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Homocysteine and Vascular Disease

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Homocysteine is a naturally occurring, sulfur-containing amino acid. Continuously formed and catabolized in vivo, its metabolism is dependent on a complex interaction of genetics and physiology (Fig. 1). Its relevance is based on the increasing recognition of the correlation between elevated levels of homocysteine and human disease.

Epidemiology

The clinical relevance of hyperhomocysteinemia (HHCY) was first recognized in the 1960's in children with homocystinuria.[1-3] This rare genetic disorder is manifest genetically by homozygous deficiencies in one of three key enzymes in the metabolism of homocysteine, with resultant increases in plasma homocysteine and urinary excretion of its metabolite homocystine. Clinical manifestations include severe atherosclerotic and thromboembolic disease. Histopathologically, this vascular disease is characterized by vascular endothelial injury,[4,5] vascular smooth muscle proliferation,[6,7] progressive arterial stenosis[8] and hemostatic changes consistent with a prothrombotic state.[9]

The relevance of these findings to non-homocystinuric individuals was initially unclear. Homocystinuria is characterized by elevations of homocysteine to a degree not seen in the general population (Fig 2). However, numerous epidemiologic studies have subsequently provided compelling evidence for an association between mild to moderate elevations of homocysteine and the development of premature coronary, cerebral and peripheral atherosclerosis and thromboembolic disease.[10-18] In 1991, Clarke et al identified HHCY as an independent risk factor for the development of coronary artery disease in a sub-study of the Physicians Health Study.[12]

An important meta-analysis by Boushey et al[19] in 1995 further quantified the magnitude of risk. In their analysis of all major studies available at that time, they found a linear, independent risk for increments in homocysteine. There were no levels above or below which an incremental rise in homocysteine did not affect cardiovascular risk. Specifically, every 5 µmol/L increment in homocysteine was found to be associated with odds ratios of 1.6 for men; (95% CI 1.4-1.7) and 1.8 for women; (95% CI 1.3-1.9) for coronary artery disease. The odds ratios were also increased for other common vascular disorders:1.5 for cerebrovascular

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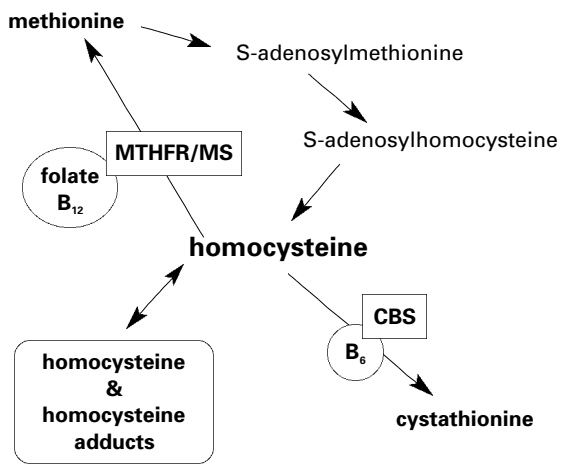
Figure 1

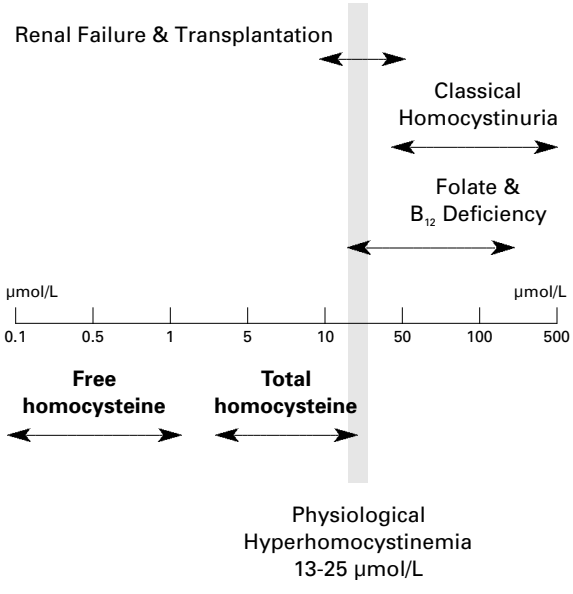


disease, and 6.8 for peripheral vascular disease. Similar odds ratios have been noted for the development of venous thromboembolism.[17]

These numbers suggest that every 5 µmol/L decrement in plasma homocysteine would be equivalent to the benefit of a 0.5 mml/L decrement in total serum cholesterol. On a population scale, at least 10% of male and 6% of female coronary artery disease-related deaths are directly attributable to elevations of homocysteine.

These numbers become even more significant if viewed from the perspective of life-years lost. HHCY is clearly associated with early disease onset, particularly in those with positive family histories.[8,17,20,21] Thus the burden of disease to society is likely even greater than suggested by the Boushey study.

Figure 2



Prevalence and Genetics of Hyperhomocysteinemia

In unselected populations, fasting plasma homocysteine values are not normally distributed, but show an upward or positive skew.[22] This skew is consistent with the presence of one or more subpopulations with elevated plasma homocysteine (Fig 2). Factors responsible for the distribution of plasma homocysteine can be genetic, physiologic or pathologic (Table 1).

Inherited mutations in one of two enzymes are important determinants of homocysteine metabolism in the general population.

Cystathionine beta synthase (CBS) catalyzes the reaction taking homocysteine to cystathionine. This enzyme requires pyridoxine as a co-factor and is an integral part of the *transsulfuration* or *pyridoxine-dependent* pathway. 33 distinct mutations have been identified,[23] with heterozygosity occurring at a prevalence of 0.5-1.5%.[3] The majority of heterozygotes will have normal fasting homocysteine levels, but can be detected with a methionine load test.[3,24] While the data is still preliminary, elevated post-methionine load homocysteine concentrations alone appear to confer significant risk.[25] This may be related to in vitro experiments which suggest that reduced enzymatic activity at the cellular level is associated with increased susceptibility of homocysteine-induced damage.[26,27]

Methylenetatrahydrofolate reductase (MTHFR) is an enzyme involved in the remethylation of homocysteine to methionine. This enzyme is folate and cobalamin (vitamin B_{12}) dependent. Logically, this pathway is known as the *remethylation* or *folate-dependent* pathway. One common and nine rare mutations of MTHFR have been identified. The common variant is known as thermolabile MTHFR or tMTHFR.[28] This variant is present in heterozygous form in 49% and homozygous form in 11% in an unselected Toronto population (D.E.C.Cole,

personal communication). A similar prevalence has been found in an urban Montreal population.[29] In Italy, 15.1% have been found to be homozygous for this variant, while 29% of those with evidence for occlusive vascular disease are homozygous.[30] The relationship between this genotype and homocysteine levels is variable and appears to be related to folate sufficiency. In particular, HHCY occurs in those individuals whose serum folate is less than the population median value.[31]

Table 1
Selected Determinants of Plasma Homocysteine*

1. **Genetic**
   - Cystathionine-beta-synthase: heterozygote mutations 0.5-1.5% {451}
   - Methionine synthase: rare
   - MTHFR: heterozygote mutations approximately 50% {403}
2. **Physiologic**
   - age: Hcy increases with increasing age {336}
   - sex: pre-and post-menopausal women have lower levels than men {247}
   - diet: related to methionine and vitamin cofactor (folate, vitamins B_6 and B_{12}) intake {437}
   - alcohol: relationship unclear {375}
3. **Pathologic**
   - vitamin deficiency: increased homocysteine concentrations {10}
   - renal disease: increase correlated with increasing serum creatinine {81}
   - transplantation: increased levels {149, 435}
   - post stroke: transiently decreased levels {341}
   - severe psoriasis: elevated levels {438}
4. **Medications**
   - oral contraceptives/hormone replacement: decreased levels {269}
   - corticosteriods: increased {159}
   - cyclosporine: increased {393}
   - smoking: increased {336}

*selected references noted for each determinant

Physiologic Factors

The concentration of homocysteine in men is approximately 25% higher than that seen in premenopausal women.[32] This difference decreases post-menopause but does not disappear.[18] Hormone replacement therapy may reduce serum homocysteine levels.[33] and there is a strong negative correlation between estradiol levels and homocysteine in post-menopausal women.[34] In pregnancy, the progressive decline in total homocysteine is largely a function of the physiologic reduction in circulating albumin, the principle reservoir of bound homocysteine.[35]

Smoking, increased age, and sedentary lifestyle have all been associated with higher homocysteine levels.[18] In particular, increasing age is associated with decreased cystathionine beta synthase activity.[36] The role of alcohol is unclear.[18,37]

Diet plays a significant role in homocysteine levels. Serum levels of homocysteine are directly related to methionine intake, and inversely related to vitamin intake.[38-40] In general diets rich in animal proteins have significantly higher methionine content than those rich in plant derived proteins.[41] In addition, high intake of fresh fruit, vegetables as well as vitamin supplements have been associated with decreased homocysteine levels.[18,38,39]

Vitamin Insufficiency

There is a clear relationship between vitamin insufficiency and homocysteine levels. Particularly in the elderly, homocysteine levels show a strong inverse relationship with vitamin B_{12} intake. Several studies confirm a high frequency of clinically silent B_{12} deficiency in this subpopulation, irrespective of gender.[42-47] In younger populations, the strongest correlation is with folate, although the elderly are also at risk for folate deficiency.[47] At intakes of less than 0.35 mg/d, increases in homocysteine levels are inversely proportional to decreases in intake.[45,48] This is not generally accompanied by signs of megaloblastic anemia, reflecting the fact that homocysteine is the most sensitive indicator of subclinical folate or B_{12} deficiency.[49-51]

Renal Failure

Homocysteine is strongly correlated with serum creatinine and renal glomerular filtration.[52-54] However, the mechanisms of homocysteine accumulation in renal failure are still not entirely clear.

A significant contributor to HHCY in chronic renal failure is likely the loss of renal catabolic capacity. With normal renal function, virtually all of the protein-bound homocysteine remains in the vascular space, and the filtered homocysteine is reclaimed by the proximal tubule. Animal experiments suggest that the intact kidney is responsible for considerable catabolism of this filtered homocysteine.[55]

Another significant factor in the maintenance of HHCY in chronic renal failure can likely be attributed to an effective cofactor deficiency. Activation of folic acid to a biologically active form requires the sequential addition of glutamyl residues to form folylpolyglutamates.[56] The length of these residues are controlled by the balance of activity between folate conjugase, which cleaves glutamyl residues, and folyl-poly-gamma-glutamate synthetase, which adds residues. Short chain folylpolyglutamates have the greatest biologic activity, while their long-chain relatives actually inhibit enzyme activity. In chronic renal failure, as yet undifferentiated inhibitors of folate conjugases have been identified. The resultant accumulation of long-chain folylpolyglutamates has been postulated as a major cause of hyperhomocysteinemia in chronic renal failure, and the reason for the requirement of high dose folate supplementation to bring levels toward normal in these patients.[57]

Transplantation

In both the renal and cardiac transplantation population, homocysteine levels are consistently noted to be elevated.[58-61] Decreased renal function appears to play a significant role as do decreased folate levels.[58-60] In addition, elevations of homocysteine have been correlated with the dose of cyclosporine,[58] and the cumulate dose of corticosteroids.[61]

Mechanisms of Disease

Investigations into the mechanisms of homocysteine-induced vascular disease have focused mainly on the vascular endothelium, though evidence of alterations in the arterial intima and media, lipid abnormalities and the development of a thrombogenic state have also been reported.

Endothelial dysfunction is common in individuals with HHCY.[62,63] Endothelial cell damage is felt by many investigators to be due to direct toxic injury by hydrogen peroxide,[64-67] which is generated from oxygen in a reaction catalyzed by homocysteine.[65] In cultured endothelial cells, homocysteine requires transition metal ions, Fe^{3+} or Cu^{2+}, to generate hydrogen peroxide.[67] These reactions could help to explain the correlation between body iron stores and the prevalence of vascular disease.

At the level of the arterial wall, homocysteine induces smooth muscle proliferation,[6,68] likely through the activity of a metabolite, homocystic acid. Homocysteine also increases sulfated glycosaminoglycans in the arterial intima, resulting in decreased solubility and increased aggregation of extracellular matrix, the binding of LDL, and increased calcification.[41] Finally, homocysteine-induced endothelial damage promotes the adherence of platelets with the release of platelet-derived growth factors.

Lipid abnormalities in HHCY include elevated plasma triglycerides,[69] and possibly increased susceptibility to oxidation of LDL.[41]

Potentially thrombogenic abnormalities associated with elevation of homocysteine include activation of Factor V, increased prothrombin activation of Factor Xa, inhibition of protein C activation, inhibition of cell surface expression of thrombomodulin,[70] and a decrease in tPA specific binding sites with a resultant 60% decrease in cell-associated tPA activity.[71]

Treatment Options: Folate

In the absence of renal failure, transplantation or severe enzymatic deficiencies, supplementation of folic acid 1 mg/d is likely adequate to afford significant reduction in fasting plasma homocysteine.[19,72] Higher doses of up to 15 mg/d have been utilized with the absence of significant adverse effects.[57,73-76]

In the setting of renal failure, significant HHCY may persist despite "low" dose supplementation. Progressively higher doses up to 15 mg/d are associated with progressive reductions in homocysteine.[57,75,76]

Theoretically, folate supplementation in the presence of undetected vitamin B_{12} deficiency could lead to the masking of hematological signs of pernicious anemia. Fears have been raised that population-based supplementation of folate could lead to an increased incidence of neurologic and/or psychiatric damage.[77] There are no recent data to support this concern. Subclinical vitamin B_{12} deficiency can be detected through elevations of methylmalonic acid,[78,79] and oral supplementation of vitamin B_{12} to maintain adequate levels is readily achievable.

Treatment Options: Pyridoxine

Pyridoxine is a cofactor for cystathionine-beta synthase. Abnormalities in this pyridoxine-dependent or transsulfuration pathway result in abnormal post-methionine load homocysteine levels, but usually normal fasting levels. Predictably, pyridoxine supplementation has little effect on fasting homocysteine levels,[75] but reduce post-methionine load elevations by up to 50%.[73]

Massive and prolonged pyridoxine ingestion has been associated with sensory and motor neuropathies. However, there have been no reports of neuropathy associated with long-term pyridoxine supplementation of less than 200 mg/d.[80]

Treatment Options: Vitamin B_{12}

Methylcobalamin or vitamin B_{12} is a cofactor for methionine synthase.[81] Japanese patients with diabetes mellitus and hyperhomocysteinemia were shown to respond to parenteral methylcobalamin with reductions in fasting homocysteine.[82] Otherwise there is little data to support the role methylcobalamin in reducing plasma homocysteine.

There are no significant safety concerns regarding methylcobalamin supplementation directly. It has been suggested as a concomitant therapy for those receiving folate supplementation to avoid subclinical vitamin B_{12} deficiency.[77]

Indications for Homocysteine Determination

At present time, comprehensive guidelines do not exist to direct clinicians in determining eligibility for homocysteine or genetic analysis. Indeed, until further data is obtained, such guidelines will necessarily be provisional.

However, a strong family history of premature vascular disease is clearly of concern both to the patient and caregiver. Provocative evidence has been presented suggesting that HHCY may be at least partially responsible for the increased risk in this population. To date, prospective longitudinal studies have not been performed to demonstrate a reduction in risk via the reduction of plasma homocysteine. However, plausible data exists suggesting that vitamin therapy should be of benefit. Given the potential benefits and near absence of significant concerns surrounding treatment, determination of fasting homocysteine levels are likely indicated in patients with a strong family history, or indeed early manifestation of vascular disease.

Summary

Homocysteine can no longer be considered an exotic metabolite of interest primarily to biochemists and geneticists. On a population scale, HHCY is responsible for a significant degree of morbidity and mortality. In the individual with elevated homocysteine, its presence confers significantly increased risk for early and severe vascular disease. Prospective, longitudinal trials are urgently needed to determine the efficacy of homocysteine-lowering treatment in the prevention of vascular disease. In the meantime, physicians and patients will have to weigh the potential though unproven benefits of treatment against the near absence of significant recognized side effects.

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Upcoming Scientific Meetings

25-29 September 96
The Royal College of Physicians and Surgeons of Canada 65th Annual Meeting
Halifax, Nova Scotia
(Royal College of Physicians and Surgeons of Canada)
Tel.: 613-730-8177

3-5 October 96
New Aspects of Drug Therapy in Cardiovascular Disease
Sophia Antipolis, France
(European Congress Organization)
Tel.: 33 92 94 76 00

19-22 October 96
1st European Research Conference on Blood Pressure and Cardiovascular Disease
Noordwijkerhout, The Netherlands
(Leeuwenhorst Congress Center)
Tel.: 31 2523 78888

24-26 October 96
New Techniques and Concepts in Cardiology
Washington, DC, USA
(American College of Cardiology)
Tel.: 301-897-5400

29 October-2 November 96
Canadian Cardiovascular Society 1996 Annual Meeting
Montreal, Quebec
(Canadian Cardiovascular Society)
Tel.: 604-681-5226

31 October-1 November 96
The Second Triennial Brigham Cardiac Valve Symposium
Boston, Massachusetts, USA
(Harvard Medical School)
Tel.: 617-432-1525

10-13 November 96
69th Scientific Session of the American Heart Association
New Orleans, Louisiana, USA
(American Heart Association)
Tel.: 214-706-1511

Abstracts of Interest

Serum total homocysteine and coronary heart disease in middle-aged British men.

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Serum total homocysteine (tHcy) levels are inversely associated with dietary intake of folic acid and B vitamins. Raised tHcy levels have been linked with coronary heart disease (CHD). We have examined the association between tHcy concentration and the subsequent risk of CHD, using a nested case control study design, within a prospective study of cardiovascular disease in British men. tHcy concentration was measured in serum samples, stored at entry to the study, from 110 incident cases of myocardial infarction and 118 controls. Cases were randomly sampled from events which occured after the first five years of follow-up. Cases and controls were frequency matched by town and age group. Levels of homocysteine [geometric mean (95% CI)] were significantly higher in cases than controls: homocysteine 13.5 (12.6 - 14.3) µmol/L vs 11.9 (11.3 - 12.6) µmol/L; p=0.005. There was a graded increase in the relative risk (odds ratio; OR) of CHD in the 2nd, 3rd and 4th quartile of tHcy (OR 1.4, 1.9, 2.2; trend p=0.006) relative to the first quartile. Adjustment for age, town, social class, body mass index, smoking, physical activity, alcohol intake, hypertensive status, serum cholesterol, and serum creatinine did not attenuate this association, (OR 2.1, 2.3, 2.7; trend p=0.04). tHcy levels were higher at baseline in men with evidence of pre-existing CHD and (as expected) adjustment for this factor attenuated the linear association between tHcy and subsequent events, trend p=0.07. The findings suggest that homocysteine is an independent risk factor for CHD with no threshold level.

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Homocysteine and Coronary Atherosclerosis

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The conventional risk factors for premature coronary artery disease include smoking, hyperlipidemia, hypertension, diabetes and a positive family history. However, many patients have precocious atherosclerosis without having any of these standard risk factors. Identification of other markers that increase the risk of coronary disease may improve our understanding of the pathophysiologic mechanisms of this disorder and allow the development of new preventive or therapeutic measures. An elevated plasma homocysteine level has recently received greater attention as an important risk factor for vascular disease, including coronary atherosclerosis. This review discusses the biochemistry of homocysteinee and the related metabolic importance of folate, vitamin B_6 (pyridoxine) and B_{12} (cobalamin) as well as a number of essential enzymes. The major factors that influence homocysteine concentration are genetic, nutritional and pathologic. The natural history, characteristic pathology and pathophysiology of homocystinuria, a syndrome of abnormal homocysteinee metabolism, are examined as the paradigm of thromboembolic disease and high circulating homocysteine concentrations. Additionally, there is a large body of experimental and clinical evidence for high plasma homocysteine to be a risk factor for vascular disease, including coronary atherosclerosis. Most important, recent research has demonstrated interventions capable of reducing plasma homocysteine, and further research will be needed to determine their impact on mortality and morbidity associated with cardiovascular disease.

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