

Homocysteine and Cardiovascular Disease: Implications for Screening and Prevention in Ireland

Ari Chodos, 5th Year Medicine

ABSTRACT

Elevated blood levels of the intermediary amino acid homocysteine have been implicated in the development of ischemic heart disease (IHD). Heart disease is a major cause of morbidity and mortality in the western world, especially so in Ireland. Thus, lowering blood homocysteine levels to prevent IHD could prove extremely valuable. Homocysteine can be lowered safely and effectively with folic acid and B-vitamin supplementation. However, while many studies have demonstrated a causal relationship between homocysteine and IHD, few have proven that lowering homocysteine will prevent adverse cardiovascular events. Even without conclusive evidence, it is reasonable to recommend folic acid and B-vitamin supplementation to individuals who may be at risk of heart disease, as well as in certain segments of the general population.

INTRODUCTION

Homocysteine (HCY) is an intermediary amino acid formed by the conversion of methionine to cysteine. Severe hyperhomocysteinemia (HHC), also known as homocystinuria, is a rare autosomal recessive disorder. Worldwide incidence is 1 in 344,000, with the highest incidence in Ireland at 1 in 65,000.¹ Clinical manifestations of this disease include developmental delay, osteoporosis, ocular abnormalities, thromboembolic disease and severe premature atherosclerosis. In 1969, McCully proposed that HCY may play a role in the pathogenesis of vascular disease in the general population.² Moderately elevated levels of homocysteine are present in five to seven percent of the population and mounting evidence suggests that this moderate HHC may be an independent risk factor for atherosclerotic vascular disease and recurrent venous thromboembolism (VTE).^{3,4} Thus, screening for HHC may be beneficial as another tool for identifying those at risk for cardiovascular disease (CVD). Since Ireland has the second highest mortality rate due to ischemic heart disease (IHD) in the European Union (as of 2002),⁵ the potential benefit of lowering HCY has great implications for the primary and secondary prevention of this disease.

Aetiology of Hyperhomocysteinemia (HHC)

The normal range of HCY levels is between 5 and 15 $\mu\text{mol/L}$. HHC is usually defined somewhat arbitrarily (for example, above the 95th percentile or two standard deviations above the mean values for fasting, healthy controls). Fasting values for moderate HHC are usually defined as 16 to 100

$\mu\text{mol/L}$ and severe HHC is defined as greater than 100 $\mu\text{mol/L}$. When HHC is defined as above the 95th percentile, five percent of the normal populations considered to have an elevated level of HCY.⁶

Homocysteine levels in the plasma are controlled by its conversion to other metabolites via one of two pathways: the transsulphation pathway or remethylation pathway. Transsulphation is mediated by the Vitamin B₆ dependent enzyme cystathione-B-synthase (CBS). Remethylation is catalyzed by methionine synthase and depends on vitamin B₁₂. The latter reaction also depends on the donation of a methyl group created in the interaction between methylene tetrahydrofolate reductase (MTHFR) and dietary folate (Figure 1).⁷

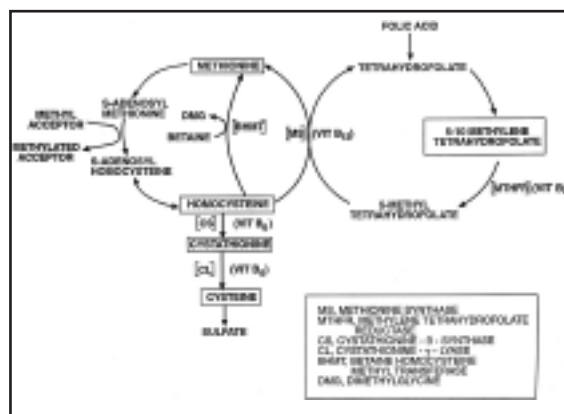


Figure 1. Interaction between MTHFR and dietary folate.⁷

Studies have shown that a variety of factors influence the plasma level of HCY in humans including nutritional deficiencies in vitamin cofactors, certain genetic polymorphisms and some medications. Deficiencies in enzyme co-

factors (folate, vitamin B₁₂, vitamin B₆) are associated with many cases of elevated HCY. Thus, it has been postulated that elevated HCY may be a marker for vitamin deficiency.^{8,9} Data from two studies on the role of vitamin deficiency in the pathogenesis of HHC suggest that low folate intake (enough to raise plasma HCY) may be common in the general population, especially in moderate consumers of alcohol.¹⁰

A genetic polymorphism in the MTHFR enzyme has also been implicated in cases of moderately elevated HCY. The defective genetic code produces a thermolabile form of the MTHFR enzyme and has a population frequency of around 10 percent.¹¹ This polymorphism is a C-T transition at nucleotide 677, causing an alanine to valine substitution.¹² This thermolabile variant reportedly leads to a 50 percent reduction in enzyme activity.¹³ However, studies have shown that high plasma HCY levels in patients with the thermolabile variant only occur in those with low serum folate levels.^{14,15} Not surprisingly, some drugs with anti-folate properties, such as methotrexate and trimethoprim, have been associated with HHC.^{16,17} Recent studies have also shown a link between elevated total plasma HCY and cigarette smoking.¹⁸

Atherothrombotic Properties of HCY

Many mechanisms have been postulated by which HCY may cause vascular injury. Experimental evidence suggests that HCY-induced atherosclerosis is the result of endothelial dysfunction and injury followed by platelet activation and thrombus formation.¹⁹ The proposed mechanisms by which HCY causes this vascular injury and subsequent atheroma/thrombus formation include the promotion of oxidative stress, leukocyte recruitment, foam cell production, smooth muscle and collagen proliferation, marked platelet accumulation and impaired nitric oxide production.

HCY is auto-oxidized when added to plasma and produces reactive oxygen species (free radicals) such as superoxide and hydrogen peroxide, which have been implicated in the direct injury of endothelial cells.²⁰⁻²³ This free radical-induced injury may expose underlying collagen and smooth muscle cells, which proliferate and promote the activation of platelets and leukocyte recruitment.^{24,25}

A by-product of HCY's auto-oxidation is an HCY-thiolactone complex, which can combine with LDL-cholesterol, forming an aggregate that is engulfed by vascular macrophages.³ This new lipid-laden macrophage is called a foam cell, which then releases its lipid into the atherosclerotic plaque.⁴ This increases the size and instability of the plaque.

Several studies have shown that HCY impairs the production of nitric oxide, an endogenous vasodilator.^{20,26} This may contribute to impaired endothelium-dependent vasodilation, which would further the development of vascular injury and atherosclerosis.

Role in Disease

The relationship between blood HCY concentration and risk of heart disease has been debated for many years. Early studies did not demonstrate a causal link between the two.²⁷ More recently, however, two meta-analyses have shown that there is a clear relationship between elevated HCY and increased disease risk, albeit a modest one. The Homocysteine Studies Collaboration was a synthesis of data from 30 prospective and retrospective studies involving 5,073 patients with ischemic heart disease events and 1,113 with stroke events. After adjustment for known cardiovascular risk factors, a 25 percent lower than normal HCY level (corrected for regression dilution bias, about 3 $\mu\text{mol/L}$ [0.41 mg/L]) was associated with an 11 percent lower IHD risk (odds ratio (OR) 0.89; 95 percent confidence interval [CI], 0.83 to 0.96) and 19 percent lower stroke risk (OR, 0.81; 95 percent CI, 0.69 to 0.95). Associations were stronger in the retrospective studies compared with the prospective ones.²⁸

Klerk *et al.* found that individuals with the MTHFR 677TT genotype are at a higher risk of coronary heart disease (CHD), particularly in the presence of low folate levels. These individuals had a 16 percent (OR, 1.16; 95 percent CI, 1.05 to 1.28) greater chance of coronary heart disease compared with people having the CC genotype. The results varied between European (OR, 1.14; 95 percent CI, 1.01 to 1.28) and North American populations (OR, 0.87; 95 percent CI, 0.73 to 1.05), which the authors attributed to higher average folate levels in North America compared to Europe.²⁹

Prevention

The results of several studies demonstrate that there may be some value in lowering HCY for the prevention of a coronary event. A randomised controlled trial (RCT) involving 553 patients who received either a combination of folic acid (1 mg/d), vitamin B₁₂ (cyanocobalamin, 400 µg/d), and vitamin B₆ (pyridoxine hydrochloride, 10 mg/d, n = 272) or placebo (n = 281) for six months found that in patients undergoing percutaneous coronary intervention after a mean follow-up of 11 months, the composite end-point (death or non-fatal myocardial infarct) was significantly lower at one year in patients treated with homocysteine-lowering therapy (15.4 percent versus 22.8 percent; relative risk (RR) 0.68; 95 percent CI 0.48 to 0.96, p = .03), primarily due to a reduced rate of target lesion revascularization (9.9 percent versus 16.0 percent; RR 0.62, 95 percent CI 0.40 to 0.97; p = 0.03).³⁰

Wald *et al.* conducted a meta-analysis to determine if there is a causal relationship between elevated HCY levels and IHD, stroke and deep vein thrombosis (DVT). They sought to quantify the effect of lowering HCY in preventing these diseases. In 72 studies where the prevalence of the MTHFR gene mutation was determined and 20 prospective studies of HCY concentration and disease risk, the authors found that there were significant associations between HCY and the diseases. The odds ratios for a 5 µmol /L increase in serum HCY were, for IHD, 1.42 (95 percent CI 1.11 to 1.84) in the genetic studies and 1.32 (1.19 to 1.45) in the prospective studies. For DVT, the OR was 1.60 (1.15 to 2.22) in the genetic studies (no prospective studies were undertaken); and, for stroke, 1.65 (0.66 to 4.13) for the genetic studies and 1.59 (1.29 to 1.96) for the prospective studies. If these data are accurate, lowering HCY levels from current levels with folic acid supplements should lead to 16 percent reduction in IHD, 25 percent reduction in DVT, and 24 percent reduction in stroke.³¹

It is recommended that patients with CHD who are homocysteinaemic be treated with folic acid, vitamin B₁₂ and vitamin B₆ supplementation.³² In addition, a diet rich in fruits, vegetables, low fat dairy products and low in saturated fat has been shown to lower HCY.³³ Vitamin supplementation with folic acid alone and fortified breakfast cereal have also been shown to lower HCY levels.^{34, 35}

Conflicting Data in HCY Studies

In terms of primary prevention, conclusive data does not exist to support the premise that lowering HCY in the general population will reduce the incidence of CVD. Until large scale RCTs are completed, these potential benefits must be extrapolated from trials demonstrating the causal relationship between HHC and CVD.

Also, there is conflicting data on the efficacy of lowering HCY in patients with established vascular disease. One RCT with 3,680 patients in the United States, Canada and Scotland found that moderate reduction of total HCY after a non-disabling cerebral infarct had no effect on vascular outcomes during the two years of follow-up.³⁶ Additionally, another RCT of folate supplementation following coronary stent placement found that patients in the treatment group had higher rates of re-stenosis and a higher percentage required re-vascularization than the control group.³⁷ The authors suggest that supplementation with folic acid, vitamin B₁₂ and vitamin B₆ may stimulate smooth muscle and matrix formation, contributing to the re-stenosis. Thus, it is recommended that HCY-lowering treatment be delayed six months in patients with CHD who have received a stent.

Other studies contradict the proposed associations between sub-optimal levels of folate, and B-vitamins and cardiovascular risk. An Australian cohort study followed 2,950 people for 29 years, although they did not measure HCY levels. They found no independent association between serum folate, red cell folate and serum vitamin B₁₂ concentrations, with mortality from cardiovascular disease after adjusting for age and other risk factors.³⁸

Screening

While most of the recent data suggest that HHC is an independent risk factor for cardiovascular disease, a strong argument for screening the general population has yet to be made.³⁹ Considering the prevalence of the thermolabile genotype (11 percent) and the relative risk of coronary heart disease in these patients, the population attributable risk (the proportion of CHD that would be eliminated if the genotype did not exist) based on screening for this variant genotype would only be one to two percent.⁴⁰ Additionally, the impact of this genotype is further

reduced simply by folic acid supplementation.

While there is an identifiable risk associated with moderately elevated blood HCY concentrations, patients do not display symptoms specific to this disorder. If a routine screening program was undertaken for HHC, these people could be identified and treated. There are, however, major problems with this type of approach. First, it has yet to be proven conclusively that lowering HCY levels will reduce disease risk. Second, elevated HCY is probably not as important a risk factor in heart disease as smoking, diabetes and hypertension. Thus, there would be questionable value in lowering HCY levels in patients who continue to smoke and have uncontrolled blood pressure and blood sugar levels. Last, assuming that there is value in lowering HCY levels to prevent heart disease, this goal may be accomplished more efficiently on a population basis with folic acid fortification of flour compared to a large-scale screening program.

Since the benefit of lowering HCY concentration on CVD and VTE disease remains unproven,⁴¹ the Atherosclerotic Vascular Disease Conference has recommended screening for HHC in patients with premature atherosclerosis, one or more CVD risk factors and in unexplained venous thrombosis.³⁹ The American Heart Association also recommends screening patients with other conditions that may be associated with high homocysteine levels including advanced age, hypothyroidism, impaired kidney function, systemic lupus erythematosus and administration of certain medications, for example, nicotinic acid, nitrous oxide exposure, theophylline, methotrexate and L-dopa.⁷

Implications for Healthcare in Ireland

With the exception of Finland, the risk of CHD is higher in Britain and Ireland than in the rest of Europe.⁴² A study comparing men in Northern Ireland with men in France found that the three-fold higher risk of CHD in Northern Ireland could not be explained by differences in conventional risk factors for atherosclerosis. Higher plasma HCY levels were discovered in the Irish population and the authors propose this difference as a possible reason for the different CHD mortality rates.⁴³ If large RCTs could demonstrate that a reduction in HCY leads to a reduction in risk for CVD, the implications for public health in Ireland and elsewhere could be substantial.

In addition to having one of the highest rates of CHD in Europe, Ireland is among the countries with the highest rates of neural tube defects in the world.⁴⁴ Both neural tube defects and CHD have been aetiologically linked to disturbed HCY metabolism. An excess of HCY or one of its metabolites is potentially toxic to the development of the fetal nervous system, as well as to the cardiovascular system later in life.²⁷ Due to varying susceptibility, however, HCY toxicity is rarely seen in individuals but rather manifests itself on a population level. Current recommendations for women of childbearing age include taking a folic acid supplement to prevent neural tube defects, possibly because folic acid lowers HCY levels. Similar recommendations for patients at risk of CHD may be warranted if evidence proves that folate intake is correlated with disease risk. Since the folate status of the average person living in Ireland is sub-optimal, folate fortification of food may have very positive results in reducing both neural tube defects and CHD in Ireland.⁴⁴

Tice *et al.* conducted a study that quantified the benefit of lowering HCY on a population basis in terms of lives, quality adjusted life-years (QALYs) and money saved. This study, conducted in the United States, estimated that flour fortification with folic acid would lead to a 13 percent reduction over 10 years in myocardial infarctions in men and eight percent reduction in women. The authors estimated that the percentage decrease in CHD deaths would be comparable to these numbers of, at the very least, reduce annual CHD mortality rates by one to three percent. If, in addition to grain fortification, all patients with known CHD were treated with 1mg of folic acid and 0.5mg of vitamin B₁₂, they projected approximately 310,000 fewer deaths would occur over a 10-year period compared with grain fortification alone.⁴⁵

Over this same 10-year period, vitamin supplementation along with grain fortification for all men 45 years or older without known CHD would save more than 300,000 QALYs and more than US\$2 billion in health care costs. For women without CHD, the preferred strategy would be to treat all women aged 55 years and older with vitamin supplements. This is projected to save more than 140,000 QALYs over 10 years. The difference in treatment age between men and women is due to the fact that men have higher baseline HCY levels and higher age-specific CHD mortality than women. This measure is intended

to maximise cost effectiveness.⁴⁵

The Tice study calculated the reduction in HCY levels after grain fortification with 140µg/100g grain for men and women aged 35 to 84 years. They estimated that these measures would increase folic acid intake 100µg per person-day. This was estimated to reduce homocysteine levels in every age category (reduction was measured at 11 percent at the most and five percent at the least), with the largest reductions in the groups with highest initial HCY levels (Table 1).⁴⁵

	Men - Percentage decrease in Myocardial Infarctions	Women - Percentage decrease in Myocardial Infarctions	Men - Percentage decrease in CHD Deaths	Women - Percentage decrease in CHD Deaths
11% reduction in HCY with liberal estimate (29%) of reduction in CHD risk	13.0	7.6	12.8	8.7
5% reduction in HCY with liberal estimate (29%) of reduction in CHD risk	6.9	3.9	6.7	4.5
11% reduction in HCY with conservative estimate (9%) of reduction in CHD risk	2.8	1.8	2.8	2.1
5% reduction in HCY with conservative estimate (9%) of reduction in CHD risk	1.4	0.9	1.4	1.0

TABLE 1. Predicted Decline in Annual CHD Events Over 10 Years Due to US Food and Drug Administration Mandated Folic Acid Fortification Based on Four Scenarios.⁴⁵

Currently in Ireland, the National Committee on Folic Acid Food Fortification, created in September 2004 by the former Minister for Health and Children, Micheal Martin, is considering recommendations from the Food Safety Authority of Ireland, which call for universal flour fortification with folic acid at 200µg per 100g of grain.⁴⁶ By their estimation, fortification at 200µg per 100g of grain would increase the daily intake of folate by 292µg for men and by 207µg for women.⁴⁴ This would be expected to produce more dramatic reductions in HCY levels than fortification with the Tice study estimates of 140µg per 100g of grain.

In 2002, heart disease in Ireland was implicated in the deaths of 6,149 people, which accounted for 20.6 percent of all causes of death that year.⁵ Using the estimates from the Tice study, this would mean that approximately 500 to 800 deaths (liberal estimate) or 60 to 180 deaths (conservative estimate) from IHD could be prevented per year with the introduction of flour fortification alone.

The Tice study is important for a number of reasons. It is one of the few studies that attempt to quantify the benefit of lowering HCY levels,

in terms of reduced mortality and money saved. Their results demonstrate the potential of a health care initiative that would, hopefully, alter the course of one of the most costly medical conditions to Western societies.

This study has some major limitations, however. Most importantly, clinical data on whether lowering HCY will reduce the risk of CVD do not exist as of yet. The authors used data from studies of vitamin therapy in a cohort of patients with homocystinuria to show biological plausibility.⁴⁷ Although data from these studies showed dramatic effects of vitamin therapy, the benefit of lowering HCY in lowering CVD risk remains theoretical.⁴⁸ The study also assumes 100 percent adherence with vitamin supplementation. Poor adherence has already been demonstrated in Irish women of childbearing age in folic acid supplementation and complete adherence is not a realistic goal in any society.⁴⁴

Even with good adherence, another problem arises. Above a certain serum folate concentration, HCY concentration ceases to be dependent on folate and shifts its dependency to vitamin B₁₂. The dependency switches back to folate when the folic acid supplementation is withdrawn.⁴⁹ Thus, it has been recommended that vitamin B₁₂ be added to fortified food as well.

CONCLUSIONS

The association of HCY with CVD has been studied extensively since the relationship was first proposed in 1969. From the wealth of data available, it seems reasonable to assume that moderately elevated HCY is an independent risk factor for cerebrovascular, peripheral vascular, coronary heart and VTE disease. Conventional risk factors such as diabetes mellitus, hypertension, smoking and hypercholesterolemia appear to be more important.

Screening for HHC in the general population is probably not warranted at this time. While there may be some value in screening those patients who are at high-risk of heart disease, it is more cost-effective to simply recommend vitamin supplementation to men older than 45 years and women older than 55 years, in addition to the high-risk patients. A policy of mandatory folic acid fortification of flour would most likely enhance the effects of a vitamin supplementation strategy.

The prospect of proving that lower HCY levels will reduce morbidity and mortality from CHD is very appealing since it has been demonstrated that serum HCY levels can be lowered fairly easily with folic acid alone or in combination with B-vitamin supplementation. Several large RCTs are underway to determine if there is a benefit from vitamin supplementation in the prevention of stroke and CVD.⁴⁷ The Vitamins to Prevent Stroke Trial (VITATOPS) is a large, randomised, double-blind, placebo-controlled trial currently underway in 19 countries that do not fortify food with folic acid and seems to be positioned to demonstrate the value (or lack thereof) of lowering HCY levels. The trial is testing HCY-lowering treatment (B-vitamins and folic acid) in patients with previous transient ischaemic attack or stroke.⁵⁰ If the benefit of lowering HCY levels for the primary prevention of CHD is demonstrated, a strategy of grain fortification and targeted vitamin supplementation would save lives and money in the long term.

Even in the absence of data on the benefit of lowering HCY to prevent CVD, folic acid

fortification in Ireland will reduce the incidence of neural tube defects. Since the introduction of fortification in the United States, the rate of neural tube defects has fallen by 19 percent.⁴⁴ The majority of the cost for mandatory fortification will be borne by the food industry. Thus, the only cost would be for complications associated with the possibility of masking vitamin B₁₂ deficiency with excessive folic acid levels. This issue was addressed in the FSAI report and the risk of this problem was deemed to be very low in light of the great benefit of a lower rate of neural tube defects.

The benefits of lowering HCY are as yet unproven. However, this should not stop doctors from expanding their recommendation of folic acid beyond women of childbearing age. Since HCY can be lowered with vitamins that are already recommended as a part of a healthy diet and the supplements of these vitamins are safe and well tolerated, it is reasonable to consider routine therapy in all men older than 45 years and in all women older than 55 years.

REFERENCES

1. Yap S, Naughten ER, Wilcken B, Wilcken DE, Boers GH. Vascular complications of severe hyperhomocysteinemia in patients with homocystinuria due to cystathionine B-synthase deficiency: Effects of homocysteine-lowering therapy. *Semin Thromb Hemost* 2000;26(3):335-40.
2. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Path* 1969;56:111-28.
3. Ueland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: Plasma levels in health, disease, and drug therapy. *J Lab Clin Med* 1989;114:473.
4. McCully KS. Homocysteine and vascular disease. *Nat Med* 1996;2:386.
5. Health Statistics 2002. Prepared by the information management unit of the Department of Health and Children.
6. Frantzen F, Faaren AL, Alfheim I, Nordhei AK. Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. *Clin Chem* 1998;44:311-6.
7. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases. A statement for healthcare professions from the Nutrition Committee, American Heart Association. *Circulation* 1999;99:178.
8. Rea IM, McMaster D, Woodside JV, et al. Community-living nonagenarians in Northern Ireland have lower plasma homocysteine but similar methylenetetrahydrofolate reductase thermolabile genotype prevalence compared to 70-89-year-old subjects. *Atherosclerosis* 2000;149:207-14.
9. Selhub J, Jacques PF, Wilson PW, et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;270:2693.
10. Ubbink JR, Vermaak WJ, van der Merwe A, et al. Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr* 1993;57:47.
11. Wald NJ. Homocysteine and ischemic heart disease: Results of a prospective study with implications regarding prevention. *Arch Int Med* 1998;158(8):862-67.
12. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111-3.
13. Kang SS, Zhou J, Wong PWK, Kowalyszyn J, Strokosch G. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. *Am J Hum Genet* 1988;43(4):414-21.
14. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase and plasma homocysteine concentrations. *Circulation* 1996;93:7-9.
15. Harmon DL, Woodside JV, Yarnell JW, et al. The common 'thermolabile' variant of methylene tetrahydrofolate reductase is a major determinant of mild hyperhomocysteinemia. *QJM* 1996;89:571.
16. Refsum H, Ueland PM, Kvinnsland S. Acute and long-term effects of high-dose methotrexate treatment on homocysteine in plasma and urine. *Cancer Res*

- 1986;46:5385.
17. Smulders YM, de Man AME, Stehouwer CDA, et al. Trimethoprim and fasting plasma homocysteine. *Lancet* 1998;352:1827.
 18. Sobczak A, Wardas W, Zielinska-Danch W, Pawlicki K. The influence of smoking on plasma homocysteine and cysteine levels in passive and active smokers. *Clin Chem Lab Med* 2004;42(4):408-14.
 19. Welch GN, Loscalzo J. Mechanisms of disease: homocysteine and atherothrombosis. *NEJM* 1998;338(15):1042-50.
 20. Stamler JS, Osborne JA, Jaraki O, et al. Adverse vascular effects homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 1993;91:308-18.
 21. Matetzky S, Freimark D, Ben-Ami S, et al. Association of elevated homocysteine levels with a higher risk of recurrent coronary events and mortality in patients with acute myocardial infarction. *Arch Intern Med* 2003;163:1933.
 22. Soinio M, Marniemi J, Laakso M, et al. Elevated plasma homocysteine level is an independent predictor of coronary heart disease events in patients with type 2 diabetes mellitus. *Ann Intern Med* 2004;140:94.
 23. Genest JJ Jr, McNamara JR, Upson B, et al. Prevalence of familial hyperhomocyst(e)inemia in men with premature coronary artery disease. *Arterioscler Thromb* 1991;11:1129.
 24. Poddar R, Sivasubramanian N, DiBello PM, et al. Homocysteine induces expression and secretion of monocyte chemoattractant protein-1 and interleukin-8 in human aortic endothelial cells: implications for vascular disease. *Circulation* 2001;103:2717.
 25. Majors A, Ehrhart LA, Pezacka EH. Homocysteine as a risk factor for vascular disease. Enhanced collagen production and accumulation by smooth muscle cells. *Arterioscler Thromb Vasc Biol* 1997;17:2074.
 26. Stuhlinger MC, Tsao PS, Her JH, et al. Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. *Circulation* 2001;104:2569.
 27. Kang DS, Rosenson RS. Overview of Homocysteine. Up To Date Online 12.3. 2004. (Accessed at www.uptodate.com).
 28. Clarke R, Collins R, Lewington S, et al. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis - Homocysteine Studies Collaboration. *JAMA* 2002;288(16):2015-22.
 29. Klerk M, Verhoef P, Clarke R, et al. MTHFR 677C-T polymorphism and risk of coronary heart disease: a meta-analysis. *JAMA* 2002;288:2023.
 30. Schnyder G, Roffi M, Flammer Y, et al. Effect of homocysteine-lowering therapy with folic acid, vitamin B(12), and vitamin B(6) on clinical outcome after percutaneous coronary intervention: the Swiss Heart study: a randomized controlled trial. *JAMA* 2002;288:973.
 31. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: Evidence on causality for a meta-analysis. *BMJ* 2002;325:1202.
 32. Kang SS. Critical points for determining moderate hyperhomocyst(e)inaemia. *Eur J Clin Invest* 1995;25:806.
 33. Appel LJ, Miller ER, Jee SH, et al. Effect of dietary patterns on serum homocysteine: results of a randomized, controlled feeding study. *Circulation* 2000;102:852.
 34. Brattstrom L, Israelsson B, Norrving B. Impaired homocysteine metabolism in early onset cerebral and peripheral occlusive arterial disease: Effects of pyridoxine and folic acid treatment. *Atherosclerosis* 1990;81:51.
 35. Neal B, MacMahon S, Ohkubo T, et al. Dose-dependent effects of folic acid on plasma homocysteine in a randomized trial conducted among 723 individuals with coronary heart disease. *Eur Heart J* 2002;23:1509.
 36. Toole JF, Manilow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: The Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* 2004;291(5):565-75.
 37. Lange H, Suryapranata H, De Luca G, et al. Folate therapy and in-stent restenosis after coronary stenting. *N Engl J Med* 2004;350:2673.
 38. Hung J, Beilby JP, Knuiman MW, Divitini M. Folate and vitamin B-12 and risk of fatal cardiovascular disease: cohort study from Busselton, Western Australia. *BMJ* 2003;326:131.
 39. Smith SC Jr, Milani RV, Arnett DK, et al. Atherosclerotic vascular disease conference: Writing Group II: risk factors. *Circulation* 2004;109:2613.
 40. Wilson PW. Homocysteine and coronary heart disease: how great is the hazard? *JAMA* 2002;288:2042.
 41. Stampfer MJ, Malinow MR. Can lowering homocysteine levels reduce cardiovascular risk? *N Engl J Med* 1995;332:328.
 42. Zarate AO. Levels and Trends, 1955-1991. International Mortality Chartbook. Hyattsville, US: DHHS, 1994.
 43. Manilow MR, Ducimetiere P, Luc G, et al. Plasma homocysteine levels and graded risk for myocardial infarction: findings in two populations at contrasting risk for coronary heart disease. *Atherosclerosis* 1996;126:27-34.
 44. Report on the mandatory fortification of flour with folic acid for the prevention of neural tube defects. Food Safety Authority of Ireland Nutrition Subcommittee. March 2003.
 45. Tice JA, Ross E, Coxson PG, et al. Cost-effectiveness of vitamin therapy to lower plasma homocysteine levels for the prevention of coronary heart disease: Effect of grain fortification and beyond. *JAMA* 2001;286(8):936-43.
 46. Department of Health and Children. Micheal Martin announces establishment of National Committee on Folic Acid Food Fortification. 29 September 2004. (Accessed at: <http://www.dohc.ie/press/releases/2004/20040929c.html>)
 47. Eikelboom JW, Lonn E, Genest J Jr, et al. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. *Ann Intern Med* 1999;131:363.
 48. Wilcken DE, Wilcken B. The natural history of vascular disease in homocystinuria and the effects of treatment. *J Inherit Metab Dis* 1997;20:295-300.
 49. Quinlivan EP, McPartlin J, McNulty H, Ward M, Strain JJ, Weir DG, Scott JM. Importance of both folic

acid and vitamin B12 in reduction of risk of vascular disease. *Lancet* 2002;359:227-28.

50. Hankey GJ, Eikelboom JW, Loh K, et al. Is there really a power shortage in clinical trials testing the "homocysteine hypothesis?" *Arterioscler Thromb Vasc Biol* 2004;24(8) e147-8.