dietary intake and by a functioning liver. Another important cofactor in this pathway is the amino acid serine, a product of betaine metabolism in the same pathway (20). This series of reactions converts homocysteine into cysteine and accounts for the fact that cysteine is not an essential dietary amino acid, provided adequate supplies of methionine are available. The transsulfuration pathway has a limited distribution, as it is only found in the liver, kidney, small intestine and pancreas (21). The homocysteine metabolism is thus highly dependent on vitamin-derived cofactors. Methionine synthase contains cobalamin as its prosthetic group and derives its methyl group from folic acid. The transsulfuration enzymes contain pyridoxal phosphate as their prosthetic group. Deficiencies of any of these vitamins (vitamin B12, folic acid

and vitamin B6) are associated with hyperhomocysteinemia (22). Since exchange of methyl and sulfur groups is implicated in the homocysteine metabolism, altered levels of homocysteine negatively affect a number of synthetic pathways in which sulfur and methyl groups are involved (23). Plasma homocysteine levels can increase as a consequence of 1) genetic mutations (18, 24; Tab. I); 2) clinical conditions; and 3) drugs (Tab. II). Increased homocysteine levels characterize chronic renal failure, since homocysteine is cleared by the kidneys, and hypovitaminosis, in particular deficiency of folic acid, cobalamin and vitamin B6. When the metabolic pathways are saturated the excess intracellular homocysteine is released into the circulation, binding plasma proteins, or eliminated through the kidneys.

HOMOCYSTEINE MEASUREMENT

Plasma total homocysteine is determined by high-performance liquid chromatography (HPLC). The normal range for plasma homocysteine levels is 5-15 $\mu\text{Mol/L}$, with ranges of 16-30 $\mu\text{Mol/L}$, 31-100 $\mu\text{Mol/L}$ and $>100 \mu\text{mol/L}$ being considered as mild, moderate and severe hyperhomocysteinemia, respectively (25). There are some considerations to take into account when measuring plasma homocysteine:

- 1) Men have higher plasma homocysteine levels than women because they have different muscular masses (26);
- 2) Smoking (27, 28) and heavy coffee consumption (28) result in higher levels of homocysteine;
- 3) High levels of homocysteine can be found in hypothyroidism because hypothyroidism decreases the hepatic levels of enzymes involved in the remethylation pathway of homocysteine (29);
- 4) Many drugs increase the level of homocysteine either by interfering with the metabolism of folate or vitamin B12 or B6 or by altering renal function, but the underlying mechanisms remain to be discovered. Some of these drugs are reported in Table II (for a complete list see refs. 25, 30);
- 5) Patients with renal failure (31, 32) and elderly people (27) have higher levels of homocysteine; this could be the result of an increased production rate, a decreased rate of removal by transsulfuration or remethylation, or a decreased excretion of homocysteine.

Before a final diagnosis of hyperhomocysteinemia is made, all the previous conditions need to be excluded.

In other situations, hyperhomocysteinemia will not be overt until the pathways of the homocysteine metabolism are stressed, as with the methionine loading test. In

TABLE I - HYPERHOMOCYSTEINEMIA AND GENETIC MUTATIONS
(18, 24)

5-10-methyltetrahydrofolate reductase (MTHFR) (human chromosome 1p43)
Methionine synthase (MS)
Methionine synthase reductase (MSR)
Cystathionine-beta synthase (CBS)

TABLE II - FACTORS INFLUENCING PLASMA HOMOCYSTEINE CONCENTRATION

Gender
Age
Muscular mass
Smoking
Coffee consumption
Thyroid function
Renal failure
Drugs:
- Fibrates
- Statins
- Niacin
- Antihypertensive agents
- Metformin
- Methotrexate
- Sulfasalazine
- Anticonvulsant drugs
- Levodopa

this test the patient is given an oral dose of 100 mg/kg methionine, followed by plasma homocysteine determination 6 hours later. The methionine loading test might be a more reliable assay because it can identify individuals who would not have hyperhomocysteinemia on a fasting sample (25).

HOMOCYSTEINE LOWERING AND CARDIOVASCULAR DISEASE

A number of experimental and clinical observations suggest a link between homocysteine and accelerated atherosclerosis. Many population studies have previously addressed the effects of multivitamin supplementation resulting in a reduction of plasma homocysteine on multiple surrogate markers of cardiovascular disease. A summary of the results is reported in Table III (33-36).

Recently, however, the results of 3 large randomized controlled trials addressing the effects of homocysteine lowering on hard cardiovascular outcomes have shed new light on the issue. Although the data generated have raised even more questions and more clinical trials are

TABLE III - RECENT CLINICAL STUDIES ON THE EFFECT OF HOMOCYSTEINE LOWERING ON SURROGATE CARDIOVASCULAR ENDPOINTS

Study, Year (reference)	Description	Treatment groups and follow-up	Outcomes and comparison	Results
The VITRO (Vitamins and Thrombosis) Study (33)	701 patients with previous deep vein thrombosis or pulmonary embolism; 341 normohomocysteinemic and 360 hyperhomocysteinemic	5 mg FA + 0.4 mg cyanocobalamin + 50 mg pyridoxine or placebo for 2.5 years	Recurrence of deep venous thrombosis or pulmonary embolism in users vs nonusers	Homocysteine lowering with multivitamin supplementation does not prevent recurrent venous thrombosis
The ASFAST (Atherosclerosis and Folic Acid Supplementation) Trial (34)	315 patients with CRF	15 mg FA or placebo for 3.6 years	Progression in carotid IMT, composite clinical endpoint of incident MI, stroke and CV deaths in users vs nonusers	High-dose FA while reducing plasma homocysteine does not slow atherosclerosis progression or improve CV morbidity and mortality in patients with CRF
The Swiss Heart Study (35)	553 patients with a history of a previous successful angioplasty of at least 1 significant coronary stenosis	FA (1 mg/day), vitamin B12 (cyanocobalamin, 400 µg/day), and vitamin B6 (pyridoxine hydrochloride, 10 mg/day) (n = 272) or placebo (n = 281) for 6 months	Composite endpoint: major adverse events (death, nonfatal MI, and need for repeat revascularization) at 6 months and 1 year in users vs nonusers	Homocysteine-lowering therapy with FA, vitamin B12 and vitamin B6 significantly decreases the incidence of major adverse events after percutaneous coronary intervention
Lange et al, 2004 (36)	636 patients who had undergone successful coronary stenting	1.2 mg FA, 48 mg B6, 60 µg B12 or placebo for 6 months	Angiographic restenosis in users vs nonusers	Restenosis rate significantly higher in the vitamin group

FA, folic acid; CRF, chronic renal failure; IMT, intima-media thickness; MI, myocardial infarction; CV, cardiovascular

ongoing, some conclusions can be drawn.

The VISP (Vitamin Intervention for Stroke Prevention) (37) trial was a secondary prevention study lasting 2 years and enrolling 3680 patients with recent ischemic stroke. The trial investigated whether B-vitamin and folic acid supplementation (2.5 mg folic acid, 25 mg B6 and 0.4 mg B12 vs 0.02 mg FA, 0.2 mg B6 and 0.4 mg B12) could prevent recurrent stroke, myocardial infarction and death. Compared to the low-dose group, treatment with high-dose multivitamin supplementation did not result in a reduced occurrence of stroke, coronary events or deaths.

The NORVIT (Norwegian Vitamin) (38) study enrolled 3749 patients with recent myocardial infarction who were randomized to receive either multivitamin supplementation (0.8 mg folic acid, 0.4 mg vitamin B12 and 40 mg vitamin B6 per day), or 0.8 mg FA+0.4 mg B12 or 40 mg B6 or placebo for 40 months. Vitamin treatment did not significantly reduce the risk of the primary endpoint (incidence of myocardial infarction, stroke and cardiac sudden death) in either group as com-

pared with placebo. In the group on multivitamin supplementation there was a significant increase in primary endpoint and nonfatal myocardial infarction while the incidence of cancer was slightly higher in the folate group.

Finally, the Hope-2 (Heart Outcomes Prevention Evaluation) trial (39) included 5522 patients with a history of vascular (coronary, peripheral or cerebrovascular) disease or diabetes and additional risk factors for atherosclerosis, irrespective of their plasma homocysteine levels, from countries with mandatory folate food fortification. Patients received a combined pill containing 2.5 mg of folic acid, 50 mg of vitamin B6 and 1 mg of vitamin B12 or placebo for 5 years. Active treatment did not significantly decrease the risk of death from cardiovascular causes, myocardial infarction, or any of the secondary outcomes (total ischemic events, death from any cause, hospitalization for unstable angina, hospitalization for congestive heart failure, revascularization, the incidence of cancer, and death from cancer). The incidence of stroke was reduced by 25%. More patients in

TABLE IV - MULTIVITAMIN SUPPLEMENTATION IN ORAL AND ENTERAL NUTRITION

Therapeutic options to lower homocysteine	Recommended daily dose in high-risk patients (mg) (42-44)	Daily requirement (mg) (45-47)	Content in commercial mixtures for EN per 1500 kcal (mg) (41)
Folic acid	0.5-5	~ 400	400 mg
Vitamin B6	10-500	2.5-4	2.5-4 mg
Vitamin B12	0.5-1	3-6	3-6 mg

EN, enteral nutrition.

the active treatment group were hospitalized for unstable angina.

The results of these studies suggest that so far there is insufficient evidence to confirm that homocysteine is a modifiable causal risk factor for acute complications of atherosclerosis or to recommend routine screening for, or treatment of, raised homocysteine concentrations with folic acid and other vitamins in order to prevent acute ischemic cardiovascular events. These data also highlight the possibility of adverse effects of high-dose B-vitamin supplementation.

METHIONINE, B VITAMINS AND FOLIC ACID IN LONG-TERM ENTERAL NUTRITION

The essential amino acid methionine, a key molecule in the metabolism of homocysteine, is normally included in mixtures for enteral nutrition in the amount of 112-133 mg/100 mL, which largely covers the minimally required amount (40). However, while vitamin status highly affects homocysteine levels, it has been shown that methionine intake does not significantly influence plasma homocysteine. Thus, hyperhomocysteinemia can be corrected by B6, B12 and folate supplementation. Commercially available preparations for enteral nutrition contain a mix of multivitamins including B6, B12 and folic acid, calculated to cover the daily requirement of each vitamin. Current indications for B vitamins and folic acid supplementation in long-term enteral nutrition suggest no need for integration (41). Since many patients on long-term enteral nutrition have a history or are at risk of cerebrovascular events, treating or not treating elevated plasma homocysteine levels may be an issue. In this case the status of plasma B6/B12 and folic acid needs to be assessed at the beginning of enteral nutrition, during intercurrent illnesses or if malabsorption is suspected, in order to correct clinical abnormalities (41); however, there is no absolute need to correct mild hyperhomocysteinemia to prevent acute vascular events in

these patients. In high-risk patients the therapeutic target for homocysteine plasma levels is <13-15 $\mu\text{mol/L}$ (25, 42-44); in this category of patients, cautious supplementation is not contraindicated in the presence of hyperhomocysteinemia to reach the therapeutic target. A side consideration is that the results of the previous studies were obtained in populations without nutritional problems and folate fortification is widespread in developed countries. In contrast, in enteral nutrition regimens, folate intake is based on the daily calculated requirement and there are no data comparing folate plasma levels in the general population versus long-term enteral nutrition. The results of the previous studies also point to the possibility of adverse effects of high-dose folic acid and B vitamin supplementation: this possibility must be taken into account especially in cancer patients on enteral nutrition. A summary of the daily requirements and doses of vitamin precursors to lower plasma homocysteine is given in Table IV.

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