



**EDUCATIONAL OBJECTIVE:** Readers will advise their patients that the hypothesis that elevated homocysteine should be treated to reduce the risk of cardiovascular disease has neither been proved nor disproved

**JOELLYN M. ABRAHAM, MD**  
Cardiovascular Medicine, Cleveland Clinic

**LESLIE CHO, MD**  
Section Head, Preventive Cardiology  
and Rehabilitation, and Director, Women's  
Cardiovascular Center, Cleveland Clinic

# The homocysteine hypothesis: Still relevant to the prevention and treatment of cardiovascular disease?

## ABSTRACT

Although evidence suggests that the homocysteine hypothesis is still relevant as a predictor of cardiovascular risk, we cannot conclude that measuring the homocysteine level is useful in guiding treatment. Furthermore, studies of primary and secondary prevention show no evidence that taking folic acid or other B vitamins lowers the risk of cardiovascular events.

## KEY POINTS

Factors that can cause the plasma homocysteine concentration to be high include deficiencies of vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, and folic acid; renal insufficiency; and genetic variants in enzymes responsible for homocysteine metabolism.

Higher plasma homocysteine levels are associated with a higher risk of cardiovascular, cerebrovascular, and peripheral arterial disease.

Supplementation of B vitamins and folic acid can lower plasma homocysteine levels.

Randomized controlled trials of supplementation to prevent cardiovascular events and other adverse outcomes have had mostly negative results. However, most patients in these trials had normal baseline plasma homocysteine levels.

Needed are randomized trials to see if supplementation improves outcomes in patients with high homocysteine levels.

doi:10.3949/ccjm.77a.10036

**P**ATIENTS OFTEN ASK primary care physicians and cardiologists about the measurement of biomarkers for cardiovascular disease and about the efficacy of preventive measures.

Although studies have shown that elevated homocysteine is a risk factor for cardiovascular and peripheral arterial disease<sup>1-3</sup> and that supplementation with folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> lowers homocysteine levels,<sup>4,5</sup> it is unclear whether such supplementation prevents cardiovascular events. As a result, there is no consensus about whose homocysteine levels should be measured and who, if anyone, should receive homocysteine-lowering therapies.

The aim of this paper is to examine whether the evidence is sufficient to recommend homocysteine testing to guide the prevention and treatment of cardiovascular disease, or to recommend using folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> for primary or secondary prevention of cardiovascular disease.

## HISTORY OF HOMOCYSTEINE AS A RISK MARKER

Homocysteine is an amino acid formed from the metabolism of methionine, an essential amino acid derived from dietary protein. Although homocysteine was first isolated by Butz and du Vigneaud in 1932,<sup>6</sup> it was not until 1964 that Gibson et al<sup>7</sup> reported that patients with homocystinuria (more about this below) had vascular anomalies and arterial thrombosis. In 1969, McCully<sup>8</sup> made the connection

**TABLE 1**

## Causes of elevated homocysteine

### Mild (15–30 $\mu\text{mol/L}$ )

Mild-moderate renal disease  
 Drug use—antiepileptic drugs, methotrexate, theophylline, niacin, immunosuppressive drugs, fibrates, levodopa, metformin (Glucophage)  
 Hypothyroidism  
 Hyperproliferative disorders, certain cancers  
 Psoriasis  
 Methylene tetrahydrofolate reductase (MTHFR) 677C>T variant  
 Mild-moderate folate or vitamin B<sub>12</sub> deficiency  
 Increasing age  
 High protein intake  
 Low intake of vegetables or fruits  
 Sickle-cell anemia

### Moderate (30–100 $\mu\text{mol/L}$ )

End-stage renal disease  
 Moderate vitamin B<sub>12</sub> deficiency  
 Severe folate deficiency  
 MTHFR 677C>T variant combined with low folic acid levels

### Severe ( $\geq 100 \mu\text{mol/L}$ )

Severe vitamin B<sub>12</sub> deficiency  
 Cystathionine beta-synthase deficiency

BASED ON INFORMATION IN REFERENCES 9, 11, AND 12

between elevated homocysteine levels and the risk of atherosclerosis.

Several possible mechanisms for the association between homocysteine and atherosclerosis have been demonstrated in experimental models. These include stimulation of smooth muscle growth, reduction in endothelial cell growth, impaired endothelial cell relaxation, decreased synthesis of high-density lipoprotein, promotion of autoimmune response, and accumulation of inflammatory monocytes in atherosclerotic plaques.<sup>3,9,10</sup>

In view of these findings, researchers have been evaluating whether homocysteine-lowering therapies decrease the risk of cardiovascular disease.

### CAUSES OF ELEVATED PLASMA HOMOCYSTEINE

An elevated plasma homocysteine level can result from many different factors, including vitamin deficiencies, renal impairment, and inborn errors of homocysteine metabolism (TABLE 1).<sup>9,11,12</sup>

**Vitamin deficiencies.** Vitamin B<sub>6</sub> (pyridoxine), vitamin B<sub>12</sub> (cyanocobalamin), and folic acid are cofactors required for homocysteine metabolism, and deficiency in any or all of these leads to disruption of the relevant metabolic pathways.

**Renal impairment.** A low glomerular filtration rate has also been correlated with an elevated plasma homocysteine concentration. This makes sense, since the kidneys perform up to 70% of the clearance of homocysteine, although a cause-and-effect relationship is unclear.<sup>13</sup>

**Inborn errors of homocysteine metabolism.** *Homocystinuria*, ie, an abnormal elevation of homocysteine in the urine, is caused by several autosomal recessive disorders. People with these genetic variations have extremely high homocysteine levels.

A deficiency in the enzyme cystathionine beta-synthase is quite rare (the incidence in newborn babies has been found to be 1 in 344,000 worldwide and 1 in 65,000 in Ireland and Australia<sup>14</sup>), but leads to homocysteine levels greater than 100  $\mu\text{mol/L}$  and often causes cardiovascular disease by the age of 30 years.<sup>15</sup>

A deficiency in the enzyme methylene tetrahydrofolate reductase (MTHFR) is a more common cause of mildly to moderately elevated plasma homocysteine levels.<sup>16</sup> The MTHFR deficiency involves a variation at position 677 in the MTHFR gene in which cytosine is replaced by thymidine (thus called C677T or 677C>T).<sup>17</sup> Ten percent of the population are homozygous for this variant (TT), 43% are heterozygous (CT), and 47% are unaffected (CC). Heterozygotes have slightly higher homocysteine levels than unaffected people, while people with the TT genotype have approximately 20% higher homocysteine levels.<sup>17</sup>

### ELEVATED HOMOCYSTEINE IS COMMON

In a study of a population in Norway from 1992 to 1993, 8.5% had mild elevations in homocysteine (plasma levels 15–29.99  $\mu\text{mol/L}$ ), 0.8% had moderate elevations (30–99.99  $\mu\text{mol/L}$ ), and 0.02% had severe elevations ( $\geq 100 \mu\text{mol/L}$ ).<sup>13,18</sup> The prevalence of hyperhomocysteinemia in the United States

TABLE 2

## Studies of homocysteine-lowering dietary factors as primary prevention

SOURCE	NO. OF PATIENTS	AGE RANGE (YEARS)	WOMEN (%)	VARIABLE OF INTEREST	OUTCOME
Cui et al (2010) <sup>23</sup>	58,730	40–79	61	Intake of folate, B <sub>6</sub> , and B <sub>12</sub>	Inverse association between dietary folate and vitamin B <sub>6</sub> and death from cardiovascular disease (multiple end points)
Liu et al (1999) <sup>24</sup>	75,521	38–63	100	Intake of whole grains	Up to 33% lower rate of fatal myocardial infarction (MI), coronary heart disease (CHD), and nonfatal MI ( <i>P</i> < .001)
Liu et al (2000) <sup>25</sup>	75,521	38–63	100	Intake of whole grains	Up to 51% lower rate of ischemic strokes ( <i>P</i> = .003)
Merchant et al (2003) <sup>26</sup>	46,036	40–75	0	B-vitamin intake	21% lower rate of peripheral arterial disease for every 400 μmol/L increase in intake (95% CI 0.64–0.96)
Rimm et al (1998) <sup>27</sup>	80,082	30–55	100	Intake of folic acid and vitamin B <sub>6</sub>	31% lower rate of nonfatal MI and fatal CHD (95% CI 0.55–0.87)

is probably much lower, given that supplementation of white flour and cereal grains with folic acid has been mandatory since 1998, but this is not well described in the literature.

### ■ HOW GREAT IS THE RISK?

Studies over the past 10 to 20 years have shown that elevated homocysteine is a marker of risk of cardiovascular disease. The association was first noted in patients with cystathionine beta-synthase deficiency, who tend to have premature cardiovascular disease.

However, studies of patients with MTHFR 677C>T have yielded mixed results. Although several meta-analyses found up to a 42% higher rate of ischemic heart disease and stroke in patients homozygous for MTHFR 677C>T (the TT genotype) than in those with the CC genotype,<sup>17,19,20</sup> two other large meta-analyses did not find an association between this variant and vascular risk.<sup>21,22</sup>

Nonetheless, in a meta-analysis of the association between homocysteine and cardiovascular disease, Wald et al<sup>17</sup> found that for every 5-μmol/L increase in serum homocysteine concentration, the risk of ischemic heart disease increased 20% to 30%.

### ■ TRIALS OF HOMOCYSTEINE-LOWERING THERAPY HAVE HAD MIXED RESULTS

#### Primary prevention of cardiovascular disease

Given the finding that treatment with folic acid lowers homocysteine—initially noted in patients with homocystinuria—researchers hypothesized that treatment with folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> would decrease the risk of cardiovascular disease.

Little evidence currently exists to guide recommendations for homocysteine-lowering therapy to prevent first attacks of cardiovascular disease. The few studies published to date have been observational studies of dietary intake (not vitamin supplementation), and many were performed before folic acid fortification was mandated for flour and cereal (TABLE 2).<sup>23–27</sup> Although the studies suggest that higher B-vitamin intake correlates with less vascular disease and its sequelae, there is uncertainty as to whether it is folic acid, vitamin B<sub>6</sub>, or vitamin B<sub>12</sub> that is responsible, and also whether supplements would provide the same protective benefit as the presence of these nutrients in a varied diet.

Thus, in its recent evaluation of novel risk markers of cardiovascular disease, the United States Preventive Services Task Force<sup>28,29</sup> does not recommend measuring the plasma homo-

**Researchers have been evaluating whether homocysteine-lowering therapies decrease the risk of cardiovascular disease**

**TABLE 3**

### Trials of B-vitamins and folic acid as secondary prevention

SOURCE	NO. OF PATIENTS	MEAN AGE (YEARS)	MEN (%)	POPULATION	MEAN CHOLESTEROL (MG/DL)	MEAN HOMOCYSTEINE (μmol/L)	BENEFIT
Albert et al (2008) <sup>4</sup>	5,442	63	0	Coronary artery disease or three risk factors for it	Not reported	12.3	No
Bassuk et al (2004) <sup>61</sup>	5,442	≥ 40	0	Coronary artery disease or three risk factors for it	To be announced	To be announced	To be announced
Bønaa et al (2006) <sup>39</sup>	2,815	63	74	After myocardial infarction	223	13.1	No
SEARCH (2010) <sup>33</sup>	12,064	64	83	Prior myocardial infarction	Not reported	13.5	No
Carrero et al (2005) <sup>40</sup>	60	63	100	Peripheral vascular disease	Not reported	13.2	Yes
Ebbing et al (2008) <sup>5</sup>	3,090	62	80	After percutaneous coronary intervention	196	11	No
Jamison et al (2007) <sup>46</sup>	2,056	66	98	Chronic kidney disease	167	21	No
Khandanpour et al (2009) <sup>36</sup>	133	70	68	Peripheral vascular disease	170	13.3	Yes
Lange et al (2004) <sup>35</sup>	636	61	77	After percutaneous coronary intervention	198	12.6	No
Liem et al (2004) <sup>41</sup>	283	59	70	Coronary artery disease	280	Not reported	No
Liem et al (2005) <sup>42</sup>	593	65	78	Coronary artery disease	178	12.1	No
Lonn et al (2006) <sup>43</sup>	5,522	69	72	Vascular disease or diabetes mellitus	186	11.8	No
Mager et al (2009) <sup>32</sup>	492	51	68	Premature coronary artery disease, elevated homocysteine	Not reported	13.2	Yes
Righetti et al (2003) <sup>11</sup>	81	64	56	End-stage renal disease	199	50.3	No
Righetti et al (2006) <sup>31</sup>	88	64	56	End-stage renal disease	196	35	Yes
Schnyder et al (2002) <sup>34</sup>	553	63	81	After percutaneous coronary intervention	213	11.3	Yes
Sydow et al (2003) <sup>44</sup>	27	67	81	Peripheral vascular disease, elevated homocysteine	Not reported	15.0	No
Toole et al (2004) <sup>45</sup>	3,680	66	63	Cerebrovascular accident	202	13.4	No
Wrone et al (2004) <sup>37</sup>	510	60	50	End-stage renal disease	184	32.9	No
Zoungas et al (2006) <sup>12</sup>	315	56	32	End-stage renal disease	201	27	No

cysteine level in the evaluation of either low-risk or intermediate-risk populations, finding no evidence that it adds any useful information in predicting major coronary events be-

yond what one could get from calculating the Framingham Risk Score. The task force also found no evidence that treating people who have elevated homocysteine levels decreases

their risk of subsequent cardiovascular events.

In addition, a recent Cochrane Database review of eight randomized controlled trials in patients at low risk did not find a lower risk of myocardial infarction (fatal or nonfatal), stroke, or death from any cause in patients receiving B-complex vitamins.<sup>30</sup>

### Secondary prevention of cardiovascular disease

Results have been mixed with regard to the ability of B vitamins to prevent cardiovascular events in patients with known cardiovascular disease (TABLE 3).<sup>4,5,11,12,31-46</sup>

**Bazzano et al**,<sup>47</sup> in a meta-analysis published in 2006, evaluated 12 randomized controlled trials of folic acid supplementation in patients with known cardiovascular disease and did not find that treated patients had better cardiovascular outcomes. The mean homocysteine level was elevated ( $> 15 \mu\text{mol/L}$ ) at baseline in only 4 of the 12 trials. However, in 1 of these 4 trials, there was no difference in outcomes comparing those with and without elevated homocysteine.<sup>31</sup>

**Albert et al**<sup>4</sup> more recently evaluated the effect of a combination pill containing folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> on cardiovascular events in women at high risk, ie, those with a history of cardiovascular disease or having three or more coronary risk factors. Treatment did not decrease the rate of the composite outcome of cardiovascular disease mortality, stroke, myocardial infarction, or coronary revascularization, although the homocysteine level decreased by a mean of 30% in the treated group. However, only 27.7% of the participants had an elevated homocysteine level. One might not expect patients to benefit from such treatment if they had normal homocysteine levels to begin with.

**Ebbing et al**,<sup>5</sup> in a trial published in 2008, investigated the effect of folic acid, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub> supplements on the risks of death from any cause and of cardiovascular events in patients undergoing coronary angiography. Outcomes were no better in the treatment group than in the control group, despite a mean decrease in homocysteine level of 19%. However, over 90% of the participants had a normal homocysteine level.

**Mager et al**,<sup>32</sup> in a study published in 2009, looked specifically at whether patients with

coronary artery disease and elevated homocysteine levels ( $> 15 \mu\text{mol/L}$ ) would benefit from folate-based vitamin therapy. In this subset, the incidence of death from any cause was lower in the treated group than in the control group (4% vs 32%,  $P < .001$ ), an association that was not present in patients with normal homocysteine levels.

The **SEARCH trial** (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine),<sup>33</sup> recently published, was a double-blind, randomized controlled trial of vitamin B<sub>12</sub> and folic acid treatment in 12,064 patients who had survived a myocardial infarction. Although those who received the vitamin therapy had a 28% reduction in homocysteine level, no clinical benefit was demonstrated. Of note, 66% of the patients had a homocysteine level lower than  $14 \mu\text{mol/L}$  at baseline.

### Restenosis after angioplasty

Results are also mixed regarding whether folic acid supplements modify the risk of restenosis after coronary angioplasty.

**Namazi et al**<sup>48</sup> evaluated the effect of folic acid supplementation on in-stent restenosis in 200 patients and found no difference between the treatment and placebo groups in the rates of either restenosis or target-vessel revascularization.

**Schnyder et al**<sup>49</sup> evaluated the effect of folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> treatment on the rate of coronary restenosis (in cases of balloon angioplasty) or in-stent restenosis (if a stent was used). Patients receiving treatment had lower rates of restenosis or in-stent restenosis (40% vs 48%,  $P = .01$ ) and of need for target-vessel revascularization (11% vs 22%,  $P = .047$ ). The mean homocysteine level was not elevated in this study either, and the researchers did not analyze the outcomes according to whether patients had high or normal homocysteine levels.

**Lange et al**<sup>35</sup> also evaluated the effect of folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> treatment on coronary in-stent restenosis. Paradoxically, the rate was higher with treatment in the overall group (mean homocysteine level  $12.2 \mu\text{mol/L}$ ), leading to a higher incidence of target-vessel revascularization. Patients who had a baseline elevation in homocysteine level

**43% of people are heterozygous for MTHFR 677C>T; 10% are homozygous**



had a nonsignificant trend toward a lower rate of in-stent restenosis.

### Cerebrovascular and peripheral arterial disease

The evidence is also mixed for using folic acid and other B vitamins to prevent cerebrovascular disease and peripheral vascular disease. Although a 2007 meta-analysis found that folic acid supplementation decreased the risk of a first stroke by 18% ( $P = .045$ ),<sup>50</sup> a later meta-analysis contradicts this finding.<sup>51</sup>

A 2009 meta-analysis found that patients with peripheral arterial disease had higher homocysteine levels than controls, but it did not find any benefit from supplementation, owing to heterogeneity of the clinical end points used.<sup>52</sup> Indeed, a 2009 Cochrane Database Systematic Review found that there were no adequate trials of the treatment of patients with peripheral vascular disease who have elevated plasma homocysteine.<sup>53</sup>

However, immediately after the Cochrane review was published, Khandanpour et al<sup>36</sup> published the results of a trial of the effect of folic acid and 5-methyltetrahydrofolate (an active form of folic acid) supplementation on the ankle-brachial pressure index and the pulse-wave velocity in patients with peripheral arterial disease. These measures improved with 16 weeks of treatment. For the ankle-brachial pressure index, the  $P$  value was less than .01 for folic acid and .009 for 5-methyltetrahydrofolate; for the pulse-wave velocity, the  $P$  value was .051 for folic acid and .011 for 5-methyltetrahydrofolate.

### Kidney disease

One could postulate that patients with end-stage renal disease or chronic kidney disease might benefit the most from folic acid supplementation, given the correlation of elevations in homocysteine levels with decline in glo-

merular filtration rate.

However, only one study found a lower rate of cardiovascular events with folic acid supplementation in dialysis patients, and the difference was not statistically significant (25% vs 36%,  $P < .08$ ).<sup>31</sup> Further, several studies found no benefit of folic acid supplementation in patients with chronic kidney disease.<sup>11,12,37</sup>

### FUTURE DIRECTIONS AND RECOMMENDATIONS

Many experts have suggested that the existing evidence indicates that the homocysteine-lowering therapies folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> do not lower the risk of cardiovascular disease.<sup>38,54–59</sup> Indeed, the American Heart Association guidelines for cardiovascular disease prevention in women do not recommend folic acid supplementation to prevent cardiovascular disease.<sup>60</sup> (Recommendations for men are the same as for women.) However, most of the clinical trials have not selected and treated patients with elevated homocysteine levels, but have instead included all patients regardless of homocysteine level.

At least two large ongoing trials are currently evaluating B-vitamin therapy for secondary prevention, but neither trial is looking specifically at patients with elevated homocysteine levels.<sup>61,62</sup>

Thus, instead of concluding that no patients could benefit from homocysteine-lowering treatment, future studies need to clarify:

- Whether patients with elevated homocysteine would benefit from such treatment
- At what level it would be appropriate to start treatment
- The appropriate target homocysteine level with treatment.

Particularly given the recent finding that folic acid supplementation may increase cancer risk,<sup>63</sup> these questions need closer scrutiny. ■

**Wald et al:**  
The risk of  
ischemic heart  
disease increases  
20%–30% with  
every 5- $\mu$ mol/L  
increase in  
homocysteine

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**ADDRESS:** Leslie Cho, MD, Women’s Cardiovascular Center, JB-1, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195; e-mail [chol@ccf.org](mailto:chol@ccf.org).