

# Therapeutical approach to plasma homocysteine and cardiovascular risk reduction

Marcello Ciaccio  
Giulia Bivona  
Chiara Bellia

Department of Medical  
Biotechnologies and Forensic  
Medicine, Faculty of Medicine,  
University of Palermo, Italy

**Abstract:** Homocysteine is a sulfur-containing amino acid produced during metabolism of methionine. Since 1969 the relationship between altered homocysteine metabolism and both coronary and peripheral atherothrombosis is known; in recent years experimental evidences have shown that elevated plasma levels of homocysteine are associated with an increased risk of atherosclerosis and cardiovascular ischemic events. Several mechanisms by which elevated homocysteine impairs vascular function have been proposed, including impairment of endothelial function, production of reactive oxygen species (ROS) and consequent oxidation of low-density lipids. Endothelial function is altered in subjects with hyperhomocysteinemia, and endothelial dysfunction is correlated with plasma levels of homocysteine. Folic acid and B vitamins, required for remethylation of homocysteine to methionine, are the most important dietary determinants of homocysteine and daily supplementation typically lowers plasma homocysteine levels; it is still unclear whether the decreased plasma levels of homocysteine through diet or drugs may be paralleled by a reduction in cardiovascular risk.

**Keywords:** homocysteine, MTHFR, cardiovascular disease, folate, B vitamin

## Introduction

The homocysteine “hypothesis of arteriosclerosis” was first proposed by McCully (1969), who observed premature atherothrombosis of the peripheral, coronary, and cerebral vasculature in children with homocystinuria, an inborn error in methionine metabolism. In 1976, Wilcken and Wilcken provided the first evidence of a relationship between abnormal homocysteine metabolism and coronary artery disease (CAD) in the general population (Wilcken and Wilcken 1976). Since these seminal observations, results from a large number of clinical and epidemiologic investigations have implied a role for homocysteine in atherosclerotic cardiovascular disease (CVD) (Welch and Loscalzo 1998; The Homocysteine Studies Collaboration 2002; Wald et al 2002, 2006). The aim of this paper was to survey the state of the art regarding homocysteine, cardiovascular risk and its potential reduction by homocysteine lowering; several studies available on Medline was selected using “homocysteine”, “CVD risk”, “folate and vitamin B therapy” as key words up to 2007. The overall strength of the evidence in these publication was evaluated according to a widely used criteria: the first level of evidence included multiple, well-designed, randomized controlled clinical trials; the second one included multiple well-designed cohort or case-control studies, or well-designed meta-analysis and the third level include smaller or less optimal designed studies or descriptive studies.

## Homocysteine metabolism

Homocysteine is a sulfur-containing amino acid produced in the metabolism of the essential amino acid methionine. Homocysteine is metabolized through two pathways: remethylation and transsulfuration. In remethylation, homocysteine acquires

Correspondence: Marcello Ciaccio  
Department of Medical Biotechnologies  
and Forensic Medicine, Via del Vespro,  
129 – Policlinico Universitario,  
90127 – Palermo, Italy  
Tel +39 091 655 32 96  
Fax +39 091 655 32 75  
Email marcello.ciaccio@unipa.it

a methyl group from methyltetrahydrofolate (MTHF) to form methionine in a vitamin B<sub>12</sub>-dependent reaction. The formation of the methyl donor MTHF depends on the presence of methyltetrahydrofolate, derived from dietary folate, and methyltetrahydrofolate reductase (MTHFR). A considerable proportion of the methionine formed in this pathway is then activated by adenosine triphosphate (ATP) to form S-adenosylmethionine (SAM). SAM serves primarily as a universal methyl donor to a variety of acceptors including nucleic acids, neurotransmitters, phospholipids, and hormones. S-adenosylhomocysteine (SAH), the byproduct of these methylation reactions, is subsequently hydrolyzed, thus generating homocysteine, which then becomes available to start a new cycle of methyl-group transfer. In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by the pyridoxal phosphate-containing enzyme, cystathionine β-synthase (CBS). Cystathionine is hydrolyzed to form cysteine, the excess of which is excreted in the urine. Thus, this transsulfuration pathway effectively catabolizes excess homocysteine which is not required for methyltransfer. Because of the existence of a cellular homocysteine export mechanism, plasma normally contains a small amount of homocysteine averaging 10 μmol/L. This export mechanism, together with transsulfuration pathway, helps maintain low intracellular concentration of this potentially cytotoxic sulfur amino acid. The occurrence of hyperhomocysteinemia indicates that homocysteine metabolism has in some way been disrupted and that the export mechanism is disposing into the blood excess homocysteine. This export mechanism limits intracellular toxicity, but leaves vascular tissue exposed to the possible deleterious effects of excess homocysteine.

## Hyper-homocysteinemia determinants

### Genetic causes

Congenital homocystinuria associated with severe hyperhomocysteinemia is caused by homozygous defects in the gene encoding for CBS. This condition is unquestionably associated with precocious atherosclerosis and extensive arterial thrombosis. In these patients, the main cause of mortality and morbidity is thromboembolism, followed by cerebrovascular accident, peripheral arterial thrombosis, and myocardial infarction. Rarely, homocystinuria is caused by low methionine synthase activity, or severe defects of MTHFR (Rosenblatt and Cooper 1990) (Table 1).

However, in 1988 has been reported that two unrelated patients with moderate hyperhomocysteinemia and low folate

**Table 1** Major determinants of serum/plasma homocysteine concentration

Homocysteinemia determinants	
Genetic factors	
	CBS deficiency ( <i>homocystinuria</i> )
	Methionine synthase deficiency ( <i>homocystinuria</i> )
	Methionine synthase reductase deficiency ( <i>homocystinuria</i> )
	MTHFR C677T (TT genotype)
Physiological determinants	
	Increasing age
	Male sex
	Pregnancy
	Postmenopausal state
	Increasing muscular mass
Lifestyle determinants	
	Folate, vitamin B <sub>12</sub> , B <sub>6</sub> , B <sub>2</sub> intake
	Smoking
	Coffee
	Exercise
Clinical conditions	
	Folate, vitamin B <sub>12</sub> , B <sub>6</sub> deficiency
	Renal failure
	Early and late stage of diabetes

levels had a variant of MTHFR that was distinguished from the normal enzyme by its lower specific activity (50%) and its thermolability (Kang et al 1988). In subsequent studies, the same author showed that MTHFR thermolability was an inherited recessive trait (Kang et al 1991). After the MTHFR gene was cloned (Goyette et al 1994), the cause of the thermolability was shown to be a common polymorphism, 677 C > T, that results in the substitution of an alanine with a valine in the catalytic domain of the enzyme. The gene frequency for 677 T > C varies among ethnic groups with the T allele having a frequency of around 30% in Europeans and Japanese but only a frequency of around 11% in Africans Americans (Schneider et al 1998). A second polymorphism, 1298 A > C, leads to the change of a glutamate to an alanine in the C-terminal regulatory domain of MTHFR and it is associated with an approximately 35% decrease in MTHFR activity, but not with thermolability (Weisberg et al 1998).

### Other causes

Mild (15–20 μmol/L) or moderate (20–50 μmol/L) degrees of hyperhomocysteinemia are generally the result of acquired disorders (Table 1). The most frequent causes are deficiencies of vitamins that are required as cofactors or substrate for homocysteine metabolism: actually, serum homocysteine levels show an inverse correlation with serum vitamin B<sub>12</sub>, B<sub>6</sub> and folate. Consequently, plasma homocysteine can be increased by various drugs and condition that interfere with folate, vitamin B<sub>12</sub> and vitamin B<sub>6</sub> metabolism.

Renal impairment commonly causes hyperhomocysteinemia, probably not because of impaired urinary excretion, which is a minor route for direct homocysteine clearance, but because of impaired metabolism of homocysteine by the kidney, the major route by which homocysteine is cleared from plasma (Bostom and Lathrop 1997).

## Homocysteinemia assessment

Homocysteine is present in plasma in four forms: about 1% circulate as the free thiol; 70%–80% is disulfide-bound to plasma proteins, chiefly albumin; the remaining 20%–30% combines with itself to form the homocysteine dimer or with other thiols, including cysteine, with which it forms the homocysteine-cysteine mixed disulfide (Ueland 1995). The term “total plasma (or serum) homocysteine” (tHcy) refers to the combined pool of all four forms of homocysteine.

In all available assays, plasma or serum is initially treated with a reducing agent that converts all Hcy species into the reduced form HcyH, which is measured either directly or after derivatization. Briefly, tHcy can be determined in serum or plasma by chromatographic methods or by enzyme and immunoassays. The chromatographic assays include wide analytical range, simultaneous determination of other compounds (other sulfur aminoacids), and sometimes lower cost than commercial reagent-based assays, but they usually require skilled staff, are labour-intensive, and throughput may be low. On the other hand, widely used enzyme and immunoassays are usually simple to perform, satisfactory analytic criteria, and give comparable results, so they are now suitable for routine laboratories (Refsum et al 2004).

The determination of tHcy can be done in a fasting state or after methionine load; post-load tHcy is probably more sensitive than the fasting tHcy to disturbances in the transsulfuration pathway such as those caused by CBS or vitamin B<sub>6</sub> deficiency (Refsum et al 1997).

## Homocysteine and cardiovascular disease

### Pathophysiologic mechanisms

The mechanisms by which elevated homocysteine impairs vascular function are not completely understood. Laboratory investigations have revealed several potential mechanisms, including impairment of endothelial function (Woo et al 1997), production of reactive oxygen species (ROS) and consequent oxidation of low-density lipids (Pfanzagl et al 2003; Hayden and Tyagi 2004), increased monocyte adhesion to the vessel wall (Welch and Loscalzo 1998), increased lipid uptake and retention (Welch and Loscalzo 1998), activation of the

inflammatory pathway (Hofmann et al 2001), stimulatory effects on smooth-muscle proliferation (Welch and Loscalzo 1998), thrombotic tendency mediated by activation of coagulation factors (Undas et al 2001), hypofibrinolysis (Lauricella et al 2006), and platelet dysfunction (Ungvari et al 2000). The atherogenic and thrombogenic potentials of homocysteine have been implicated in promoting endothelial dysfunction induced by acute hyperhomocysteinemia after methionine loading in human subjects (Bellamy et al 1998), facilitating the progression of atherosclerotic plaque in apolipoprotein E-deficient mice (Hofmann et al 2001), promotion of prothrombotic state (Welch and Loscalzo 1998), and exacerbation of intimal hyperplasia and restenosis after balloon injury of arteries (Morita et al 2001; Cook et al 2002). These findings provide a coherent and biologically plausible basis for a direct role for homocysteine in promoting atherothrombosis.

### Hyperhomocysteinemia and cardiovascular risk

The results of early cross-sectional and case-control studies strongly support that tHcy measured in serum or plasma is a strong predictor of cardiovascular disease risk (Ueland and Refsum 1989; Boushey et al 1995; Refsum et al 1998; Hankey and Eikelboom 1999). Since 1992, however, the results of several large, well-conducted prospective studies in which blood samples were collected before the cardiovascular event showed weaker relations and gave a less consistent picture (Christen et al 2000; Ueland et al 2000). Some prospective studies showed a strong association between tHcy and cardiovascular disease (Arnesen et al 1995; Perry et al 1995; Wald et al 1998; Bots et al 1999; Ridker et al 1999), some found weaker association (Stampfer et al 1992; Giles et al 1998; Stehouwer et al 1998; Ubbink et al 1998), and others, including the Multiple Risk Factor Intervention Trial and the Atherosclerosis Risk in Communities Study, failed to find any significant associations (Evans et al 1997; Folsom et al 1998). The reasons for these conflicting results have not been fully explored, but may be related to differences in diet, lifestyle, and other cardiovascular risk factors, and to characteristics including length of follow-up and blood sample handling and storage. Notably, prospective studies of patient populations known to be at high risk of cardiovascular events consistently report strong positive association between tHcy and cardiovascular morbidity or mortality (Nygård et al 1997; Moustapha et al 1998; Kark et al 1999; Stehouwer et al 1999; Taylor et al 1999). In follow up studies of the Framingham cohort tHcy was shown to have strong and significant associations of similar strength

with both all-cause and cardiovascular disease (Bostom et al 1999). The results of several investigations have been compiled in a large meta-analysis conducted by The Homocysteine Studies Collaboration (The Homocysteine Studies Collaboration 2002); the aim of this collaborative meta-analysis was to combine individual participant data from 12 prospective and 18 retrospective studies from 1966 to 1999 to produce reliable estimates of the associations of tHcy with ischemic heart disease (IHD) and stroke. A total of 5,073 coronary artery disease events and 1,113 stroke events were observed among 16,786 healthy individuals. The results showed that among prospective studies of individuals with no history of cardiovascular disease, and after appropriate adjustment for known cardiovascular risk factors and correction for regression dilution bias, a 25% lower usual homocysteine level was associated with about an 11% lower IHD and about a 19% lower stroke risk. Moreover, the risk of IHD and stroke associated with homocysteine levels was significantly weaker in the prospective studies than the retrospective studies; this result may reflect bias in retrospective studies caused by difficulties of selecting appropriate controls, the effects of changes in treatment, renal function, or other factors after the onset of disease that produce increases in homocysteine concentrations among the cases.

### Impact of homocysteinemia lowering on cardiovascular disease

Increases in homocysteinemia are common and can easily be corrected with safe and inexpensive therapy. Folic acid and B vitamins, required for remethylation of homocysteine to methionine, are the most important dietary determinants of homocysteine. Daily supplementation with 0.5–5.0 mg of folic acid typically lowers plasma homocysteine levels by about 25%; vitamin B<sub>12</sub> supplementation of at least 0.4 mg daily further lowers levels by about 7%, and vitamin B<sub>6</sub> supplements may be particularly important in lowering homocysteine after methionine loading (Homocysteine Lowering Trialists' Collaboration 2002). These observations have formed the basis of large-scale intervention trials that are seeking to determine whether lowering homocysteine concentrations through B vitamin supplementation can decrease cardiovascular risk in healthy subjects or improve survival in patients with coronary heart disease. The effects of prolonged administration of folate combined with vitamins B<sub>6</sub> and B<sub>12</sub> on cardiovascular risk have been analyzed in a large, prospective, randomized clinical trial (The Heart Outcomes Prevention Evaluation (HOPE) 2 Investigators

2006). In this study, 2758 subjects were randomly assigned to active treatment with folic acid, vitamin B<sub>12</sub> and B<sub>6</sub> versus 2764 assigned to placebo; the primary study outcome was the composite of death from cardiovascular causes, myocardial infarction and stroke. Results demonstrate that daily administration lowered homocysteine levels significantly but did not reduce the incidence of the primary outcome during a mean follow-up period of five years. These results are consistent with those of the Norwegian Vitamin (NORVIT) trial (Bonna et al 2006); this trial evaluated 3749 patients with recent myocardial infarction and found no significant beneficial effects of combined treatment with folate and vitamin B<sub>12</sub>, with or without vitamin B<sub>6</sub>, in spite of adequate homocysteine lowering. Similarly, there was no treatment benefit in the Vitamin Intervention for Stroke Prevention (VISP) study and in a smaller trial conducted in 593 patients with stable coronary heart disease in the Netherlands (Liem et al 2003; Toole et al 2004). A plausible explanation for the discordance between the epidemiology of homocysteine and the results of the clinical trials may be related to inherent limitations of observational studies.

### Conclusions

Although several studies focusing on the role of homocysteine in cardiovascular disease have been conducted, there isn't fully agreement on this topic. Homocysteine levels are related to renal dysfunction, smoking, elevated blood pressure, and other cardiovascular risk factors; moreover, homocysteine levels are higher in people with atherosclerosis than in those without. Therefore, homocysteine could be a marker, but not a cause, of vascular disease, and the epidemiologic data could be the result of residual confounding that cannot be fully adjusted for, of reverse causality, or both. Routine screening for elevated homocysteinemia is not yet recommended. However, screening may be advisable for individuals who manifest atherothrombotic disease that is out of proportion to their traditional risk factors or who have a family history of premature atherosclerotic disease.

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