

Homocysteine Metabolism: Nutritional Modulation and Impact on Health and Disease

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Abstract

Interest and research into the causes and treatment of hyperhomocysteinemia has increased dramatically in recent years, as increased plasma homocysteine has joined smoking, dyslipidemia, hypertension, and obesity as an independent risk factor for cardiovascular disease. In addition, elevated homocysteine levels have been implicated in a number of other clinical conditions, including neural tube defects, spontaneous abortion, placental abruption, low birth weight, renal failure, rheumatoid arthritis, alcoholism, osteoporosis, neuropsychiatric disorders, non-insulin-dependent diabetes, and complications of diabetes. Homocysteine is an intermediate metabolite of methionine metabolism and is itself metabolized by two pathways: the re-methylation pathway, which regenerates methionine, and the trans-sulfuration pathway, which degrades homocysteine into cysteine and then taurine. Because homocysteine is located at this metabolic crossroad, it impacts all methyl- and sulfur-group metabolism occurring in the body. Consequently, elevated levels of homocysteine would be expected to negatively impact the biosynthesis of all of the following: S-adenosylmethionine, carnitine, chondroitin sulfates, coenzyme A, coenzyme Q10, creatine, cysteine, dimethylglycine, epinephrine, glucosamine sulfate, glutathione, glycine, melatonin, pantethine, phosphatidylcholine, phosphatidylserine, serine, and taurine. Nutritional intervention with the cofactors required for optimal metabolism of the methionine-homocysteine pathways offers a new, integrated possibility for primary prevention and treatment. Supplementation with betaine, vitamin B12, folic acid, and vitamin B6 assists in optimizing methyl- and sulfur-group metabolism, and might play a significant role in the prevention and treatment of a wide array of clinical conditions.

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Introduction

Hyperhomocysteinemia has received increasing attention during the past decade and has joined smoking, dyslipidemia, hypertension, and obesity as an independent risk factor for cardiovascular disease. In addition to its role in cardiovascular disease, increased homocysteine levels have been implicated in a variety of other clinical conditions, including neural tube defects, spontaneous abortion, placental abruption, low birth weight, renal failure, non-insulin-dependent diabetes and complications of diabetes, rheumatoid arthritis, alcoholism, osteoporosis, and neuropsychiatric disorders (See Table 1).

Studies of healthy men and women indicate that certain acquired and genetic determinants can impact total plasma homocysteine. Women tend to have lower basal levels than men,¹ and neither contraceptives nor hormone replacement therapy seem to significantly alter the levels.² However, in post-menopausal women, hormone replacement therapy might slightly decrease elevated homocysteine concentrations. No significant lowering effect was observed in women with low homocysteine levels.³ Generally, homocysteine concentrations are significantly higher in postmenopausal women than in premenopausal women; however, the above-mentioned sex differences in homocysteine concentrations persist even in elderly populations.^{4, 5, 6} The anti-estrogen drug tamoxifen, used in the long-term treatment of breast-cancer patients, is reported to decrease homocysteine levels in postmenopausal women with breast cancer.⁷ Epidemiological evidence has shown homocysteine levels to be 45% lower in westernized adult black South Africans than in age-matched white adults, revealing racial genetic differences in homocysteine metabolism.⁸

Nutrition impacts homocysteine concentrations in both men and women. Those individuals in the lowest quartiles for serum folate and vitamin B12 (nutrients which impact homocysteine metabolism) have significantly higher concentrations of homocysteine, and men in the lowest quartile of serum pyridoxal 5'-phosphate (P5P—the bioactive form of vitamin B6) also have increased homocysteine concentrations.²

An association between coffee consumption and the concentration of total

homocysteine in plasma has been reported. A marked positive dose-response relation between coffee consumption and plasma homocysteine levels was observed. The relationship was most marked in males and females consuming greater than 8 cups of coffee per day. The combination of cigarette smoking and high coffee intake was associated with particularly high homocysteine concentrations.⁹

Chronic alcohol ingestion has also been associated with increased homocysteine levels.^{10, 11}

Table 1. Conditions Associated with Elevated Levels of Homocysteine

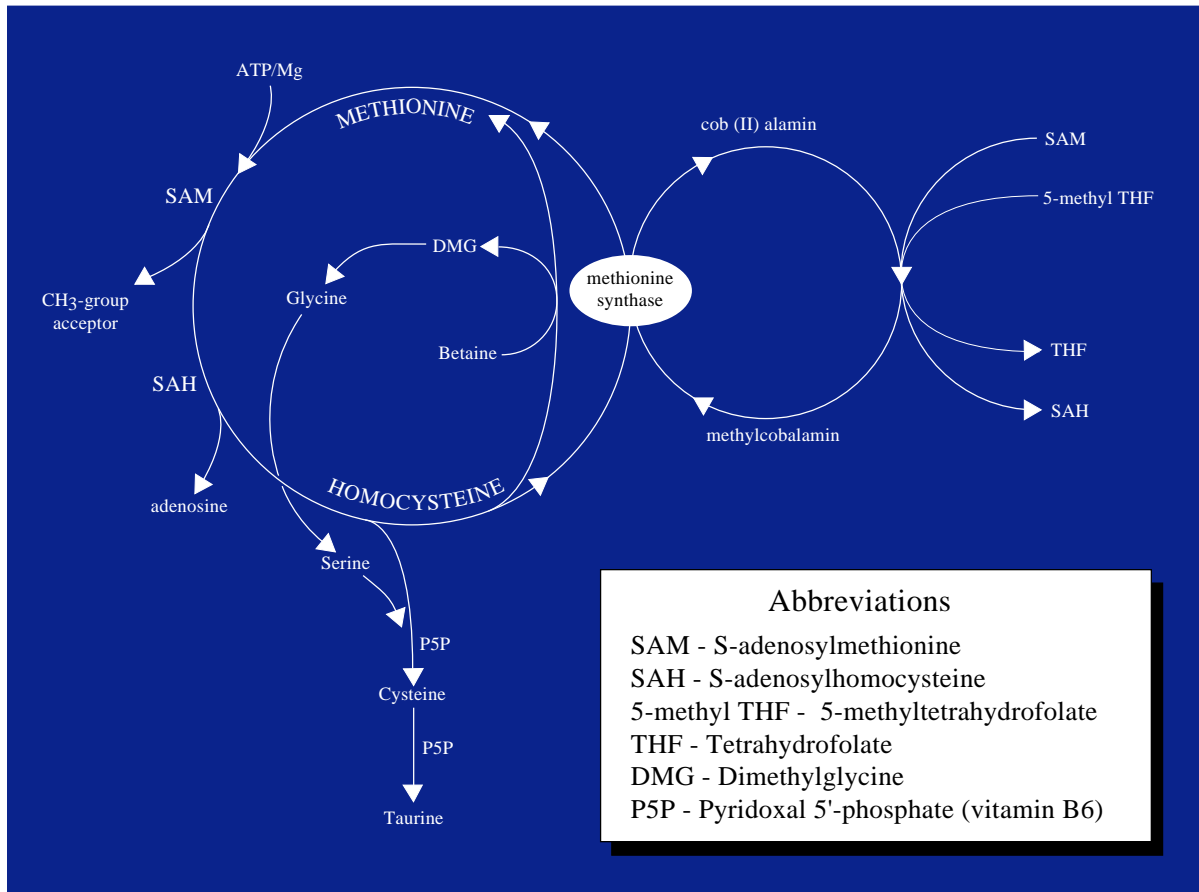
Alcoholism	Non-insulin-dependent Diabetes Mellitus
Alzheimer's Disease	Osteoporosis
Cognitive Decline	Parkinson's Disease
Coronary Artery Disease	Peripheral Vascular Disease
Deep Vein Thrombosis	Placental Abruption
Depression	Renal Failure
Diabetic Nephropathy	Retinal Vascular Occlusion
Diabetic Retinopathy	Rheumatoid Arthritis
Intermittent Claudication	Schizophrenia
Multiple Sclerosis	Spontaneous Abortion
Myocardial Infarction	Stroke
Neural Tube Defects	

Homocysteine Metabolism

Metabolism of the amino acid methionine, a limiting amino acid in the synthesis of many proteins, affects several biochemical pathways involving the production of nutrients which are essential to the optimal functioning of the cardiovascular, skeletal, and nervous systems.

Homocysteine is an intermediate product of methionine metabolism and is itself metabolized by two pathways: the re-methylation pathway, which regenerates methionine, and the trans-sulfuration pathway, which degrades

Figure 1. Homocysteine Metabolism

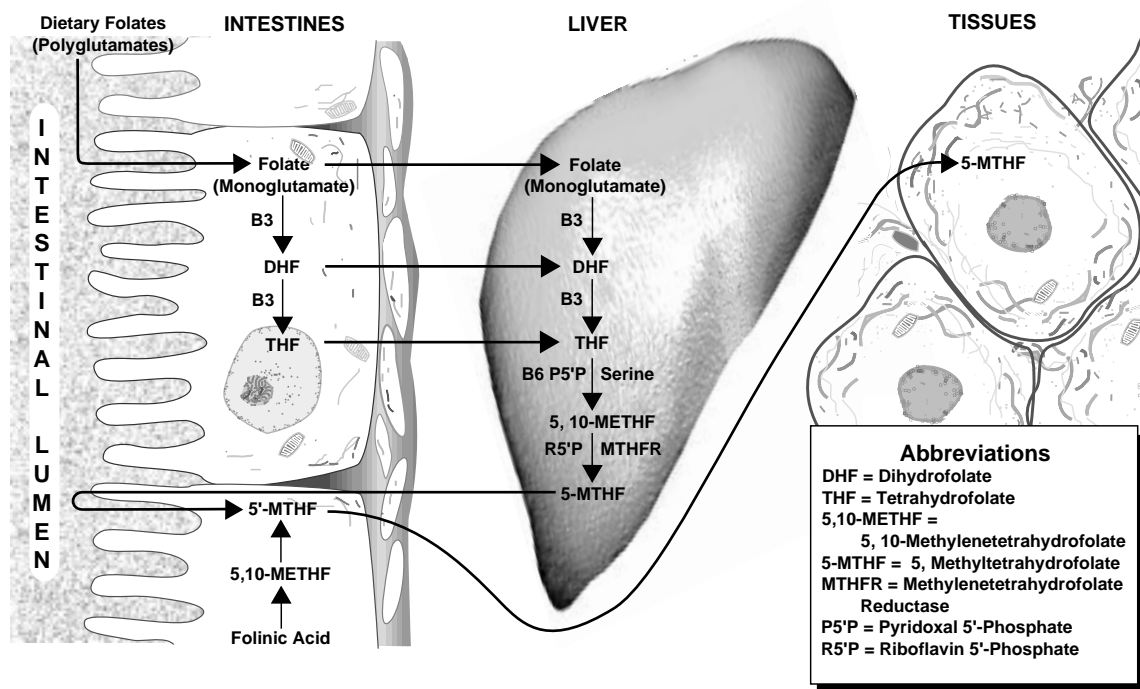


homocysteine into cysteine and then taurine. In essence, the intermediate metabolite homocysteine is located at a critical metabolic crossroad, and therefore both directly and indirectly impacts all methyl and sulfur group metabolism occurring in the body. Experiments have demonstrated if high enough levels of homocysteine and adenosine accumulate in the cell, all methylation reactions are completely inhibited.¹²

The re-methylation pathway (see Figure 1) is comprised of two intersecting biochemical pathways, and results in the transfer of a methyl group (CH₃) to homocysteine by either methylcobalamin or betaine (trimethylglycine). Methylcobalamin originally receives its methyl group from S-adenosylmethionine (SAM) or 5-methyltetrahydrofolate (5-methylTHF), an active form of folic acid. After re-methylation, methionine can be re-utilized to produce SAM, the body's "universal

methyl donor," which participates in several key metabolic pathways, including methylation of DNA and myelin, synthesis of carnitine, coenzyme Q10, creatine, epinephrine, melatonin, methylcobalamin, and phosphatidylcholine, as well as phase II methylation detoxification reactions.

The trans-sulfuration pathway of methionine/homocysteine degradation (see Figure 1) produces the amino acids cysteine and taurine, which are important nutrients for cardiac health, hepatic detoxification, cholesterol excretion, bile salt formation, and glutathione production. This pathway is dependent on adequate dietary intake and hepatic conversion of vitamin B6 into its active form, P5P. Also necessary is the amino acid serine, a downline metabolite generated from betaine via the homocysteine-remethylation pathway.

Figure 2. Absorption and Activation of Folic Acid

In addition to 5-methylTHF, methylcobalamin, betaine, and P5P, N-acetylcysteine has been reported to significantly lower homocysteine levels.¹³

Methyltetrahydrofolate

Folates function as carbon donors in the synthesis of serine from glycine, directly in the synthesis of purines and pyrimidine bases, indirectly in the synthesis of transfer RNA, and as a methyl donor to create methylcobalamin which is used for re-methylation of homocysteine to methionine. Synthesis of the active forms of folic acid is a complex process requiring several enzymes, as well as adequate supplies of niacin, riboflavin 5'-phosphate (R5P), P5P, and serine as cofactors (see Figure 2).

The formation of 5, 10-methylenetetrahydrofolate (5, 10-methyleneTHF) is of central importance, being the precursor of the metabolically-active 5-methylTHF, which is involved in homocysteine metabolism. Folic acid (5-formylTHF), available

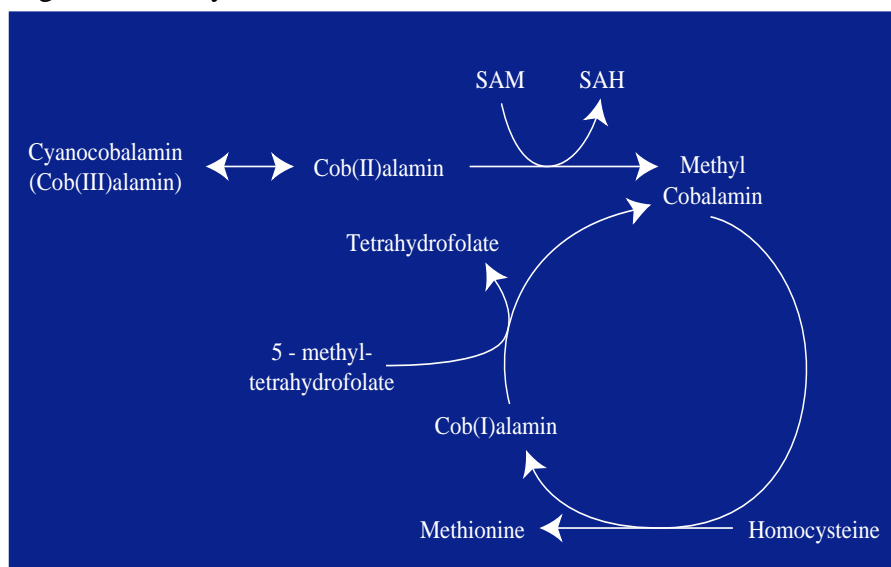
supplementally as calcium folinate—also known as leucovorin calcium—is an immediate precursor to 5, 10 methyleneTHF. Folic acid can correct deficiencies of the active forms of folic acid, is more stable than folic acid, has a longer half-life in the body, and readily crosses the blood-brain barrier.¹⁴

Methylcobalamin

Although the basic cobalamin molecule is only synthesized by micro-organisms, all mammalian cells can convert it into the co-enzymes adenosylcobalamin and methylcobalamin. Adenosylcobalamin is the primary form in cellular tissues, where it is retained in the mitochondria. Methylcobalamin predominates in blood plasma and certain other body fluids, and in cells is found in the cytosol.

Methylcobalamin's only known biological function in humans is in the re-methylation of homocysteine to methionine via the enzyme methionine synthase. In order to originally form methylcobalamin from cyanocobalamin or other Cob(III)alamin or

Figure 3. Methylcobalamin Metabolism



methylenetetrahydrofolate, which can be converted to 5-methylTHF. (See Figure 4)

Betaine supplementation has been shown to reduce homocysteine levels while resulting in modest increases of plasma serine and cysteine levels.¹⁵ This stimulation of betaine-dependent homocysteine remethylation and the subsequent decrease in plasma homocysteine can be maintained as long as supplemental betaine is taken.¹⁶

Cob(II)alamin precursors, SAM must be available to supply a methyl group. Once methylcobalamin is formed, it functions in the regeneration of methionine by transferring its methyl group to homocysteine. Methylcobalamin can then be regenerated by 5-methylTHF (see Figure 3).

Betaine

The metabolic pathways of betaine, methionine, methylcobalamin, and 5-methylTHF are interrelated, intersecting at the regeneration of methionine from homocysteine. Since tetrahydrofolate and its derivatives can only cross the mitochondrial membrane very slowly, regeneration of methionine inside the mitochondria relies heavily on recovery of a methyl group from betaine.

Betaine donates one of its three methyl groups to homocysteine, via the enzyme betaine:homocysteine methyltransferase, resulting in the regeneration of methionine. After donation of the methyl group, one molecule of dimethylglycine (DMG) remains. This molecule is oxidized to glycine and two molecules of formaldehyde by riboflavin-dependent enzymes. The formaldehyde can combine with tetrahydrofolate within the mitochondria to generate one of the active forms of folic acid,

Pyridoxal 5' -Phosphate

P5P is the active coenzyme form of vitamin B6. This cofactor is involved in myriad biological processes, including the transsulfuration pathway of homocysteine. This degradation pathway involves a two-step process resulting in the formation of cystathionine and its subsequent cleavage to cysteine. Both of the enzymes involved, cystathionine synthase and cystathioninase, require P5P as a cofactor. The first step in the degradation of homocysteine, via cystathionine synthase, also requires serine, a downstream metabolite of betaine.

Once cysteine is generated it can be directed into several pathways, including synthesis of glutathione, acetylCoA, and taurine. There are three known pathways from cysteine to taurine; all require P5P.

Homocysteine's Impact on Key Nutrients

Because of its central role in sulfur and methyl group metabolism, elevated levels of homocysteine would be expected to negatively impact the biosynthesis of each of the following: SAM, carnitine, chondroitin sulfates, coenzyme A, coenzyme Q10,

creatine, cysteine, dimethylglycine, epinephrine, glucosamine sulfate, glutathione, glycine, melatonin, pantethine, phosphatidylcholine, phosphatidylserine, serine, and taurine.

S-Adenosylmethionine

Methionine is a component of many proteins and cannot be manufactured from other dietary amino acids. It serves as a source of available sulfur for the synthesis of both cysteine and taurine, and, as SAM, it is the most important methyl-group donor in cellular metabolism.

SAM is formed by the transfer of an adenosyl group from ATP to the sulfur atom of methionine. This reaction requires magnesium as a cofactor. When methyl groups are transferred from SAM, S-adenosylhomocysteine is formed, which is then hydrolyzed to release the adenosine and results in the formation of homocysteine.

SAM is known to be utilized in the synthesis of the following compounds: carnitine, coenzyme Q10, creatine, methylcobalamin from Cob(III)alamin, 1-methylnicotinamide, N-methyltryptamine, phosphatidylcholine, and polyamines. It is also utilized in numerous other methylation reactions, including hepatic phase II detoxification.

Carnitine

A trimethylated amino acid roughly similar in structure to choline, carnitine is a cofactor for transformation of free, long-chain fatty acids into acylcarnitines, and their transport into the mitochondrial matrix, where they undergo beta-oxidation for cellular energy production. Mitochondrial fatty acid oxidation is the primary fuel source in heart and skeletal muscle. Synthesis of carnitine begins with the methylation of the amino acid L-lysine by SAM. Methionine, magnesium, vitamin C, iron, P5P, and niacin, along with the cofactors responsible for regenerating SAM from

homocysteine (5-methylTHF, methylcobalamin, and betaine) are required for optimal carnitine synthesis.

A pivotal enzyme in carnitine synthesis, betaine aldehyde dehydrogenase is the same enzyme responsible for synthesis of betaine from choline. Two recent studies suggest this enzyme has a preference for choline-betaine conversion, and choline supplementation might decrease carnitine synthesis; therefore, it might be of greater benefit to supplement with betaine rather than its precursor, choline.^{17, 18}

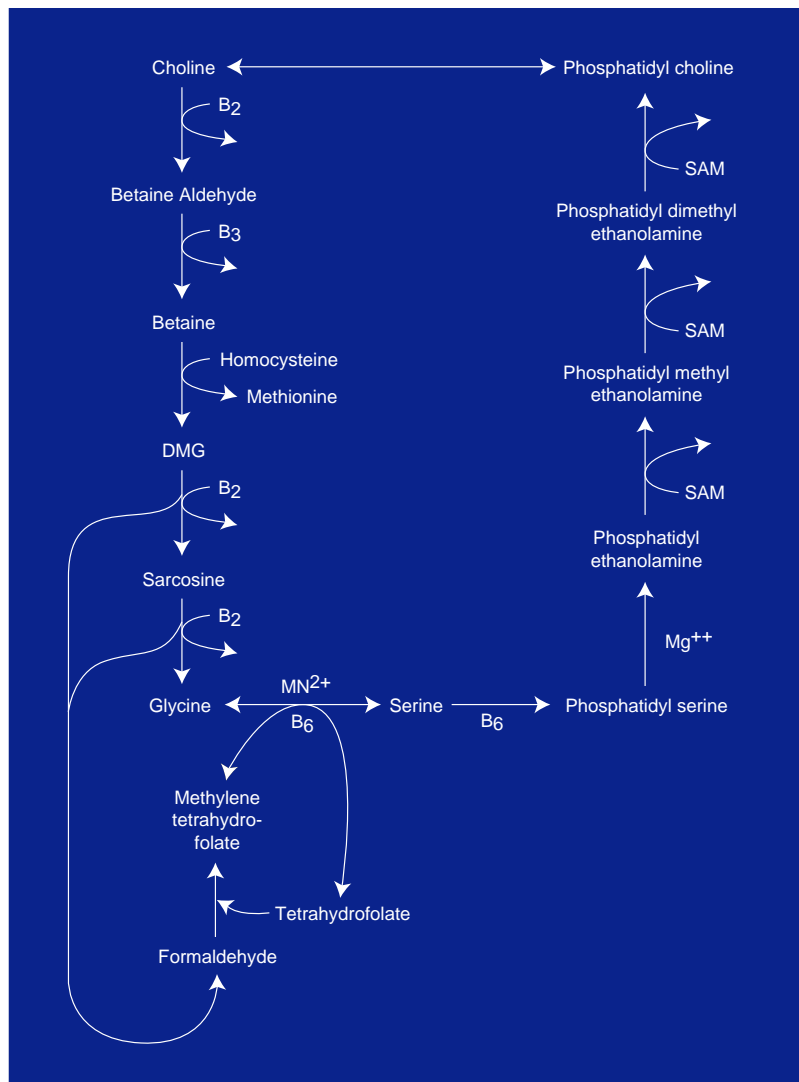
Chondroitin Sulfates, Glucosamine Sulfate and other Sulfated Proteoglycans

Proteoglycans are amino sugars found in all tissues, but are in the highest concentration in cartilage, tendons, ligaments, synovial fluid, skin, finger- and toenails, heart valves, and the basement membrane of all blood vessels. Perhaps the most widely known of the amino sugars are the chondroitin sulfates and glucosamine sulfate.

Chondroitin sulfates are primarily composed of alternating residues of N-acetyl-D-galactosamine and D-glucuronate. Sulfate residues are present on C-4 of the galactosamine residues in one type of chondroitin and on C-6 in another. Glucosamine sulfate is a simple molecule composed of glucose, the amino acid glutamine, and a sulfate group. Other sulfated proteoglycans include dermatan sulfates, keratan sulfates, and heparin sulfates.

High levels of homocysteine are likely to negatively impact the formation of the sulfated amino sugars because, although some sulfates are present in the diet, the sulfoxidation of cysteine is an important source of sulfate molecules. The sulfoxidation pathway proceeds through the toxic intermediate sulfite and requires molybdenum as a cofactor.

Figure 4. Betaine / Phosphatidylcholine Loop



body has sub-optimal levels of cysteine. Because of this, even in the presence of adequate levels of pantothenic acid, it is possible to have inadequate biosynthesis of acetyl coenzyme A. The disulfate form of pantothenic acid, known as pantethine, as opposed to pantothenic acid, bypasses cysteine conjugation and decarboxylation. This might account for some of the clinical benefits seen with pantethine supplementation which have not been reproduced with supplementation of pantothenic acid.

Coenzyme Q10

Coenzyme Q10 (CoQ10) is a fat-soluble quinone occurring in the mitochondria of every cell. The primary biochemical action of CoQ10 is as a cofactor in the electron transport chain, the biochemical pathway responsible for generating adenosine triphosphate (ATP). Since most cellular functions are dependent on an adequate supply of ATP, CoQ10 is essential for the health of virtually all human tissues and organs.

Biosynthesis of CoQ10 begins with the amino acid tyrosine. Pantothenic acid, P5P, and vitamin C are all required for the initial steps in its synthesis. An isoprenyl side chain from farnesyl diphosphate, an intermediate in cholesterol synthesis, is then added. In two of the final steps in the synthesis of CoQ10, methyl groups are provided by SAM. Adequate dietary methionine and a sufficient supply of the nutrients required for re-methylation of homocysteine to methionine (5-methylTHF, methylcobalamin, and betaine) are required to generate sufficient SAM for optimal synthesis of CoQ10.

Coenzyme A

Coenzyme A consists of an adenine nucleotide and phosphopantetheine. Contained within the structure of this coenzyme is pantothenic acid; however, the reactive component of the molecule is a sulfhydryl group which is not contained within the vitamin. In order to form the sulfhydryl-containing molecule (pantetheine), pantothenic acid must combine with cysteamine. Cysteamine is formed through conjugation and decarboxylation reactions of cysteine. As was previously discussed, the metabolic stagnation caused by elevated homocysteine levels indicates the

Creatine

In humans, over 95% of total creatine content is located in skeletal muscle, of which approximately one-third is in its free form as creatine, also known as methylguanidinoacetic acid, while the remainder is present in a phosphorylated form as creatine phosphate (also called phosphocreatine). Creatine phosphate is utilized within skeletal muscle for storing high energy phosphate bonds.

Creatine is formed in the liver, kidney, and pancreas by the combination of arginine and glycine, which produce guanidinoacetate. A methyl group from SAM is then transferred, resulting in the formation of creatine. The byproduct of this reaction, S-adenosyl-homocysteine, is subsequently hydrolyzed into homocysteine and adenosine.

Epinephrine and Melatonin

Derivatives of the aromatic amino acids L-tyrosine and L-tryptophan, epinephrine and melatonin require methylation for biosynthesis of their down-line metabolites.

The biosynthesis of catecholamines begins with the amino acid L-tyrosine and proceeds through dopa and dopamine, resulting in the formation of norepinephrine, the neurotransmitter substance found in the majority of sympathetic nerve terminals, as well as in some synapses of the central nervous system. In the chromaffin cells of the adrenal medulla, a methyl group is provided by SAM, resulting in the formation of epinephrine from norepinephrine. A number of metabolites are formed from degradation of both norepinephrine and epinephrine. Catecholamine degradation proceeds independently, in addition to in conjunction with monoamine oxidase, by catechol-O-methyltransferase. This enzyme catalyzes the transfer of a methyl group donated by SAM and, depending on the substrate, results in the formation of homovanillic acid, normetanephrine, and metanephrine.

Formation of melatonin from L-tryptophan proceeds through 5-hydroxy-tryptophan, serotonin, and N-acetylserotonin. Melatonin is then formed in the pineal gland by the donation of a methyl group. 5-Methoxytryptamine, an alternate metabolite of serotonin, also requires the addition of a methyl group.

Since elevated homocysteine results in sub-optimal synthesis of SAM, some impact on aromatic amino acid derivatives will occur. The exact nature of the impact on catecholamine metabolites is still unclear, due to SAM's role in both synthesis and degradation. It appears likely the biochemical stagnation associated with elevated levels of homocysteine would negatively impact melatonin synthesis.

Phosphatidylcholine

Dietary choline is derived primarily from phosphatidylcholine (PC), a component of lecithin. After absorption by the intestinal mucosa, PC is metabolized to choline in the liver by the enzyme phospholipase D. Most choline is re-phosphorylated to PC; however, a small amount is carried to the brain via the blood stream, where it is converted to the neurotransmitter acetylcholine. If PC or choline are lacking in the diet they can be synthesized from phosphatidylserine and phosphatidylethanolamine. Synthesis of PC is dependent on the availability of SAM as a methyl donor, since synthesis involves the transfer of methyl groups from three SAM molecules to phosphatidylethanolamine in order to generate one molecule of PC (see Figure 4).

Taurine

Taurine is a unique amino acid because it carries a sulfonic acid group ($-\text{SO}_3\text{H}$) instead of a carboxyl group ($-\text{CO}_2\text{H}$). Taurine is biosynthesized from methionine or from cysteine via the trans-sulfuration pathway (see Figure 1). As discussed previously, homocysteine can be re-methylated to form

methionine; however, it can also be degraded to form cysteine. Once cysteine is generated it can be directed into several different pathways, including synthesis of glutathione, acetyl-CoA, 3'-phosphate 5'-phosphosulfate (PAPS), and taurine. Degradation involves a two-step process, resulting in formation of cystathionine and its subsequent cleavage to cysteine. Both of the enzymes involved require P5P as a cofactor, and the committed first step in the degradation of homocysteine, utilizing cystathionine synthase, also requires serine. In humans, defects in both of these enzymatic reactions occur.

Homocysteine and Phase II Detoxification Reactions

Because homocysteine is a critical intermediate in both methyl and sulfur group metabolism, elevated levels could indicate nutrient deficiencies which might compromise function in virtually all phase II detoxification reactions.

Amino acid conjugation reactions require either glycine, glutamine, or taurine. Glycine functions in the conjugation of aromatic acids (e.g., benzoic acid to hippuric acid). Elevated levels of homocysteine might indicate reduced nutritional levels of betaine and subsequently its downline metabolite glycine. Taurine functions in acylations (e.g., bile conjugation). As discussed, optimal taurine synthesis requires proper movement of homocysteine into its degradation pathway. There are no known interactions between glutamine and homocysteine.

Sulfur conjugation requires N-acetylcysteine (NAC), glutathione (GSH), PAPS, or methionine/cysteine. NAC is used for mercapturic acid synthesis and is involved in detoxification of a wide variety of compounds including aromatic hydrocarbons, some phenols, halides, esters, epoxides, and caffeine. GSH is involved in dismutation reactions of organic nitrates (e.g.,

nitroglycerin). PAPS is utilized in sulfate ester synthesis, mostly with phenols, and some aliphatic alcohols (e.g., ethanol), and aromatic amines. Methionine and cysteine are used in cyanide-thiocyanate detoxification. A portion of the inorganic sulfur needed for the formation of all these compounds passes through the homocysteine cycle.

Alkylation reactions require SAM, methylcobalamin, or 5-methylTHF. These compounds provide methyl groups to detoxify compounds containing OH, SH, or NH₂ groups. Examples of these reactions include norepinephrine to epinephrine, epinephrine to metanephrine, guanidoacetic acid to creatine, and N-acetylserotonin to melatonin.

Other phase II detoxification reactions which might be impacted by elevated homocysteine as a biological marker of reduced nutrient formation include acetylation by acetylcoenzyme A, which requires cysteine as a source of its cysteamine component; and the use of carnitine for the conversion of valproic acid to valpropylcarnitine

Homocysteine and Heart Disease

A significant component in the pathogenesis, prevention, and treatment of heart disease involves the amino acid homocysteine. Increased blood levels of homocysteine are correlated with significantly increased risk of coronary artery disease (CAD),¹⁹⁻²² myocardial infarction,^{23,24} peripheral occlusive disease,²⁵⁻²⁸ cerebral occlusive disease,^{25, 28} and retinal vascular occlusion.²⁹

For over 25 years researchers have known inborn errors of homocysteine metabolism result in high levels of homocysteine in the blood and severe atherosclerotic disease. We now know, even within the range which is considered normal (4-16 $\mu\text{mol/L}$), there is a graded increase in risk for CAD. In a study of 304 patients with CAD vs. controls, Robinson et al found the odds ratio for CAD increased as plasma homocysteine increased, even within

the normal range. A 5 $\mu\text{mol/L}$ increase in plasma homocysteine was correlated with an increase in the odds ratio of 2.4 ($p < .001$), with no “threshold effect.”²²

Data gathered by Boers³⁰ from a number of studies indicated that, after a methionine load test, mild hyperhomocysteinemia occurred in 21%, 24%, and 32% of patients with CAD, cerebrovascular disease, and peripheral vascular disease, respectively. Selhub et al found the incidence of hyperhomocysteinemia ($>14 \mu\text{mol/L}$ by their definition), in a group of 1160 elderly individuals (ages 67-96) in the Framingham Heart Study, to be 29.3%. The study also indicated plasma homocysteine levels increase with age.²⁵

Homocysteine facilitates the generation of hydrogen peroxide.³¹ By creating oxidative damage to LDL cholesterol and endothelial cell membranes, hydrogen peroxide can then catalyze injury to vascular endothelium.^{31, 32}

Nitric oxide and other oxides of nitrogen released by endothelial cells (also known as endothelium-derived relaxing factor, or EDRF) protect endothelial cells from damage by reacting with homocysteine, forming S-nitrosohomocysteine, which inhibits hydrogen peroxide formation. However, as homocysteine levels increase, this protective mechanism can become overloaded, allowing damage to endothelial cells to occur.³²⁻³⁴ Because of the role of sulfate compounds in the formation of amino sugars needed to form the basement membrane of blood vessels, high levels of homocysteine are likely to contribute to the formation of blood vessels which are more susceptible to oxidative stress.³⁴ The end result of the combination of oxidative damage and endothelial collagen instability is the formation of atherosclerotic plaques.

Re-methylation of homocysteine and the subsequent formation of SAM is critical for biosynthesis of L-carnitine, CoQ10, and

creatine. Similarly, the trans-sulfuration pathway must be functioning properly for optimal biosynthesis of cysteine, GSH, pantethine, and taurine. All of these nutrients are used clinically to either reduce oxidative stress, improve risk factor markers, or treat heart disease.

Decreased plasma folate levels are correlated with increased levels of homocysteine, and a subsequent increased incidence of CAD. In a 15-year Canadian study of CAD mortality in 5056 men and women 35-79 years of age, lower serum folate levels were correlated with a significantly increased risk of fatal CAD.³⁵ In a cohort from the Framingham Heart Study, concentrations of folate and P5P were inversely correlated with homocysteine levels and the risk of extracranial carotid-artery stenosis.²⁵ Low P5P and low vitamin B12 have also been linked with hyperhomocysteinemia and a significantly increased risk of CAD.²²

If a dietary deficiency or an increased demand, resulting from genetic biochemical individuality, exists for 5-methylTHF, methylcobalamin, P5P, or betaine, treatment with these micro-nutrients should reduce homocysteine levels. Several studies utilizing folic acid, B6, B12, and betaine either alone or in combination have demonstrated the ability of these nutrients to normalize homocysteine levels.^{15, 26, 28, 36, 37} In a recent placebo-controlled clinical study of 100 men with hyperhomocysteinemia, oral therapy with 650 mcg folic acid, 400 mcg vitamin B12, 10 mg vitamin B6, or a combination of the three nutrients was given daily for six weeks. Plasma homocysteine was reduced 41.7% ($p < 0.001$) during folate therapy and 14.8% ($p < 0.01$) during B12 therapy, while 10 mg B6 did not reduce plasma homocysteine significantly. The combination worked synergistically to reduce homocysteine levels 49.8%.³⁸ In 68 patients with recent myocardial infarction, 18% had increased plasma homocysteine. Oral folate therapy (2.5 mg) reduced this hyperhomocysteinemia in 94% of treated patients (mean decrease 27%).²³

A deficiency of the P5P dependent enzyme cystathione synthase is the most common genetic abnormality affecting the transsulfuration pathway of homocysteine breakdown. Fortunately, B6 supplementation stimulates this enzyme and, in combination with betaine, corrects the hyperhomocysteinemia in these individuals.^{15, 36}

Homocysteine and Peripheral Vascular Disease

Elevated homocysteine levels have been established as an independent risk factor for intermittent claudication (IC) and deep vein thrombosis. Elevated homocysteine levels corresponded with an increased incidence of intermittent claudication and decreased serum folate levels in a study of 78 patients with IC.³⁹ A four-fold increase in risk of peripheral vascular disease was noted in individuals with hyperhomocysteinemia compared to people with normal homocysteine levels.⁴⁰ A group of researchers in the Netherlands found high homocysteine levels to be a significant risk factor for deep-vein thrombosis, with a stronger relationship among women than men.⁴¹

An increased risk of peripheral vascular occlusion has been noted in women taking oral contraceptives, which might be linked to the significantly increased homocysteine levels in women so affected. Oral contraceptives can cause declines or deficiencies in vitamins B6, B12, and folate, nutrients integral to the processing of homocysteine. Laboratory assessment of plasma homocysteine levels might be helpful to detect women who are predisposed to peripheral vascular occlusion while on oral contraceptives.⁴²

In a group of 48 patients with peripheral atherosclerotic vascular disease, 50% had abnormally high fasting plasma homocysteine levels, while 100% had abnormal plasma homocysteine after a methionine load. Treatment with 5 mg folic acid and 250 mg pyridoxine for 12 weeks normalized 95% of the fasting

levels and 100% of post-load homocysteine levels.²⁶

Homocysteine and Stroke

Stroke patients have significantly elevated homocysteine levels compared to age-matched controls,⁴³ with a linear relationship existing between risk of stroke and homocysteine levels.⁴⁴ Decreased blood folate concentrations in stroke patients might be a possible cause of the observed hyperhomocysteinemia.⁴⁵

Homocysteine and Pregnancy

Biochemical enzyme defects and nutritional deficiencies are receiving increasing attention for their role in causing neural tube defects (NTD) as well as other negative pregnancy outcomes, including spontaneous abortion, placental abruption (infarct), pre-term delivery, and low infant birth weight. Recent evidence has suggested derangement of methionine-homocysteine metabolism could be the underlying mechanism of pathogenesis of neural tube defects and might be the mechanism of prevention observed with folic acid supplementation.^{46, 47} A low dietary intake of folic acid increases the risk for delivery of a child with an NTD, and periconceptional folic acid supplementation reduces the NTD occurrence.⁴⁸⁻⁵⁴ Supplemental folic acid intake also results in increased infant birth weight and improved Apgar scores, along with a concomitant decreased incidence of fetal growth retardation and maternal infections.⁵⁵⁻⁵⁸ A derangement in methionine-homocysteine metabolism has also been correlated with recurrent miscarriage and placental infarcts (abruption).⁵⁹

The amino acid homocysteine, when elevated, might be a teratogenic agent contributing to congenital defects of the heart and neural tube. Evidence from experimental animals lends support to this belief. When avian embryos were fed homocysteine to raise serum homocysteine to over 150 nmol/ml,

dysmorphogenesis of the heart and neural tube, as well as of the ventral wall, were observed.⁶⁰

Because homocysteine metabolism, through the re-methylation and transsulfuration pathways, affects several biochemical pathways involving the production of nutrients which are essential to the optimal functioning of the cardiovascular, skeletal, and nervous systems, it is not surprising these other nutrients have been linked to complications of pregnancy in animal models and humans. Low plasma vitamin B12 levels have been shown to be an independent risk factor for NTD.^{61,62} Methionine supplementation has been shown to reduce the incidence of NTD by 41% in an animal model.^{63,64} This evidence indicates that a disturbance in the re-methylation pathway, with a subsequent decrease in SAM, might be a contributing factor to these complications of pregnancy. Phosphatidylcholine, due to its role as a precursor to acetylcholine and choline, is acknowledged as a critical nutrient for brain and nerve development and function.⁶⁵⁻⁶⁷ Since the metabolic pathways of choline (via betaine), methionine, methylcobalamin, and 5-methylTHF are interrelated, intersecting at the regeneration of methionine from homocysteine, a disturbance in the metabolism of either of these two methyl-donor pathways, due to limited availability of key nutrients or decreased enzyme activity, will have a direct impact on the body's ability to optimize SAM levels.

Evidence suggests women with a history of NTD-affected pregnancies have altered folic acid metabolism.⁶⁸⁻⁷¹ Patients with a severe congenital deficiency of the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR), which is needed for the formation of 5-methylTHF, have reduced levels of both methionine and SAM in the cerebrospinal fluid, and show demyelination in the brain and degeneration of the spinal cord.^{2,72} Because of its direct impact in the activation of folic acid to its methyl derivative, a milder version of

this enzyme defect is also strongly suspected to increase incidence of NTD.⁷³

High vitamin A intake during the first two months of pregnancy is associated with a several-fold higher incidence of birth defects.^{74,75} Although the mechanism of action remains to be elicited, in an animal model the activity of hepatic MTHFR is suppressed with high vitamin A levels,⁷⁶ suggesting its teratogenic effect during early pregnancy might be associated with subsequent derangement in the re-methylation of homocysteine.

Since a significant correlation has been found between higher homocysteine levels in women experiencing placental abruption, infarction, and spontaneous abortion than in control women, and since homocysteine and CoQ10 synthesis are both dependent on the methionine-SAM-homocysteine pathway, it is possible low CoQ10 and elevated homocysteine, independently found in complicated pregnancy, might also in fact be found to be related conditions.^{77,78}

Homocysteine and the Nervous System

In addition to the known impact of homocysteine on the cardiovascular system and micro-nutrient biochemical pathways, numerous diseases of the nervous system are correlated with high homocysteine levels and alterations in B12, folate, or B6 metabolism, including depression, schizophrenia, multiple sclerosis, Parkinson's disease, Alzheimer's disease, and cognitive decline in the elderly.

Methylation reactions via SAM, including methylation of DNA and myelin, are vitally important in the CNS. The neurologic complications of vitamin B12 deficiency are thought to be due to a reduction of activity of the B12-dependent enzyme methionine synthase, and the subsequent reduction of SAM production. The CNS lacks the alternate betaine pathway of homocysteine remethylation; therefore, if methionine

synthase is inactivated, the CNS has a greatly reduced methylation capacity.⁷⁹ Other causes of reduced methionine synthase activity include folic acid deficiency and nitrous oxide anesthesia exposure.⁸⁰

Homocysteine has also been found to be a neurotoxin, especially in conditions in which glycine levels are elevated, including head trauma, stroke,⁸¹ and B12 deficiency. Homocysteine interacts with the N-methyl-D-aspartate receptor, causing excessive calcium influx and free radical production, resulting in neurotoxicity.⁸¹ The neurotoxic effects of homocysteine and/or reduced methylation reactions in the CNS contribute to the mental symptomatology seen in B12 and folate deficiency. Increased homocysteine levels can also be seen in schizophrenics.⁸²

Significant deficiencies in B12 and folate are common in the elderly population, and can contribute to a decline in cognitive function.⁸³⁻⁸⁵ An investigation of cognitive ability in older men (ages 54-81) found poorer spatial copying skills in those individuals with higher homocysteine levels. Better memory performance was correlated with higher vitamin B6 levels.⁸⁶

B12 deficiency and increasing severity of cognitive impairment has been seen in Alzheimer's disease (AD) patients compared to controls and patients with other dementias.⁸⁷ In a study of 52 AD patients, 50 hospitalized non-demented controls, and 49 elderly subjects living at home, patients with AD were found to have the highest homocysteine levels and the highest methylmalonic acid (an indicator of B12 deficiency) levels.⁸⁸ In a study of 741 psychogeriatric patients, high plasma homocysteine levels were found in demented and non-demented patients; however, only demented patients also had lower blood folate concentrations compared to controls. Patients with concomitant vascular disease had significantly higher plasma homocysteine than those without diagnosed vascular disease.

Significantly higher homocysteine levels, compared to controls, have also been found in Parkinson's patients.⁸⁹

Homocysteine's effects on neurotransmitter metabolism, along with its potential reduction of methylation reactions, could be a contributing factor to the etiology of depression. Folate and B12 deficiency can cause neuropsychiatric symptoms, including dementia and depression. Although no studies have been performed to date investigating depression, folate and B12 deficiency, and homocysteine levels, the information regarding these deficiencies and methionine synthase inhibition suggests this connection will be revealed in the future. SAM is used therapeutically as an antidepressant in Europe,^{90,91} and was the third most popular antidepressant treatment in Italy in 1995.⁹¹ As yet, SAM is not available as a supplement in the United States.

Methylation of myelin basic protein is vital to maintenance of the myelin sheath. The worst-case scenario of folate and B12 deficiency includes demyelination of the posterior and lateral columns of the spinal cord, a disease process called subacute combined degeneration of the spinal cord (SCD).⁷⁹ SCD can also be precipitated by nitrous oxide anesthesia, which causes an irreversible oxidation of the cobalt moiety of the B12 molecule and the subsequent inhibition of methionine synthase activity, a decrease in homocysteine re-methylation, and decreased SAM production.⁸⁰ This has been treated using supplemental methionine, which further supports the theory of a nitrous oxide-induced biochemical block at methionine synthase.⁹² Particularly at risk for this condition are B12-deficient individuals who visit their dentist and receive nitrous oxide.^{80,93}

Abnormal methylcobalamin metabolism is a proposed mechanism for the pathophysiology of the demyelinating disease multiple sclerosis (MS). Deficiency of vitamin B12 has been linked to some MS cases, and it is suggested dietary deficiency, or more likely,

a defect in R-protein-mediated absorption or methylation of B12, might be a significant contributor to MS pathogenesis.⁹⁴

Patients with congenital MTHFR deficiency, which is needed for the formation of 5-methylTHF, have reduced levels of both methionine and SAM in the cerebrospinal fluid (CSF) and show demyelination in the brain and degeneration of the spinal cord. Methionine is effective in the treatment of some of these patients; however, betaine was shown to restore CSF SAM levels to normal and to prevent the progress of neurological symptoms in all patients in whom it was tried.⁹⁵

Homocysteine and Diabetes Mellitus

Homocysteine levels appear to be lower in individuals with type I diabetes mellitus. Forty-one type I diabetic subjects (age 34.8 ± 12 yr, duration of illness; 10.7 ± 11.1 yr) were compared to 40 age-matched control subjects (age 34.2 ± 9.1 yr). Following an overnight fast, homocysteine was significantly lower ($p = 0.0001$) in the diabetic group (6.8 ± 2.2) than in controls (9.5 ± 2.9). This difference was apparent in male and female subgroups.⁹⁶ However, increased levels of homocysteine have been reported in type I diabetics with proliferative retinopathy⁹⁷ and nephropathy.^{97, 98}

Evidence to date suggests metabolism of homocysteine is also impaired in patients with non-insulin-dependent diabetes mellitus (NIDDM). Following a methionine load, hyperhomocysteinemia occurred with significantly greater frequency in patients with NIDDM (39%) as compared with age-matched controls (7%). The area under the curve over 24 hours, reflecting the total period of exposure to increased homocysteine, was also elevated with greater frequency in patients with NIDDM and macrovascular disease (33%) as compared with controls (0%). The authors concluded hyperhomocysteinemia is associated with macrovascular disease in a

significant proportion of patients with NIDDM.⁹⁹ Araki et al also reported increased homocysteine levels correlate with the occurrence of macroangiopathy in patients with NIDDM. Intramuscular injection of 1000 micrograms methylcobalamin daily for three weeks reduced the elevated plasma levels of homocysteine in these individuals.¹⁰⁰

Elevated homocysteine levels appear to be a risk factor for diabetic retinopathy. This might be due to a point mutation on the gene for the enzyme MTHFR,^{101, 102} as a significantly higher percentage of diabetics with retinopathy exhibit this mutation.¹⁰² Elevated homocysteine levels cause cell injury to the small vessels, which might contribute to development of retinopathy and cardiovascular macroangiopathy.¹⁰¹

Homocysteine and Rheumatoid Arthritis

Elevated total homocysteine levels have been reported in patients with rheumatoid arthritis (RA). Twenty-eight patients with RA and 20 healthy age-matched control subjects were assessed for homocysteine levels, while fasting and in response to a methionine challenge. Fasting levels were 33% higher in RA patients than in controls. Four hours following the methionine challenge, the increase in plasma homocysteine concentration was also higher in patients with rheumatoid arthritis.¹⁰³ Another study found statistically significant increases in homocysteine in RA patients ($p = 0.003$), with 20% of the patients having homocysteine levels above the reference range.¹⁰⁴ A mechanism for this increased homocysteine in RA patients has not been elucidated. Penicillamine, a common sulfhydryl-containing arthritis treatment, has been found to lower elevated homocysteine levels *in vivo*.¹⁰⁵ Further investigation into both the prevalence of hyperhomocysteinemia and the mechanism of action impacting rheumatoid arthritis is needed.

Homocysteine and Kidney Failure

Because homocysteine is cleared by the kidneys, chronic renal failure, as well as absolute or relative deficiencies of 5-methylTHF, methylcobalamin, P5P, or betaine, results in increased homocysteine levels. In 176 patients with end-stage renal disease on peritoneal- or hemodialysis, homocysteine concentrations averaged $26.6 \pm 1.5 \mu\text{mol/L}$ in patients with renal failure as compared to $10.1 \pm 1.7 \mu\text{mol/L}$ in normals. Abnormal values exceeded the 95th percentile for normal controls in 149 of the patients with renal failure.¹⁰⁶ Data also indicates plasma homocysteine values represent an independent risk factor for vascular events in patients on peritoneal- and hemodialysis. Patients with a homocysteine concentration in the upper two quintiles ($> 27.8 \mu\text{mol/L}$) had an independent odds ratio of 2.9 (CI, 1.4 to 5.8; $P = .007$) of vascular complications. B vitamin levels were also lower in patients with vascular complications than in those without.¹⁰⁷

Homocysteine and Ethanol Ingestion

Chronic alcoholism is known to interfere with one-carbon metabolism. Because of this, it is not surprising to find mean serum homocysteine concentrations twice as high in chronic alcoholics as compared to nondrinkers ($p < 0.001$). Beer consumers have lower concentrations of homocysteine than drinkers of wine or spirits ($p = 0.05$). In chronic alcoholics, serum P5P and red blood cell folate concentrations have been shown to be significantly lower than in control subjects.¹⁰ Hultberg et al reported a significantly higher concentration of plasma homocysteine, compared with controls, in 42 active alcoholics hospitalized for detoxification. In another group of 16 alcoholics, abstaining from ethanol ingestion, plasma homocysteine did not deviate from levels found in controls.¹¹

Feeding ethanol to rats produces prompt inhibition of methionine synthase as well as a subsequent increase in activity of betaine homocysteine methyltransferase. Despite the inhibition of methionine synthase, the enhanced betaine homocysteine methyltransferase pathway utilizes hepatic betaine pools to maintain levels of SAM.¹⁰⁸ Results indicate ethanol feeding produces a significant SAM loss in the first week, with a return to normal SAM levels the second week. Betaine feeding enhances hepatic betaine pools in control as well as ethanol-fed animals, attenuates the early SAM loss in ethanol-fed animals, produces an early increase in betaine homocysteine methyltransferase activity, and generates increased SAM levels in both control and ethanol-fed groups.¹⁰⁹ Minimal supplemental dietary betaine at the 0.5% level generates SAM twofold in control animals and fivefold in ethanol-fed rats. Concomitant with betaine-generated SAM, ethanol-induced hepatic fatty infiltration was ameliorated.¹²¹ Betaine supplementation also reduces the accumulation of hepatic triglycerides produced after ethanol ingestion.¹⁰⁹

Homocysteine and Gout

Although homocysteine levels have been positively correlated with increased uric acid levels,^{2, 110, 111} no studies exist to date which have investigated homocysteine levels in gout patients. It is possible increased uric acid levels in gout are due to decreased SAM production because of the reduction in homocysteine recycling. The excess adenosine, which would have reacted with methionine to form SAM, is degraded to form uric acid as its end product.

Niacin is contraindicated in gout, as it competes with uric acid for excretion.¹¹² In animal studies, increased levels of S-adenosylhomocysteine (SAH), and thus homocysteine, cause significant reductions in SAM-dependent methylation reactions.¹² Therefore, since degradation of the niacin-containing coenzyme nicotinamide adenine

dinucleotide (NAD) is dependent on methylation by SAM, and SAM activity is severely reduced in hyperhomocysteinemia, niacin levels might be higher in these people, resulting in less uric acid excretion, higher uric acid levels, and increased gout symptoms in susceptible individuals.

Additionally, one study indicates niacin supplementation increases homocysteine levels. In the Arterial Disease Multiple Intervention Trial,¹¹³ niacin supplementation of less than one gram per day increased serum homocysteine levels by 55% over an 18-week period. A similar outcome was noted in an animal study, which took the investigation one step further by adding vitamin B6 to the regimen. B6-supplemented animals showed a reversal of the niacin-induced hyperhomocysteinemia compared to non-B6-supplemented animals.¹¹⁴ Extrapolating from this research, it seems prudent to supplement vitamin B6 if high-dose niacin is to be used for hyperlipidemia treatment. And, since niacin needs to be methylated for its degradation, providing the methyl-donating nutrients betaine, B12, and folate also makes sense.

Homocysteine and Osteoporosis

Homocystinuria due to cystathionine synthase deficiency is an autosomal recessive error of sulfur amino acid metabolism characterized clinically by lens dislocation, mental retardation, skeletal abnormalities, and thromboembolic phenomena.¹¹⁵ Individuals with this enzyme deficiency have increased concentrations of homocysteine and decreased concentrations of cysteine and its disulfide form, cystine. In children with homocystinuria, osteoporosis is a common presenting symptom.¹¹⁶ Because of the role of sulfur compounds in formation of sulfated amino sugars, disturbed cross-linking of collagen has been proposed as a possible mechanism of action. Lubec et al examined 10 patients with homocystinuria. They found synthesis of

collagen was normal; however, they reported a significant reduction of cross-links in the group with homocystinuria.¹¹⁷

Because of the correlation between homocystinuria and osteoporosis in children with this amino acidopathy, and because of the increase in homocysteine concentrations in postmenopausal woman, several authors have implied elevated homocysteine levels contribute to postmenopausal osteoporosis. To date, no evidence is available which demonstrates homocysteine levels are higher in postmenopausal women with osteoporosis than in age-matched controls.

Diagnostic Considerations

Many of the studies referenced herein have used 12-16 $\mu\text{Mol/L}$ as the upper limit of the normal range for homocysteine levels. We probably will see this level drop, as we did with cholesterol testing, since, even within the "normal" range, the relative risk of atherosclerotic cardiovascular disease, and most likely other disease processes, increases as homocysteine levels increase. A number of clinical laboratories currently perform plasma homocysteine determinations, either alone or combined with a cardiovascular panel. Additionally, either elevated creatinine or uric acid levels on a blood chemistry panel might warrant further investigation for elevated homocysteine levels.

A one-time, fasting determination of plasma homocysteine will show hyperhomocysteinemia in clear-cut cases. Other cases will not be uncovered unless the pathways of homocysteine metabolism are stressed, as with the methionine loading test. An oral dosage of 100mg/kg methionine is given, followed by a plasma homocysteine determination six hours later. The methionine loading test might be a more reliable assay, as it can reveal those individuals who would not have hyperhomocysteinemia on a fasting sample.

Conclusion

Elevated homocysteine levels have been confirmed as an independent risk factor for atherosclerotic cardiovascular disease, and are implicated in a number of other vascular, neuropsychiatric, renal, skeletal, perinatal, and endocrine diseases. Supplementation with the nutrient cofactors required for optimal functioning of the methionine/homocysteine metabolic pathways significantly impacts homocysteine levels, and offers a new integrated possibility for primary prevention. Betaine, vitamin B12, folic acid, and vitamin B6 assist in optimizing methyl and sulfur group metabolism and their use might play a significant role in the prevention and treatment of a wide array of clinical conditions. With the current emphasis on homocysteine research (over 1000 articles on homocysteine published in the scientific literature in the past five years) it is very likely that these disease connections will be further confirmed and others will be revealed in the months and years to come.

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