Homocysteine as a risk factor for cardiovascular disease: should we (still) worry about it?

David Faeh[a,b], Arnaud Chiolero[a], Fred Paccaud[a]

[a] University Institute for Social and Preventive Medicine, Lausanne, Switzerland
[b] Department of Physiology, University of Lausanne, Lausanne, Switzerland

Summary

Cardiovascular disease (CVD) is the leading cause of death worldwide. CVD is causally related to "classical" risk factors such as elevated blood pressure, cholesterol, or glucose level and smoking. A causal role in the development of CVD is also suggested for numerous other factors, including an elevated plasma homocysteine concentration. Variation of homocysteinaemia is mainly due to genetic mutations and/or vitamin deficiency. The homocysteine concentration can be lowered with folate. Vitamin supplementation has thus been proposed in individuals with hyperhomocysteinaemia in order to reduce their CVD risk. On the other hand, population-based studies show little or no association between moderate hyperhomocysteinaemia and CVD risk. Nor has any randomised clinical trial clearly proven the efficacy of lowering the homocysteine concentration as a means of lowering the incidence of CVD. Hence at present it is inappropriate to recommend screening and treatment of hyperhomocysteinaemia in asymptomatic persons with or without other CVD risk. Until new evidence is available, clinicians should focus on better control of the "classical" risk factors for CVD.

Key words: homocysteine; cardiovascular disease; risk factor; treatment; screening

Introduction

Smoking, high blood pressure, elevated serum cholesterol and elevated serum glucose are major modifiable risk factors for cardiovascular disease (CVD) [1]. Numerous other risk factors – or risk markers for CVD – have been identified, including homocysteine [1]. The aetiopathological role of these "new" risk factors/markers in the development of CVD is fiercely debated. How far these factors add value to the "classical" risk factors for the prediction or the prevention of CVD morbidity/mortality risk is uncertain. Others argue that only 50% of CVD can be explained by "classical" risk factors, and that "new" risk factors could significantly boost CVD predictive power [2]. However, this claim has been widely criticised [3, 4] by authors who show that up to three quarters of coronary heart disease (CHD) events, if not more, could be attributed to "classical" risk factors [5, 6]. To be considered clinically relevant, a risk factor should add significantly to the predictive value of "classical" risk factors for CVD and its treatment should be associated with a proven reduction in CVD risk. Alternatively, a new risk factor can also be meaningful when it is easier (or cheaper) to obtain than a classical risk factor.

Numerous observational studies have shown that high homocysteine concentrations are associated with a heightened risk of CVD [7–10]. Since homocysteine levels can be reduced with B vitamins, some authors recommend treatment when the homocysteine concentration exceeds 10 µmol/L [11–16]. Typically, folate was proposed as part of the debated "polypill", a combination of various drugs (i.e. three blood pressure lowering drugs, a statin, aspirin, and folate), which would reduce the risk of ischaemic heart disease events and strokes by more than 80% in persons presenting with CVD or aged 55 years or more [17]. Another proposed strategy is systematic screening for hyperhomocysteinaemia and, if necessary, correction by vitamin supplementation [14–16, 18]. However, to qualify as a screening tool, a risk factor should be strongly and causally associated with the target disease [19], and many authors doubt whether such a relationship between homocysteine and CVD exists [7, 20–25].

We reviewed the available evidence concerning the association between serum homocysteine and CVD, with the objective of discussing the pertinence of screening, treatment, and prevention of

hyperhomocysteinaemia in a general and/or in a selected clinical population. This review does not refer to supplementation of folic acid to reduce the risk of neural tube defects, which must be discussed separately [26].

Homocysteine metabolism

Homocysteine, a sulphur-containing amino acid, is an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine. Homocysteine is predominantly metabolised via two pathways (figure 1). The enzyme N^5, N^{10}-methylenetetrahydrofolate reductase (MTHFR) [24] converts homocysteine to methionine. When MTHFR activity is low, homocysteine transformation to methionine is impaired and homocysteine accumulates in the blood. The activity of MTHFR is strongly dependent on the presence of the two vitamins folate (vitamin B_9) and cobalamin (vitamin B_{12}). The conversion of homocysteine to cysteine is catalysed by cystathionine, -synthase (C,S), an enzyme with pyridoxine (vitamin B_6) as an essential cofactor [24]. Similarly to MTHFR, low C,S activity translates into increased homocysteine concentrations.
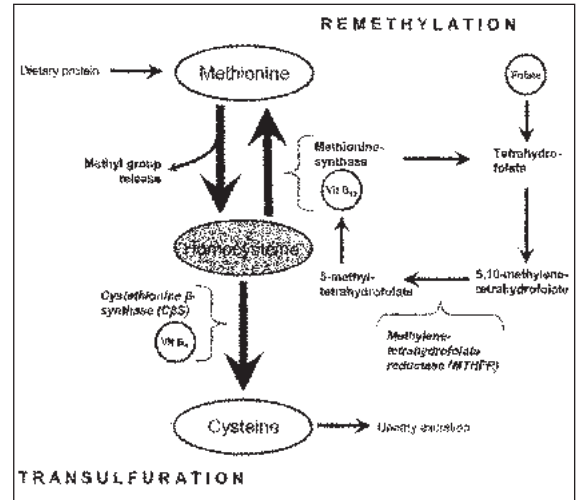
Figure 1

Major metabolic pathways of homocysteine.

Possible causes of hyperhomocysteinaemia

There are two types of hyperhomocysteinaemia: (1) rare, severe forms are due to major genetic mutations of the enzymes implicated in homocysteine metabolism; (2) the more common forms result in moderately elevated homocysteine levels related to a pathogenesis that includes genetic and environmental factors.

The homozygous mutation of C,S can cause severe hyperhomocysteinaemia with homocysteine concentrations of up to 40-fold normal levels [24]. This disease occurs in approximately one of 100,000 live births [27]. When untreated, a vascular event (stroke, myocardial infarction, other thromboembolic complication) occurs in about half of these patients before the age of 30 [28]. Another cause of rare, genetically mediated severe hyperhomocysteinaemia is due to homozygous mutations of MTHFR [24, 29]. Similarly, persons with these mutations have premature CVD, and vitamin treatment may reduce the risk of CVD in patients with some enzyme function [24].

Some genetic mutations may only mildly impair homocysteine metabolism. Among the known mutations of MTHFR the two best characterised are A1298C and C677T (missense mutations) [30]. Only C677T may lead to increased homocysteine levels. This point mutation (C-to-T substitution at nucleotide 677) in the gene for MTHFR is associated with a thermolabile MTHFR variant having approx. half-normal activity. Together with a low folate intake, patients with this mutation may have moderate or intermediate hyperhomocysteinaemia [24, 31]. MTHFR mutations can be found in about 30% of individuals with moderately increased homocysteine levels, and in 70% of subjects with intermediate hyperhomocysteinaemia [21]. Transcobalamin is a protein which is required for vitamin B_{12} uptake and metabolism. A common genetic polymorphism of transcobalamin (missense mutation: TC 776C3ÆG) can also be responsible for increased homocysteine levels [32].

Low intake of folate, vitamin B_{12}, and to a lesser extent vitamin B_6 are associated with increased homocysteine levels independently of any genetic mutation [18, 24]. In addition, several diseases such as renal and thyroid dysfunction, cancer, psoriasis, and diabetes may be associated with moderately elevated homocysteine concentrations [18, 24]. Finally, various drugs, alcohol, tobacco, and coffee consumption, as well as older age and menopause, are associated with high homocysteine levels [18, 24] (table 1).

Table 1

Causes of hyperhomocysteinaemia of different degrees.

	Moderate hyperhomocysteinaemia	Intermediate hyperhomocysteinaemia	Severe hyperhomocysteinaemia
Blood concentration	12–30 µmol/L	31–100 µmol/L	>100 µmol/L
Mutations and polymorphisms	Homozygous mutation (677C→T) of methylenetetrahydrofolate reductase (MTHFR)	Homozygous mutation of (677C→T) MTHFR	
	Heterozygous mutation (I278T and G307S) of cystathionine β-synthase (CβS)	Mutations (e.g. P1297L) leading to vitamin B_{12} deficiency	Homozygous mutation (I278T and G307S) of CβS
	Mutation (TC 776C→G) of transcobalamin		
Nutrition	Deficiencies in folate, Vitamin B_{12}, B_6, cholin, serin	Deficiencies in folate, Vitamin B_{12}, B_6, cholin, serin	Severe deficiencies in folate, Vitamin B_{12}, B_6, cholin, serin
	Increased intake in methionine		
Diseases	Renal impairment		
	Pernicious anaemia		
	Hypothyroidism		
	Various cancers		
	Psoriasis		
	Diabetes		
Drugs	Folate antagonists	Folate antagonists	
	Vitamin B_6 antagonists		
Others	Tobacco	Alcohol abuse	
	Alcohol		
	Coffee		
	Menopause		
	Male sex		
	Old age		

Prevalence of hyperhomocysteinaemia

Mean homocysteine levels and the prevalence of hyperhomocysteinaemia vary significantly between populations, and probably depend on age, diet, and genetic background. Also, the definition of hyperhomocysteinaemia differs between studies [33–35]. Moreover, differences in folate intake, in particular food fortification with folate, may also add to the variations between populations. Most studies indicate prevalences of hyperhomocysteinaemia above 10% in developed countries (table 2). Currently, no data are available for Switzerland.

Homocysteine and the risk of vascular and other diseases

In 1969, McCully described premature atherosclerosis in children with homocysteinuria [36]. Homocysteinuria is a rare autosomal recessive condition marked by major alterations in the activity of MTHFR, CβS, and an enzme implicated in vitamin B_{12} metabolism, resulting in markedly increased homocysteine levels [36]. In 1976, Wilcken found higher homocysteine levels due to abnormalities in methionine metabolism in coronary artery disease patients compared to healthy controls (normal coronarography) [37]. Meanwhile, a substantial amount of data from case-control and cohort studies has been gathered which supports a relationship between moderately elevated homocysteine levels and the risk of CVD (coronary heart, cerebrovascular, and peripheral artery diseases) [7–10]. More recently, hyperhomocysteinaemia has been shown to be associated with a higher risk of venous thrombosis [38, 39]. Other studies suggest that elevated homocysteine concentrations may be associated with alterations in mental health [40], e.g. cognitive impairment [41, 42], dementia [43], depression [44, 45] or Alzheimer's [43, 46, 47] and Parkinson's disease [48].

In vitro studies indicate that homocysteine may have a harmful effect on endothelial cells, increase coagulability, and have a proliferative effect on smooth muscle cells [49–54]. However, homocysteine doses given in many in vitro studies (up to 200 µmol and more) far exceed pathological homocysteine levels in humans [50–52]. Another

Table 2

Mean homocysteine levels and prevalence of hyperhomocysteinaemia in different populations.

Region [reference]	Publication year	Sample size	Age of participants (years)	Mean homocysteine levels	Cut-off for hyperhomocysteinaemia	Prevalence of hyperhomocysteinaemia
Europe [35]	2005	25,489	>20	M: 12.4 μmol/L (range: 9.0–15.9), F: 10,6 μmol/L (range: 8.7–15.1)	Total homocysteinaemia >15 μmol/L	11%
United States [33]	2000	2,491	60–95	10.1 μmol/L (before food fortification with folate) 9.4 μmol/L (after food fortification with folate)	Total homocysteinaemia >13 μmol/L	18.7% (before food fortification with folate) 9.8% (after food fortification with folate)
Australia (Sydney) [105]	2004	2,963	≥50	n. a.	Total homocysteinaemia >15 μmol/L	M: 20.8%, F: 13.7%
Iran (Tehran) [106]	2006	1,214	25–64	M: 19.02 μmol/L, F: 14.05 μmol/L	Total homocysteinaemia >15 μmol/L	M: 73.1%, F: 41.07%
Singapore [34]	2000	726	30–69	Indians: M: 16.2 μmol/L, F: 11.5 μmol/L, Malays: M: 15.0 μmol/L, F: 12.5 μmol/L, Chinese: M: 15.3 μmol/L, F: 12.2 μmol/L.	Total homocysteinaemia >14 μmol/L	Indians: M: 60.0%, F: 21.9%, Malays: M 53.9, F: 37.8%, Chinese: M: 56.6, F: 30.6%
Canada (Ontario) [107]	2000	711	Mean: 58.4	M: 9.3 μmol/L F: 8.3 μmol/L	Total homocysteinaemia >15 μmol/L	n. a.
Australia [108]	1999	365	Mean: 42	M: 14.4 μmol/L, F: 11.9 μmol/L	Total homocysteinaemia ≥15 μmol/L	24%
Argentina (Buenos Aires) [109]	2002	196	>65	M: 15.0 μmol/L, F: 12.3 μmol/L	Total homocysteinaemia ≥95th (M: 11.4 μmol/L, F: 10.4 μmol/L)	M: 76.2%, F: 66.4%
Korea [110]	2002	195	23–72	M: 11.2 μmol/L, F: 9.2 μmol/L	Total homocysteinaemia ≥5 μmol/L	M: 10.1%, F: 2.1%

M: male, F: female n.a.: not available

point is related to the prooxidative properties of homocysteine which may induce oxidative stress. On the one hand, this leads to impaired synthesis of nitric oxide (NO, a potent vasodilator) and other vasoactive substances, resulting in endothelial dysfunction [52, 55–57]. On the other hand, homocysteine-induced oxidative stress favours proatherogenic transformation of lipoproteins [57] and induces production of inflammatory factors such as TNF-alpha [58], thus accelerating atherosclerosis in animal models [58, 59]. In mice with a genetic deficiency of CβS, hyperhomocysteinaemia was associated with impaired endothelial function [60] and abnormal lipid metabolism [61]. Abnormal lipid deposition in the aorta was observed in mice with mutation of the MTHFR gene [62]. Finally, increased homocysteine levels could also cause an imbalance in coagulation/thrombolytic factors towards a prothrombotic state [63, 64].

Homocysteine can be lowered by supplementation with folate, vitamin B_6, and B_{12} [65]. Supplementation with these vitamins is inexpensive, safe, and effective in normalising hyperhomocysteinaemia [66–68]. In patients with markedly increased homocysteine levels, vitamin treatment was associated with a decrease in CVD risk in a controlled trial [69]. In individuals with moderate hyperhomocysteinaemia, vitamin supplementation may lower homocysteine concentrations [70, 71] when daily folate intake exceeds 400 μg/day [11]. Some authors suggest that such a correction of elevated homocysteine levels could reduce the relative risk of CVD by approximately 10% in the general population, and up to 25% in high risk groups [7, 72]. Foods rich in folate include green leafy vegetables, wheat germ, and sprouts, but beans, citrus fruits, and liver are also good sources. It is difficult to achieve an intake of ≥400 μg/day of folate with a normal diet. For instance, this would require eating 560 g of broccoli or 1.2 kilo of oranges daily [73], suggesting that vitamin supplementation or food fortification with folate (e.g. flour) would be necessary to obtain the appropriate daily supply [11, 66, 67, 74]. Voluntary fortification in an adult Australian population was associated with a 38% increase in mean serum folate and a 21% decrease in mean homocysteine [68], corresponding to a supposed reduction in ischaemic heart disease (IHD) and stroke of 13% and 20% respectively [68].

Homocysteine and CVD: cause, consequence, or merely a marker?

For several reasons, a causal role for homocysteine in the development of CVD is still debated. First, the association between homocysteine and CVD is not marked. A recent meta-analysis of 30 observational studies including 5,073 subjects with IHD and 1,113 with stroke found that a 25% lower homocysteine level was associated with an 11% lower relative risk of IHD and a 19% lower relative risk of stroke. The authors of the study concluded that elevated homocysteine was at most a modest independent predictor of IHD and stroke in healthy populations [9]. Another meta-analysis which included 57 studies found low correlations between homocysteine concentrations and coronary heart and cerebrovascular disease [10].

Secondly, randomised controlled studies have not consistently shown that supplementation with folate reduces CVD risk. A randomised controlled trial including high risk CVD patients failed to show a beneficial effect from homocysteine-lowering therapy on CVD markers. Supplementation with folic acid in patients with type 2 diabetes and mild hyperhomocysteinaemia was effective in reducing homocysteine concentrations, but was not associated with any improvement in biochemical markers of endothelial dysfunction or inflammation [75]. Furthermore, homocysteine-lowering therapy in bare-metal stented patients may have moderate benefits at best, or even adverse effects, on restenosis [76, 77]. To our knowledge no similar study has been carried out with drug-eluting stents.

These conflicting findings are in line with studies in individuals with a mutation in the gene coding for MTHFR. If homocysteine was causally linked to CVD, subjects with this gene mutation should be more prone to CVD. Two meta-analyses including 6,000 participants each have been carried out to asses this question [78]. They found no significant (OR: 1.12 [95% CI, 0.92 to 1.37]) [21], or only a weak association (OR: 1.22 [95% CI, 1.01 to 1.47]) [78] between mutations in MTHFR and CVD. Still, these meta-analyses may not have had sufficient statistical power to show an effect of hyperhomocysteinaemia on CVD [7]. However, a recent large meta-analysis including 26,000 cases of CHD and 31,183 controls confirmed the lack of statistically significant association between MTHFR mutations and CHD in subjects living in Europe (OR: 1.08 [95% CI, 0.99 to 1.18]), Asia (OR: 1.23 [95% CI, 0.94 to 1.62]), North America (OR: 0.93 [95% CI, 0.80 to 1.10]) and Australia (OR: 1.04 [95% CI, 0.73 to 1.49]) [25]. In this meta-analysis, the only significant associations were found in the Middle East (OR: 2.61

[95% CI, 1.81 to 3.75]) and Japan (OR: 1.71 [95% CI, 1.23 to 2.37]) [25].

Another meta-analysis including 111 studies showed that among 15,635 healthy subjects (without previous CVD), the mean difference in homocysteine concentration between TT and CC homozygotes was 1.93 µmol/L (95% CI, 1.38–2.47). In a complementary genetic meta-analysis (n = 13,928), the odds ratio for stroke was 1.26 (95% CI, 1.14 to 1.40) for TT versus CC homozygotes, an increased risk consistent with the difference in homocysteine level between polymorphisms [79]. The polymorphism being distributed randomly at the time of gamete formation (Mendelian randomisation [74]), its association with stroke should not be confounded by any other factors (smoking, previous CVD, etc). However, heterogeneity for the effect of the polymorphisms was found: the association was lower in North American studies, which may suggest that gene-environment interactions should be considered [79]. In addition, the size of the effect was small compared with classical CVD risk factors. Finally, studies utilising Mendelian randomisation should be interpreted with caution, as discussed by Nitsch et al. [80].

On another note, recent studies suggest that homocysteine levels may increase secondary to the occurrence of CVD and/or due to the presence of atherosclerosis. For example, subjects with reduced renal function have elevated homocysteine concentrations, which suggests that vascular disease, which may impair renal function, could cause hyperhomocysteinaemia [81, 82]. However, other findings show that hyperhomocysteinaemia is a predictor of CVD in patients with renal failure [83] as well as chronic stable renal transplant recipients [84], independently of renal function. This contradicts the assumption that hyperhomocysteinaemia is caused by renal dysfunction. Hyperhomocysteinaemia may also be secondary to myocardial infarction or stroke [85–90]. In one study, patients had low homocysteine levels during, and briefly after, myocardial infarction or stroke, and had higher homocysteine concentrations later during the convalescent phase [90].

Finally, the association between CVD and homocysteine may result from deficiency of B vitamins per se. In fact, homocysteine may only alter vascular reactivity when folate is simultaneously low. On the other hand, folate is associated with an alteration in vascular reactivity without any change in homocysteine concentrations [91, 92]. In addition, B vitamins were shown to reduce homocysteine without improving endothelial dysfunction or hypercoagulability [93].

More results are needed

To date, no evidence is available which links a reduction in serum homocysteine to a decrease in CVD morbidity or mortality [72, 94]. Results of four large-scale randomised controlled trials are currently available, all of which were carried out in CVD patients (table 3). In one trial, 3,680 stroke patients were randomised to either high or low dose vitamin supplementation. High vitamin supplementation had no significant effect (RR: 1.0 [95% CI, 0.8–1.1]) on vascular outcomes after two years, despite a significant reduction in homocysteine levels of 2 µmol/L [95]. Similar reductions in homocysteine concentrations (2.4 µmol/L) were found in the "HOPE-2" study after treatment with 2.5 mg folic acid, 50 mg vitamin B_6 and 1 mg vitamin B_{12} for five years. In this trial, including 5,522 patients with CVD or diabetes, homocysteine-lowering treatment failed to reduce the risk of major cardiovascular events (RR for death from cardiovascular causes: 0.96 [95% CI, 0.81–1.13], RR for myocardial infarction: 0.98 [95% CI: 0.85–1.14]) [96]. In another trial, 636 patients undergoing bare-metal stenting were randomised to either high doses of B vitamins (1.2 mg folic acid, 48 mg vitamin B_6, and 60 µg vitamin B_{12}) for 6 months, or placebo. The administration of high doses of B vitamins lowered homocysteine concentrations significantly, but tended to increase the risk of restenosis (34.5% vs. 26.5%, P = 0.05) [77]. In the "NORVIT" trial 3,749 CVD patients participated. Homocysteine-lowering treatment with folic acid (0.8 mg) plus vitamin B_{12} (0.4 mg), with or without high doses of vitamin B_6 (40 mg) did not lower the risk of recurrent CVD or death after acute myocardial infarction compared with placebo (RR for folic acid plus vitamin B_{12}: 1.08 [95% CI, 0.93–1.25]; RR for folic acid plus vitamin B_{12} with vitamin B_6: 1.22 [95% CI, 1.00–1.50]). The latter association suggests a trend (P = 0.05) towards higher risk in the treated group. Consequently, and in line with the previous study, the authors suggest a harmful effect from a treatment of this kind after acute myocardial infarction [97]. Additional clinical trials are ongoing (table 3). Given the weak association between homocysteine and CVD, sufficient power to detect an effect necessitates the inclusion of a very large number of patients [7]. However, even if these trials show a favourable effect from vitamin supplementation on clinical endpoints, they will not distinguish the effects of vitamin supplementation per se from the effect of lowering homocysteine concentrations. In addition, it may be increasingly difficult to carry out clinical trials on homocysteine, since food fortification with folate has been introduced in several countries such as the United States, Canada [98, 99] and Australia [68]. In these populations, the mean homocysteine concentration has decreased significantly since the introduction of food fortification with folate, and identification of subjects with low folate intake and an associated hyperhomocystinemia may present a challenge [33].

Screening and treatment of hyperhomocysteinaemia

Regular fruit and vegetable intake may have only a moderate impact on elevated homocysteine levels [11, 70, 72, 100, 101]. Hence some authors recommend supplementation with vitamins, e.g. 200–800 µg folate, 3–30 µg vitamin B_{12}, and 2–6 mg vitamin B_6 for patients with manifest CVD or high CVD risk (hypertension, dyslipidaemia, diabetes, smoking, family history of premature atherosclerosis), with hyperhomocysteinaemia (>12 µmol/L), and for populations at high risk for vitamin deficiency, in order to target serum homocysteine <10 µmol/L [11–16, 18, 72].

Numerous reports do either not support [9, 10, 25, 94, 102] or discourage [76, 77, 97, 103] these proposals. The current recommendations of major health agencies or other groups regarding screening, prevention and treatment of hyperhomocysteinaemia in the general population or in selected clinical populations are prudent (table 4). None of the agencies recommends homocysteine-reducing vitamin supplementation or screening for hyperhomocysteinaemia. The American Heart Association recently states as part of its diet and lifestyle recommendations [104]: "Available evidence is inadequate to recommend folate and other B vitamin supplements as a means to reduce CVD risk at this time".

Therefore, we would suggest:

1 No routine determination of homocysteine in the general population.

2 No routine determination of homocysteine in high risk CVD patients.

3 If homocysteine has been measured and found elevated in patients with low CVD risk, no treatment is recommended but increased intake of fruit and vegetables (at least five servings per day) and regular physical activity is recommended. No further blood homocysteine determinations are necessary.

4 For individuals with a high homocysteine level and a high CVD risk:

a. Ensure control of "classical" CVD risk factors (e.g. tobacco, lipids, blood pressure and diabetes).

Table 3

Randomised clinical trials of homocysteine reduction therapy in cardiovascular patients.

Study name [reference]	Region	Sample size	Study population	Baseline Homocysteine levels	Studied outcome	Intervention	Main result(s)
Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine [SEARCH] [111]	United Kingdom (Oxford)	12,064	Patients with myocardial infarction; Population without mandatory folate fortification of food	n.a.	Myocardial infarction	2×2 factorial design: folic acid, 2 mg/d, and vitamin B_{12}, 1 mg/d, compared with placebo; simvastatin, 80 mg/d, compared with 20 mg/d	Ongoing study
Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease [PACIFIC] Study [112]	Australia	10,000	Patients with coronary heart disease; Population with voluntary folate fortification of food	10.9 and 11.1 µmol/L	Arterial vascular disease	3×3 factorial design: folic acid, 0.2 or 2 mg/d, compared with placebo; angiotensin-converting enzyme inhibitor at 2 doses compared with placebo	Ongoing study
Vitamins to Prevent Stroke [VITATOPS] Study [113, 114]	Australia	8,000	Patients with stroke or transient ischaemic attack; Populations with and without mandatory folate fortification of food	12.8 and 13.4 µmol/L	Stroke	Folic acid, 2 mg/d, plus vitamin B_6, 25 mg/d, plus vitamin B_{12}, 0.4 mg/d, compared with placebo	Ongoing study
Heart Outcomes Prevention Evaluation [HOPE-2] Study [96]	Canada	5,522	Patients (age: >55 years) with vascular disease or diabetes; Populations with and without mandatory folate fortification of food	12.2 µmol/L	Cardiovascular events	Folic acid, 2.5 mg/d; vitamin B_6, 50 mg/d; plus vitamin B_{12}, 1 mg/d	No reduction of major cardiovascular events (RR for death from cardiovascular causes: 0.96 [95% CI, 0.81–1.13], RR for myocardial infarction: 0.98 [95% CI: 0.85 –1.14])
Women's Antioxidant and Cardiovascular Disease Study [WACS] [115]	United States	5,442	Female health professionals (age: >40 years); Population with mandatory folate fortification of food	n.a.	Myocardial infarction, stroke, coronary revascularization and total CVD mortality	Folic acid, 2.5 mg/d, plus vitamin B_6, 50 mg/d, plus vitamin B_{12}, 1 mg/d, compared with placebo	Ongoing study
Folic Acid for Vascular Outcome Reduction In Transplantation (FAVORIT) trial [116]	United States	4,000	Renal transplant recipients; Population without mandatory folate fortification of food	17.4 µmol/L	Cardiovascular events	Folic acid, 5.0 or 0 mg/d, vitamin B_6, 50 or 1.4 mg/d and vitamin B_{12} 1 or 0.002 mg/d	Ongoing study
Norwegian Study of Homocysteine Lowering with B-Vitamins in Myocardial Infarction [NORVIT] [97]	Norway	3,749	Patients with acute myocardial infarction within 7 days before randomisation; Population without mandatory folate fortification of food	12.9-13.3 µmol/L	Myocardial infarction	2×2 factorial design: folic acid, 0.8 mg/d, vitamin B_{12}, 0.4 mg, vitamin B_6, 40 mg/d; folic acid, 0.8 mg/d, vitamin B_{12}, 0.4 mg; vitamin B_6, 40 mg/d; placebo	No reduction of the risk of recurrent cardiovascular disease after acute myocardial infarction (RR for folic acid plus vitamin B_{12}: 1.08 [95% CI, 0.93–1.25]). Possible harmful effect from combined B vitamin treatment (RR for folic acid plus vitamin B_{12} with vitamin B_6: 1.22 [95% CI, 1.00– 1.50])
Vitamins in Stroke Prevention [VISP] Trial [95]	United States	3,680	Patients with nondisabling cerebral infarction; Mainly populations with mandatory folate fortification of food	13.4 µmol/L	Stroke	Folic acid, 2.5 mg/d, vitamin B_6, 25 mg/d, and vitamin B_{12}, 0.4 mg/d, compared with folic acid, 0.02 mg/d, plus vitamin B_6, 0.2 mg/d, plus vitamin B_{12}, 0.06 mg/d	No effect of homocysteine lowering treatment on vascular outcomes despite moderate reduction of homocysteine concentration (RR: 1.0 [95% CI, 0.8 to 1.1])

Table 3 cont.

Study name [reference]	Region	Sample size	Study population	Baseline Homocysteine levels	Studied outcome	Intervention	Main result(s)
Western Norway B-vitamin Intervention Trial (WENBIT) [117]	Norway	3,000	Patients with coronary artery disease. Population without mandatory folate fortification of food	11.1 μmol/L	Cardiovascular events	2×2 factorial design: folic acid, 0.8 mg/d, vitamin B_{12}, 0.4 mg, vitamin B_6, 40 mg/d; folic acid, 0.8 mg/d, vitamin B_{12}, 0.4 mg; vitamin B_6, 40 mg/d; placebo	Ongoing study
SU.FOL.OM3 study [118]	France	3,000	Patients with myocardial infarction, unstable angina pectoris or ischaemic stroke. Population without mandatory folate fortification of food	n.a.	Recurrent ischaemic diseases	folate (in the natural 5-methyl-tetrahydrofolate form) in combination with vitamin B_6 and B_{12} and/or omega-3 fatty acids and/or placebo	Ongoing study
Homocysteinaemia in kidney and end stage renal disease (HOST) study [119]	United States	2,000	Patients with end stage renal disease or advanced chronic kidney disease. Population without mandatory folate fortification of food	n.a.	All cause mortality, myocardial infarction, stroke, amputation of a lower extremity	Folic acid, 40 mg/d, vitamin B_6, 100 mg, vitamin B_{12}, 2 mg compared with placebo	Ongoing study
Cambridge Heart Antioxidant Study [CHAOS-2] [120]	United Kingdom	1,882	Cardiovascular patients. Population without mandatory folate fortification of food	n.a.	Myocardial infarction, unstable angina	Folic acid, 5 mg/d, compared with placebo	Ongoing study
Folate therapy and in-stent restenosis after coronary stenting [77]	Germany (Bremen) & Netherlands (Zwolle)	636	Bare-metal coronary stented patients. Population without mandatory folate fortification of food	12.2 and 12.9 μmol/L	Restenosis	Folic acid, 1 mg, vitamin B_6, 5 mg and vitamin B_{12}, 1 mg intravenous bolus, then folic acid, 1.2 mg/d plus vitamin B_6, 48 mg/d plus vitamin B_{12}, 0.06 mg/d compared with placebo	Homocysteine lowering treatment may increase the risk of in-stent restenosis (34.5% vs. 26.5% in placebo, $P = 0.05$) and the need for target-vessel revascularisation (15.8% vs. 10.6% in placebo, $P = 0.05$)
Transplant Study [103]	Italy	51	De novo heart transplant recipients (mean age: 53 years). Population without mandatory folate fortification of food	17.9 μmol/L	Coronary allograft vasculopathy (CAV)	Folic acid, 15 mg/d compared with placebo	Despite being effective in preventing hyperhomocysteinaemia after heart transplantation, folate therapy does not affect early CAV onset.

n.a.: not available

Table 4

Recommendations for screening, prevention and treatment of hyper-homocysteinaemia.

U.S. Preventive Services Task Force (USPSTF): (…) the USPSTF concludes that the evidence is insufficient to recommend for or against the use of supplements of vitamins A, C, or E; multivitamins with folate; or antioxidant combinations for the prevention of cancer or cardiovascular disease (…) [121].
International Task Force for Prevention of Coronary Heart Disease: (…) At present, there is insufficient evidence to recommend measuring homocysteine levels in the general population. Homocysteine itself may not be an independent risk factor, since levels correlate with renal function, smoking, and levels of fibrinogen and C-reactive protein, which are known markers of increased risk for CHD. In addition, the risk threshold is unclear and recommended cut-off levels vary between 12 and 18 µmol/L. Treatment with folate, vitamin B_6 and vitamin B_{12} lowers homocysteine levels, but no trial evidence exists that this treatment reduces cardiovascular event rates. (…) [122]
AHA (American Heart Association) / CDC (Centers of Disease Control and Prevention): (…) Screening for homocysteine levels in the blood may be useful in patients with a personal or family history of CVD but who don't have the well-established risk factors (smoking, high blood cholesterol, high blood pressure, physical inactivity, obesity and diabetes). Although evidence for the benefit of lowering homocysteine levels is lacking, patients at high risk should be strongly advised to be sure to get enough folate and vitamins B_6 and B_{12} in their diet. They should eat at least five servings of fruits and green, leafy vegetables daily. (…) [123]
National Guideline Clearinghouse: There is insufficient evidence to recommend for or against screening for hyperhomocysteinaemia in the general population. There is insufficient evidence to recommend for or against screening for hyperhomocysteinaemia in high-risk populations, however, screening may identify individuals at higher risk of developing coronary artery disease, leading to aggressive risk factor modification. There is insufficient evidence to recommend for or against treatment of hyperhomocysteinaemia with vitamin therapy. [124]

b. Recommend increased intake of fruit and vegetables (at least five servings per day) and regular physical activity.

c. No clear evidence for or against oral supplementation with folate, vitamins B_6, and B_{12} if "a" and "b" are not achievable.

d. Further blood homocysteine determinations are disputable.

Conclusion

Homocysteine is associated with a risk of cardiovascular and cerebrovascular diseases and venous thrombosis. However, there are insufficient and inconsistent data from which to draw conclusions as to whether homocysteine is causally linked with these diseases. In addition, there is currently no evidence that homocysteine-lowering therapy has a beneficial effect on CVD risk. The current body of evidence further precludes recommending screening for homocysteine at population level or food-fortification with B vitamins to reduce overall CVD risk. For clinicians, the objective should be to reduce the overall CVD risk in hyperhomocysteinemic individuals by controlling "classical" risk factors such as smoking, elevated blood pressure, adverse lipid profiles and diabetes, and by promoting a healthy lifestyle.

Search strategy and selection criteria

Publications of interest were randomised controlled trials, animal and in vitro studies, literature reviews, meta-analysis and epidemiological studies (cohort or case-control studies). Publications were identified by systematic searches of MEDLINE 1980–2006 using the following keywords per se and in combination: "homocysteine", "hyperhomocysteinaemia", "folic acid", "vitamins", "deficiency", "cardiovascular risk factor", "cardiovascular disease", "treatment" and "supplementation". Although we concentrated on recent findings we also included some older research milestones.

Correspondence:
David Faeh
Institut universitaire de médecine sociale
et préventive (IUMSP)
Rue du Bugnon 17
CH-1005 Lausanne
Switzerland
E-Mail: david.faeh@unil.ch

References

1 Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. Circulation. 2001;104(22):2746–53.

2 Syme SL. Rethinking disease: where do we go from here? Ann Epidemiol. 1996;6(5):463–8.

3 Magnus P, Beaglehole R. The real contribution of the major risk factors to the coronary epidemics: time to end the "only-50%" myth. Arch Intern Med. 2001;161(22):2657–60.

4 Beaglehole R, Magnus P. The search for new risk factors for coronary heart disease: occupational therapy for epidemiologists? Int J Epidemiol. 2002;31(6):1117–22; author reply 1134–1115W.

5 Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). Jama. 1986;256(20):2823–8.

6 Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97(18):1837–47.

7 Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr. 2000;72(2):324–32.

8 Kaplan ED. Association between homocyst(e)ine levels and risk of vascular events. Drugs Today. (Barc) 2003;39(3):175–92.

9 The Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. Jama. 2002;288(16):2015–22.

10 Ford ES, Smith SJ, Stroup DF, Steinberg KK, Mueller PW, Thacker SB. Homocyst(e)ine and cardiovascular disease: a systematic review of the evidence with special emphasis on case-control studies and nested case-control studies. Int J Epidemiol. 2002;31(1):59–70.

11 Beitz R, Mensink GB, Fischer B, Thamm M. Vitamins – dietary intake and intake from dietary supplements in Germany. Eur J Clin Nutr. 2002;56(6):539–45.

12 Genest J Jr. Hyperhomocyst(e)inemia – determining factors and treatment. Can J Cardiol. 1999;15(Suppl B):35B–38B.

13 Graham I. Homocysteine in health and disease. Ann Intern Med. 1999;131(5):387–88.

14 Moghadasian MH, McManus BM, Frohlich JJ. Homocyst(e)ine and coronary artery disease. Clinical evidence and genetic and metabolic background. Arch Intern Med. 1997;157(20): 2299–308.

15 Oakley GP Jr. Eat right and take a multivitamin. N Engl J Med. 1998;338(15):1060–1.

16 Peterson JC, Spence JD. Vitamins and progression of atherosclerosis in hyper-homocyst(e)inaemia. Lancet. 1998;351 (9098):263.

17 Wald NJ, Law MR. A strategy to reduce cardiovascular disease by more than 80%. BMJ. 2003;326(7404):1419.

18 Stanger O, Herrmann W, Pietrzik K, Fowler B, Geisel J, Dierkes J, et al. Clinical use and rational management of homocysteine, folic acid, and B vitamins in cardiovascular and thrombotic diseases. Z Kardiol. 2004;93(6):439–53.

19 Wald NJ, Hackshaw AK, Frost CD. When can a risk factor be used as a worthwhile screening test? BMJ. 1999;319(7224): 1562–5.

20 Wilcken DE, Wang XL, Wilcken B. Methylenetetrahydrofolate reductase (MTHFR) mutation, homocyst(e)ine, and coronary artery disease. Circulation. 1997;96(8):2738–40.

21 Brattstrom L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinaemia but not to vascular disease: the result of a meta-analysis. Circulation. 1998;98(23):2520–6.

22 Bunout D, Hirsch S. Are we losing homocysteine as a cardiovascular risk factor? Nutrition. 2005;21(10):1068–9.

23 Guilland JC, Favier A, Potier de Courcy G, Galan P, Hercberg S. Hyperhomocysteinaemia: an independent risk factor or a simple marker of vascular disease? 2. Epidemiological data. Pathol Biol. (Paris) 2003;51(2):111–21.

24 Guilland JC, Favier A, Potier de Courcy G, Galan P, Hercberg S. Hyperhomocysteinaemia: an independent risk factor or a simple marker of vascular disease?. 1. Basic data. Pathol Biol. (Paris) 2003;51(2):101–10.

25 Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ. 2005;331(7524):1053.

26 Eichholzer M, Tönz O, Zimmermann R. Folic acid: a public-health challenge. Lancet. 2006;367:1352–61.

27 1. American College of Medical Genetics. Newborn Screening: Toward a Uniform Screening Panel and System. Final Report, March 8, 2005. http://mchb.hrsa.gov/screening/ (accessed: 2 May 2006).

28 Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, et al. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet. 1985; 37(1):1–31.

29 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10(1):111–3.

30 Friso S, Girelli D, Trabetti E, Stranieri C, Olivieri O, Tinazzi E, et al. A1298C methylenetetrahydrofolate reductase mutation and coronary artery disease: relationships with C677T polymorphism and homocysteine/folate metabolism. Clin Exp Med. 2002;2(1):7–12.

31 Malinow MR, Nieto FJ, Kruger WD, Duell PB, Hess DL, Gluckman RA, et al. The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. Arterioscler Thromb Vasc Biol. 1997;17(6):1157–62.

32 von Castel-Dunwoody KM, Kauwell GP, Shelnutt KP, Vaughn JD, Griffin ER, Maneval DR, et al. Transcobalamin 776C->G polymorphism negatively affects vitamin B-12 metabolism. Am J Clin Nutr. 2005;81(6):1436–41.

33 Selhub J, Jacques PF, Bostom AG, Wilson PW, Rosenberg IH. Relationship between plasma homocysteine and vitamin status in the Framingham study population. Impact of folic acid fortification. Public Health Rev. 2000;28(1-4):117–45.

34 Hughes K, Ong CN. Homocysteine, folate, vitamin B_{12}, and cardiovascular risk in Indians, Malays, and Chinese in Singapore. J Epidemiol Community Health. 2000;54(1):31–4.

35 de Bree A, van der Put NM, Mennen LI, Verschuren WM, Blom HJ, Galan P, et al. Prevalences of hyperhomocysteinaemia, unfavorable cholesterol profile and hypertension in European populations. Eur J Clin Nutr. 2005;59(4):480–8.

36 McCully KS. Vascular pathology of homocysteinaemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol. 1969;56(1):111–28.

37 Wilcken DE, Wilcken B. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. J Clin Invest. 1976;57(4):1079–82.

38 Ray JG. Meta-analysis of hyperhomocysteinaemia as a risk factor for venous thromboembolic disease. Arch Intern Med. 1998;158(19):2101–6.

39 den Heijer M, Rosendaal FR, Blom HJ, Gerrits WB, Bos GM. Hyperhomocysteinaemia and venous thrombosis: a meta-analysis. Thromb Haemost. 1998;80(6):874–7.

40 Bottiglieri T. Homocysteine and folate metabolism in depression. Prog Neuropsychopharmacol Biol Psychiatry 2005.

41 Tucker KL, Qiao N, Scott T, Rosenberg I, Spiro A, 3rd. High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study. Am J Clin Nutr. 2005;82(3):627–35.

42 Ravaglia G, Forti P, Maioli F, Scali RC, Saccheitti L, Talerico T, et al. Homocysteine and cognitive performance in healthy elderly subjects. Arch Gerontol Geriatr. Suppl 2004;(9):349–57.

43 Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, et al. Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr. 2005;82(3):636–43.

44 Bottiglieri T. Homocysteine and folate metabolism in depression. Prog Neuropsychopharmacol Biol Psychiatry. 2005;29(7): 1103–12.

45 Ramos MI, Allen LH, Haan MN, Green R, Miller JW. Plasma folate concentrations are associated with depressive symptoms in elderly Latina women despite folic acid fortification. Am J Clin Nutr. 2004;80(4):1024–8.

46 Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med. 2002;346(7): 476–83.

47 Morris MS. Homocysteine and Alzheimer's disease. Lancet Neurol. 2003;2(7):425–8.

48 O'Suilleabhain PE, Sung V, Hernandez C, Lacritz L, Dewey RB Jr, Bottiglieri T, et al. Elevated plasma homocysteine level in patients with Parkinson disease: motor, affective, and cognitive associations. Arch Neurol. 2004;61(6):865–8.

49 Vignini A, Nanetti L, Bacchetti T, Ferretti G, Curatola G, Mazzanti L. Modification induced by homocysteine and low-density lipoprotein on human aortic endothelial cells: an in vitro study. J Clin Endocrinol Metab. 2004;89(9):4558–61.

50 Su SJ, Huang LW, Pai LS, Liu HW, Chang KL. Homocysteine at pathophysiologic concentrations activates human monocyte and induces cytokine expression and inhibits macrophage migration inhibitory factor expression. Nutrition. 2005;21(10): 994–1002.

51 Demuth K, Atger V, Borderie D, Benoit MO, Sauvaget D, Lotersztajn S, et al. Homocysteine decreases endothelin-1 production by cultured human endothelial cells. Eur J Biochem. 1999;263(2):367–76.

52 Zhang X, Li H, Jin H, Ebin Z, Brodsky S, Goligorsky MS. Effects of homocysteine on endothelial nitric oxide production. Am J Physiol Renal Physiol. 2000;279(4):F671–78.

53 Brown JC, Rosenquist TH, Monaghan DT. ERK2 activation by homocysteine in vascular smooth muscle cells. Biochem Biophys Res Commun. 1998;251(3):669–76.

54 Fryer RH, Wilson BD, Gubler DB, Fitzgerald LA, Rodgers GM. Homocysteine, a risk factor for premature vascular disease and thrombosis, induces tissue factor activity in endothelial cells. Arterioscler Thromb. 1993;13(9):1327–33.

55 Upchurch GR Jr, Welch GN, Fabian AJ, Freedman JE, Johnson JL, Keaney JF Jr, et al. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. J Biol Chem 1997;272(27):17012–7.

56 Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, et al. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. J Clin Invest. 1993;91(1):308–18.

57 Foncea R, Carvajal C, Almarza C, Leighton F. Endothelial cell oxidative stress and signal transduction. Biol Res. 2000;33(2): 89–96.

58 Hofmann MA, Lalla E, Lu Y, Gleason MR, Wolf BM, Tanji N, et al. Hyperhomocysteinaemia enhances vascular inflammation and accelerates atherosclerosis in a murine model. J Clin Invest. 2001;107(6):675–83.

59 Bellamy MF, McDowell IF. Putative mechanisms for vascular damage by homocysteine. J Inherit Metab Dis. 1997;20(2):307–15.

60 Lentz SR, Erger RA, Dayal S, Maeda N, Malinow MR, Heistad DD, et al. Folate dependence of hyperhomocysteinaemia and vascular dysfunction in cystathionine beta-synthase-deficient mice. Am J Physiol Heart Circ Physiol. 2000;279(3): H970–5.

61 Namekata K, Enokido Y, Ishii I, Nagai Y, Harada T, Kimura H. Abnormal lipid metabolism in cystathionine beta-synthase-deficient mice, an animal model for hyperhomocysteinaemia. J Biol Chem. 2004;279(51):52961–9.

62 Chen Z, Karaplis AC, Ackerman SL, Pogribny IP, Melnyk S, Lussier-Cacan S, et al. Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinaemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. Hum Mol Genet. 2001;10(5):433–43.

63 Harpel PC, Zhang X, Borth W. Homocysteine and hemostasis: pathogenic mechanisms predisposing to thrombosis. J Nutr. 1996;126(4 Suppl):1285S–9S.

64 Lentz SR. Mechanisms of homocysteine-induced atherothrombosis. J Thromb Haemost. 2005;3(8):1646–54.

65 Wald DS, Bishop L, Wald NJ, Law M, Hennessy E, Weir D, et al. Randomized trial of folic acid supplementation and serum homocysteine levels. Arch Intern Med. 2001;161(5):695–700.

66 Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. Jama. 1995;274(13):1049–57.

67 Tucker KL, Mahnken B, Wilson PW, Jacques P, Selhub J. Folic acid fortification of the food supply. Potential benefits and risks for the elderly population. Jama. 1996;276(23):1879–85.

68 Hickling S, Hung J, Knuiman M, Jamrozik K, McQuillan B, Beilby J, et al. Impact of voluntary folate fortification on plasma homocysteine and serum folate in Australia from 1995 to 2001: a population based cohort study. J Epidemiol Community Health. 2005;59(5):371–6.

69 Yap S, Boers GH, Wilcken B, Wilcken DE, Brenton DP, Lee PJ, et al. Vascular outcome in patients with homocystinuria due to cystathionine beta-synthase deficiency treated chronically: a multicenter observational study. Arterioscler Thromb Vasc Biol. 2001;21(12):2080–5.

70 Moat SJ, Hill MH, McDowell IF, Pullin CH, Ashfield-Watt PA, Clark ZE, et al. Reduction in plasma total homocysteine through increasing folate intake in healthy individuals is not associated with changes in measures of antioxidant activity or oxidant damage. Eur J Clin Nutr. 2003;57(3):483–9.

71 Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. BMJ. 1998;316(7135):894–8.

72 Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation. 1999;99(1):178–82.

73 National Nutrient Database for Standard Reference of the US Department of Agriculture (USDA, release 18). http://www.nal.usda.gov/fnic/foodcomp/srch/search.htm (accessed: 20 March 2006).

74 de Bree A, van Dusseldorp M, Brouwer IA, van het Hof KH, Steegers-Theunissen RP. Folate intake in Europe: recommended, actual and desired intake. Eur J Clin Nutr. 1997; 51(10):643–60.

75 Spoelstra-de MA, Brouwer CB, Terheggen F, Bollen JM, Stehouwer CD, Smulders YM. No effect of folic acid on markers of endothelial dysfunction or inflammation in patients with type 2 diabetes mellitus and mild hyperhomocysteinaemia. Neth J Med. 2004;62(7):246–53.

76 De Luca G, Suryapranata H, Gregorio G, Lange H, Chiariello M. Homocysteine and its effects on in-stent restenosis. Circulation. 2005;112(19):e307–311.

77 Lange H, Suryapranata H, De Luca G, Borner C, Dille J, Kallmayer K, et al. Folate therapy and in-stent restenosis after coronary stenting. N Engl J Med. 2004;350(26):2673–81.

78 Kluijtmans LA, Kastelein JJ, Lindemans J, Boers GH, Heil SG, Bruschke AV, et al. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. Circulation. 1997;96(8): 2573–7.

79 Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. Lancet. 2005;365(9455):224–32.

80 Nitsch D, Molokhia M, Smeeth L, DeStavola BL, Whittaker JC, Leon DA. Limits to causal inference based on Mendelian randomization: a comparison with randomized controlled trials. Am J Epidemiol. 2006;163(5):397–403.

81 Bostom AG, Culleton BF. Hyperhomocysteinaemia in chronic renal disease. J Am Soc Nephrol. 1999;10(4):891–900.

82 Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. Jama. 1995;274(19):1526–33.

83 Bostom AG. Homocysteine: "expensive creatinine" or important modifiable risk factor for arteriosclerotic outcomes in renal transplant recipients? J Am Soc Nephrol. 2000;11(1):149–51.

84 Ducloux D, Motte G, Challier B, Gibey R, Chalopin JM. Serum total homocysteine and cardiovascular disease occurrence in chronic, stable renal transplant recipients: a prospective study. J Am Soc Nephrol. 2000;11(1):134–7.

85 Egerton W, Silberberg J, Crooks R, Ray C, Xie L, Dudman N. Serial measures of plasma homocyst(e)ine after acute myocardial infarction. Am J Cardiol. 1996;77(9):759–61.

86 Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, et al. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12, and folate. Am J Epidemiol. 1996;143(9):845–59.

87 Landgren F, Israelsson B, Lindgren A, Hultberg B, Andersson A, Brattstrom L. Plasma homocysteine in acute myocardial infarction: homocysteine-lowering effect of folic acid. J Intern Med. 1995;237(4):381–8.

88 Hultberg B, Andersson A, Lindgren A. Marginal folate deficiency as a possible cause of hyperhomocystinaemia in stroke patients. Eur J Clin Chem Clin Biochem. 1997;35(1):25–8.

89 Loehrer FM, Angst CP, Haefeli WE, Jordan PP, Ritz R, Fowler B. Low whole-blood S-adenosylmethionine and correlation between 5-methyltetrahydrofolate and homocysteine in coronary artery disease. Arterioscler Thromb Vasc Biol. 1996;16(6): 727–33.

90 Lindgren A, Brattstrom L, Norrving B, Hultberg B, Andersson A, Johansson BB. Plasma homocysteine in the acute and convalescent phases after stroke. Stroke. 1995;26(5):795–800.

91 Hirsch S, Ronco AM, Vasquez M, de la Maza MP, Garrido A, Barrera G, et al. Hyperhomocysteinaemia in healthy young men and elderly men with normal serum folate concentration is not associated with poor vascular reactivity or oxidative stress. J Nutr. 2004;134(7):1832–5.

92 Hirsch S, de la Maza P, Mendoza L, Petermann M, Glasinovic A, Paulinelli P, et al. Endothelial function in healthy younger and older hyperhomocysteinemic subjects. J Am Geriatr Soc. 2002;50(6):1019–23.

93 Dusitanond P, Eikelboom JW, Hankey GJ, Thom J, Gilmore G, Loh K, et al. Homocysteine-lowering treatment with folic acid, cobalamin, and pyridoxine does not reduce blood markers of inflammation, endothelial dysfunction, or hypercoagulability in patients with previous transient ischemic attack or stroke: a randomized substudy of the VITATOPS trial. Stroke. 2005;36(1):144–6.

94 Hackam DG, Anand SS. Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. Jama. 2003;290(7):932–40.

95 Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, 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(5):565–75.

96 Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. N Engl J Med. 2006;354(15):1567–77.

97 Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med. 2006; 354(15):1578–88.

98 Eichholzer M, Tonz O, Zimmermann R. Folic acid: a public-health challenge. Lancet. 2006;367(9519):1352–61.

99 Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999–2000. Am J Clin Nutr. 2005;82(2):442–50.

100 Husemoen LL, Thomsen TF, Fenger M, Jorgensen T. Changes in lifestyle and total homocysteine in relation to MTHFR(C677T) genotype: the Inter99 study. Eur J Clin Nutr 2005.

101 Rousseau AS, Robin S, Roussel AM, Ducros V, Margaritis I. Plasma homocysteine is related to folate intake but not training status. Nutr Metab Cardiovasc Dis. 2005;15(2):125–33.

102 Hankey GJ. Secondary prevention of recurrent stroke. Stroke. 2005;36(2):218–21.

103 Potena L, Grigioni F, Magnani G, Ortolani P, Coccolo F, Sassi S, et al. Homocysteine-lowering therapy and early progression of transplant vasculopathy: a prospective, randomized, IVUS-based study. Am J Transplant. 2005;5(9):2258–64.

104 Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation. 2006;114(1): 82–96.

105 Flood VM, Webb KL, Smith W, Rochtchina E, Mitchell P. Prevalence of low serum folate, red cell folate, serum vitamin B_{12} and elevated homocysteine. Asia Pac J Clin Nutr. 2004; 13(Suppl):S85.

106 Fakhrzadeh H, Ghotbi S, Pourebrahim R, Nouri M, Heshmat R, Bandarian F, et al. Total plasma homocysteine, folate, and vitamin B_{12} status in healthy Iranian adults: the Tehran homocysteine survey (2003-2004)/ a cross – sectional population based study. BMC Public Health. 2006;6(1):29.

107 Ray JG, Cole DE, Boss SC. An Ontario-wide study of vitamin B_{12}, serum folate, and red cell folate levels in relation to plasma homocysteine: is a preventable public health issue on the rise? Clin Biochem. 2000;33(5):337–43.

108 Shaw JT, McWhinney B, Tate JR, Kesting JB, Marczak M, Purdie D, et al. Plasma homocysteine levels in indigenous Australians. Med J Aust. 1999;170(1):19–22.

109 Janson JJ, Galarza CR, Murua A, Quintana I, Przygoda PA, Waisman G, et al. Prevalence of hyperhomocysteinemia in an elderly population. Am J Hypertens. 2002;15(5):394–7.

110 Lim HS, Heo YR. Plasma total homocysteine, folate, and vitamin B_{12} status in Korean adults. J Nutr Sci Vitaminol. (Tokyo) 2002;48(4):290–7.

111 MacMahon M, Kirkpatrick C, Cummings CE, Clayton A, Robinson PJ, Tomiak RH, et al. A pilot study with simvastatin and folic acid/vitamin B_{12} in preparation for the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH). Nutr Metab Cardiovasc Dis. 2000;10(4):195–203.

112 Neal B, MacMahon S, Ohkubo T, Tonkin A, Wilcken D. Dose-dependent effects of folic acid on plasma homocysteine in a randomized trial conducted among 723 individuals with coronary heart disease. Eur Heart J. 2002;23(19):1509–15.

113 The VITATOPS (Vitamins to Prevent Stroke) Trial: rationale and design of an international, large, simple, randomised trial of homocysteine-lowering multivitamin therapy in patients with recent transient ischaemic attack or stroke. Cerebrovasc Dis. 2002;13(2):120–6.

114 Hankey GJ, Eikelboom JW, Loh K, Tang M, Pizzi J, Thom J, et al. Sustained homocysteine-lowering effect over time of folic acid-based multivitamin therapy in stroke patients despite increasing folate status in the population. Cerebrovasc Dis. 2005;19(2):110–6.

115 Bassuk SS, Albert CM, Cook NR, Zaharris E, MacFadyen JG, Danielson E, et al. The Women's Antioxidant Cardiovascular Study: design and baseline characteristics of participants. J Womens Health. (Larchmt) 2004;13(1):99–117.

116 Bostom AG, Carpenter MA, Kusek JW, Hunsicker LG, Pfeffer MA, Levey AS, et al. Rationale and design of the Folic Acid for Vascular Outcome Reduction In Transplantation (FA-VORIT) trial. Am Heart J. 2006;152(3):448–53.

117 Bleie O, Refsum H, Ueland PM, Vollset SE, Guttormsen AB, Nexo E, et al. Changes in basal and postmethionine load concentrations of total homocysteine and cystathionine after B vitamin intervention. Am J Clin Nutr. 2004;80(3):641–8.

118 Galan P, de Bree A, Mennen L, Potier de Courcy G, Preziozi P, Bertrais S, et al. Background and rationale of the SU.FOL.OM3 study: double-blind randomized placebo-controlled secondary prevention trial to test the impact of supplementation with folate, vitamin B_6 and B_{12} and/or omega-3 fatty acids on the prevention of recurrent ischemic events in subjects with atherosclerosis in the coronary or cerebral arteries. J Nutr Health Aging. 2003;7(6):428–35.

119 Jamison RL, Hartigan P, Gaziano JM, Fortmann SP, Goldfarb DS, Haroldson JA, et al. Design and statistical issues in the homocysteinemia in kidney and end stage renal disease (HOST) study. Clin Trials. 2004;1(5):451–60.

120 Baker F, Picton D, Blackwood S, Hunt J, Erskine M, Dyas M. Blinded comparison of folic acid and placebo in patients with ischemic heart disease: an outcome trial. Circulation. 2002;106(3642 (abstract)).

121 U.S. Preventive Services Task Force. Routine vitamin supplementation to prevent cancer and cardiovascular disease: recommendations and rationale. Am Fam Physician. 2003;68 (12):2422–4.

122 International Task Force for Prevention of Coronary Heart Disease. http://www.chd-taskforce.de/guidelines/kap23.htm (accessed: 3 May 2006).

123 AHA (American Heart Association) & CDC (Centers of Disease Control and Prevention). http://www.americanheart. org/presenter.jhtml?identifier=4677 (accessed: 3 May 2006).

124 National Guideline Clearinghouse. Preventive health care: screening and management of hyperhomocysteinemia for the prevention of coronary artery disease events. http://guideline.gov/summary/pdf.aspx?doc_id=2702&stat=1&string= (accessed: 3 May 2006). 2000.

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses

- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

Impact factor Swiss Medical Weekly

EMH
Editores Medicorum Helveticorum
FMH SCHWABE

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts: submission@smw.ch
Letters to the editor: letters@smw.ch
Editorial Board: red@smw.ch
Internet: http://www.smw.ch