Effect of vitamin supplementation on hyperhomocysteinemia and cardiovascular risk reduction

Chiara Bellia, Giulia Bivona, Bruna Lo Sasso, Marcello Ciaccio
Chair of Clinical Biochemistry, Department of Medical Biotechnologies and Forensic Medicine, Faculty of Medicine, University of Palermo, Italy

ABSTRACT

Homocysteine is a sulfur-containing aminoacid produced during methionine metabolism. Since 1969 the relationship between altered homocysteine metabolism and both coronary and peripheral atherotrombosis has been known; in recent years, experimental evidence has shown that elevated plasma concentrations of homocysteine are associated with an increased risk of atherosclerosis and cardiovascular ischemic events. Several mechanisms by which elevated homocysteine concentrations impair vascular function have been proposed, including impairment of endothelial function, production of reactive oxygen species and consequent oxidation of low-density lipoproteins. Folate and B vitamins, required for remethylation of homocysteine to methionine, are the most important dietary determinants of homocysteinemia and daily supplementation typically lowers plasma homocysteine concentrations. Recently, large-scale intervention trials have been conducted to determine whether lowering homocysteine concentrations through B vitamins supplementation can decrease cardiovascular risk in healthy subjects or improve survival in patients with coronary heart disease. Some of these trials found no significant beneficial effects of combined treatment with folate and vitamin B_{12}, with or without vitamin B_6, in spite of significant homocysteine lowering. In conclusion, it is still unclear whether decreasing plasma concentrations of homocysteine through diet or vitamin supplementation may be paralleled by a reduction in cardiovascular risk.

HOMOCYSTEINE METABOLISM

Homocysteine is a sulphur-containing aminoacid produced in the metabolism of the essential aminoacid methionine. Homocysteine can be produced by two different pathways: the transulfuration and the remethylation pathway (Figure 1). In remethylation, homocysteine acquires a methyl group from methyltethrahydrofolate (MTHF) to form methionine in a vitamin B_{12}-dependent reaction. The formation of the methyl donor MTHF occurs in all tissue and it depends on the presence of methylentethrahydrofolate, derived from dietary folate, and methyltetrahydrofolate reductase (MTHFR). Methionine is then activated by ATP to form S-adenosylmethionine (SAM), which is an universal methyl donor. The by-product of methylation is S-adenosylhomocysteine, which is subsequently hydrolyzed, regenerating homocysteine. In the transulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by cystathionine β-synthase (CBS). Cystathionine is hydrolyzed to form cysteine, the excess of which is excreted in the urine. Thus, the transulfuration pathway effectively catabolyzes the excess of homocysteine, which is not required for methyl transfer. Homocysteine exceeding the metabolic requests of the cell is exported in the extracellular space by an export mechanism (1); actually, this export mechanism, together with transulfuration pathway, helps maintaining low intracellular concentrations of this potentially cytotoxic sulfur amino acid, but leaves vascular tissue exposed to the possible deleterious effects of homocysteine excess. The occurrence of hyperhomocysteinemia indicates that homocysteine metabolism has been disrupted in some way and that the export mechanism is disposing into the blood homocysteine excess.

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, forming the homocysteine-cysteine mixed disulfide (2). The term "total plasma (or serum) homocysteine" (tHcy) refers to the combined pool of all four forms of homocysteine. The determination of tHcy can be performed in a fasting state or after methionine load; post-load tHcy is probably more sensitive than the fasting tHcy to disturbances in the transulfuration pathway, such as those caused by CBS or vitamin B_6 deficiency (3).

Determinants of plasma homocysteine include genetic, physiologic and lifestyle factor, various disease and drugs (Table 1) (4). Among genetic factors, congenital homocystinuria, a rare congenital metabolic disease caused by a mutation in the gene encoding for CBS, is the most severe cause of hyperhomocysteinemia. This condition is unquestionably associated with precocious atherosclerosis and extensive arterial thrombosis. Other genetic causes of severe hyperhomocysteinemia include homozygous deficiency of MTHFR, deficiency of methionine synthase and methionine synthase reductase (5). The most common genetic defect associated with moderate

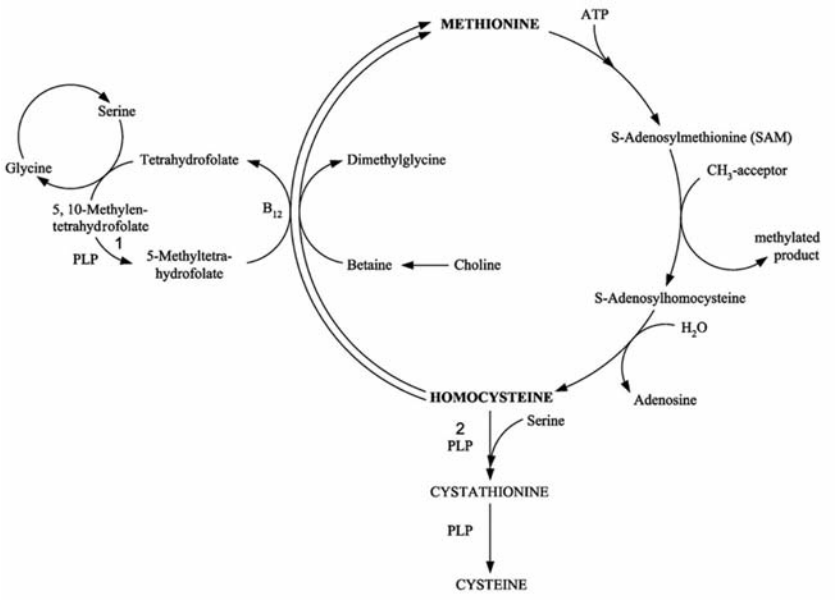


Figure 1
Homocysteine metabolism pathways. 1: Methyltetrahydrofolate reductase (MTHFR); 2: Cystathionine β-synthase (CBS). PLP, pyridoxal-phosphate (active form of vitamin B_6).

Table 1
Major determinants of plasma homocysteine concentrations

Hyperhomocysteinemia determinants
Genetic factors
CBS deficiency[a]
Methionine synthase deficiency[a]
Methionine synthase reductase deficiency[a]
MTHFR deficiency[a]
MTHFR 677TT genotype[b]
MTHFR 1298CC genotype[b]
Physiologic determinants
Increasing age
Renal function
Male sex
Pregnancy
Postmenopausal state
Increasing muscular mass
Lifestyle determinants
Folate, vitamin B_{12}, B_6, B_2 intake
Smoking
Coffee
Physical exercise
Clinical conditions
Folate, vitamin B_{12}, B_6 deficiency
Renal failure

[a]Involved in homocystinuria pathogenesis, [b]involved in mild hyperhomocysteinemia.
CBS, cystathionine β-synthase; MTHFR, methyltetrahydrofolate reductase.

hyperhomocysteinemia is the point mutation C677T in the gene encoding for MTHFR enzyme: this substitution is associated with a thermolabile variant of the enzyme, which has about half of the physiological activity (6, 7). Individuals with the genotype 677TT usually have about 25% higher homocysteinemia than those with the CC genotype (8), but the increase depends on folate status (9).

One of the most relevant cause of hyperhomocysteinemia is vitamin deficiency, which is usually a result of poor diet, such as in vegetarians. Indeed, vegetarians, and particularly vegans who exclude from their diet also dairy products and eggs, show a deficiency especially in vitamin B_{12}, which occurs in substantial amounts only in foods derived from animals. Other causes of folate and cobalamin deficiency are malabsorption, alcoholism, or use of certain drugs. It is also common during pregnancy and in the elderly. Vitamin deficit predisposes to hyperhomocysteinemia with an inverse relationship (10-14). The independent association between nutrients and homocysteinemia was studied in the Framingham Heart study: after controlling for age, sex, and other vitamins, plasma homocysteinemia exhibited a strong, nonlinear inverse correlation with plasma folate and a weaker inverse association with vitamin B_{12} and B_6 (15).

Several clinical conditions are associated with hyperhomocysteinemia; one of the most significant is renal failure. There is an inverse relationship between homocysteinemia and renal function (16). Most dialysis patients have hyperhomocysteinemia and folic acid supplementation efficiently reduces homocysteinemia in these patients, although higher doses are required and homocysteinemia could remain high also after an effective treatment (17, 18). These limitations are probably due to the role of kidney in homocysteine catabolism.

PATHOPHYSIOLOGIC MECHANISM OF HYPERHOMOCYSTEINEMIA

The hypothesis that hyperhomocysteinemia has a pathogenic role in vascular disease arises from several cases of homocystinuria first observed by McCully: subjects affected by homocystinuria had a higher risk of premature, frequently fatal, thromboembolic events and autopsy evidence of vascular disease (19). 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), although results remain contradictory (20-22).

Several mechanisms have been proposed to explain the link between hyperhomocysteinemia and CVD: these include generation of reactive oxygen species (ROS), coagulation cascade activation, endothelial dysfunction, production of inflammatory mediators (23). Many studies on animal models show that moderate hyperhomocysteinemia leads to accelerated development of atherosclerosis (24, 25). The atherogenetic effect of hyperhomocysteine is quite variable and it depends on factors such as diet, sex, severity and duration of hyperhomocysteinemia. Cell culture studies demonstrate that homocysteine can promote vascular smooth muscle cell growth (26) and adhesion of leukocyte such as monocytes and T cells to endothelial cells (27). Moreover, it has been demonstrated that homocysteine promotes inflammation by increasing production of several proinflammatory mediators, including interleukin-8 and monocyte chemoattractant protein-1 (28). It has also been suggested that homocysteine can affect hemostasis leading to a prothrombotic state. Homocysteine activates platelets and promote expression of the CD40/CD40L from activated platelets (29, 30). Recently, Wilson et al. have shown that apoE-deficient mice exhibit accelerated arterial thrombosis and endothelial dysfunction when fed hyperhomocysteinemic diet (31). In particular, there was no effect on platelet activation, while results suggested that the mechanism of accelerated thrombosis may be related to diminished production of endothelium-derived nitric oxide (NO) or other prothrombotic factors.

The mechanism by which hyperhomocysteinemia induces endothelial dysfunction is not completely understood, but there are strong evidence that it includes impairment of vasodilatation mediated by endothelium-derived NO. There are several evidences that hyperhomocysteinemia led to a dose dependent increase of intracellular ROS generation and to a decrease of endothelial NO release in different experimental settings (32-36). Another potential mechanism for endothelial dysfunction during hyperhomocysteinemia is inhibition of NO production caused by asymmetric dimethylarginine (ADMA), an endogenous endothelial nitric oxide synthase (eNOS) inhibitor (37, 38): in human subjects plasma concentrations of ADMA increase rapidly after acute methionine loading and elevation of plasma ADMA correlates with impairment of endothelium-dependent relaxation (39). Elevation of ADMA in hyperhomocysteinemia may be caused by decreased catabolism of ADMA by dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that hydrolyze ADMA to citrulline and methylamines, providing a potential link between hyperhomocysteinemia and the increase in ADMA (40). Dayal et al. have shown that hyperhomocysteinemia in mice cystathionine beta-synthase (CBS-/+) causes tissue-specific decreases in the expression of DDHA, although in this experimental setting downregulation of DDHA does not result in a decrease of plasma ADMA concentrations (41).

EPIDEMIOLOGICAL STUDIES

The hypothesis that hyperhomocysteinemia was a cardiovascular risk factor came from several retrospective case-control studies (42-45). After these first evidences, prospective observational studies, including cohort and nested case-control studies, have been performed, suggesting that hyperhomocysteinemia is a graded, independent risk factor for CVD. Nygard et al. found a strong, graded association between plasma homocysteine concentrations and overall mortality in patients with angiographically confirmed coronary artery disease (CAD), although adjustment for confounding factors, such as serum creatinine, left ventricular ejection fraction and history with respect to myocardial infarction, weakened the predictive power of homocysteine somewhat (46). In contrast, a nested case-control study involving 712 men participating in the Multiple Risk Factor Intervention Trial (MRFIT) showed no association of homocysteine plasma concentrations with CAD (47). Similarly, the Atherosclerosis Risk in Communities (ARIC) study, a prospective case-cohort study conducted over 3.3 years, revealed that homocysteinemia is not independently associated with CAD in men after adjustment for age and race, while only a weak association was found in women (48). Moreover, Ridker et al. found that in a cohort of 14916 initially healthy subjects, among which 140 subsequently developed peripheral artery disease (PAD) during a 9-years follow up, non significant baseline elevation of homocysteinemia was observed (49).

Data from a large meta-analysis revealed that stronger association was observed in retrospective studies of homocysteine measured in blood collected after the onset of CVD than in prospective studies, among individuals who had no history of CVD at the time of collecting sample; however, among prospective studies, after appropriate adjustment for known cardiovascular risk factors and correction for regression dilution bias, a 25% lower homocysteine values were associated with about 11% lower ischemic heart disease and about a 19% lower stroke risk (21). Interestingly, results from the sixth examination cycle of the Framingham Offspring study reveal that homocysteine is included in the panel of most informative biomarkers for

predicting death, whereas it is not useful for predicting major cardiovascular events (50). Overall, inconsistent results have been obtained, so it is still questionable if hyperhomocysteinemia has a causal role in the development of CVD or if it might be merely a marker of disease.

EFFECTS OF VITAMIN SUPPLEMENTATION ON CARDIOVASCULAR RISK

Given the uncertain results of prospective studies, great attention was addressed to large clinical trials testing the effect of vitamin supplementation on hyperhomocysteinemia and cardiovascular risk. The VISP (Vitamin intervention for stroke prevention) trial involved 3680 individuals who experienced a non-disabling ischemic stroke; the aim of the study was to evaluate the effects of high- and low dose therapy with folic acid, vitamin B_6 and vitamin B_{12} on recurrent stroke considered as primary outcome and CAD and death as secondary outcome. During a 2-years follow-up, Authors found a moderate reduction of plasma homocysteine, but no effects on vascular outcomes (51). Authors suggested that results could be affected by low baseline values of homocysteinemia (10.5 μmol/L, the 25th percentile for North America stroke population) and the short period of follow-up. Analogous results were obtained when considering subjects with previous myocardial infarction: the NORVIT trial, a multicenter, randomised, double-blind trial, involved 3749 subjects who had an acute myocardial infarction; participants were randomised to receive different combinations of folic acid, vitamin B_{12}, vitamin B_6, or placebo; the primary endpoint was a composite of recurrent myocardial infarction, stroke, and sudden death attributed to CAD during a mean follow-up of 40 months. None of the treatments significantly reduced the risk of primary or secondary outcomes, despite a substantial reduction (27%) in plasma homocysteine observed in patients receiving folic acid (52).

Overall, it seems that vitamin supplementation with folic acid and B vitamins is not harmful in secondary prevention of stroke or myocardial infarction. At the same time of NORVIT trial, similar results from another large-scale intervention study were published: the HOPE-2 trial included individuals with history of vascular disease (coronary, cerebrovascular or peripheral) or diabetes and additional risk factors for atherosclerosis, irrespective of their homocysteine plasma concentrations; individuals were treated with folic acid, vitamin B_6 and B_{12} for an average of 5 years; a composite of death from cardiovascular causes, myocardial infarction and stroke was considered as primary outcome (53). In accordance with previous results, the Authors found that the treatment decreased homocysteinemia, but had no effects on patient outcome. Interestingly, when each component of the primary composite outcome was analyzed separately, there was a reduction of risk of stroke in the active-treatment group [relative risk (RR) 0.75, confidence interval (CI): 0.59-0.97)], although the Authors interpreted this result with caution for the low number of stroke cases included in the study and the lack of adjustment for the multiplicity of outcomes compared. In accordance to results of HOPE-2 trial on stroke risk reduction, in a recent meta-analysis Wang et al. found that folic acid supplementation, regardless of B vitamins inclusion, reduces the risk in individuals without history of stroke (RR 0.75, CI: 0.62-0.94), when the treatment is longer than 36 months (RR 0.71, CI: 0.57-0.97), and when homocysteine plasma concentrations are reduced of 20% or more (RR 0.77, CI: 0.63-0.94), suggesting an inverse relationship between the concentrations of homocysteine and the RR of stroke (54). Following studies have suggested that hyperhomocysteinemia is also associated with stroke subtypes, especially small vessel disease, at least in UK black population after adjusting for age, gender, vascular risk factors, vitamin levels and renal function (55).

Both in observational studies and in randomized trials, women were underrepresented; given the paucity of data on women and the known influence of estrogens on homocysteine plasma concentrations, Albert et al. designed a randomised trial aimed at testing if therapy with folic acid, vitamin B_6 and B_{12} lowered the risk of CVD during a 7-year follow-up among 2721 women who had history of CVD or at least 3 cardiovascular risk factors (56). In this study, patients receiving active treatment had the similar cardiovascular risk compared with placebo group (RR for primary endpoint: 1.03, CI: 0.90-1.19); at the end of follow-up, Authors observed a decrease of homocysteine plasma concentrations by 18.5%.

Most trials were designed in pre-existing CVD population as a secondary prevention strategy, but it is possible that folic acid supplementation has a greater protective effect in primary rather than secondary prevention: at this regard, Authors from the BVAIT research group found that in a population of about 500 subjects with a low cardiovascular risk and homocysteinemia >8.5 μmol/L, a 3 year treatment with folic acid, vitamin B_{12} and B_6 significantly reduced progression of early-stage subclinical atherosclerosis, evaluated as carotid artery intima-media thickness, but had no effect on progression of aortic or coronary artery calcification, measured by multidetector spiral computerized tomography (57).

It is well known that homocysteinemia is elevated in kidney disease, and renal dysfunction is recognized as a risk factor for CVD. To assess the potential benefit of homocysteinemia lowering on mortality and CVD in individuals with renal impairment, Jamison et al. (58) designed a randomized controlled trial involving 2056 subjects with advanced chronic kidney disease or end stage renal disease that were treated with folic acid and B vitamins during a 3-year follow-up; primary outcome was death from any cause, while secondary outcomes comprised time to myocardial infarction, stroke, and amputation of lower extremity. In this experimental setting, vitamin supplementation decreased homocysteinemia, but failed to decrease the risk for

neither primary nor secondary endpoints, suggesting that the treatment give no benefit even in this population (58). Analogous results were obtained from HOPE-2 when individuals with chronic kidney disease were considered in the trial: there were no significant treatment benefit on death from cardiovascular causes, myocardial infarction, and stroke; incidence of CVD increased only with decreasing of glomerular filtration rate (59). Moreover, in a recent post-hoc analysis, Potter et al. found that, after adjustment for any single marker of renal function, homocysteinemia lost its significance as a predictor of the vascular measurements (carotid intima-media thickness as well as flow-mediated dilatation of the brachial artery) (60). On the basis of these evidences, some Authors suggested that renal dysfunction may account for the epidemiologic association between mild hyperhomocysteinemia and increased cardiovascular risk (61).

CONCLUSIONS

Overall, hyperhomocysteinemia is associated with an increased incidence of cardiovascular events, as shown by prospective studies, but intervention trials failed to show a benefit from folic acid and B vitamin supplementation; these evidences justified many to conclude that lowering homocysteinemia is ineffective in preventing vascular disease. Although these considerations are due, future studies should take more into account the influence of renal function; moreover, there are important pathophysiological differences between myocardial infarction and cerebral infarction, so it is possible that homocysteine lowering could be effective in reduce the risk of stroke, but not the CAD one, as supported by some trials (54, 62). Another hypothesis is that folic acid therapy is not beneficial for vascular disease; if it is true, new efforts will be required to identify other therapeutic approach for lowering hyperhomocysteinemia, such as methionine restricted diet, to further reduce the risk of vascular events. Until these questionable points remain unsolved, lowering homocysteinemia with folic acid and B vitamins cannot be recommended for prevention of CVD, although hyperhomocysteinemia remains an interesting field of study as unconventional cardiovascular risk factor.

REFERENCES

1. Christensen B, Refsum H, Vintermyr O, et al. Homocysteine export from cells cultured in the presence of physiological or superfluous levels of methionine: methionine loading of non-transformed, transformed, proliferating, and quiescent cells in culture. J Cell Physiol 1991;146:52-62.
2. Ueland PM. Homocysteine species as components of plasma redox thiol status. Clin Chem 1995;41:340-2.
3. Refsum H, Fiskerstrand T, Guttormsen AB, et al. Assessment of homocysteine status. J Inher Metab Dis 1997;20:286-94.
4. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendation about total homocysteine determinations: an expert opinion. Clin Chem 2004;50:3-32.
5. Rosenblatt DS, Cooper BA. Inherited disorders of vitamin B_{12} utilization. Bioessays 1990;12:331-4.
6. Kang SS, Zhou J, Wong PWK, et al. Intermediate homocysteinemia: a thermolabile variant of methylentethrahydrofolate reductase. Am J Hum Genet 1988;43:414-21.
7. Kang SS, Wong PWK, Susmano A, et al. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Gen 1991; 48:536-45.
8. Brattstrom L, Wilcken DE, Ohrvik J, et al. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation 1998;98:2520-6.
9. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996;93:7-9.
10. Dudman NP, Wilcken DE, Wang J, et al. Disordered methionine/homocysteine metabolism in premature vascular disease. Its occurrence, cofactor therapy, and enzymology. Arterioscler Thromb 1993;13:1253-60.
11. Kang SS, Wong PW, Norusis M. Homocysteinemia due to folate deficiency. Metabolism 1987;36:458-62.
12. Stabler SP, Marcell PD, Podell ER, et al. Elevation of total homocysteine in the serum of patients with cobalamin or folate deficiency detected by capillary gas chromatography-mass spectrometry. J Clin Invest 1988; 81:466-74.
13. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988;318:1720-8.
14. Klee GG. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B(12) and folate. Clin Chem 2000;46:1277-83.
15. Selhub J, Jacques PF, Wilson PW, et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693-8.
16. Bostom AG, Culleton BF. Hyperhomocysteinemia in chronic renal disease. J Am Soc Nephrol 1999;10:891-900.
17. Sunder-Plassmann G, Födinger M, Buchmayer H, et al. Effect of high dose folic acid therapy on hyperhomocysteinemia in hemodialysis patients: results of the Vienna multicenter study. J Am Soc Neprol 2000;11:1106-16.
18. Tremblay R, Bonnardeaux A, Geadah D, et al. Hyperhomocysteinemia in hemodialysis patients: effects of 12-months supplementation with hydrosoluble vitamins. Kidney Int 2000;58:851-8.
19. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111-28.
20. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042-50.
21. The Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke. A meta-analysis. JAMA 2002;288:2015-22.
22. Wald DS, Morris JK, Law M, et al. Folic acid, homocysteine, and cardiovascular disease: judging causality in the face of inconclusive trial evidence. Br Med J 2006;333:1114-7.
23. Austin RC, Lentz SR, Werstuck GH. Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease. Cell Death Differ 2004;11:S56-64.
24. Hofmann MA, Lalla E, Lu Y, et al. Hyperhomocysteinemia

enhances vascular inflammation and accelerates atherosclerosis in a murine model. J Clin Invest 2001; 107:675-83.
25. Zhou J, Møller J, Danielsen CC, et al. Dietary supplementation with methionine and homocysteine promotes early atherosclerosis but not plaque rupture in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 2001; 21:1470-6.
26. Tsai JC, Perrella MA, Yoshizumi M, et al. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proc Natl Acad Sci USA 1994;91: 6369-73.
27. O. Postea, F. Krotz, A. Henger, et al. Stereospecific and redoxsensitive increase in monocyte adhesion to endothelial cells by homocysteine. Arterioscler Thromb Vasc Biol 2006;26:508-13.
28. Poddar R, Sivasubramanian N, DiBello PM, et al. Homocysteine induces expression and secretion of monocyte chemoattractant protein-1 and interleukin-8 in human aortic endothelial cells: implications for vascular disease. Circulation 2001;103:2717-23.
29. Mohan IV, Jagroop IA, Mikhailidis DP, et al. Homocysteine activates platelets in vitro. Clin Appl Thromb Hemost 2008; 14:8-18.
30. Prontera C, Martelli N, Evangelista V, et al. Homocysteine modulates the CD40/CD40L system. J Am Coll Cardiol 2007;49:2182-90.
31. Wilson KM, McCow RB, Leo L, et al. Prothrombotic effects of hyperhomocysteinemia and hypercholesterolemia in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 2007; 27:233-40.
32. Zhang X, Li H, Jin H, et al. Effects of homocysteine on endothelial nitric oxide production. Am J Physiol Renal Physiol 2000;279:F671-8.
33. Topal G, Brunet A, Millanvoye E, et al. Homocysteine induces oxidative stress by uncoupling of NO synthase activity through reduction of tetrahydrobiopterin. Free Radic Biol Med 2004;36:1532-41.
34. Au-Yeung KK, Woo CW, Sung FL, et al. Hyperhomocysteinemia activates nuclear factor-kappaB in endothelial cells via oxidative stress. Circ Res 2004;94:28-36.
35. Holven KB, Holm T, Aukrust P, et al. Effect of folic acid treatment on endothelium-dependent vasodilatation and nitric oxide-derived end products in hyperhomocysteinemic subjects. Am J Med 2001;110:536-42.
36. Wei-gou Z, Shan L, Le-qing L, et al. Vascular oxidative stress increase dendritic cell adhesion and transmigration induced by homocysteine. Cell Immunol 2009;254:110-6.
37. Daval S, Lentz SR. ADMA and hyperhomocysteinemia. Vasc Med 2005;10:S27-33.
38. Stuhlinger MC, Stanger O. Asymmetric dimethyl-L-arginine (ADMA): a possible link between homocyst(e)ine and endothelial dysfunction. Curr Drug Metab 2005;6:3-14.
39. Böger RH, Lentz SR, Bode-Böger SM, et al. Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperhomocyst(e)inemia in humans. Clin Sci 2001;100:161-7.
40. Hong L, Fast W. Inhibition of human dimethylarginine dimethylaminohydrolase-1 by S-nitroso-L-homocysteine and hydrogen peroxide. Analysis, quantification, and implications for hyperhomocysteinemia. J Biol Chem 2007;282:34684-92.
41. Dayal S, Rodionov RN, Arning E, et al. Tissue-specific downregulation of dimethylarginine dimethylaminohydrolase in hyperhomocysteinemia. Am J Physiol Heart Circ Physiol 2008;295:H816-25.
42. Wilcken DE, Wilcken B. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. J Clin Invest 1976;57:1079-82.
43. Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991;324:1149-55.
44. Boushey CJ, Beresford SA, Omenn GS, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274:1049-57.
45. Graham IM, Daly LE, Refsum HM, et al. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 1997;277:1775-81.
46. Nygard O, Nordrehaug JE, Refsum H, et al. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337:230-6.
47. Evans RW, Shaten J, Hempel JD, et al. Homocyst(e)ine and risk of cardiovascular disease in the Multiple Risk Factor Intervention Trial. Arterioscler Thromb Vasc Biol 1997;17:1947-53.
48. Folsom AR, Nieto FJ, McGovern PG, et al. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphism, and B vitamins. The atherosclerosis risk in communities (ARIC) study. Circulation 1998;98:204-10.
49. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis. A comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA 2001;285:2481-5.
50. Wang T, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med 2006;355:2631-9.
51. Tool JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death. The vitamin intervention for stroke prevention (VISP) randomized controlled trial. JAMA 2004;291:565-75.
52. Bonaa KH, Njolstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med 2006;354:1578-88.
53. Lonn E, Yufsum S, Arnold MJO, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. N Engl J Med 2006;354:1567-77.
54. Wang X, Qin X, Demirtas H, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. Lancet 2007;369:1876-82.
55. Khan U, Crossley C, Kalra L, et al. Homocysteine and its relationship to stroke subtypes in a UK black population. Stroke 2008;39:2943-9.
56. Albert CM, Cook NR, Gaziano JM, et al. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease. A randomized trial. JAMA 2008;299:2027-36.
57. Hodis HN, Mack WJ, Dustin L, et al. High-dose B vitamin supplementation and progression of subclinical atherosclerosis: a randomized controlled trial. Stroke 2009;40:730-6.
58. Jamison RL, Hartigan P, Kaufman JS, et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal

disease. A randomized controlled trial. JAMA 2007;298:1163-70.
59. Mann JF, Sheridan P, McQueen MJ, et al. Homocysteine lowering with folic acid and B vitamins in people with chronic kidney disease-Results of the renal HOPE-2 study. Neprol Dial Transplant 2008;23:645-53.
60. Potter K, Hankey GJ, Green DJ, et al. Homocysteine or renal impairment. Which is the real cardiovascular risk factor? Arterioscler Thromb Vasc Biol 2008;28:1158-64.
61. Milani RV, Lavie CJ. Homocysteine: the Rubik's cube of cardiovascular risk factors. Mayo Clin Proc 2008;83: 1200-2.
62. Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? Lancet Neurol 2007;6:830-8.