The Journal of the American Nutraceutical Association

Vol. 7, No. 1, Winter 2004 **www.ana-jana.org** **Reprint**

REVIEW ARTICLE

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A Peer-Reviewed Journal on Nutraceuticals and Nutrition

Editor-in-Chief
Mark Houston, M.D.

ISSN-1521-4524

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ABSTRACT

As an emerging independent risk factor for cardiovascular disease and other aging diseases such as Alzheimer's, homocysteine-related research has generated a vast amount of literature and sparked a vigorous debate over the past decade. In fact, a comprehensive textbook is now available describing the role of homocysteine in health and disease.[3] This review will survey the history of homocysteine research, the rationale for considering homocysteine as a causative agent rather than just a marker for vascular diseases, and review the intervention trials for lowering homocysteine in patients.

INTRODUCTION

Homocysteine is a sulfur amino acid and a normal intermediate in methionine metabolism. When excess homocysteine is produced in the body and not readily converted into methionine or cysteine, it is excreted out of the tightly regulated cell environment into the blood. It is the role of the liver and kidney to remove excess homocysteine from the blood. In many individuals with inborn errors of homocysteine metabolism, kidney or liver disease, nutrient deficiencies, or concomitant ingestion of certain pharmaceuticals, homocysteine levels can rise beyond normal levels and lead to adverse health outcomes.

The role of elevated blood homocysteine levels in clinical practice is still being debated. The central question is, whether it is clinically beneficial to measure for and treat elevated levels of homocysteine?[1,2] While some may consider homocysteine simply as a marker but not a treatable causative agent, or perhaps ignore homocysteine as an innocuous metabolite coincidental to other treatable risk factors, the weight of the scientific evidence suggests otherwise.

HISTORICAL PERSPECTIVE

In the early 1960s, researchers described several inborn errors of homocysteine metabolism in young children that led to extremely high levels of homocysteine that in turn resulted in mental retardation and early death, often caused by some cardiovascular event. After postmortem examinations of many similar cases, Kilmer McCully, MD, noted an emerging pattern of arteriosclerosis due to formation of fibrous plaques and loss of elasticity. McCully's unique training in biochemistry and pathology, along with his inclination to be curious, placed him in a unique position to pioneer a new theory in cardiovascular research. He concluded, as did others, that severely elevated levels of homocysteine were directly responsible for the various vascular lesions in individuals with genetic defects in homocysteine metabolism. He further postulated that moderately elevated homocysteine due to heterozygous mutations in homocysteine-related genes or poor vitamin status would

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also lead to increased risk of cardiovascular disease in the general population.[4] Since his new theory questioned the role of cholesterol and other lipids in the genesis of arteriosclerosis, finding acceptance within the mainstream medical community was difficult; eventually, his 28-year affiliation with Harvard Medical School (undergraduate through assistant professor) came to an end when he was unable to "prove" the theory to the satisfaction of some of his colleagues. While employed as a pathologist at the Veterans Affairs Medical Center in Providence, Rhode Island, he continued his work through the past several decades. A colorful description of both the homocysteine theory of cardiovascular disease and the history behind its discovery can be found in McCully's book *The Homocysteine Revolution* (Keats Publishing).

By the early 1990s, elevated homocysteine was being considered an independent risk factor for cardiovascular disease (along with cholesterol and other lipid markers, age, gender, smoking status, obesity, hypertension, and diabetes). A prospective study of male physicians in 1992 found that acute myocardial infarction (MI) or death due to coronary disease was statistically related to increased homocysteine levels, after adjusting for other risk factors.[5] In 1995, a key meta-analysis was published by *JAMA* in which 27 studies involving over 4,000 subjects concluded that homocysteine was an independent risk factor for cardiovascular disease (CVD) and estimated that 10% of the population's CVD risk is attributable to elevated homocysteine.[6] In total, nearly 100 retrospective and prospective clinical studies link homocysteine levels with increased risk of cardiovascular outcomes, and numerous reviews of the literature are available.[7-11]

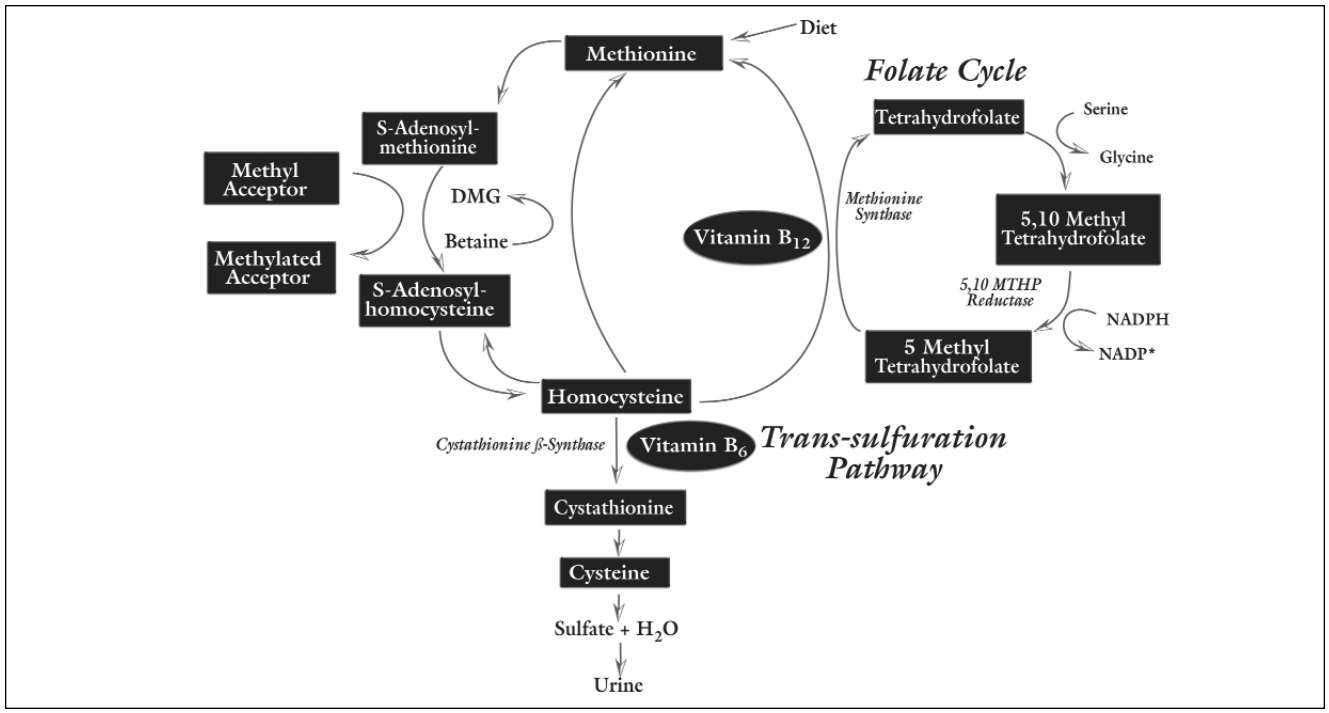
According to a recent meta-analysis of the data, a causal relationship between homocysteine and cardiovascular disease is highly likely.[12] The authors conclude that lowering plasma total homocysteine 3 µmol/L (micromoles per liter) would reduce the risk of ischemic heart disease by 16%, deep vein thrombosis by 25%, and stroke by 24%.

HOMOCYSTEINE METABOLISM

Figure 1 shows the basic metabolic pathways concerning homocysteine. Homocysteine is an intermediate in methionine metabolism, with the methionine derived primarily from dietary protein. This pathway involves the formation of S-adenosylmethionine (SAM), which subsequently transfers a methyl group to any number of several methyl acceptor molecules (DNA, proteins, neurotransmitters) and forms adenosylhomocysteine, which is subsequently converted to homocysteine.

Homocysteine is then either converted back to methionine by remethylation or further metabolized to cysteine via the trans-sulfuration pathway. Remethylation primarily occurs when a methyl group is transferred from methyltetrahydrofolate (MTHF), the active form of the folic acid/folate cycle, by a methyltransferase enzyme requiring cobalamin (vitamin B_{12}) as a necessary cofactor. A secondary remethylation pathway, active primarily in liver and kidney cells, uses trimethylglycine (betaine) as the methyl donor. The trans-sulfuration pathway requires two enzymatic reactions, both of which

Figure 1. Homocysteine Metabolism



require the cofactor pyridoxal-5-phosphate, the active form of vitamin B_6.

MEASURING HOMOCYSTEINE LEVELS

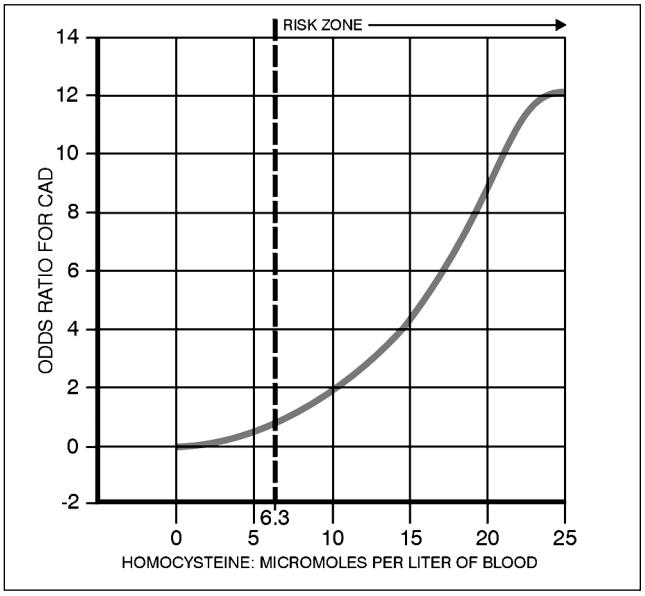
Homocysteine (Hcy) levels can easily be measured in most laboratories that test for other blood chemicals. It's important to follow the instructions provided by the lab to ensure consistent homocysteine measurements. Often, incorrect values are a result of poor collection, poor post-collection procedures (not centrifuging or storing on ice soon enough), non-fasting conditions, and posture (10% lower in supine compared to sitting).[151] Average fasting plasma total homocysteine for "healthy" subjects in the current folic-acid-fortified US population is between 6 and 12 μmol/L. In normal subjects, 75% of total plasma Hcy (tHcy) is bound to various proteins (primarily albumen) via disulfide bonds. The remaining 25% free Hcy is found mostly as oxidized homocysteine dimers (homocystine) or as homocysteine-cysteine heterodimers, while only about 1–2% is in the reduced state. Because of the many forms of homocysteine, tHcy was often termed "Homocyst(e)ine" in the literature to account for these multiple forms. Currently, most studies concerning Hcy levels have primarily focused on tHcy levels and not on free Hcy or free/bound ratios. Some advocate for the use of free Hcy as a marker rather than tHcy, or even intracellular levels rather than plasma levels, although more research in humans needs to be conducted as other species such as rats have 65–75% of tHcy as free Hcy. A complete review with recommendations concerning testing homocysteine levels in various patient groups has recently been published.[152]

HYPERHOMOCYSTEINEMIA—RISK FACTOR ASSESSMENT

Increased Mortality

Elevated plasma tHcy is an independent risk factor for cardiovascular-related as well as non-cardiovascular-related mortality.[31,13] In a prospective cohort study following 2,127 men and 2,639 women for over 4 years, increasing levels of plasma tHcy were directly related with increasing mortality.[13] The population was divided into quintiles based on initial plasma tHcy (5.1–-8.9, 9.0–11.9, 12.0–14.9, 15.0–19.9, >20 μmol/L) and followed for survival. After adjusting for other cardiovascular risk factors, the overall mortality ratio was 1, 1.33, 2.02, 2.48, and 3.56 for the 5 quintiles. The authors conclude that after multivariate adjustment, a 5 μmol/L increase in tHcy increased all-cause mortality by 49%, cardiovascular mortality 50%, cancer mortality 26%, and non-cancer, non-cardiovascular mortality 104%. This data suggests that the level of homocysteine likely to result in a low risk for mortality is below 9 and perhaps even lower. Figure 2 shows a graph of increasing coronary artery disease (CAD) risk based on mathematical modeling of

Figure 2. Increased Risk of CAD



CAD patients and control subjects.[146] The model suggests that the relative risk surpasses 1 when tHcy is 6.5 μmol/L, and continues to increase in a near linear fashion until plasma levels reach 20 μmol/L or more.

Increased Acute Coronary Syndromes

Sufficient epidemiological evidence now exists to conclude that moderately elevated homocysteine increases the risk of cardiovascular events. However, what about acute coronary events following admissions with either unstable angina or myocardial infarction? This was measured in 440 consecutive admissions to a coronary care unit.[14] As each patient was diagnosed and treated, baseline homocysteine levels were recorded. Of the patients surviving the first 28 days (in which 9.3% of the MI patients died), there was a statistical decrease in event-free survival in patients with tHcy above 12 μmol/L (nearly 4 times more events from the highest to the lowest quintiles). This data, as well as data from the MRFIT trial and the Hordaland homocysteine study, suggest that homocysteine levels may be better predictors for recurrent cardiovascular events than for primary cardiovascular events.[15,153] Additionally, according to researchers from the Framingham Heart Study, hyperhomocysteinemia is also an independent risk factor for congestive heart failure in patients without prior cardiovascular events.[108]

Increased Stroke Risk

As cerebrovascular events are similar in many ways to cardiovascular events, it should not be surprising that homocysteine is also an independent risk factor for ischemic stroke.[16] Following a cohort from the Framingham study, those individuals in the highest quartile (>14.24 μmol/L) had a relative risk of 1.8 compared to the lowest

quartile (<9.25 μmol/L) in incidence of stroke over a 10-year follow-up.[32] Even with these data, there is not complete agreement on whether tHcy is causal or coincidental with incidence of stroke.[17-19] One report showed that in 75 patients who experienced an ischemic stroke event, there was nearly a 12-fold increased risk of bad recovery (Rankin Scale) in those with tHcy levels above 15 μmol/L.[20] This is confirmed by other reports of increased recurrent stroke based on increasing homocysteine levels.[21] Furthermore, increased dietary intake of folic acid and vitamin B_{12} (but not B_6) is inversely related to reduction in stroke risk.[147]

Risk of Hypertension

The relationship between homocysteine and hypertension is less understood.[22] As many of the risk factors for hypertension and other cardiovascular diseases overlap, it is difficult to deduce when one is a risk factor for the other. However, the mechanisms by which homocysteine is thought to affect the vascular endothelium are consistent with in vitro research and known mechanisms for hypertension.[23] Data suggest that elevated tHcy is an independent risk factor for primary hypertension as well as primary pulmonary hypertension.[23,24] Hypertensive patients typically have higher homocysteine than normotensive controls,[25] a condition exacerbated by smoking[26] and menopause.[27]

In addition, the Dietary Approaches to Stop Hypertension (DASH) diet, recommended to hypertensive patients, is beneficial for lowering blood pressure as well as homocysteine.[28] This diet is high in fruit and vegetable consumption and recommends low dairy and meat fat intake. Physicians should note that thiazide diuretics, some of the first medications given to hypertensive patients, significantly raise homocysteine levels, which may nullify some of the benefit gained by these medications.[29]

Risk of Cognitive Disorders and Dementia

In February of 2002, the *New England Journal of Medicine* published a landmark study that concluded that increased plasma homocysteine is a strong, independent risk factor for developing dementia and Alzheimer's disease.[30] Taking data from 1,092 participants in the Framingham Study cohort, the study found that the risk for Alzheimer's dementia doubled (average 8-year follow-up) when plasma tHcy exceeded 14 μmol/L. These results confirmed previous smaller studies published concerning cognitive decline and dementias related to serum homocysteine levels.[33,34,35,40,42] However, there was disagreement over these data, and others disputed the direct connection between Hcy and dementias, preferring to interpret the data as coincidental.[36,37]

In two separate community studies, increasing serum homocysteine levels was inversely related to how well healthy elderly subjects performed on the Mini-mental State Examination, widely used to measure cognitive impairment in elderly patients.[38,39] Mild cognitive impairment is considered one of the leading risk factors for dementias and specifically Alzheimer's disease.[41]

While the mechanism is not fully understood, many of the same processes may be at work in cerebrovascular tissue and neurons as are proposed for arterial endothelial damage. Alzheimer patients have higher plasma homocysteine levels, but they also have higher levels of asymmetric dimethylarginine and decreased concentrations of nitric oxide, two risk factors for cardiovascular disease related to the oxidative affects of homocysteine and perhaps emerging risk factors for dementia.[43] It is known that patients with either mild cognitive impairments or Alzheimer's disease have similarly and severely reduced levels of all major antioxidants.[44] How much homocysteine plays in the reduction of these plasma antioxidants remains to be seen; however, in vitro research on oligodendrocytes suggests that homocysteine increases the neuronal cytotoxic effect of amyloid beta-peptides.[45] A recently-published report suggests that a protein called transthyretin (prealbumin) becomes amyloidogenic and potentially a factor in dementia when bound to homocysteine.[46] Another interesting finding is that treating patients with hyperhomocysteinemia and mild cognitive impairment with folic acid, B_6, and B_{12} improves the function of the blood-brain barrier.[47]

Homocysteine and Diabetes

While no specific causal relationship has been attributed to onset or risk of type II diabetes and homocysteine levels, the fact that both are strong independent risk factors for cardiovascular disease has led researchers to study what relationship they have in overall risk for diabetic patients. Type II diabetics have cardiovascular mortality rates 2 to 4 times that of non-diabetic controls, and diabetic patients with hyperhomocysteinemia (tHcy above 14 μmol/L) have a 2-fold higher risk of mortality than other diabetic patients (tHcy below 14 μmol/L). For each 5 μmol/L increase in serum tHcy, the risk of 5-year mortality rose by 17% in non-diabetics and 60% in diabetic subjects.[48] Looking further at these data, this same group concluded that homocysteine increases the risk of retinopathies in diabetic subjects, but is not correlated to increased risk in non-diabetic subjects.[49] There may also be a connection between gestational diabetes and homocysteine levels, a relationship that could result in several different types of birth defects.[50]

Whatever the relationship between homocysteine and diabetes is, it seems that improved glycemic control lowers homocysteine levels in diabetic patients. In 95 type II diabetics followed for 3 years, those patients with improving glycemic control measured by glycosylated hemoglobin (%HbA1c) had lower homocysteine levels than those with increased HbA1c levels.[51] It is possible then, that one way

diabetes increases cardiovascular risk is by increasing homocysteine levels, although the means by which this occurs is unknown at present.[78] Measuring and treating diabetic patients for elevated homocysteine levels may increase the benefit of improving glycemic control in the same population. Ironically, metformin, one of the leading oral hypoglycemic drugs used to treat type II diabetics, decreases plasma folate and vitamin B_{12} levels and increases homocysteine levels.[52] Certainly one could consider lifestyle and nutraceutical interventions prior to drug therapy for this and other reasons.

Homocysteine and Cancer Risk

For the past several years, a link has been established between certain cancers and elevated plasma homocysteine. It is a bit early in the cycle of data collection to know how much can be attributed to high homocysteine, and how much to lower folate, B_6, or B_{12} levels; or perhaps even to a genetic predisposition causative to both phenomenon. That said, some groups find a strong predictive relationship between tumor growth and homocysteine levels.[53] Other researchers have developed theories by which homocysteine affects carcinogenesis via estrogen-induced pathways[54] or DNA damage.[55] As there is limited data at this time on the long-term effects of therapeutically lowering homocysteine levels and preventing or treating various cancers, little more can be said at this time.

Homocysteine and Kidney Disorders

Normal kidney metabolism and filtration plays a prominent role in removing homocysteine from the blood: thus, hyperhomocysteinemia is common in patients with chronic renal insufficiency and is nearly ubiquitous in patients with end-stage renal disease, who have up to a 30 times higher risk of cardiovascular-related death than the general population.[56] Likewise, renal transplant recipients typically have elevated homocysteine levels.[57] These groups of patients are often targeted for treatment with homocysteine-lowering therapies discussed later in this review.

It is important to note that only free (unbound) homocysteine is filtered and metabolized by the kidney. As this represents only 25% of the plasma tHcy levels in humans, one way to increase kidney filtration efficiencies in patients with normal kidney function may be to stimulate the conversion of bound Hcy to free Hcy. This has been clinically proven by giving patients N-acetylcysteine (NAC), a thiol compound that directly, or through increased glutathione levels, breaks homocysteine-protein disulfide bonds.

Homocysteine and the Risk for Other Conditions

Numerous other diseases have been linked to elevated homocysteine levels, including deep-vein thrombosis,[58,59] neural-tube and other birth defects,[60,61] peripheral arterial occlusive disease,[62,63] Parkinson's disease,[64] and polycystic ovarian disease.[65,66]

POSSIBLE MECHANISM ATTRIBUTED TO HOMOCYSTEINE

In order to consider homocysteine a causative rather than coincidental factor, plausible mechanisms for homocysteine action must be presented and proved. The most common and plausible mechanisms are briefly outlined here.

Oxidative Damage

Much of the endothelial dysfunction attributed to homocysteine is thought to occur primarily from oxidative stress.[82,97] This is also one of the proposed mechanisms for DNA damage and carcinogenesis.[55] In one study, 17 healthy volunteers were given methionine (100 mg/kg) to induce elevated homocysteine levels, which immediately led to vascular endothelial dysfunction measured by brachial artery flow-mediated dilation (a nitric-oxide-mediated process).[98] This rapid onset of endothelial dysfunction was prevented when these same subjects consumed vitamin C (1 g/day oral) for 1 week prior to the test. This is strong evidence that oxidation is part of the mechanism attributed to homocysteine, and perhaps explains one of the many benefits of antioxidant therapy for vascular dysfunction.[99,100]

Relation to Other Risk Factors

If homocysteine directly increases other cardiovascular risk factors or reduces beneficial factors, this may contribute to the increase in cardiovascular risk. Studies have shown that homocysteine suppresses the vasodilator nitric oxide, perhaps by increasing the levels of asymmetric dimethylarginine (ADMA), a strong inhibitor of endothelial nitric oxide synthase (eNOS) and strong independent risk factor for cardiovascular disease, although the relationship is still debated.[90-94] If indeed this is true, this could certainly account for dramatic changes in vascular endothelial compliance and platelet coagulation changes that promote cardiovascular disease. Also, some reports show that homocysteine is capable of increasing the activity of HMG-CoA reductase, which results in increased cholesterol synthesis.[95,96]

Vascular Smooth Muscle Cell Proliferation

In numerous in vitro studies, homocysteine was able to trigger proliferation of vascular smooth muscle cells,[101,102,103] an effect attenuated by folic acid.[104,105] By increasing vascular smooth muscle proliferation, the arterial lumen space will be more narrow, typically considered deleterious for CAD. This mechanism, along with endothelial cell cytotoxicity, is thought to be a leading cause of vascular lesions triggered by hyperhomocysteinemia.[103]

Causes for Hyperhomocysteinemia

The various diseases for which homocysteine is a risk factor or marker and the potential mechanisms by which homocysteine may be a causative factor have been previously reviewed. Below is a review of the factors that predispose or cause elevated homocysteine levels. Table 1 summarizes this information.

Diet and Lifestyle Factors

It is obvious from the metabolism of homocysteine (Figure 1) that when the required metabolic cofactors folic acid, vitamin B_6, or vitamin B_{12} are suboptimal in the diet, homocysteine levels may elevate. In fact, hyperhomocysteinemia can be induced in monkeys simply by increasing methionine and decreasing folic acid and choline, the precursor of betaine, from their normal diet.[67] Numerous human epidemiological studies have shown homocysteine levels correlate inversely and closely with plasma folate levels and less so with vitamin B_{12} and B_6 levels.[68,69,70]

The DASH diet, promoted for lowering hypertension, also significantly lowers homocysteine levels, presumably because it promotes higher intake of fruits and vegetables, providing more folic acid and vitamin B_6 and lower amounts of methionine.[28] Interestingly, while increasing fruit and vegetable intake seems to lower homocysteine levels,[71] strict vegetarians are often at risk for hyperhomocysteinemia due to low plasma B_{12} levels.[72,73] Coffee consumption ($\geq$4 cups/day) seems linked with moderate elevations in homocysteine,[74,75] although this effect can apparently be countered by supplementing with 200 μg/day of folic acid.[76] Moderate levels of alcohol consumption (even wine) may raise homocysteine levels,[77] although some reports claim that moderate beer consumption may actually lower homocysteine levels.[79] As with nearly every other cardiovascular risk factor, smoking cigarettes is linked with elevated levels of homocysteine.[80,81]

Genetic Defects in Homocysteine Metabolism

The metabolism of homocysteine is dependent on one of several enzymes, a methyl donor, and several nutrient cofactors. All of these pathways are therefore ultimately controlled by the genes encoding the various metabolic enzymes, and, as with any gene, there are inborn errors that affect the efficiency by which homocysteine can be metabolized. Three main errors that have become clinically important are cystathionine β-synthase deficiency (CBS—see Figure 1), inborn errors of cobalamin metabolism or absorption, and inborn errors in folate metabolism. As most of these particular defects are beyond the scope of this review, those wanting further information should consult specific references.[3,82,83,84] Because of its importance, one will be covered below.

Mutations in the gene encoding for the enzyme methylenetetrahydrofolate reductase (MTHFR) are well known in the literature. This enzyme is responsible for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (MTHF or 5MTHF), the active folate that donates its methyl group to homocysteine to make methionine (Figure 1). Certain rare defects in this gene render the enzyme completely dysfunctional, and individuals with this defect are noted for extremely high homocysteine, homocystinuria, brain damage, and childhood cardiovascular disease. An extremely common mutation in the MTHFR gene, known as a polymorphism because it occurs at greater than 1% in most populations, results when a cytosine is replaced by a thymine at base pair number 677 (C677T). This poly-

Table 1. Factors Causing Hyperhomocysteinemia

General
Increased age
Male gender
Menopause (HRT may lower homocysteine)
Lifestyle factors
Smoking
High consumption of coffee
Alcohol consumption (moderate beer intake may be beneficial)
Diet
Low consumption of fruits and vegetables
No consumption of multivitamins
Low intake of folic acid, vitamin B_6, vitamin B_{12}
High intake of methionine-containing proteins
Diseases or Inherited Causes
Cystathionine β-Synthase deficiency
5MTHFR errors
Methionine synthase deficiencies
Chronic renal failure
Diabetes
Hypothyroidism
Psoriasis
Certain malignancies
Malabsorption syndrome
Rheumatoid arthritis
Helicobacter pylori infection
Drugs that increase Homocysteine
Some antiepileptic drugs (phenobarbitol, valproate, phenytoin etc)
Diuretic therapy
Methotrexate
Nitrous oxide
Cholestyramine
Fibric acid derivatives (fenofibrate)
Estrogen-containing oral contraceptives
Metformin
Niacin
Theophylline
Sulfasalazine

morphism leads to an alanine to valine change in the enzyme, which results in a 55–65% loss in enzyme activity. Individuals with errors in both alleles (TT homozygous) may realize this level of enzyme activity reduction, while those with a C677T change in one allele (CT heterozygous) will have only a 25% loss in activity compared to a CC homozygous individual.[85,86] The frequency of this polymorphism is very low in some populations (<1% in those of African descent) and very high in others (11–15% in Anglo-Americans, and >20% in Italian, Hispanic, and Columbians). As half the homocysteine is metabolized by remethylation to methionine, this polymorphism is often associated with elevated homocysteine levels, although adequate folate levels minimize this significantly.[87,88] A complete meta-analysis of the C677T polymorphism effect on the risk for heart disease has recently been published.[111]

DRUGS THAT INCREASE HOMOCYSTEINE

Many pharmaceuticals commonly prescribed to patients have the unintended consequence of increasing plasma homocysteine levels (see Table 1). Many do so by impairing folate metabolism or absorption: oral contraceptives, methotrexate, certain anticonvulsants, sulfasalazine, and metformin.[3,52] Several, such as thiazide diuretics, cholestyramine, and fibric acid derivatives like fenofibrate,[29,89,150,157] are used to lower risk of cardiovascular disease.

HOMOCYSTEINE-LOWERING THERAPIES

If the debate over whether moderate hyperhomocysteinemia is a causative agent for various diseases is relatively convoluted, the treatment that effectively lowers homocysteine levels is, conversely, fairly straightforward.

Folic Acid

The fact that about half the body's homocysteine is metabolized by remethylation to methionine, a process involving a folate-derived methyl donor, makes folic acid an obvious choice for treatment. In fact, the connection between blood folate levels, homocysteine, and the incidence of neural tube defects was so compelling that the FDA mandated the fortification of all enriched flour, rice, pasta, and grains (140 µg/100 g) in the US since January 1998. This moderate increase in folate consumption has had an impact on the population in terms of increasing plasma folate levels and decreasing baseline homocysteine levels.[106,107] Whether these modest decreases in homocysteine will reduce cardiovascular risk in the overall population is still to be seen, but fortification (which can result in nearly 200 µg/day of folic acid) is unlikely to be therapeutic in patients with coronary heart disease with elevated homocysteine.[109] These patients typically need at least 800 µg or more per day of supplemental folic acid to achieve a meaningful decrease in homocysteine.[110]

In general, one can reasonably predict that adequate supplementation (0.5–5 mg/day) will result in a 25% reduction of homocysteine levels.[112] One group determined that in order to ensure that 95% of the elderly population is without risk due to folate deficiency, intake of 925 µg/day would need to be consumed.[113] Interestingly, once weekly doses of 2.8 milligrams had the equivalent homocysteine-lowering effect as daily doses of 400 µg in one group of women studied.[114]

High-Dose Folic Acid

While moderate supplementation of folic acid supplementation is successful in lowering homocysteine in the vast majority of the population, many individuals with cardiovascular disease and kidney disease (including renal transplant patients or patients on hemodialysis) are refractory to these lower levels and require significantly higher levels of folic acid supplementation; 2 to 15 mg/day are often used.[115-117] As most of these studies were done in combination with other vitamins, they will be discussed in the "combination treatments" section of this review. It's interesting to note that nearly all large intervention trials currently assessing the role of folic acid in combination with other vitamins for the reduction of homocysteine and cardiovascular risk used at least 2 mg/day of folic acid. When using these higher doses, additional vitamin B_{12} is usually recommended to prevent a masked B_{12} deficiency. While no dose-related clinical trial has apparently been published to determine the amount of B_{12} needed when supplementing high doses of folic acid, 1 mg oral doses of B_{12} are considered the standard for prophylactic and treatment of B_{12} deficiency and are preferable to intramuscular injections.[154] Patients taking high doses of folic acid on dialysis for end-stage renal disease are often resistant to oral doses of B_{12} but respond well to 1 mg IV doses of B_{12}.[155] This same B_{12} dose (i.m.) had no effect on homocysteine in similar patients when no supplemental folic acid was used.[156]

Forms of Folic Acid

Synthetic folic acid taken in supplement form or fortification has nearly doubled the bioavailability of folates within foods.[3] Available forms include folic acid, folinic acid (formyl tetrahydrofolate), and 5-methyltetrahydrofolate (5-MTHF). With the understanding that some individuals have genetic-related difficulties producing the methylated folate forms, several groups have looked at whether 5-MTHF or folinic acid would have more therapeutic benefit with respect to lowering homocysteine in various populations. In a recent study, 160 healthy women were given either 400 µg/day of folic acid or 480 µg/day (equimolar amount) of 5-MTHF.[118] Blood samples were collected at baseline, 4 weeks, and 8 weeks, and measured for tHcy. In these women, folic acid was significantly better at lowering homocysteine than 5-MTHF. In women homozygous for the C677T polymorphism, in whom one would logically expect 5-MTHF to perform better than folic acid, the folic acid sup-

plement reduced homocysteine better than the 5-MTHF. Another study in healthy adults using low doses (100 µg equivalents) of folic acid and 5-MTHF to mimic fortification levels showed similar results.[119] These two compounds seem to have similar bioavailabilities in humans.[120] In hemodialysis patients taking 15 mg/day of folic acid, equivalent high doses of 5-MTHF were of no additional benefit, and although both were beneficial, neither could fully normalize the elevated levels in these patients.[121] More research needs to be conducted to see if there is a patient population that could benefit from the use of folate forms other than folic acid, the form used in nearly all the research to date.

Vitamin B_{12} and Vitamin B_6

Unlike folic acid, which acts as a substrate in the remethylation reaction, vitamins B_{12} and B_6 act as cofactors for the enzymes responsible for remethylation and transsulferation, respectively (see Figure 1). While we know that each plays a role in keeping homocysteine levels from elevating, it is difficult to assess their role independently as most intervention trials include folic acid as well. However, when individuals are deficient in either B_{12} or B_6, both may have significant homocysteine-lowering effects when supplemented.[122-125] Vitamin B_{12} deficiency is quite common in vegetarians and in the elderly and is often detected by elevated homocysteine levels.[154] One meta-analysis, which concluded that folic acid provides a 25% drop in homocysteine, reported that additional B_{12} (avg. 0.5 mg/day) would produce an additional 7% reduction in tHcy and B_6 (avg. 16.5 mg/day) had negligible benefits.[112] Likewise, use of folic acid with vitamin B_6 and B_{12} typically reduces homocysteine in a way that suggests synergistic effects.

Clinical trials involving monotherapy of vitamin B_{12}, except in populations with noted deficiencies, is uncommon. Typical oral vitamin B_{12} doses range from 200 µg to 2 mg per day when added to folic acid therapy. As mentioned previously, 1 mg oral daily doses are considered safe, cost effective, and significant in eliminating B_{12}-related homocysteine elevations. The most commonly used forms of vitamin B_{12} in the literature are cyanocobalamin and methylcobalamin.

Concerning vitamin B_6, in one study, 120 mg of vitamin B_6 had similar homocysteine-lowering effects as 300 µg of folic acid (17% vs 20%, 32% when combined) in apparently healthy subjects.[158] While these data differ from previous trials showing much less benefit attributed to B_6 monotherapy, these authors attribute the success to providing this dose in 3 divided daily doses (40x3). In elderly patients without B_{12} deficiency (22 subjects) and previously supplemented with 400 µg folic acid daily (19.6% drop in tHcy), as little as 1.6 mg/day of vitamin B_6 led to an additional 7.5% reduction in tHcy levels. Most combined vitamin therapy studies for homocysteine reduction provide between 10–50 mg pyridoxine.

Combination Treatment and Clinical Outcomes

In most homocysteine-lowering intervention trials, a combination of folic acid, B_6, and B_{12} is used and compared to placebo. And while homocysteine levels are consistently lowered, the important question is whether clinical outcomes are changed. The following is a review of several recent clinical studies concerning clinical outcomes.

Improved vascular endothelial function was demonstrated by measuring brachial artery flow-mediated dilation in coronary heart disease patients (89 males) given 5 mg folic acid and 1 mg vitamin B_{12} daily for 8 weeks.[127] In these patients, tHcy levels fell from an average of 13.0 to 9.3 µmol/L in these 8 weeks, while flow-mediated dilation improved from 2.5% to 4.0% at the same time (placebo group showed no improvement in either). The authors believe that because flow-mediated dilation is mediated through nitric oxide (NO), and homocysteine is known to lower NO levels, this is one of the likely mechanisms attributed to this therapy. Additionally, the authors believe it's the reduced unbound form of homocysteine, which accounts for only about 1–2% of tHcy, that may be the culprit in endothelial damage.[128] Other groups have confirmed that lowering homocysteine by folic acid therapy alone (5 mg) has a benefit on vascular compliance.[131]

As mentioned previously, renal-transplant recipients (RTRs) are noted for elevated homocysteine levels and increased risk for CAD. A group of 56 RTRs with elevated homocysteine were randomly assigned to either placebo or vitamin supplementation (folic acid 5 mg/day, B_6 50 mg/day, B_{12} 400 µg/day) and followed for 6 months.[129] In the vitamin group, homocysteine levels fell from an average of 21.8 (range 15.5–76.6) to 9.3 (5.8–13.0), while the placebo group saw no change (pre 20.5, post 20.7). Additionally, these patients were measured for carotid intima-media thickness (cIMT), considered to be a marker for atherosclerotic changes and an independent risk factor for myocardial infarction and stroke. In 6 months, RTRs receiving vitamin therapy had an average 32% reduction in cIMT, while those on placebo had an increase of 23%. Another study reported that 5 mg of folic acid with 250 mg of B_6 for 2 years in healthy siblings of patients with premature atherothrombotic disease, decreased occurrence of abnormal exercise electrocardiography tests, which is consistent with a decreased risk of atherosclerotic coronary events.[130] These data suggest that outcomes, apart from merely lowering homocysteine, are measurable in these patients.

Another way this can be assessed is to measure outcomes after interventions such as angioplasty. Such was the case in the Swiss Heart Study.[132] Post-angioplasty patients (556 subjects) were randomized to receive either placebo or a vitamin combination (1 mg folic, 400 µg B_{12}, and 10 mg B_6) and followed for 1 year. After adjusting for potential confounders, at the end of 1 year the group taking the vita-

min combination had a 34% reduction in risk compared to the placebo group (combined risks for death, non-fatal myocardial infarction, and need for repeat revascularization). Event-free survival and decreased rate of restenosis (re-narrowing after angioplasty) was previously shown with the same moderate doses of vitamins.[133] Additional studies by these authors have led them to conclude that plasma homocysteine is an independent predictor of mortality, non-fatal MI, target lesion revascularization, and overall adverse late outcome after successful coronary angioplasty.[134] These data suggest that measuring and treating elevated homocysteine levels in patients with previous CAD is likely to have positive outcomes.

One of the largest and most recent intervention trials to date, however, showed somewhat equivocal results in preventing cardiovascular events. The recent results of the vitamin intervention for stroke prevention (VISP) trial published in *JAMA* are likely to stir the homocysteine debate.[153] This multi-center study of over 3,500 patients assessed whether the addition of daily doses of folic acid (2.5 mg), B_6 (25 mg), and B_{12} (400 μg) were able to prevent recurrent cerebral infarctions and cardiovascular events in patients admitted with nondisabling cerebral infarctions. In all parameters measured, the treatment group had similar outcomes to the control group when followed for 2 years. The authors provide several compelling reasons for the lack of effectiveness of the multivitamin approach in this study. First, the inclusion criteria resulted in patients with an average homocysteine level of 13.4 μmol/L, considered by most laboratories to be within normal ranges. The authors state that the modest decrease in homocysteine in the vitamin group of 2 μmol/L would have required a larger patient sample to reach statistical significance. Another confounder to this study was that during the trial (1996–2003), the US began their folic acid fortification program, which may have increased the control group folic acid intake 10-fold. Regardless, several more studies are in progress and more will need to be done that include patients with higher baseline tHcy, higher levels of vitamin therapy, and longer duration to compare with these results.

Betaine (TMG)

The use of supplemental betaine (trimethyglycine) is also a potential treatment option as both kidney and liver cells express an enzyme that allows for the remethylation of homocysteine using betaine as a methyl donor. Fewer studies, however, have assessed the use of betaine in large patient studies. A recent small trial (n=12) of subjects with mildly elevated homocysteine showed that 6 g/day of betaine had only about 65% of the homocysteine-lowering capacity compared to 800 μg/day folic acid.[135] On the other hand, in these subjects, betaine was able to blunt the homocysteine rise due to methionine loading, while folic acid was not. The clinical implications of this are yet to be determined. Other studies have shown very small decreases in homocysteine when similar doses are given to obese patients,[136] although doses much higher than this are helpful in lowering homocysteine in patients with homocystinuria, where betaine use is more common.[137] While there are other health benefits for consuming betaine, and low-dose ingestion (1.5 g/day) has moderate homocysteine-lowering effects,[148] at this point it would seem to be a second-line therapy for reducing homocysteine, and as a monotherapy would need to be consumed in excess of 6 g/day.

N-Acetylcysteine (NAC)

NAC has been shown to increase plasma free homocysteine, the form removed by the kidney, by breaking the disulfide link of the bound forms.[139-141] A dose-response curve is apparent with oral doses, showing benefits are higher with doses of 1,800 mg/day,[142] while lower doses often do not show statistical improvements.[144] Hemodialysis patients often do not respond to even high oral NAC doses,[143,145] but 5 grams of NAC provided to ESRD patients during dialysis resulted in significantly lower homocysteine levels and greatly improved endothelial function ($p<0.01$) as measured by fingertip photoplethysmography.[149] At present, the use of high oral doses of NAC may be a potential addition to regimens containing the multivitamin approach outlined previously. Patients with impaired kidney function are not likely to benefit from this approach, however, unless NAC is used during dialysis.

SUMMARY

While additional studies are needed to determine how significant the overall benefit will be in measuring and treating homocysteine levels in the clinical setting, enough evidence is available to suggest ignoring homocysteine levels in patients at risk for cardiovascular disease would be unwise. Knowing base levels of homocysteine in all adult patients may simply be an easy way to measure folate, B_6, and B_{12} status, especially important in those with the C667T polymorphism in the MTHFR gene.

This review presents data showing homocysteine as an independent risk factor for cardiovascular and numerous other diseases, and provides plausible mechanisms by which homocysteine may play causative roles in many of them. As treatment of hyperhomocysteinemia with folic acid, vitamin B_{12}, and vitamin B_6 is extremely successful in a majority of these patients, physicians should consider these nutraceuticals as viable options for lowering homocysteine levels in patients with elevated levels.

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