

# HOMOCYSTEINE: A RISK FACTOR WORTH TREATING

**A**s 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.

Homocysteine is a sulfur amino acid and a normal intermediate in methionine metabolism. When excess homocysteine is made 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 in-born 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 consider homocysteine as simply a marker but not a treatable causative agent, or ignore homocysteine as an innocuous metabolite that is coincidental to other treatable risk factors; the weight of the scientific evidence suggests otherwise.

## Brief Historical Perspective

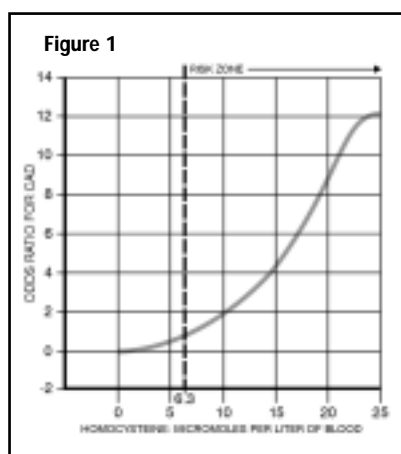
In the early 1960's several inborn errors of homocysteine metabolism were described in young children leading to extremely high levels of homocysteine—resulting in mental retardation and early death, caused usually by some cardiovascular event. After examining many cases and performing autopsies on several young people, Kilmer McCully concluded, as did others, that the severely elevated levels of homocysteine were directly responsible for the various vascular

lesions in these individuals and he further postulated that **moderately elevated** homocysteine due to heterozygous mutations in homocysteine related genes or poor vitamin status would also lead to increased risk of cardiovascular disease (4).

By the early 1990's, 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 there are nearly 100 retrospective and prospective clinical studies linking homocysteine levels and increase risk of cardiovascular outcomes and numerous reviews of the literature 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 homocysteine 3  $\mu\text{mol/L}$  would reduce the risk of ischemic heart disease by 16%, deep vein thrombosis by 25% and stroke by 24%.



## IN MY OPINION

Saw palmetto extracts lower urinary symptoms in patients diagnosed with benign prostatic hyperplasia (BPH). This is a truthful statement and can be shown by reliable clinical trials published in peer-reviewed journals. And even though saw palmetto extracts are classified as dietary supplements if a manufacturer were to produce a product with the above statement on the label (or in other point of purchase literature) the FDA would consider this an unapproved drug. Why? Because for FDA, a drug is not the substance per se, but what can be said about the substance. If the substance is intended to diagnose, prevent or cure a known disease, then it is a drug and requires FDA drug approval. This is why pharmaceutical companies spend as much time inventing new diseases as they do new drugs. That said, dietary supplements (nutraceuticals as they might be called) are substances, we are told, **not intended** to diagnose, prevent or cure any known disease. Apparently saw palmetto is fine when it is being used by persons without BPH, or for those who treat BPH **unintentionally**. With this type of logic water is fine when used "to support normal hydration" but becomes an unapproved drug when recommended to hydrate persons with diarrhea.

As it turns out, this may be one of the big issues facing the use of nutraceuticals in the future and it involves the First Amendment to the Constitution. The question is whether FDA has the right to curtail free speech of certain words (those involving diseases) when they are used truthfully regardless of the substance's FDA approval status. This is one of the major stumbling blocks preventing research of proprietary dietary supplement products. Who wants to do a multi-center, double-blind study showing their product significantly lowers BPH symptoms when all they can legally say is "Supports normal prostate function- not intended to cure.....etc." This is not only false, but ludicrous. FDA must be called to task for this double standard and allow claims to be made which reflect the real benefit of products being sold, independent of whether those claims include a disease. Until then, we will be at the mercy of looming new diseases invented to sell us more pharmaceuticals.\*

\* These comments are not intended to diagnose, cure, or treat the overwhelming conflict of interest between the FDA approval process and Big Pharma.

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### Homocysteine Metabolism

Figure 2 (p.6) shows the basic metabolic pathways concerning homocysteine. Homocysteine is an intermediate in methionine metabolism, the latter being 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 transsulfuration 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 B12) as a necessary cofactor. A secondary remethylation pathway, active primarily in liver and kidney cells, uses trimethylglycine (a.k.a. betaine) as the methyl donor. The transsulfuration pathway requires two enzymatic reactions, both of which require the cofactor pyridoxal-5-phosphate- the active form of vitamin B6.

### Measuring Homocysteine Levels

Homocysteine (Hcy) levels can easily be measured in most laboratories which test for other blood chemicals. It is 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), or non-fasting conditions. Average fasting plasma total homocysteine for "healthy" subjects in the current folic acid fortified U.S. population is between 6 and 12  $\mu\text{mol/L}$  (or  $\mu\text{M}$ ). 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 of the 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.

### Hyperhomocysteinemia- Risk Factor Assessment

#### Increased Mortality

Elevated plasma tHcy is an independent risk factor for cardiovascular as well as non-cardiovascular mortality (31, 13). In a prospective cohort study following 2127 men and 2639 women for over 4 years, increasing levels of plasma tHcy was directly related with increasing mortality. 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,  $>20 \mu\text{mol/L}$ ) 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  $\mu\text{mol/L}$  increase in tHcy increased all cause mortality by 49%, cardiovascular mortality 50%, cancer mortality 26% and non-cancer,



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non-cardiovascular mortality 104%. This data suggests that the level of homocysteine which is likely to result in a low risk for mortality is below 9 and perhaps even lower. Figure 1 shows a graph of increasing coronary artery disease (CAD) risk (both fatal and non-fatal) summarized from the various prospective trials available. The data suggest that the relative risk surpasses one at 6.5  $\mu\text{mol/L}$ , and continues to increase in a near linear fashion until plasma levels of 20  $\mu\text{mol/L}$  or more.

### Increased Acute Coronary Syndromes

We have sufficient epidemiological evidence 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). Each patient was diagnosed and treated and 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  $\mu\text{mol/L}$  (nearly 4 times more events from the highest to the lowest quintiles). This data, as well as data from the MRFIT trial (15), suggest that homocysteine may be an even better predictor for the recurrence of cardiovascular events than for a first cardiovascular event. Additionally, according to researchers from the Framingham Heart Study, hyperhomocysteinemia is also an independent risk factor for congestive heart failure even 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 (32), those individuals in the highest quartile ( $>14.24 \mu\text{mol/L}$ ) had a relative risk of 1.8 compared to the lowest quartile ( $<9.25 \mu\text{mol/L}$ ) in incidence of stroke over a 10 year follow-up. Even with these data, there is not complete agreement on whether tHcy is causal or coincidental with incidence of stroke (17,18,19). One report showed that in 75 patients who experienced an ischemic stroke event, there was nearly a 12 fold increase risk of bad recovery (Rankin Scale) in patients with tHcy levels above 15  $\mu\text{mol/L}$  (20). This is confirmed by other reports of increased recurrent stroke based on increasing homocysteine levels (21).

### 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 are thought to affect the vascular endothelium are consistent with *in vitro* research and known mechanisms for hypertension (23). Data suggests 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 any benefit gained by the medication (29).

### Risk of Cognitive Disorders and Dementia

In February of 2002, the New England Journal of Medicine published a landmark study in which they concluded that increased plasma homocysteine is a strong, independent risk factor for developing dementia and Alzheimer's disease (30). Taking data from 1092 participants in the Framingham Study cohort, they found that the risk for Alzheimer's dementia doubled (average 8 year follow-up) when the plasma tHcy exceeded 14  $\mu\text{mol/L}$ . These results confirmed smaller studies published previously concerning cognitive decline and dementias related to serum homocysteine levels (33,34,35,40,42), but others dispute 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). Of course, mild cognitive impairments is 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 (see section Possible Mechanisms for Homocysteine). Alzheimer patients have higher plasma homocysteine levels, but they also have higher levels of asymmetric dimethylarginine and decreased concentrations of nitric oxide (43), two risk factors for cardiovascular disease related to the oxidative affects of homocysteine and perhaps emerging risk factors for dementia. 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 very recent published report also suggests that a protein called transthyretin (prealbumin) becomes amyloidogenic and potentially a factor in dementia when it is bound to homocysteine (46). Another interesting finding is that treating patients with hyperhomocysteinemia and mild cognitive impairment with folic acid, B6 and B12 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 of these are strong independent risk factors for cardiovascular disease has lead 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  $\mu\text{mol/L}$ ) have a 2-fold higher risk of mortality than other diabetic patients (tHcy below 14  $\mu\text{mol/L}$ ). For each 5  $\mu\text{mol/L}$  increase in serum tHcy, the risk

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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 after 3 years (51). It is possible then, that one of ways by which 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 B12 levels and increases homocysteine levels (52). Certainly one should consider lifestyle and nutraceutical interventions prior to drug therapy for this and other reasons (see The Standard Vol. 5 #1 for natural ways to treat type II diabetes and metabolic syndrome).

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### 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, B6 or B12 levels; or perhaps even to a genetic predisposition that is causative to both phenomenon. That said, some groups find a strong predictive relationship between tumor growth and homocysteine levels (53) and others have developed theories by which homocysteine affect carcinogenesis via estrogen-induced pathways (54) or DNA damage (55). As there is little information on the long-term affects of therapeutically lowering homocysteine levels and preventing or treating various cancers, little more can be said at this time.

### Homocysteine and Kidney Disorders (56)

Normal kidney metabolism and filtration plays a prominent role in removing homocysteine from the blood. Not surprising then, hyperhomocysteinemia is very 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. Likewise, renal transplant recipients typically have elevated homocysteine levels (57). These groups of patients are often targeted for treatment with homocysteine lowering therapies, the results of which are covered in another section (Homocysteine Lowering Therapies)

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 can

break homocysteine-protein disulfide bonds. See NAC heading in "Homocysteine Lowering Therapies" section for more details.

### Homocysteine and the Risk for Other Conditions

To exhaust the list of conditions that have been linked to elevated homocysteine levels would be futile. Here are some that may be of interest: 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 tested. The most common and plausible mechanism 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 (98), 17 healthy volunteers were given methionine (100mg/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). 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 some of the increase in cardiovascular risk. Studies have shown that homocysteine suppresses the vasodilator nitric oxide (90,91), perhaps by increasing the levels of asymmetric dimethylarginine (ADMA), a strong inhibitor of the endothelial nitric oxide synthase (eNOS) and strong independent risk factor for cardiovascular disease (92), although the relationship is still debated (93,94). If indeed this is true, this could certainly account for dramatic changes in vascular endothelial compliance and platelet coagulation changes which 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 which is attenuated by folic acid (104,105). By increasing vascular smooth muscle proliferation, the arterial lumen space will be more narrow- typically considered to be deleterious for CAD. This mechanism, along with endothelial cell cytotoxicity (103) is thought to be a leading cause of vascular lesions triggered by hyperhomocysteinemia.



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**Causes for Hyperhomocysteinemia**

We have reviewed the various diseases for which homocysteine is a risk factor or marker and the potential mechanisms by which homocysteine may be a causative factor. Here we will briefly review the factors which predispose or cause elevated homocysteine levels. Table 1 summarized this information.

**Diet and Lifestyle Factors**

It is obvious from the metabolism of homocysteine (Fig 2) that if the required metabolic cofactors folic acid, vitamin B6 or vitamin B12 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 B12 and B6 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 B6 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 B12 levels (72,73). Coffee consumption (≥4 cups/day) seems to be linked with moderate elevations in homocysteine (74,75), although this effect can apparently be countered by supplementing with 200mg/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. The three main errors that have become clinically important are cystathionine

β-synthase deficiency (CBS- see Figure 2), 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 concerning these should consult specific references (3,82,83,84). One of these is worth a further mention however.

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 (Fig. 2). Certain rare defects in this gene render the enzyme completely dysfunctional and these individuals 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 polymorphism 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 of 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).

**Pharmaceuticals that Increase Homocysteine (3,29,52,89)**

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

**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 straight forward.

**Folic Acid**

The fact that about half of the homocysteine is

<b>Table 1:</b>	
<b>Factors Causing Hyperhomocysteinemia</b>	
<b>General</b>	<ul style="list-style-type: none"> <li>• Increased Age</li> <li>• Male Gender</li> <li>• Menopause (HRT may lower homocysteine)</li> </ul>
<b>Lifestyle factors</b>	<ul style="list-style-type: none"> <li>• Smoking</li> <li>• High consumption of coffee</li> <li>• Alcohol consumption (moderate beer intake may be beneficial)</li> </ul>
<b>Diet</b>	<ul style="list-style-type: none"> <li>• Low consumption of fruits &amp; vegetables</li> <li>• No consumption of multivitamins</li> <li>• Low intake of folic acid, vitamin B6, vitamin B12</li> <li>• High intake of methionine containing proteins</li> </ul>
<b>Diseases or Inherited Causes</b>	<ul style="list-style-type: none"> <li>• Cystathionine β-Synthase deficiency</li> <li>• 5MTHFR errors</li> <li>• Methionine synthase deficiencies</li> <li>• Chronic Renal Failure</li> <li>• Diabetes</li> <li>• Hypothyroidism</li> <li>• Psoriasis</li> <li>• Certain malignancies</li> <li>• Malabsorption syndrome</li> <li>• Rheumatoid Arthritis</li> </ul>
<b>Drugs that increase Homocysteine</b>	<ul style="list-style-type: none"> <li>• Some anti-epileptic drugs (phenobarbital, valproate, phenytoin etc)</li> <li>• Methotrexate</li> <li>• Nitrous oxide</li> <li>• Cholestyramine</li> <li>• Fibric acid derivatives</li> <li>• Estrogen-containing oral contraceptives</li> <li>• Metformin</li> <li>• Niacin</li> <li>• Theophylline</li> <li>• Sulfasalazine</li> </ul>

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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 (140µg/100g) 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 meaningful drops in homocysteine (110).

In general, one can reasonably predict that adequate supplementation (0.5-5mg/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 was without risk due to folate deficiency, intake of 925 µg/day would need to be consumed (113). Interestingly, once weekly doses of nearly 3grams had the equivalent homocysteine lowering effect as daily doses of 400 µg in one group of women studied (114).

2mg/day of folic acid. When using these higher doses of folic acid, additional vitamin B12 is usually recommended to prevent a masked B12 deficiency.

### Forms of Folic Acid

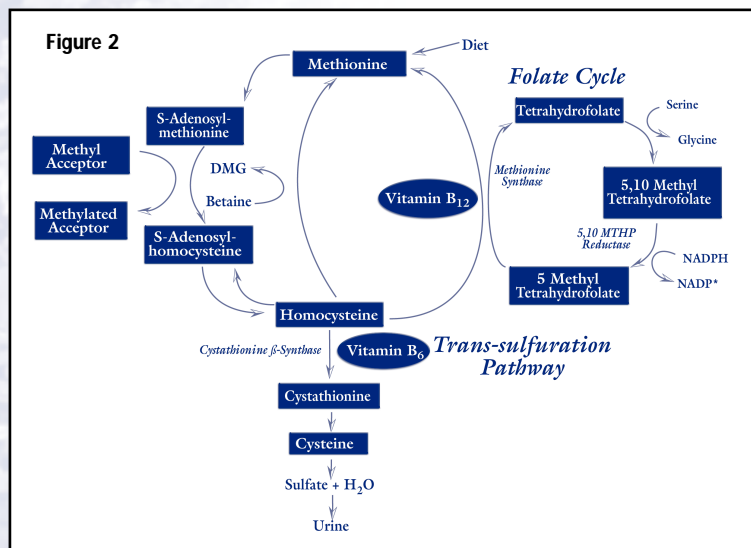
Synthetic folic acid taken in supplement form or fortification has nearly double the bioavailability as folates within foods (3-Pg.271). Available forms include folic acid, folinic acid (formyl tetrahydrofolate) and 5-methyltetrahydrofolate (5-MTHF). With the understanding that some individuals have genetic-related difficulties producing the methylated folate forms, several groups have looked at whether 5-MTHF or folinic acid would be 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 be perform better than folic acid, the folic acid supplement reduced homocysteine better than the 5-MTHF. One other 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 which could benefit from the use of folate forms other than folic acid, the form used in nearly all the research to date.

### Vitamin B12 and Vitamin B6

Unlike folic acid, which acts as a substrate in the remethylation reaction, vitamins B12 and B6 act as cofactors for the enzymes responsible for remethylation and transsulfuration, respectively (See Fig. 2). 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. One meta-analysis, which concluded that

folic acid provides a 25% drop in homocysteine, reported that additional B12 (avg. 0.5 mg/day) would produce an additional 7% reduction in tHcy and B6 (avg. 16.5 mg/day) had negligible benefits (112). However, when individuals are deficient in either B12 or B6, as is common in the elderly and those with poor dietary nutrition, both B12 and B6 may have significant benefit (122-125). Likewise, use of folic acid with vitamin B6 and B12 typically reduces homocysteine in a way that suggests synergistic effects (see below). Typical vitamin B12 doses range from 200 µg-2 mg per day and vitamin B6 doses from 10-100 mg per day. The most commonly used form of vitamin B12 in the literature is cyanocobalamin (although many clinicians prefer to use methylcobalamin); while for vitamin B6, it is pyridoxine hydrochloride.

Figure 2



### 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, 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 (2-15 mg/day are often used) (115-117). As most of these studies were done in combination with other vitamins, these studies will be discussed in the "Combination Treatments" below. It is interesting to note that nearly all of the large intervention trials currently assessing the role of folic acid (in combination with other vitamins) for the reduction of homocysteine and cardiovascular risk use at least



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### Combination Treatment and Clinical Outcomes

In most homocysteine lowering intervention trials a combination of folic acid, B6 and B12 is used and compared to placebo. And while homocysteine levels are consistently lowered, the important question is whether clinical outcomes are changed. Let us review a few of the more recent clinical studies concerning clinical outcomes.

Improved vascular endothelial function was demonstrated by measuring brachial artery flow-mediated dilation in coronary heart disease patients given 5mg folic acid and 1mg vitamin B12 daily for 8 weeks (127). In these patients, tHcy levels went from an average of 13.0 to 9.3 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 NO and homocysteine is known to lower NO levels, this is one of the likely mechanisms attributed to this therapy. Additionally, these authors believe it is the reduced unbound form of homocysteine (which accounts for only about 1-2% of the tHcy) that may be the culprit in endothelial damage (128). Other groups have confirmed that lowering homocysteine by folic acid therapy alone (5mg) has a benefit on vascular compliance (131).

As we mentioned previously, renal-transplant recipients (RTRs) are noted for elevated homocysteine and increased risk for CAD. A group of 56 RTRs with elevated homocysteine were randomly assigned to either placebo or vitamin supplementation (folic acid 5mg/day, B6 50 mg/day, B12 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 250mg of B6 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 suggests 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). 556 post-angioplasty patients were randomized to receive either placebo or a vitamin combination (1mg folic, 400 µg B12 and 10mg B6) and followed for 1 year. After adjusting for potential confounders, at the end of one year the group taking the vitamin 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 lead them to conclude that plasma homocysteine is an independent predictor of mortality, nonfatal 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.

### Betaine (TMG)

The use of supplemental betaine (trimethylglycine) is also a potential treatment option as both kidney and liver cells express an enzyme which 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 of patients with elevated homocysteine showed that 6 grams/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 beneficial in patients with homocystinuria, where betaine use is more common (137). While there are other health benefits for consuming betaine, 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 6g/day.

### N-Acetyl Cysteine (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 daily 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 NAC doses (143,145). At present, the use of high doses of NAC may be a potential addition to regimens containing the multivitamin approach outlined above. Patients with impaired kidney function are not likely to benefit from this approach however.

### Summary

While there is much yet to be done 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, B6 and B12 status, especially important in those with the C667T polymorphism in the MTHFR gene.

We have presented data showing homocysteine as an independent risk factor for numerous and various diseases, and included plausible mechanisms by which homocysteine may play causative roles in many of them. As treatment of hyperhomocysteinemia with folic acid, vitamin B12 and vitamin B6 is extremely successful in a majority of these patients, there is little reason not to consider this a target for lowering cardiovascular risk. If, by chance, homocysteine is merely an innocuous marker and coincidental with other modifiable risk factors- evidence suggests that the multi-vitamin approach which lowers homocysteine significantly reduces other measures of cardiovascular risk outcomes. While this is unlikely to be independent of homocysteine lowering, the benefit will be realized nonetheless.





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