

Homocysteine and Neurologic Disease

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Over the last 10 years, there has been an explosion of interest in homocysteine, a sulfur-containing amino acid that occupies a central location in the metabolic pathways of thiol compounds. This interest is primarily because of the realization that hyperhomocysteinemia is an important risk factor for vascular disease, including stroke, independent of long-recognized factors such as hyperlipidemia, hypertension, diabetes mellitus, and smoking. Since elevated homocysteine levels can often be normalized by supplementing the diet with folic acid (folate), pyridoxine hydrochloride (vitamin B₆), and cyanocobalamin (vitamin B₁₂), these observations raise the exciting possibility that this inexpensive and well-tolerated therapy may be effective in decreasing the incidence of vascular disease. In addition to its association with cerebrovascular disease, homocysteine may play a role in neurodegenerative disorders, even if only as a marker of functional vitamin B₁₂ deficiency. Homocysteine is also important to neurologists since most anticonvulsants raise homocysteine levels, an effect that may explain the teratogenic effects of these drugs. Practical knowledge concerning some details of homocysteine metabolism, the diagnosis of hyperhomocysteinemia, and the use of polyvitamin therapy to lower homocysteine levels will be increasingly important in the treatment of patients with neurologic disease.

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HOMOCYSTEINE AND NEUROLOGIC DISEASE

In 1969, McCully¹ reported the autopsy findings of a 7-week-old infant who died as a result of very high plasma levels of homocysteine, due to a rare defect in the *cbLC* gene in the vitamin B₁₂ (cyanocobalamin) metabolic pathway. At autopsy, the infant's arteries exhibited advanced atherosclerotic lesions, with pathologic features that reminded McCully of vascular lesions seen in children with homocystinuria due to cystathionine β-synthase (CBS) deficiency. Since the 2 inborn errors of metabolism had in common marked elevations in homocysteine levels but differed in the concentration of methionine (low in *cbLC* but high in CBS deficiency) and cystathionine (high *cbLC* but low in CBS deficiency), McCully hypothesized that "the arterial damage found in association with both diseases is attributed to the meta-

bolic effects of increased concentrations of homocysteine, homocystine, or a derivative of homocysteine."¹ This hypothesis was largely ignored for 20 years, but during the last decade, a wealth of epidemiologic evidence has made it evident that even moderate elevations in plasma homocysteine levels, in the range of 15 to 20 μmol/L, are a common and an important risk factor for vascular disease.²⁻⁶ Since elevated homocysteine in principle is more readily treatable (with vitamin supplements) than other vascular risk factors (such as diabetes mellitus, hypertension, hyperlipidemia, and smoking, which often require pharmacologic intervention), these observations have generated much excitement in the medical community as well as in the lay press and the Internet.⁷

The realization that homocysteine is a risk factor for vascular disease coincided with observations initially made by Lindenbaum et al⁸ that homocysteine (along with methylmalonic acid) was a sensitive indicator of subtle vitamin B₁₂ deficiency in patients presenting with dementia, psy-

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chosis, ataxia, or neuropathy. The likelihood that elevated homocysteine levels play a role in other, more commonly occurring dementias has received attention during the past decade.⁹⁻¹³

The goal of this review is to discuss the features of homocysteine metabolism and the epidemiologic factors that are relevant to clinical neurologists. First, we briefly review homocysteine metabolism and the acquired and genetic conditions resulting in hyperhomocysteinemia. Second, we will evaluate the data linking elevated homocysteine levels to cerebrovascular disease and discuss therapeutic interventions aimed at lowering homocysteine levels. Third, we will review the recent reports linking elevated homocysteine levels with dementia. Last, we will briefly review the importance of homocysteine metabolism in patients with epilepsy.

HOMOCYSTEINE METABOLISM

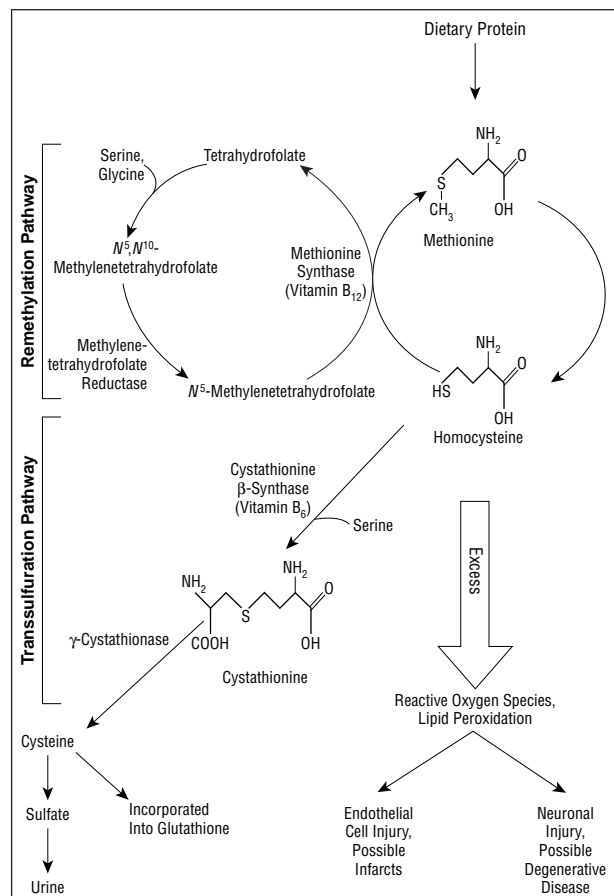
Homocysteine is a thiol-containing amino acid that is formed by demethylation of methionine. Homocysteine is metabolized by 2 major pathways: remethylation and transsulfuration (Figure). In the remethylation cycle, homocysteine is converted back to methionine by a reaction catalyzed by methionine synthase (MS). This enzyme requires vitamin B₁₂ as a cofactor and uses N⁵-methyltetrahydrofolate as the methyl donor. The remethylation process is primarily responsible for setting basal homocysteine levels.¹⁴ Under conditions in which an excess of methionine is present or if cysteine synthesis is required, homocysteine enters the transsulfuration pathway. Homocysteine first condenses with serine to form cystathionine, a reaction catalyzed by CBS, an enzyme that uses vitamin B₆ (pyridoxal phosphate) as a cofactor. Cystathionine is subsequently hydrolyzed to form cysteine, a reaction catalyzed by γ-cystathionase. Cysteine can in turn be incorporated into glutathione or further metabolized to sulfate and excreted in the urine.

Several technical issues of practical importance to clinicians need discussion here. First, homocysteine should be measured in blood samples that are refrigerated immediately after collection, since levels markedly increase if whole blood is maintained at room temperature for several hours.¹⁵ Second, the timing of sample collection is important. While homocysteine levels are usually obtained in the fasting state, another approach is to measure elevations in homocysteine levels after an oral methionine dose. This method is more cumbersome and has been performed less frequently than the fasting method, but up to 40% of patients with hyperhomocysteinemia have elevated levels only after a methionine load.¹⁶ Since both fasting and postmethionine load (PML) hyperhomocysteinemia are independently associated with an increased risk of atherothrombotic disease,^{17,18} measuring homocysteine in the fasting state alone misses a substantial number of patients for whom it is an important risk factor.¹⁸

FACTORS CONTROLLING HOMOCYSTEINE LEVELS

Vitamin Deficiencies

Elevations in plasma homocysteine levels result from a complex interaction of acquired and genetic factors.



Homocysteine metabolism showing remethylation and transsulfuration pathways and the epidemiologic factors that may occur at elevated homocysteine levels.

Among the acquired factors, quantitatively, the most important is the relative deficiency of folate, vitamin B₆, and vitamin B₁₂. Selhub et al² have estimated that two thirds of the cases of hyperhomocysteinemia (measured in randomly obtained samples) are due to relatively low levels of these vitamins. Epidemiologically, the vitamin with levels that are most inversely correlated to hyperhomocysteinemia is folate.²⁻⁴ Folate consumption has recently increased substantially in the United States. Since January 1, 1998, the US Food and Drug Administration has required that all enriched flour, rice, pasta, cornmeal, and other grain products contain at least 140 µg of folate per 100 g. This level of fortification, which was chosen with the goal of preventing neural tube defects, is estimated to increase the dietary folate intake of most adults by 100 µg/d. How this low level of fortification will affect homocysteine levels in patients at risk for vascular disease is not fully known. Malinow et al,¹⁹ studying patients at risk for vascular disease, found that levels of supplementation in the order of 500 µg/d were required to obtain significant reductions. On the other hand, Jacques et al²⁰ found that foodstuff fortification lowered homocysteine levels by 7% in the general population and that the percentage of subjects with homocysteine levels greater than 13 µmol/L decreased by almost 50%. The effect of folate supplementation of food on PML homocysteine levels has not been studied.

Other Acquired Factors

After vitamin concentrations, the factor most closely associated with elevated homocysteine levels is old age. Homocysteine levels are relatively stable through the first 4 decades of life and then rise sharply, particularly after age 70.^{2,21,22} Male sex is associated with higher homocysteine levels at all ages except in the very old.²¹ Pregnancy lowers homocysteine levels,²³ and estrogen replacement therapy in postmenopausal women results in significantly lowered homocysteine levels.²⁴ Renal insufficiency markedly raises homocysteine levels,²⁵ either through decreased clearance or slower metabolism of homocysteine. Coffee consumption of 4 or more cups per day also raises homocysteine levels,²⁶ while protein consumption of more than 75 g/d lowers fasting homocysteine levels.²⁶ Drugs such as methotrexate, 6-azauridine, nicotinic acid, and bile acid sequestrants cause elevations in homocysteine levels.^{27,28} Of particular importance to neurologists, intrathecal methotrexate has been associated with marked elevations of homocysteine levels in the cerebrospinal fluid, which is associated with therapy-induced leukoencephalopathy.²⁹ Additionally, most classic anticonvulsants raise homocysteine levels,^{30,31} as discussed in more detail below.

Genetic Factors Controlling Homocysteine Levels

Severe homocysteinemia with homocystinuria was first identified in cases of rare inborn errors of metabolism characterized by marked elevations of plasma and urine homocysteine concentrations. The most common of these is the deficiency of CBS, the homozygous form of which occurs in approximately 1 in 200 000 live births and is associated with fasting plasma homocysteine concentrations of up to 200 $\mu\text{mol/L}$. Clinical manifestations include mental retardation, thromboembolism, seizures, premature atherosclerosis, skeletal deformities, and ectopia lentis.³² The heterozygote state is estimated to occur in 1% to 2% of the population,¹⁸ and these patients have mild elevations of fasting homocysteine (usually 20-30 $\mu\text{mol/L}$), but recent epidemiologic studies suggest that they are at increased risk for premature atherosclerosis.¹⁸

Homozygous deficiency of N^5,N^{10} -methylene tetrahydrofolate reductase (MTHFR) is rare and results in severe hyperhomocysteinemia and early death.³³ A much more common mutation in the *MTHFR* gene is a C-to-T transition at codon 677 that substitutes a valine for an alanine. This polymorphism is common, and the prevalence of homozygotes is 5% to 10%, at least in white populations. Patients homozygous for the *C677T* mutation have slight elevations in homocysteine levels and are at increased risk for premature vascular disease.^{34,35} Recent observations suggest that patients with the *V/V* MTHFR genotype have higher folate requirements than individuals with a normal genotype.

HOMOCYSTEINE AND VASCULAR DISEASE

Since the initial hypothesis by McCully¹ 30 years ago, much epidemiologic evidence from prospective cohort,

cross-sectional, and case-control studies, encompassing more than 100 studies involving more than 12 000 patients, has confirmed the relationship between elevated homocysteine levels and vascular disease.^{36,37} Although the precise pathophysiological link has yet to be elucidated, it is clear that hyperhomocysteinemia is an independent risk factor for coronary artery disease, cerebrovascular disease, peripheral vascular disease, and venous thrombosis.^{5,17,32,36,38,39} It has been estimated that 10% of the vascular disease risk in the general population is due to homocysteine.¹⁷

The prevalence of hyperhomocysteinemia is almost twice as high when based on homocysteine measurements taken after a methionine load as when based solely on fasting levels.³² Both fasting and PML hyperhomocysteinemia are independently associated with increased risk of vascular disease in case-control studies.^{17,32} While fasting hyperhomocysteinemia was related to low plasma levels of folate and vitamin B₁₂, there was no such relationship for abnormal increases in homocysteine levels after a methionine challenge.

Hyperhomocysteinemia appears to be more closely associated with microvascular stroke. Fassbender et al¹⁰ found that small-vessel cerebrovascular disease, diagnosed clinically by the presence of findings of multi-infarct dementia or gait disorders and the presence of white matter lesions on computed tomographic or magnetic resonance imaging scans, was strongly associated with elevated homocysteine levels, while patients with isolated large vessel disease but without microvascular disease did not have significantly higher homocysteine levels. Evers et al⁴⁰ also found that an elevated homocysteine level was associated with cerebral microangiopathy but not with cardioembolic or macroangiopathic mechanisms.

The pathophysiological mechanisms by which hyperhomocysteinemia causes atherothrombosis are unknown. Several mechanisms have been proposed,^{32,36} a complete review of which is beyond the scope of this review. A leading hypothesis is that homocysteine is rapidly oxidized to homocystine, mixed disulfides, and homocysteine lactone when released into plasma, a reaction that produces reactive oxygen species, which have been implicated in the development of atherosclerosis. Importantly, some of these toxic products are also believed to play a role in the pathogenesis of neurodegenerative diseases.⁴¹ Of particular importance to neurologic disease is that homocysteine⁴² and its metabolite homocysteic acid⁴³ are potent agonists for *N*-methyl-D-aspartate receptors and are neurotoxic to cultured neurons at concentrations that are likely reached in the central nervous system after breakdown of the blood-brain barrier.

EFFECTS OF VITAMIN THERAPY IN LOWERING HOMOCYSTEINE LEVELS

There has been much interest in using polyvitamin therapy to lower homocysteine concentrations in the general population and in patients at risk for vascular disease. A recently published meta-analysis of such 12 randomized trials⁴⁴ concluded that daily intake of multivitamin preparations containing folic acid, 0.5 to 5.0 mg, and cyanocobalamin, 0.5 mg, would lower homocysteine con-

centrations by approximately one third. These findings are encouraging, since they indicate that hyperhomocysteinemia is reversible by an inexpensive and well-tolerated therapy. Some have argued that isolated folic acid supplementation may mask hematologic signs of vitamin B₁₂ deficiency,⁴⁵ potentially delaying therapy for other serious complications, such as neuropathy and dementia. This theoretical concern, which has not been confirmed in practice, would be obviated if cyanocobalamin, 0.1 to 1.0 mg, were included in the vitamin supplements.⁴⁴

These observations have prompted the use of vitamin therapy for prevention of cerebrovascular disease in randomized trials. Two of these trials are ongoing in North America. The first of these is the Vitamin Intervention for Stroke Prevention trial, a multicenter, double-blind, randomized trial that seeks to enroll 3600 patients. It is attempting to determine whether high-dose multivitamins reduce the risk of recurrent cerebral or myocardial infarction in hyperhomocysteinemic patients who have had a nondisabling stroke. The second trial started in early 1998. The Trials of Prevention of Cognitive Decline in Women, part of the Women's Health Study, have randomized 3445 healthy women 65 years of age or older to receive a multivitamin containing folic acid, pyridoxine hydrochloride, and cyanocobalamin or placebo. Investigators plan to assess cognitive function at 2-year intervals by a telephone interview. Other large, controlled clinical trials studying the effectiveness of homocysteine-lowering therapy in vascular disease are under way in Europe and Australia.³⁷ Results of these well-designed trials will add important information to this critical issue.

HOMOCYSTEINE AND NEURODEGENERATIVE DISEASES

The association of vitamin deficiency with dementing illnesses has been noted for more than 50 years.⁴⁶ Severe deficiency of vitamin B₁₂ causes confusion, dementia, and neurologic damage, such as myelitis and peripheral neuropathy, a syndrome that has been called *subacute combined degeneration*.⁴⁷ The study by Lindenbaum et al⁸ established that in patients with subtle vitamin B₁₂ deficiency and neuropsychiatric symptoms, such as dementia, personality changes, psychosis, and ataxia, homocysteine levels (as well as methylmalonic acid levels) were a sensitive marker of vitamin B₁₂ deficiency. Folate deficiency was also associated with dementing and neuropsychiatric illnesses early on.⁴³ In the mid-1980s, several studies also found that patients with Alzheimer-type dementia as well as other dementing illnesses frequently had low serum vitamin B₁₂ and/or folate levels,^{46,48,49} an association that grew stronger when hyperhomocysteinemia was used as a surrogate for vitamin B₁₂ deficiency.^{9,50} More recently, 2 large, population-based studies of healthy older adults, one done in the 1980s (subjects were men and women >60 years old)⁵¹ and the second done in the 1990s (subjects were men between the ages of 54 and 81 years),⁵² revealed an association of subtle but significant deficits in neurocognitive tests with low levels of vitamins B₁₂, vitamin B₆, or folate. In the second study,⁵² homocysteine levels were measured and were found to be a stron-

ger predictor of poor performance than low vitamin concentrations. The association between homocysteine and poor cognitive performance could not be explained by clinical diagnosis of vascular disease in their population of healthy elderly subjects. A recent pathologic analysis of the Alzheimer disease (AD) Nun Study⁵³ found that low plasma folate levels were correlated with the severity of neocortical atrophy.

Three recent case-control studies, 2 from the United Kingdom^{11,12} and 1 from Sweden,¹³ have reported a correlation between AD and high homocysteine levels. The most rigorous of these studies was by Clarke et al,¹¹ who evaluated 164 AD patients referred to the Oxford Project to Investigate Memory and Ageing, 76 of whom had pathologic confirmation of the diagnosis AD. They found a significant difference ($P < .05$) in the distribution of total homocysteine levels between AD patients and age-matched controls, which became more significant ($P < .001$) if only pathologically confirmed cases were analyzed. The odds ratio for AD in subjects with homocysteine concentrations in the upper third percentile was 2.0 and was as high as 4.5 when only the pathologically confirmed cases were analyzed. These findings indicate that the association between elevated homocysteine levels and dementia is not simply a reflection of increased risk of multi-infarct dementia.

Homocysteine may also be an important factor in Parkinson disease. Kuhn et al^{54,55} found a 2-fold elevation of plasma homocysteine concentrations in patients treated with levodopa compared with untreated parkinsonian patients and age-matched controls. Hyperhomocysteinemia in this setting probably results from the increased production of S-adenosylhomocysteine during the metabolism of levodopa by catechol-O-methyltransferase. Whether levodopa-induced homocysteine elevation is associated with increased risk of vascular disease or cognitive decline in parkinsonian patients remains to be established.

HOMOCYSTEINE IN EPILEPSY

Systemic administration of high doses of homocysteine in animals produce convulsive seizures,^{56,57} a fact that has been exploited in models of experimental epilepsy. Furthermore, up to 20% of patients with homozygous CBS deficiency have seizures,⁵⁸ and the high plasma concentrations of homocysteine in these patients (usually 50-200 $\mu\text{mol/L}$) may contribute to epilepsy.⁵⁹ Whether less severe hyperhomocysteinemia (15-20 $\mu\text{mol/L}$) predisposes patients to epilepsy has not been established.

Homocysteine relates to 2 additional important issues in the management of patients with epilepsy. First, most anticonvulsants lower plasma folate levels,^{60,61} and as a result, almost half of patients treated with anticonvulsants had homocysteine levels sufficiently elevated to put them at high risk for vascular disease.^{30,31} Arteriosclerosis is an important issue for patients requiring long-term anticonvulsant therapy, particularly given the rising incidence of epilepsy in older age groups. The effectiveness of polyvitamin therapy in lowering homocysteine levels in the setting of anticonvulsant use has not been directly studied.

A second issue relates to putative teratogenic effects of high homocysteine levels. There is an increased risk of major congenital malformations in children whose mothers receive anticonvulsants during the first trimester.⁶² While the mechanism of teratogenicity in folate deficiency is unclear, recent data implicate elevations in homocysteine.⁶³⁻⁶⁵ First, fasting or PML hyperhomocysteinemia is commonly found in women who have given birth to infants with neural tube defects.^{66,67} Second, the C677T mutation in the MTHFR gene significantly increases the risk of neural tube defects.^{68,69} Finally, amniotic fluid homocysteine levels were found to be significantly higher in pregnancies complicated by neural tube defects.⁷⁰ Observations such as these led to a practice parameter recently promulgated by the American Academy of Neurology, recommending that all women of childbearing potential who are taking anticonvulsants consume at least 0.4 mg/d of folic acid.⁶² Whether this or higher doses of folic acid are effective in lowering homocysteine levels or in decreasing the incidence of neural tube defects in epileptic women has not been studied. It is also unclear whether cyanocobalamin and pyridoxine hydrochloride supplements are necessary for this population.

CONCLUSIONS AND PRACTICAL APPLICATIONS

There is strong epidemiologic evidence indicating that elevated plasma homocysteine levels constitute an important risk factor for vascular disease, including ischemic stroke. Hyperhomocysteinemia appears to be independent of other factors, such as smoking, hypertension, diabetes mellitus, and hyperlipidemia, and since homocysteine levels can usually be lowered by polyvitamin therapy, which is inexpensive, well tolerated, and perceived to be "natural," this association has attracted much attention in the medical as well as the lay community. Neurologists must also consider the relationship of homocysteine levels to dementing illnesses and to anticonvulsant use as well as to the documented usefulness of homocysteine measurements as a sensitive indicator of functional vitamin B₁₂ deficiency in neuropsychiatric diseases. The efficacy of polyvitamin therapy in these settings is less well established, and there is a need for clinical research in this area.

Given the intense public interest in medicine and nutrition, many patients will not wait for definitive studies, and practicing neurologists will be faced with the need to give advice based on limited information. The first practical question relates to when homocysteine levels should be measured in neurologic practice. It seems reasonable to measure homocysteine levels in patients presenting with cerebral infarction, especially microvascular infarction, at a young age or when other cerebrovascular risk factors are not prominent. This information may refine risk assessment and raises the question of whether vitamin therapy should be prescribed for patients with high homocysteine levels. Answers to the latter question must await the results of randomized clinical trials that are currently under way. While some have argued in favor of prescribing multivitamin supplements without measuring homocysteine levels, the effectiveness of that

practice is unknown, and some patients may require higher than normal vitamin doses to normalize homocysteine concentrations. These issues and others will be the focus of intense clinical investigations over the next decade.

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