Homocysteine:
The Cholesterol of the Twenty-First Century?

Steven L. Leach, MD

Internal Medicine Grand Rounds
University of Texas
Southwestern Medical Center at Dallas

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The association of vascular disease with elevated homocysteine was first proposed 35 years ago by Kilmer McCully. In 1969 he published an autopsy study of a seven-week old child who had died as a result of a defect in cobalamin metabolism.[1] The metabolic abnormality stemming from this defect consisted of homocystinuria, cystathionuria, and methylmalonic aciduria. The pathological findings, which included extensive focal vascular abnormalities, were strikingly similar to those found in patients with cystathionine-β-synthase deficiency, the cause of classical homocystinuria. Given that homocystinuria was the common metabolic abnormality, he postulated that homocysteine was the direct cause of the vascular findings. Six years later, McCully and Wilson, formally published the "homocysteine theory of arteriosclerosis."[2]

Homocysteine (Hcy) is now recognized as a risk factor for coronary artery disease, cerebrovascular disease, peripheral vascular disease, venous thrombosis, dementia, Alzheimer's disease and cerebral microvascular disease. Yet 35 years after it was first postulated, the actual contribution of homocysteine to vascular disease is still uncertain and the scientific community has been slow to adopt homocysteine as a standard component of cardiovascular risk assessment.[3,4,5] The lack of exuberance in the scientific community has not dampened interest in the popular press. Popular books and internet web sites tout its significance, claiming that "homocysteine is 40 times more predictive than cholesterol in assessing cardiovascular disease risk,"[6] with some deeming it the cholesterol of the twenty-first century.

Homocysteine Metabolism[7,8]

The sulfur-containing amino acid homocysteine was first described in 1931 by Vincent du Vigneaud after isolating it from a bladder stone. As a result of his extensive studies of organic sulfur compounds, he was awarded the Nobel Prize in chemistry in 1955.

Homocysteine is unique among amino acids in that it is not incorporated directly into proteins. Rather, it is an intermediary in the methylation cycle. Methionine, which is both a precursor and a metabolite of homocysteine, is the hub of the only methyl-donating pathway in humans.[9] Methylation is essential to the function of many biomolecules, such as DNA, creatine, proteins, phospholipids and neurotransmitters.

The metabolism of homocysteine is depicted in Figure 1. In biological systems, high energy methyl groups are created when methionine is catalytically combined with adenosine to form S-adenosylmethionine (SAM) by the enzyme methionine S-adenosyltransferase. After donation of its methyl group, SAM is converted to S-adenosylhomocysteine (SAH), which is then hydrolyzed to adenosine and homocysteine. Further metabolism of homocysteine then occurs by one of two pathways, the transulferation pathway or the remethylation pathway.

In remethylation, homocysteine is enzymatically converted back to methionine by methionine synthase, an enzyme that requires vitamin B_{12} (cobalamin) as an essential cofactor. The methyl donor in this reaction is the folate analogue, N-5-methyltetrahydrofolate (N5-MTHF). This reaction takes place in essentially all tissues. An alternate pathway derives a methyl group from betaine and is catalyzed by betaine-

Steven L. Leach, MD
Associate Professor of Internal Medicine
Division of General Internal Medicine
Interests: Preventive Medicine

homocysteine methyltransferase. This pathway is primarily restricted to the liver and is also vitamin B_{12} dependent.[7]

In the transulferation pathway, homocysteine condenses with serine via a reaction catalyzed by cystathionine β-synthase (CβS) to form cystathionine. Cystathionine is cleaved to cysteine and α-ketoglutarate by cystathionase-γ-lyase. Both of these reactions are vitamin B_6-dependent.

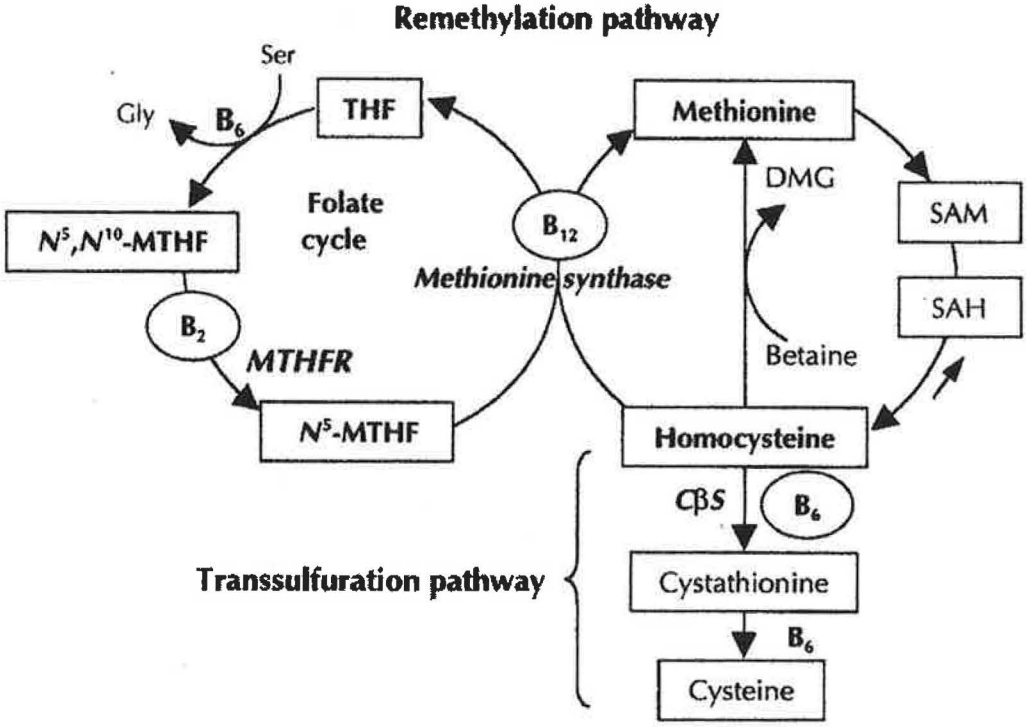


Fig. 1: Biochemical pathways of homocysteine metabolism. Ser = serine; Gly = glycine; MTHF = methylenetetrahydrofolate; MTHFR = N^5,N^{10}-methylenetetrahydrofolate reductase; THF = tetrahydrofolate; SAM = *S*-adenosylmethionine; SAH = *S*-adenosylhomocysteine; DMG = dimethylglycine; CβS = cystathionineβ-synthase.

Booth GL et al. CMAJ 2000;163:1-9.

Because of its dependence on folate, remethylation is tightly linked to the folate cycle. After sequential reduction reactions, dietary folic acid (pteroylpolyglutamic acid) enters the cycle as tetrayhydrofolate (THF). THF is converted to N5,N10-methylenetetrahydrofolate (N5,N10-MTHF) by the addition of a single carbon unit donated by serine in a vitamin B_6-dependent process. N5,N10-MTHF is then converted to N5-methyltetrahydrofolate (N5-MTHF) by methylenetetrahydrofolate reductase (MTHFR) which is dependent on vitamin B_2. Donation of the methyl group of N5-MTHF to homocysteine results in the regeneration of THF.

SAM is the primary regulator of both the remethylation and transulferation pathways and is heavily influenced by nutritional factors. It is both an allosteric inhibitor of MTHFR and an activator of CβS. When methionine intake is high, SAM levels are elevated, resulting in inhibition of MTHFR. The consequent reduction in N5-

MTHF, the essential substrate for methionine synthase activity, prevents remethylation. Concomitantly, CβS is activated, promoting increased transulferation. Conversely, when methionine intake is low, intracellular levels of SAM are insufficient to inhibit MTHFR, which results in increased remethylation. Simultaneously, transulferation is reduced due to failure to activate CβS. Remethylation is the primary modulator of fasting homocysteine levels, whereas the transulfuration pathway appears to modulate postprandial homocysteine levels.

Intracellular levels of homocysteine are influenced by many factors. These include enzyme deficiencies (MTHFR, CβS, methionine synthase), defects in cobalamin metabolism and transport, and cofactor deficiencies (folate, cobalamin, vitamin B_6, vitamin B_2). One of the most common defects occurs in the MTHFR enzyme. In population studies, about 10% of Caucasians are homozygous for the C677T polymorphism of the MTHFR enzyme, which produces a heat labile mutant associated with increased homocysteine levels.

Table 1. Determinants of plasma total homocysteine

Causes / determinants	Effect[a]	Evidence
Genetic factors		
Homocystinuria[b]	↑↑↑	II / III
Heterozygosity for CBS defects[c]	↑	III
Down syndrome	↓	III
MTHFR 677C→T (homozygosity)	↑	II
Other polymorphisms	(↑) ↓	III
Physiologic determinants		
Increasing age	(↑)	II
Male sex	(↑)	II
Pregnancy	↓	II
Postmenopausal state	(↑)	II
Renal function, reduced GFR[d]	(↑)	II
Increasing muscle mass	(↑)	III
Lifestyle determinants		
Vitamin intake (folate, B_{12}, B_6, B_2)	↓	I / II
Smoking	(↑)	II
Coffee	(↑)	I
Ethanol intake	↑↓	II
Exercise	(↑) ↓	II
Clinical conditions		
Folate deficiency	↑↑	I / II
Cobalamin deficiency	↑↑↑	II
Vitamin B_6 deficiency[c]	↑	III
Renal failure	↑↑	II
Hyperproliferative disorders	↑	III
Hypothyroidism	↑	III
Hyperthyroidism	↓	III
Early stage of diabetes	↓	II
Late stage of diabetes	↑	II

[a] ↓ = decrease in tHcy; (↑) = increase within the reference interval; ↑, ↑↑, and ↑↑↑ = moderate (15-30 μmol/L), intermediate (30-100 μmol/L), and severe hyperhomocysteinemia (>100 μmol/L), respectively.
[b] Homocystinuria includes deficiency in CBS, MTHFR, methionine synthase, or methionine synthase reductase and defects in intracellular cobalamin metabolism (see Fig. 1).
[c] In individuals with Vitamin B_6 deficiency or mild defects in CBS, the fasting concentrations are usually within the reference interval, whereas the post-methionine-load tHcy concentrations are often increased.
[d] GFR, glomerular filtration rate.
Refsum et al. Clin Chem 2004;50(1):3-32.

Plasma homocysteine is the result of a cellular export mechanism that functions to reduce intracellular levels. Although homocysteine levels are elevated in renal disease, only a trivial amount of is excreted in the urine.[10] The apparent cause of this elevation is decreased renal tubular metabolism, since the kidneys, along with the liver, play a key role in catabolism via transulferation.[11] Enzymes of the transulferation pathway are not expressed in vascular tissue, prompting some to suggest that this may place vascular tissue at particular risk of the effects of hyperhomocysteinemia.

About 70-80% of homocysteine in plasma is bound to protein, primarily albumin, via disulfide bonds. The remaining free homocysteine is largely found in oxidized disulfides, such as homocysteine-cysteine-dimers, or the homocysteine dimer known as homocystine. Only about 1% of the homocysteine

measured in blood samples is in the free, reduced state. In addition, as a result of intramolecular condensation, it can form homocysteine-thiolactone, or it may be nitrosylated to S-nitroso-homocysteine. As an indication of the relative preponderance of dimers in plasma, homocysteine is often written "homocyst(e)ine." Homocysteine, largely due to its sulfhydryl group, is one of the most reactive amino acids in biological systems and may be a source of "oxidative stress." During laboratory measurement, bound homocysteine is liberated from disulfide bonds by the addition of a strong reducing agent, yielding a measurement of total homocysteine (tHcy).[12]

Table 2. Drug effects on plasma total homocysteine

Class	Drug	tHcy	Possible mechanism
Folate antagonists	Methotrexate	↑	Inhibition of DHFR[b]
	Trimethoprim	↑	Inhibition of DHFR
	Anticonvulsants (inducers)	↑	Inhibition of polyglutamation, folate depletion
	Cholestyramine	(↑)	Inhibition of folate absorption
Cobalamin antagonists	Nitrous oxide	↑↑	Inactivation of methionine synthase
	Nitric oxide	ND	Inactivation of methionine synthase
	Metformin	(↑)	Inhibition of cobalamin absorption
	H2-receptor antagonists	ND	Inhibition of cobalamin absorption
	Omeprazole	ND	Inhibition of cobalamin absorption
Vitamin B_6 antagonists[c]	Niacin	↑	Inhibition of pyridoxal kinase
	Azauridine	↑	Inhibition of pyridoxal kinase
	Isoniazid	ND	Inhibition of pyridoxal kinase
	Theophylline	↑	Inhibition of pyridoxal kinase
Hcy production	Adenosine analogs	↓	Inhibition of AdoHcy hydrolase
	Creatine	↓[d]	Reduced creatinine (and Hcy) synthesis
	L-Dopa	↑	Substrate for AdoMet-dependent COMT
Sulfhydryl compounds	D-Penicillamine	↓	Disulfide exchange, displacement
	N-Acetylcysteine	↓	Disulfide exchange, displacement
	Mesna	↓	Disulfide exchange, displacement
Sex steroids and related compounds	Estrogens (postmenopausal)	↓	Not known, interference with vitamin function
	Androgens	(↑)	Increased muscle mass/creatinine synthesis
	Tamoxifen	↓	Not known, estrogen effect?
	Aminoglutethimide	↑	Induction of liver metabolism
Other	Betaine	↓	Enhancement of remethylation
	Cyclosporin A	↑	Impaired renal function
	Simvastatin	↓	Not known
	Fibrates	↑	Renal impairment, altered creatinine metabolism
	Diuretics	↑	Reduced glomerular filtration rate?

[a] The data are based on systematic review of the literature [modified from Ref. (262)]. With few exceptions, the level of experience for each drug is III (Table 1), but the known effects of these drugs support the findings.
[b] DHFR, dihydrofolate reductase; ND, not determined; AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; COMT, catecholamine-*O*-methyltransferase.
[c] Vitamin B_6 antagonists predominantly affect post-methionine-load tHcy concentrations.
[d] Marginal or absent effect.
Refsum et al. Clin Chem 2004;50(1):3-32.

Serum levels of homocysteine are affected by numerous factors, some of which are listed in Table 1. Homocysteine levels increase with age and male sex, and decrease during pregnancy. Elevated levels are associated with hypothyroidism, renal impairment, diabetes, the metabolic syndrome, B vitamin deficiencies and coffee consumption. In addition, several common medications are known to increase levels. These are identified in Table 2, along with proposed mechanisms.

Small meals do not affect homocysteine concentrations in healthy adults, but protein rich meals may increase plasma homocysteine levels as much as 10-15%.[13] Consequently, fasting samples are recommended. Alternately, methionine loading

(typically with 100 mg methionine per kg) 2-6 hours prior to measurement provides useful information about homocysteine metabolism. As many as 39% of persons with normal fasting homocysteine levels may have abnormal postprandial values, with elevated cardiovascular disease risk.[14] Day-to-day variation in fasting homocysteine levels is small, so a single measurement is sufficient in healthy individuals.[15] Samples obtained in the supine position may be 10% lower than those obtained in the sitting position, possibly due to the change in the distribution of albumin.[16] After blood collection, but prior to removal of red blood cells, there is a time- and temperature-dependent increase of homocysteine of about 1 μmol/l/h at room temperature.[17] Once red cells are removed, homocysteine in plasma or serum is stable. Several different methods of determining homocysteine levels are available, but there is considerable variation between methods and between labs.[12] Serum levels are also directly and independently related to concentrations of creatinine[18] and albumin.[19] Based on standard methods, reference ranges for homocysteine are typically 5 to 15 μmol/l, although "ideal" levels are often considered less than 10 μmol/l. Elevations between 15 to 30, 30 to 100, and >100 are considered moderate, intermediate and severe, respectively.[20]

Homocystinuria

Insights into the potential biological effects of elevated homocysteine can be gleaned from the rare genetic and metabolic disturbances that result in homocystinuria. Classical homocystinuria, typically associated with plasma homocysteine levels as high as 400-500 μmol/l, is caused by cystathionine β-synthase deficiency. However, it can also be caused by any of several defects in cobalamin metabolism or mutations in the MTHFR or methionine synthase genes.

The clinical presentation of CβS deficiency is variable, but characterized by four major types of manifestations: ocular, skeletal, CNS and vascular.[21] Ectopia lentis, myopia and glaucoma are common and typically severe. Late manifestations include retinal detachment/degeneration, optic nerve atrophy and cataracts. Osteoporosis occurs almost invariably, frequently in childhood, and commonly associated with pathologic fractures. Similar to Marfan's patients, homocystinuria results in elongation of the long bones, arachnodactyly, *genu valgum*, *pes cavus*, and *pectus excavatum*. Developmental delay and mental retardation are common and may be associated with seizures, psychiatric abnormalities or cerebrovascular accidents. Thrombophlebitis and pulmonary embolism are the most common vascular events. Thrombosis of large and medium-sized arteries is also common, particularly in renal and carotid arteries.

Homocysteine and Cardiovascular Disease

The first controlled study investigating the link between moderate homocsysteinemia and vascular disease was published by Wilcken and Wilcken in 1976.[22] The significance of this study is that it demonstrated for the first time that the manifestations of severe homocysteinemia may have a corollary in persons with much smaller elevations. Since then, over a hundred population studies investigating the relationship have been published. Some of these have had very dramatic findings.

The earliest studies, largely retrospective, and were highly suggestive of a significant positive association. In a widely-quoted meta analysis of 17 epidemiologic studies (mostly retrospective) published in 1995, Boushey and colleagues concluded that

a 5 μmol/l increase in homocysteine levels resulted in a combined odds ratio (OR) for CHD of 1.6 (95% CI, 1.4 to 1.7) for men and 1.8 (95% CI, 1.4 to 2.3) for women.[23] They calculated that 10% of the population's CAD risk was attributable to homocysteine and concluded that 13,500 to 50,000 CAD deaths per year could be avoided by folic acid supplementation.

However, more recent prospective studies have had variable results. Of the 23 studies reviewed for this paper, sixteen[24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39] have had positive results and seven[40,41,42,43,44,45,46] have reported negative results. This includes positive findings in important subpopulations that include dialysis patients,[26] diabetics[35] and postmenopausal women.[30]

A careful review of these studies yields valuable insights. Some studies suggest a threshold effect. The US Physicians Study showed a positive association for the top 5% (>15.8 μmol/l) of homocysteine distribution compared to the lower 90% (<14.1 μmol/l).[27] The Framingham Study found an adjusted OR for cardiovascular disease mortality of 1.52 (95% CI, 1.16 to 1.98) for those in the top quartile of distribution compared to the lower three (non-fasting cutoff of 14.26 μmol/l).[38] Likewise, the British Heart Study showed an increased risk for the highest quintile (>16.5 μmol/l) when compared to the lower three (adjusted OR 1.75, 95% CI, 1.2 to 2.55), but failed to show an association when examined for linear effects.[36] In contrast, the British United Provident Association (BUPA) study revealed a continuous dose-response relationship based on quartiles, using the lowest as the referent (<10.25 μmol/l), with adjusted OR and 95% CI of 1.43 (1.07 to 1.92) for homocysteine 10.25 to 12.32 μmol/l, 1.46 (1.08 to 1.97) for homocysteine 12.33 to 15.16 μmol/l, and 2.90 (2.04 to 4.12) for homocysteine ≥15.17 μmol/l.[28]

Some have suggested that the strength of the association is greatest in studies of shorter duration. In the US Physicians Study, the positive findings at five years[27] were no longer statistically significant at 7.5 years.[47]

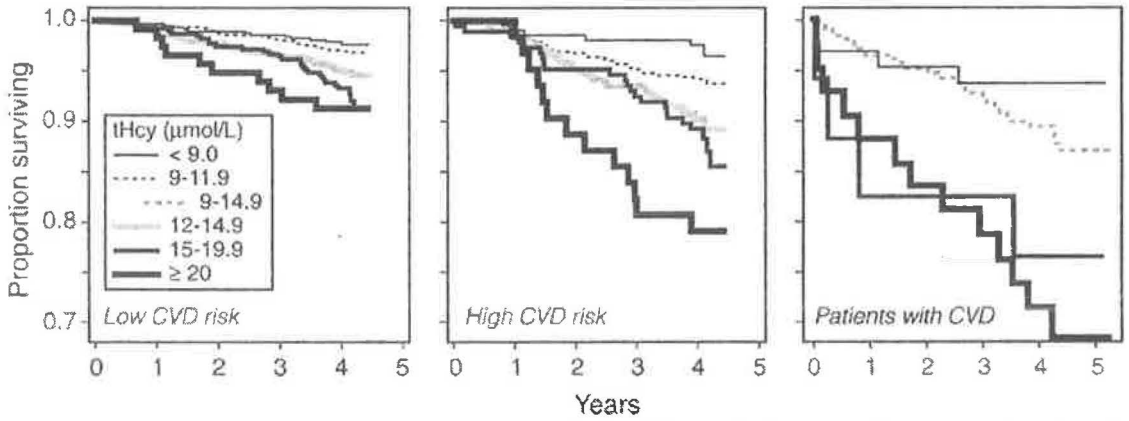


Fig. 2. Prediction of mortality by plasma tHcy, depicted as Kaplan-Meier survival curves, in three groups characterized by different CVD risk profiles. Refsum H et al. Clin Chem 2004;50(1):3-32.

The association of hyperhomocysteinemia and cardiac events is particularly strong in persons with established coronary disease.[24,29,37,39] Knekt and colleagues demonstrated that elevated homocysteine levels are predictive of secondary coronary events in men with heart disease (RR 2.23, 95% CI, 1.03 to 4.85 for highest quintile compared to lowest), but not in men free of disease at baseline.[29] Figure 2 is summary

data combined from studies of Nygard[24] and Vollset,[33] demonstrating the relative risk in populations with differing CVD risk profiles. This finding, along with the finding that homocysteine levels are higher in convalescence after myocardial infarction (Figure 3)[48] or stroke[49,50] than before, has prompted some to suggest that elevated homocysteine is a result of vascular disease rather than a cause.

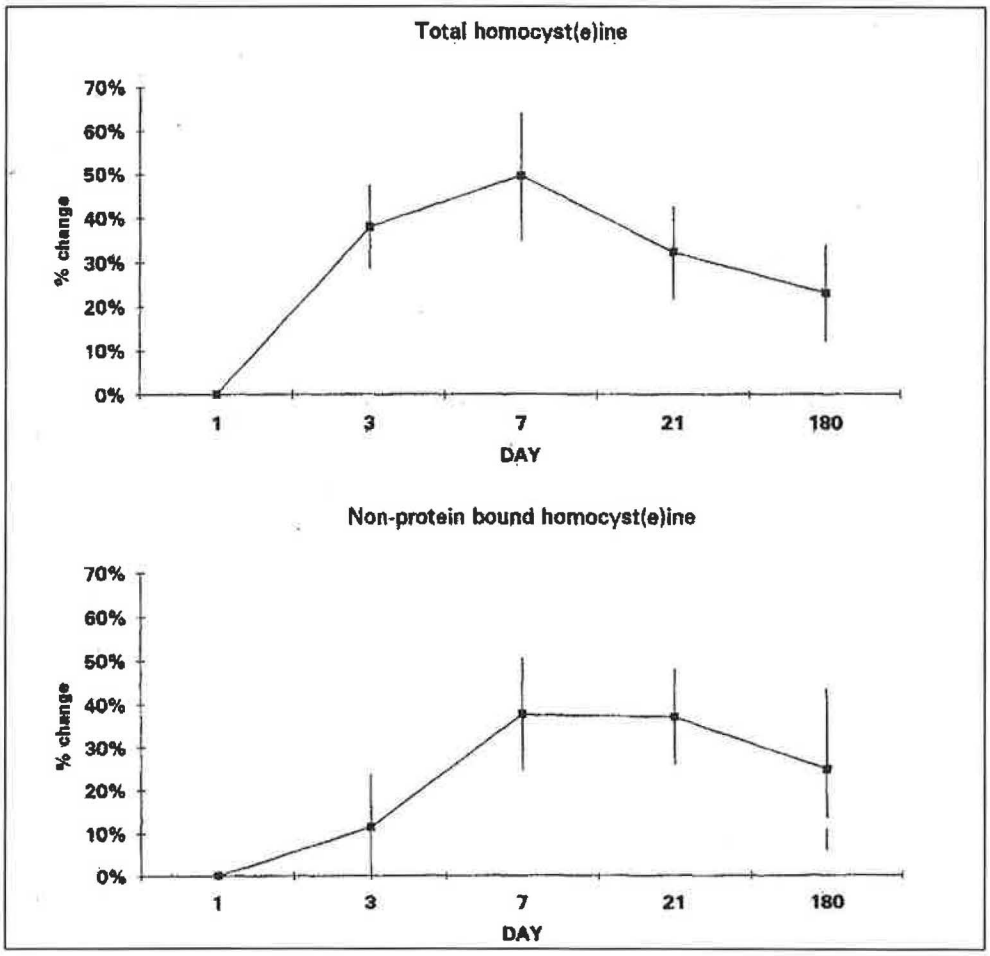


Figure 3. Percent change in homocysteine levels after myocardial infarction

Lindgren A et al. Stroke 1995;26:795-800.

Among the negative studies are notably three American studies—MRFIT,[42] ARIC (Atherosclerosis Risk in Communities Study),[40] and the US Physicians Study (7.5 year follow up which trended to but did not reach significance).[47] In a separate nested-case control study of the US Physicians Study, Verhoef and colleagues attempted to assess the atherogenic effect of homocysteine using the development of angina or revascularization as an endpoint.[41] They found no association.

Two more recent meta-analyses were published in 2002. In their review of 16 prospective studies on homocysteine and ischemic heart disease, Wald and colleagues calculated that the adjusted OR for a 5 µmol/l increase in homocysteine was 1.23 (95% CI, 1.14 to 1.32), or 1.32 (95% CI, 1.19 to 1.45) after correction for dilution bias (Figure 4).[51] Since therapeutic trials typically reduce homocysteine by about 25%, they calculated the OR for a 3 µmol/l decrease in homocysteine to be 0.84 (95% CI, 0.80 to 0.89).

The Homocysteine Studies Collaboration reviewed 18 retrospective studies and 12 prospective studies of the relationship between homocysteine and CAD (Figure 5).[52] Before correction for confounding factors, the odds ratio for a 25% reduction in total homocystiene (roughly equivalent to 3 µmol/) was 0.87 (95% CI, 0.82 to 0.92) in prospective studies and 0.71 (95% CI, 0.68 to 0.75) in retrospective studies with population controls. This dramatic difference underscores the potential hazard of over-reliance on retrospective data. After adjustment for known cardiovascular risk factors and dilution bias, the prospective studies yielded an OR of 0.89 (95% CI, 0.83-0.96) for a 25% lowering of homocysteine. This 11% risk reduction in prospective studies was much lower than what was found in the retrospective studies.

Based on the positive associations of hyperhomocysteinemia and CAD in the studies noted above, Omenn and colleagues calculated a "best estimate" for risk. Comparing the relative risks of elevated homocysteine with those of total cholesterol, they concluded that the risk of total homocysteine greater than 15 versus less than 10 is similar to the difference between serum total cholesterol of 275 and 189 mg/dl.[53]

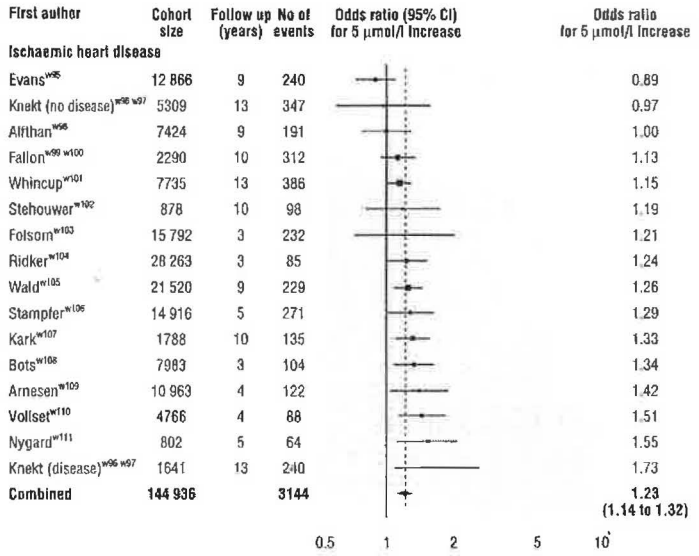


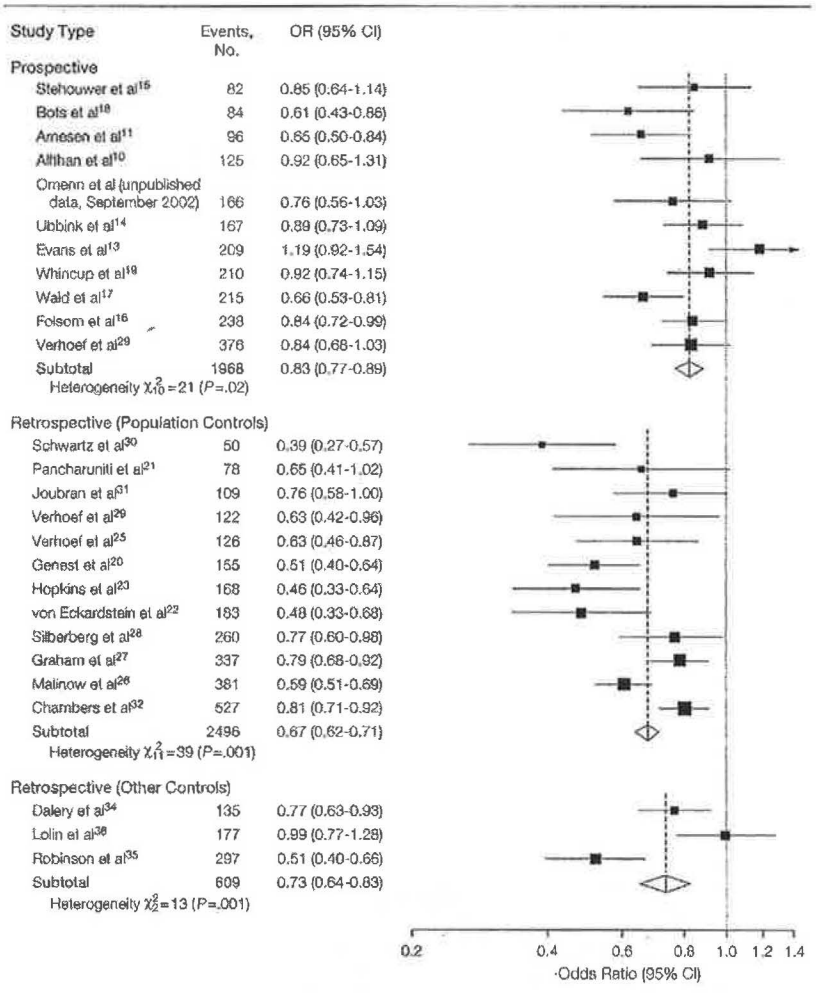
Fig 4 Results of prospective studies of serum homocysteine concentration and ischaemic heart disease: values are odds ratios (95% confidence intervals) for a 5 uMol/l increase in serum homocysteine, adjusted for age, sex, smoking, cholesterol concentration, and blood pressure (except in one study, adjusted for age and sex alone) but not for regrssion dilution bias. Wald DS et al. BMJ 2002;325:1202-1208

Other studies have attempted to evaluate the risk of developing vascular disease in persons at high risk for hyperhomocysteinemia, such as those with the C677T mutation of the MTHFR enzyme. This mutation results in about a 20-25% increase in serum homocysteine levels. It is also very common, with about 10% of the population being homozygous TT, 47% heterozygous CT and 43% homozygous unaffected CC.[51] Brattström et al, found that the odds ratio for developing vascular disease in homozygous affected (TT) persons when compared to homozygous unaffected persons (CC) trended toward increased risk but did not reach significance at 1.12 (95% CI, 0.92-1.37), in spite of a 2.6 µmol/l increase in total homocysteine.[54]

More recently, however, Wald and colleagues reviewed forty-six studies of the relationship between the C677T mutation in the MTHFR gene and ischemic heart

disease.[51] They found that the TT genotype conferred an odds ratio of 1.21 (95% CI 1.06 to 1.39) compared to phenotype CC. In the 33 studies that reported homocysteine levels, the mean difference between genotypes TT and CC was 2.7 μmol/l.

Figure 5. Odds Ratios of Ischemic Heart Disease for a 25% Lower Usual Homocysteine Level in Individual Studies



Data were adjusted for study, sex, and age at enrollment and were corrected for regression dilution. The size of the square is inversely proportional to the variance of the log odds ratio (OR). The horizontal lines represent the 95% confidence intervals (CIs). The combined ORs in the subtotals for each study design and their 95% CIs are indicated by the diamonds.

Clarke R, et al. JAMA 2002;288:2015-2023.

In a similar study on the C677T polymorphism of the MTHFR gene, Klerk and colleagues found somewhat less robust results.[55] They combined both prospective and retrospective studies. Overall, TT genotype compared with CC yielded an odds ratio of 1.16 (95% CI 1.05-1.28) for having CAD. Interestingly, when studies were grouped by continent, the OR for CAD in Europe was 1.14 (95% CI, 1.01-1.28), but in North America, the OR was 0.87 (95% CI, 0.73-1.05). This intercontinental variation suggests that dietary factors and folate status may play a significant role. In another subanalysis, they stratified cases according to folate status. There was no significant correlation with CAD for genotype TT with high folate status, but there was a significant, graded correlation for all phenotypes in cases with low folate status.

Other studies have evaluated vitamin status and the risk of cardiovascular disease. Robinson and colleagues found that RBC folate below the lowest decile (<513 nmol/L) increased the risk of vascular disease which was in part attributed to elevated homocysteine.[56] They also found an association with B_6 below the lowest quintile (<23.3 nmol/L). Others have found no association between RBC folate levels and vitamin B_{12} status.[57]

Homocysteine and Neurological Diseases

The relationship between homocysteine and cerebrovascular disease has been likewise frought with conflicting data, but the preponderance of data suggests that the association may be even more significant when compared to CAD. Elevated homocysteine levels have been implicated in stroke (including hemorrhagic, embolic and thrombotic),[58] recurrent stroke,[59] silent brain infarction,[60] small vessel disease (lacunar infarcts and white matter disease)[61] and most recently Alzheimer's dementia.[62,63,64,65]

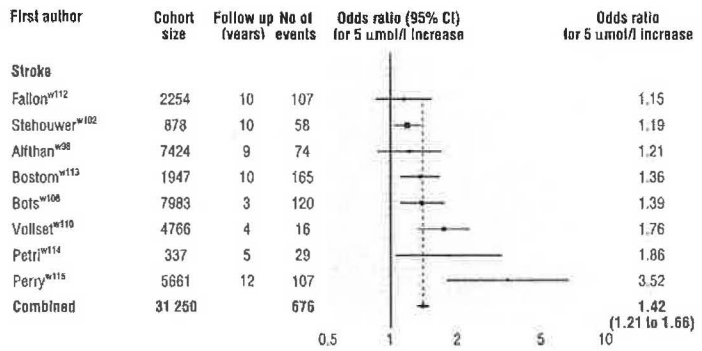


Fig 6 Results of prospective studies of serum homoscyteine concentration and stroke: values are odds ratios (95% confidence intervals) for a 5 uMol/l increase in serum homocysteine, adjusted for age, sex, smoking, cholesterol concentration, and blood pressure (except in one study, adjusted for age and sex alone) but not for regression dilution bias.

Wald DS et al. BMJ 2002;325:1202-1208.

Boushey's meta-analysis of mostly retrospective studies suggested an odds ratio of 1.5 (95% CI, 1.3 to 1.9) for a 5 μmol/l increase in homocysteine.[23] In an analysis of 8 prospective studies, Wald and colleagues found that the OR for a 5 μmol/l increase in homocysteine was 1.42 (95% CI, 1.21 to 1.66) (Figure 6).[51] The Homocysteine Studies Collaboration concluded that the OR for stroke based on a 25% lowering of homocysteine (about 3 μmol/l) is 0.81 (95% CI, 0.69 to 0.95) when corrected for other established vascular risk factors.[52] Folic acid intake was inversely associated with the risk of ischemic stroke, but not hemorrhagic, with a RR of 0.71 (95% CI, 0.52 to 0.96) for those in the highest quintile of intake versus those in the lowest.[66] Vitamin B_{12} intake, but not B_6, is also inversely related to stroke risk.

Some studies suggest that homocysteine is a much stronger predictor of risk for small vessel disease than other subtypes of stroke, although this has not been confirmed in others. Hassan and colleagues studied both isolated lacunar infarction and ischemic leukoaraiosis (periventricular white matter disease).[61] Total homocysteine levels were highest in patients with ischemic leukoaraiosis (mean 15.15 μmol/l), intermediate in lacunar infarction (mean 13.14 μmol/l) and lowest in controls (mean 12.01 μmol/l). The extent of disease was also positively correlated with leukoaraiosis and large focal lesions, but not with small lesions. Prospective studies of the association of homocysteine and cerebral small vessel disease are currently not available.

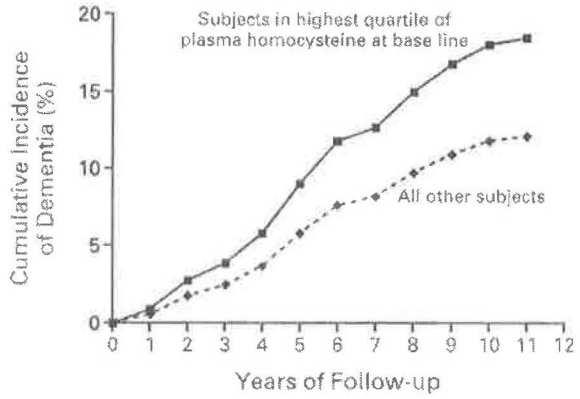


Figure 7. Crude Cumulative Incidence of Dementia among Subjects with Base-Line Plasma Homocysteine Levels in the Highest Age-Specific Quartile and among All Other Subjects.

The 75th percentile of the plasma homocysteine level (the cut-off point for quartile 4) was 13.2 μmol per liter for subjects 65 to 69 years old, 13.8 μmol per liter for subjects 70 to 74 years old, 14.5 μmol per liter for subjects 75 to 79 years old, 16.5 μmol per liter for subjects 80 to 84 years old, 19.3 μmol per liter for subjects 85 to 89 years old, and 26.6 μmol per liter for subjects 90 to 95 years old.

Seshadri S,et al. N Engl J Med 2002;346:476-483.

The association of elevated homocysteine and dementia has been studied in cross-sectional, case-control and prospective studies, most of which have demonstrated a graded, direct relationship. With an average of 2.7 years of follow up, the prospective Rotterdam Study found no association between baseline homocysteine levels and a decline in Mini-Mental Status Examination.[67] However, more recently a cohort of the Framingham Study was prospectively studied and found a very significant, graded association (Figure 7).[65] The RR for developing dementia for those in the highest quartile of total homocysteine compared to all other quartiles was 1.9 (CI, 1.3 to 2.9). When adjusted for vitamin status, the RR increased to 2.5 (CI, 1.5 to 4.4) and the relationship was strongest for those with sustained elevations of homocysteine as determined by measurements at baseline and 8 years prior to baseline. In this study, a positive relationship was found only after four years of follow up, suggesting that the follow up in the Rotterdam Study was too short. The study concluded that a 5 μmol/l increase in homocysteine resulted in a 40% increased risk of developing Alzheimer's disease.

Biological Plausibility

The line of reasoning upon which McCully based his "homocysteine theory of arteriosclerosis" remains compelling today. Three autosomal recessive disorders result in profound serum elevations of homocysteine and homocystinuria. All of these are associated with premature vascular disease, with half developing clinically significant disease by age 30.[21]

Numerous mechanisms have been suggested to explain the association of hyperhomocysteinemia and vascular disease. Broadly, these can be categorized as 1) endothelial dysfunction, 2) procoagulant effects, and 3) lipid oxidation.

The vascular endothelium is involved in the modulation of vascular tone, initiation of coagulation and fibrinolysis and the generation of inflammatory mediators. Endothelial dysfunction refers to a disruption in any of these processes and may be manifested by any of a constellation of findings that promote atherogenesis, such as decreased production of nitric oxide and inappropriate vasoconstriction, over-production of monocyte adhesion molecules, disruption of the normal ratio of plasminogen activator inhibitor (PAI) to tissue plasminogen activiator (tPA), increased platelet adhesiveness and activation of smooth muscle cells.

Evidence that elevated homocysteine causes endothelial injury and dysfunction comes from numerous lines of research. Under physiologic conditions, homocysteine is

susceptible to oxidation, producing reactive species such as hydrogen peroxide, superoxide anions and hydroxyl radicals. These species have been implicated in the peroxidation of endothelial cell membranes and in the inactivation of NO, resulting in impaired endothelial function. [68]

In cell culture, homocysteine induces apoptotic death in human endothelial cells.[69] Homocysteine induces cell death and potentiates amyloid β-peptide toxicity in neurons.[70] It stimulates vascular smooth muscle cell growth[71] and increases collagen synthesis in smooth muscle cells.[72] Several *in vivo* studies have demonstrated significantly impaired endothelium dependent vascular dilation (assessed by flow-mediated dilation) in patients with elevated total homocysteine, which can be corrected with folic acid supplementation.[73,74] Others have found elevated circulating levels of thrombomodulin and von Willebrand factors as biochemical markers of endothelial injury.[75] One of the criticisms of many of these studies is that they were done with supraphysiologic concentrations of homocysteine.

Numerous studies suggest various mechanisms by which homocysteine contributes to a procoagulant state. Homocysteine has been shown to inhibit the activity of anticoagulant factors such as thrombomodulin, antithrombin III and protein C.[76] It has been shown to increase activity of factors V, X, and XII and activate endothelial cell tissue factor.[77] It may also increase platelet aggregation and modulate tissue plasminogen activator binding to its endothelial receptor, annexin II.[78]

The effect of homocysteine on LDL peroxidation is less well-defined. Prelimary data suggest that homocysteine-thiolactone may react with the amino groups of LDL cholesterol, resulting in increased uptake by macrophages and promoting the development of foam cells and the deposition of lipids in atheromas. Studies, both *in vitro* and *in vivo,* suggest that homocysteine can both inhibit and promote LDL oxidation, depending on the concentration. For instance, Voutilainen showed that markers of lipid peroxidation are elevated in men with homocysteinemia,[79] whereas Halvorsen demonstrated increased oxidation at levels <6 μmol/l and that high levels were protective.[80]

Treatment Studies

The fact that vitamin therapy can lower serum homocysteine levels is well established. In a meta-analysis, Clarke and Armitage showed that folic acid supplementation in the range of 0.5 to 5 mg daily reduced homocysteine concentrations by 25% (95% CI, 23 to 28%).[81] The addition of vitamin B_{12} (mean 0.5 mg/day) reduced homocysteine levels an additional 7% (95% CI, 3 to 10%). Although low vitamin B_6 status has been demonstrated to be an independent risk factor for cardiovascular disease, the effects of supplementation have been inconsistent.[82] In renal transplant patients, B_6 has been shown to decrease post-methionine load homocysteine levels by 22%.[83] Furthermore, *in vitro* studies have shown that folic acid improves endothelial function, independent of its effect on homocysteine.[84]

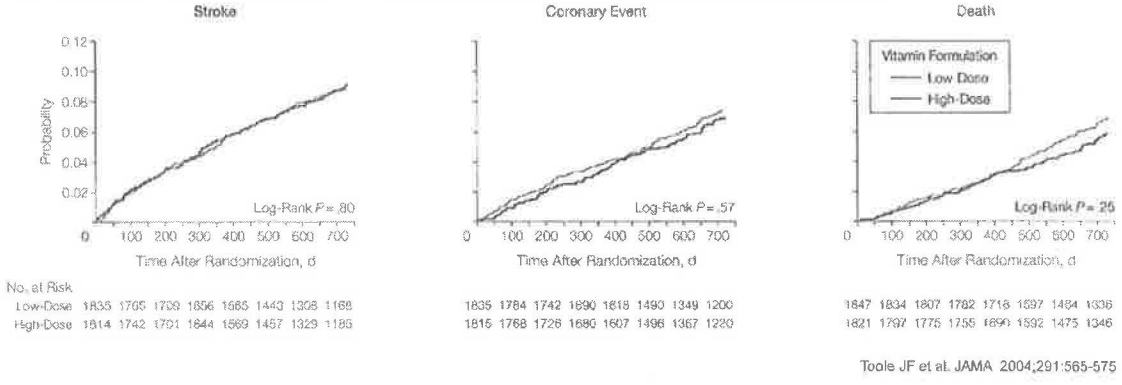
In 2001, Schnyder and colleagues published a compelling paper reporting that the lowering of plasma homocysteine levels significantly improved restenosis rates in patients undergoing PTCA.[85] Two hundred and five patients were randomized in a double blind fashion to either combination vitamin therapy (folic acid 1mg, vitamin B_{12} 400 μg, pyridoxine 10 mg) or placebo for six months. They found that plasma

homocysteine levels were lowered on average from 11.1 to 7.2 in the treatment group. Overall, the rate of restenosis was 19.6% in the treatment group vs. 37.6% in the placebo group and the need for target lesion revascularization was 10.8% vs. 22.3%. However, in stented vessels there was no difference. The same group reported on an expanded cohort of 553 patients on the same protocol with 1 year of follow up, again demonstrating a significantly decreased rate of restenosis compared to placebo (9.9% vs. 16.0%; P=0.03).[86]

Others have not been able to reproduce this finding. In the FACIT trial which included 626 patients, folate therapy increased in-stent restenosis.[87] In a study reported by Baker and colleagues, 1882 patients were immediately randomized following a positive angiogram to either 5 mg of folic acid or placebo for 2 years. Endpoints were non-fatal myocardial infarction, cardiovascular death, or unplanned revascularization. Treatment reduced average homocysteine levels from 11.2 to 9.7, but there was no difference in the composite outcome (RR 0.97: 95% CI, 0.72 to 1.29).[88]

Liem and colleagues studied the effects of folic acid supplementation in patients with stable CAD on statin therapy. Five hundred ninety-three patients were randomized to receive either 0.5 mg/day of folic acid or placebo. After 2 years of therapy no significant differences were found for the composite endpoints of overall mortality, sudden death, MI, stroke or major vascular surgery.[89] In another study, Liem and colleagues reported findings of folic acid supplementation when added to statin therapy following acute MI.[90] Two hundred eighty-three patients were enrolled with an average cholesterol of 251. All received fluvastatin 40 mg daily. In addition, 140 patients received folic acid 5 mg every other day or placebo. After one year there was no difference between the primary endpoints (sudden death, fatal recurrent MI, fatal stroke and other cardiovascular deaths).

Figure 8. Probability of Stroke, Coronary Event, or Death Over Time, by Treatment Group



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The largest randomized-controlled trial published to date is the Vitamin Intervention for Stroke Prevention (VISP) Trial which reported results in February of this year.[91] Although homocysteine levels were decreased by an average of 2.3 μmol/l in the treatment group (folate 2.5 mg, pyridoxine 25 mg and cobalamin 0.4 mg), there was no reduction in recurrence of stroke, coronary heart disease events or death after 2 years of treatment (Figure 8). Two other large randomized controlled trials, the Women's

Antioxidant Cardiovascular Disease Study (WACS) and the Heart Outcomes Prevention Evaluation (HOPE-2) are currently underway in North America.

Issues Regarding Supplementation

Although the role of folic acid in the treatment and prevention of vascular disease is unclear, its biological importance is well established. The recommended daily allowance of folate has been established by the Food and Nutrition Board of the Institute of Medicine. Historically, this value was derived from the amount of folic acid needed to prevent anemia due to folate deficiency. In the past, this value has been as low as 200 μg/day, but more recently has been increased to 400 μg/day.[92] The recommended upper intake level has been set at 1 mg/day, largely due to the concern of masking neurological complications in persons with vitamin B_{12} deficiency. The normal U.S. diet typically provides from 50 to 500 μg of absorbable folate per day, with the average being about 250 μg.[93] Folic acid given in supplement form (pteroylmonoglutamic acid) is more bioavailable than food folate (pteroylpolyglutamic acid). RDA's are given in *dietary folate equivalents* (DFE). One DFE (1 μg) of food folate is equal to 0.6 μg of folic acid from fortified food or supplements taken with food, or 0.5 μg of a supplement taken on an empty stomach.[94,95,96]

In the recognition of the evidence linking neural tube defects in the fetus with folate deficiency, the FDA and the U.S. Public Health Service recommended in 1992 that all women of child bearing age consume 400 μg/day of supplemental folic acid with the goal of attaining 1 mg daily of dietary folate equivalence. However, failure to achieve anticipated goals with this policy resulted in the 1996 decision of the FDA to require that all enriched flour, rice, pasta, cornmeal or other grain products contain 140 μg of folic acid per 100 grams.[97] It was estimated that this amount of fortification would increase the daily intake of folic acid in women of childbearing age by 80 to 100 μg per day and 70 to 120 μg in middle-aged and older adults. The regulation went into effect January 1, 1998. Studies suggest that actual folate intake has increased more than anticipated, by about 215 to 240 μg/day.[98] As a result, neural tube defects have decreased by 25%.[99] This dramatic effect on neurological development, along with the association of low folate and Alzheimer's Disease, creates great promise in the minds of those desiring to prevent dementia.[100]

Although there was no stated intention to improve cardiovascular risk by lower homocysteine levels, this may be an additional effect of the regulation. Jacques and colleagues were able to assess the effects of fortification in a cohort of subjects enrolled in the Framingham Offspring Study.[93] Among participants who did not use B vitamin supplements, plasma folate increased 117% (4.6 to 10.0 ng/ml). The prevalence of low folate concentrations (defined as serum levels < 3 ng/ml) decreased by 92% (22 to 1.7). Fasting homocysteine levels decreased by 7% (10.1 to 9.4 μmol/l) and the prevalence of high homocysteine levels (defined as homocysteine > 13 μmol/l) decreased by 48% (18.7 to 9.8). In the cohort that was taking B vitamin supplements, plasma folate levels also increased significantly (11.7 to 18.9 μg/ml). Interestingly, fasting total homocysteine levels increased in this group by 8% (7.9 to 8.5 μmol/l), but remained in a range associated with the lowest risk of developing vascular disease. All of these measures had highly significant P values. Bostom and colleagues evaluated the homocysteine lowering effect of folic acid supplementation in the era of fortified cereal. They found that 2.5 mg

of folic acid only reduced total homocysteine level by about 1.0 μmol/l. Consequently, studies initiated prior to fortification, such as the VISP study, anticipated much larger reductions in homocysteine and consequently may be underpowered now.[101]

Not everyone believes that folic acid supplementation is innocuous. There have been associations with increased rates of spontaneous abortion. Others have suggested a link to autism. More common is the concern that supplementation will mask B_{12} deficiency. To date, there is no evidence to support this.[102] In a study of ESRD patients on dialysis, doses as high as 16 mg/day have been given without apparent adverse effects.[103] Others have reported serious side effects, including sleep disturbance, mental status changes, malaise, irritability, excitability, overactivity, exacerbation of seizures, nausea and gastric distention.[104] Other rare events include angioedema, urticaria, anaphylaxis and zinc depletion.[105,106]

Current Recommendations

Routine testing of homocysteine in cardiovascular risk assessment is currently not recommended by any major professional organization.[3,4,5] Although the potential treatment is safe and inexpensive, screening is not. The list price for a serum homocysteine level at Mayo reference lab is $145. Furthermore, the addition of homocysteine to assessment of traditional risk factors has thus far not improved predictability of future coronary events in those getting appropriate lipid management.[89,90,107] Consequently, it is essential that providers continue to focus on proper management of the risk factors for which treatment is known to have a beneficial effect.

However, in recognition of the strong association in population studies, there is reasonable consensus that certain high risk individuals may benefit from testing and treatment. The American Heart Association suggests evaluating those at high risk, such as those with a family history of premature CAD, renal failure, hypothyroidism, malnutrition, malabsorption or systemic lupus erythematosis, as well as those taking certain medications such as theophylline, bile acid-binding resins, methotrexate, L-dopa or recent exposure to nitrous oxide. Fasting homocysteine concentrations of less than 10 μg/mol/l are considered ideal. The statement concludes:

> Until results of controlled clinical trials become available, population-wide screening is not recommended, and emphasis should be placed on meeting current RDAs for folate, as well as vitamins B_6 and B_{12}, by intake of vegetables, fruits, legumes, meats, fish, and fortified grains and cereals.[3]

Although the emphasis on improving folate status by increasing dietary folate seems reasonable, it has been shown to be ineffective. Cuskelly and colleagues showed that dietary recommendations to increase folate consumption were unsuccessful at increasing red cell folate.[108] Consequently, some have advocated the "eat right and take a vitamin" approach.[92] In the Framingham Study, 90 percent of those who were not taking a multivitamin had homoycsteine levels above the low, normal base line associated with adequate blood folate.[109] Based on current population data, Tice and colleagues calculated that the consumption of a vitamin supplement in addition to grain fortification may result in 310,000 fewer deaths over 10 years and save more than $2 billion.[110] In

fact, the Nurses Healthy Study showed an association between multivitamin use and reduced coronary events after 14 years, but the benefit was not seen in the initial analysis at 8 years. [111] Those reporting the lowest risk were those who took supplements most days of the week for at least five years. However, no benefit in cardiovascular mortality was seen in the Physicians Health Study at 4 years.[112] It is impossible to estimate a potential benefit for neurological disease at this time, but this may be the most compelling reason to pursue supplementation.

Future Directions

Defining the actual contribution of homocysteine to vascular and neurological diseases is certain to be more difficult to ascertain in an era of folic acid supplementation. Several large population studies (Table 3) are currently underway but may be underpowered as a result of the smaller differences in homocysteine levels between treatment groups and controls. Consequently, studies in smaller target populations may be particularly helpful.

Table 3. Large-scale randomized trials of homocysteine lowering therapy

Study	Population	Start Date	Sample Size
Bergen Vitamin Study	Stroke (Norway)	1997	2000
Cambridge Heart Antioxidant Study (CHAOS-2)	MI, Unstable angina (UK)	1998	4000
Heart Outcomes Prevention Evaluation (HOPE-2)	Arterial Disease (Canada)	1999	5000
Norwegian Study of Homocysteine Lowering with B-Vitamins in Myocardial Infarction (NORVIT)	MI (Norway)	1998	3000
Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease (PACIFIC)	Arterial Disease (Australia)	1998	10,000
Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH)	MI (UK)	1998	12,000
Vitamins in Stroke Prevention (VISP)	Stroke (US)	1998	3600
VITAmins TO Prevent Stroke Study (VITATOPS)	Stroke (Australia)	1999	5000
Women's Antioxidant and Cardiovascular Disease Study (WACS)	Vascular disease (US)	1998	8000
Vitamins and ThROmbosis Trial (VITRO)	Venous Thrombosis (Netherlands	2000	600

Adapted from Boot et al. CMAJ 2000;163:1-9.

One such target population is patients with Parkinson's disease. Standard treatment of Parkinson's disease with levodopa has been shown to increase homocysteine levels.[113] Elevated plasma homocysteine levels are associated with measurable motor, cognitive affective findings in these patients.[114] This population provides an excellent opportunity to study the effectiveness of treatment in this population at high risk. A study addressing this is ongoing at this institution now.

Another potential target group is renal transplant patients. Bostom and Culleton have shown that renal transplant recipients have much higher baseline levels of homocysteine when compared to patients with coronary artery disease without renal disease. Hence, they will be less likely to be affected by dietary supplements and are more likely to reveal an effect of treatment if one is present.[10]

In the meantime, until convincing treatment data is available, a general recommendation for the screening of homocysteine cannot be justified.

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