

Homocysteine and Atherosclerotic Disease: The Epidemiologic Evidence

S MSaw,*^{MBBS, MPH, PhD}

Abstract

Homocysteine is a sulphur-containing amino acid formed during metabolism by one of two pathways by remethylation and transsulfuration. Altered homocysteine metabolism may be implicated as a factor in atherosclerosis, cerebrovascular disease or peripheral vascular disease. It is postulated that homocysteine may damage endothelial cells or acts as a direct causal factor in the thromboembolic process. Several studies have reported that there are a number of factors that may influence levels of homocysteine in humans. Serum homocysteine levels may be associated with low levels of folate, vitamin B6 and vitamin B12. These studies showed that serum homocysteine levels were higher in men and older adults, and some showed that there was a direct relationship between homocysteine and cigarette smoking, diabetes, obesity, and hypertension. Subjects who consume larger amounts of coffee were also noted to have higher serum homocysteine levels. Several cross-sectional, case-control, and cohort studies have linked homocysteinaemia with cardiovascular disease morbidity and mortality. In the Framingham Heart Study, the cohort study in Tromsø, Norway, and the Atherosclerosis Risk in Communities (ARIC) Study, homocysteine levels were found to be higher in adults with asymptomatic or symptomatic coronary artery disease. In the British Regional Heart Study, homocysteine levels were found to be significantly higher in patients with stroke. Thus, there are suggestions that vitamin therapy and alteration of lifestyle habits such as cigarette smoking may lower homocysteine levels. There may be less coronary heart disease morbidity and mortality with lower homocysteine levels.

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Introduction

Homocysteine is a thiol-containing amino acid formed during the metabolism of methionine. The metabolic fate of homocysteine may be influenced by the alterations in the activities of serum folate, pyridoxine and cobalamin.¹ Therefore, a wide variation of serum homocysteine levels have been observed depending on the vitamin supplementation intake of the patients. McCully² reported about 25 years ago that a child dying of homocystinuria, and cystathioninuria secondary to abnormal cobalamin metabolism exhibited arterial lesions. There was thus some suggestion that markedly elevated plasma homocysteine concentrations may be responsible for premature occlusive vascular disease. Some individuals may have a rare genetic form of homocystinuria which is associated with thromboembolic complications at an early age which includes thrombosis of arteries, veins and sinuses. The individuals may also have some form of mental retardation and other abnormalities resembling Marfan's syndrome. Early observations of children with defects in homocysteine metabolism and rapidly progressing arteriosclerotic disease suggest a possible atherogenic effect of homocysteine. Recent studies

have demonstrated that homocysteine damages endothelial cells and stimulates growth of cultured smooth muscle cells.³

Evidence from epidemiologic studies have supported a possible association between homocysteine levels and vascular events such as coronary heart disease as well as stroke incidence and mortality.⁴⁻⁷ It has been postulated that homocysteine acts as a direct causal factor for the arteriosclerotic and thromboembolic processes. There is increasing evidence that homocysteine may affect the coagulation system and resistance of endothelium to thrombosis. Furthermore, we have found that homocysteine levels may vary with vitamin supplement intake, cigarette smoking, alcohol, and history of medical illnesses such as diabetes mellitus and hypertension.⁸⁻¹⁰ This paper summarises the correlates of homocysteine and the role of homocysteine in the pathogenesis of coronary heart disease.

Serum Levels of Homocysteinaemia

Rapid HPLC (high-performance liquid chromatography) with fluorescence may be used to determine total homocysteine levels.¹¹ Frozen samples of up to 10 years

* Assistant Professor

Department of Community, Occupational, and Family Medicine
National University of Singapore

Address for Reprints: Dr Saw Seang Mei, Department of Community, Occupational and Family Medicine, National University of Singapore, Lower Kent Ridge Road (MD 3) Faculty of Medicine, 16 Medical Drive, Singapore 117597.

may be used as levels do not differ with fresh samples.¹² Several case-control studies of homocysteinuria as a risk factor for vascular disease have reported geometric mean of 10.6 $\mu\text{mol/l}$ in Whites in Boston, a geometric mean of 13.7 $\mu\text{mol/l}$ in middle-aged men in the United Kingdom, and arithmetic mean of 12.7 $\mu\text{mol/l}$ in males in Norway. The geometric means of the controls in the three studies were 9.6, 11.9, and arithmetic mean of 11.3 $\mu\text{mol/l}$, respectively. A WHO project of total serum homocysteine levels in 11 countries (using the same analytical techniques) showed a range of mean plasma homocysteine levels from 7.1 $\mu\text{mol/l}$ in Germany to 10.7 in Kuopio.⁵ Only one Asian country, Japan, was included in the study and the mean plasma homocysteine level was approximately 7.5 $\mu\text{mol/l}$. There is little information on homocysteine levels in other Asian populations where the risks of coronary artery disease may be lower.

Correlates of Homocysteine

Vitamins

There are several studies that support the hypothesis that serum homocysteine levels are associated with vitamin intake.⁸⁻¹⁰ Even though homocysteine may be atherogenic, it may also be a marker of folate and vitamin B6 status. Homocysteine is derived from an amino acid, methionine, which is high in protein rich food. In a case-control study of plasma homocysteine and atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) Study, plasma homocysteine levels were lower among users of vitamin supplement products compared to non-users.⁹ Cold fortified breakfast cereals are strongly correlated with plasma homocysteine levels. Plasma homocysteine levels were inversely associated with intakes of folate, vitamin B6, and vitamin B12. There was also an inverse association of plasma homocysteine with thiamin, riboflavin, calcium, phosphorus and iron. In a study of 349 ambulatory Caucasian men aged 19 to 71 years in South Africa, fasting venous blood samples with ethylenediaminetetraacetic acid (EDTA) as anticoagulant were obtained.¹³ There was a high prevalence of subclinical folate, vitamin B12, and vitamin B6 deficiencies in men with hyperhomocysteinaemia. Seventy-two patients with mild hyperhomocysteinaemia and cardiovascular disease were treated with vitamin B6 plus folic acid therapy for 6 weeks.¹⁴ The postload plasma homocysteine concentration was normalised in 66 of the 72 patients. However, there was no placebo control group and long-term effects were not investigated.

The serum specimens of 1160 adults aged 67 to 96 years from the original Framingham Heart Study cohort were examined. There was a strong inverse association between homocysteine levels and plasma folate, B12, pyridoxal 5'-phosphate (PLP) and age.¹⁵ In the Boston Health Study, 130 cases of first myocardial infarction

and 118 population controls, less than 76 years of age, were evaluated for associations between the cofactors of homocysteine metabolism and the risk of myocardial infarction.¹⁶ There is an inverse association between plasma and intake levels of vitamins B6, B12 and folate with plasma levels of homocysteine, adjusted for age and sex. Folate was the most important determinant of plasma homocysteine levels even in subjects with adequate nutritional status. Homocysteine levels were lower in women. An age and sex adjusted odd ratio of 4.66 (95% confidence interval 1.9-11.4) was observed for individuals with plasma homocysteine levels higher than 80th percentile compared to individuals below 20th percentile.

Other Variables

Other variables that may be related to homocysteinuria include cigarette smoking, diabetes, obesity, and hypertension which are known risk factors for coronary heart disease.⁸ In the ARIC Study, 15,800 men and women between 45 to 64 years were sampled and homocysteine was found to show a positive association with age, systolic and diastolic pressures, waist-to-hip ratio, and fibrinogen with high density lipoprotein (HDL) cholesterol showing an inverse association.¹⁷ One limitation of the inferences is that complex probability sampling techniques were used which may limit potential comparability with the rest of the population. Two hundred forty-four randomly selected residents of two towns in Sweden were investigated for plasma homocysteine and serum folate, B12, and creatinine levels. Homocysteine levels were related to increasing age, and lower serum folate, vitamin B12, and creatinine levels. Users of multivitamins had lower homocysteine levels than non-users (10.5 μmol compared to 13.5 μmol , respectively).¹⁸

Coffee Drinking

Several studies have linked coffee intake to coronary heart disease. In a study of 1040 male medical students who were followed for 28 to 44 years, the relative risk of developing coronary heart disease in students associated with drinking 5 cups of coffee per day was 2.94 (1.27, 6.81) at baseline, 5.52 (1.31, 23.18) for average, and 1.95 (0.86, 4.40) for most recent intake after adjustment for baseline age, serum cholesterol levels, calendar time, and the time-dependent covariates such as number of cigarettes, body mass index and incident hypertension and diabetes.¹⁹ Nygard et al²⁰ reported a positive dose-response relationship between cigarette smoking and coffee drinking with total plasma homocysteine levels in the Hordaland Homocysteine Study cohort, established from April 1992 to April 1993, where 16,175 subjects were given a mailed questionnaire and gave blood specimens. Since coffee intake has been implicated to be associated with coronary heart disease and congenital malformations, a link between coffee intake and homo-

cysteine levels may suggest strategies for future prevention of the health outcomes related to coffee intake.

In summary, hyperhomocysteinaemia may be related to nutritional, genetic, pharmacological, and other factors. Hyperhomocysteinaemia is associated with a low serum PLP (pyridoxal-5-phosphate, coenzyme of vitamin B6), low folic acid, low vitamin B12, and low vitamin B6.¹³⁻¹⁸ Thus, vitamin supplements, which are relatively inexpensive, may lower homocysteine levels. The correlates of homocysteinuria have been examined in the West but the nature of the correlates has not been closely examined in detail in Asia where the levels of specific correlates may be different.

Morbidity and Mortality Associated with Homocysteinaemia

Hyperhomocysteinaemia may result in vascular manifestations as first suggested by Wilcken and Wilcken in 1976.²¹ Homocysteine is a highly reactive amino acid which is toxic to vascular endothelium, can potentiate auto-oxidation of low-density lipoprotein cholesterol and promote thrombosis.^{2,17} Further research on whether there is a threshold concentration of homocysteine that may affect vascular injury is needed.

The evidence for homocysteine concentrations and disease morbidity and mortality is from cross-sectional cohort and case-control studies (Table I). Moghadasian et al⁸ reviewed 25 case-control studies where the homocysteine levels were consistently higher (about 26%) in patients with atherosclerotic disease compared to controls in 23 case-control studies. A meta-analysis of fasting levels of homocysteine estimated an odds ratio to be about 1.8 (95% CI, 1.6-2.0), 2.3 (95% CI, 1.8-2.9), and 6.8 (95% CI, 2.9-15.8) for coronary artery disease, cerebrovascular disease, and peripheral vascular disease, respectively.²²

A cross-sectional study of 1041 elderly subjects (418 men and 623 women, age 67 to 96 years) from the Framingham Heart Study showed that there was a relationship between maximal degree of stenosis of the extracranial carotid arteries and serum homocysteine levels.²³ The odds ratio of stenosis of >25% was 2.0 (95%

CI, 1.4-2.9) for subjects with highest compared to lowest plasma concentrations, after adjustment for sex, age, plasma high-density lipoprotein cholesterol concentration, systolic blood pressure, and smoking status. Similar findings were found in a nested case-control study of 122 cases of myocardial infarction and 478 controls was conducted in a population-based cohort in Tromsø, Norway.²⁴ Serum total homocysteine level is an independent predictor of the risk of myocardial infarction in the general population. The relative risk for a 4 µmol/l increase in serum homocysteine was 1.41 (95% CI 1.16-1.71) and adjusting for possible confounders reduced the relative risk to 1.32 (95% CI 1.05-1.65).

The relationship between homocysteine levels and carotid artery intimal wall thickness was examined in the ARIC Study. A matched case-control study of 287 pairs of asymptomatic adults, the odds ratio for having a thickened carotid artery wall was 3.15 for subjects in the top quintile of plasma homocysteine levels (>10.5 µmol/l) compared with those in the bottom quintile (<5.88 µmol/l).¹⁷ Case-control study of 750 cases of atherosclerotic vascular disease (cardiac, cerebral and peripheral) and 800 controls (random population samples, family practice registers, and occupational registers) in 9 European countries of individuals younger than 60 years showed that the relative risk for vascular disease in the top fifth compared with the bottom four fifths of the control fasting total homocysteine distribution was 2.2 (95% CI, 1.6-2.9).²⁵ There was a strong interaction between smoking and hypertension with serum homocysteine levels. Case-control study of 101 white males aged 30 to 50 years with angiographically demonstrated coronary heart disease and 108 white males similarly aged was investigated in Alabama, Georgia.²⁶ The odds ratio of coronary artery disease was 1.4 (95% CI, 1.0-2.0), adjusting for age, cholesterol, body mass index, smoking, hypertension and diabetes. Serum creatinine, LDL cholesterol and protein were positively correlated with plasma homocysteine levels.

Serum homocysteine may be related to other vascular disorders such as stroke. A nested case-control study within the British Regional Heart Study cohort of gen-

TABLE I: SUMMARY OF EPIDEMIOLOGIC STUDIES OF HOMOCYSTEINE AS A RISK FACTOR FOR ATHEROTHROMBOTIC DISEASE

Reference (year)	Study design	Number of subjects	Health outcome of interest	Adjusted relative risk/Odds ratio
Selhub et al ²³ (1995)	Cross-sectional	1041	Maximal degree of stenosis of extracranial carotid arteries	2.0 (95% CI, 1.4-2.9)
Arnesen et al ²⁴ (1995)	Nested case-control	600	Myocardial infarction	1.32 (95% CI, 1.05-1.65)
Malinow et al ¹⁷ (1993)	Matched case-control	574	Thickened carotid artery wall	3.15
Graham et al ²⁵ (1997)	Case-control	1550	Atherosclerotic vascular disease	2.2 (95% CI, 1.6-2.9)
Pancharuniti et al ²⁶ (1994)	Case-control	209	Coronary artery disease	1.4 (95% CI, 1.0-2.0)

CI: confidence interval

eral practice from 18 towns in the United Kingdom was conducted in 1978 and 1980.²⁷ Homocysteine levels were measured in 107 stroke cases and 118 controls and found to be significantly higher in stroke cases than controls (geometric mean 13.7 versus 11.9). These findings suggest that homocysteine is a strong and independent risk factor for stroke.

Conclusion

Homocysteine molecules or a related cofactor may promote atherothrombosis. There is evidence from case-control and cohort studies that there is a positive association between serum homocysteine levels and coronary heart disease, cerebrovascular disease, and peripheral vascular disease. There is also some evidence that certain lifestyle factors such as cigarette smoking, lack of physical exercise, obesity, diabetes mellitus, and hypertension may affect homocysteine levels. Serum homocysteine levels may be correlated with low folate, B12, and B6 levels. There are suggestions that current intake levels of folate, B6, and B12 are too low to protect against the development of coronary heart disease. More epidemiologic evidence is needed before we may evaluate the role of vitamin therapy in preventing the progression of vascular disease. The optimal dose and route of administration may be determined in further randomised clinical trials.

Further studies in Asian populations may be needed to evaluate the levels of homocysteine, the average folate, B6, and B12 intake in various populations, and the possible factors related to homocysteine in Asian populations. Cohort and case-control studies that examine the relationship between homocysteine, folate, vitamin B6, and vitamin B12, and coronary heart disease in Asian populations may give us more information on the possible causes of coronary heart disease morbidity and mortality.

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