Homocysteine, vitamins, and vascular disease prevention[1–3]

Kilmer S McCully

ABSTRACT

In mid-20th century United States, deaths from vascular disease reached a peak incidence in 1955, but little was known about the underlying causes of this epidemic of disease. The significance of homocysteine in human disease was unknown until 1962, when cases of homocystinuria were first associated with vascular disease. Analysis of an archival case of homocystinuria from 1933 and a case of cobalamin C disease from 1968 led to the conclusion that homocysteine causes vascular disease by a direct effect of the amino acid on arterial cells and tissues. The homocysteine theory of arteriosclerosis attributes one of the underlying causes of vascular disease to elevation of blood homocysteine concentrations as the result of dietary, genetic, metabolic, hormonal, or toxic factors. Dietary deficiency of vitamin B-6 and folic acid and absorptive deficiency of vitamin B-12, which result from traditional food processing or abnormal absorption of B vitamins, are important factors in causing elevations in blood homocysteine. Numerous clinical and epidemiologic studies have established elevated blood homocysteine as a potent independent risk factor for vascular disease in the general population. Dietary improvement, providing abundant vitamin B-6, folic acid, and cobalamin, may prevent vascular disease by lowering blood homocysteine. The dramatic decline in cardiovascular mortality in the United States since 1950 may possibly be attributable in part to voluntary fortification of the food supply with vitamin B-6 and folic acid. Fortification of the US food supply with folic acid in 1998, as mandated by the US Food and Drug Administration, was associated with a further decline in mortality from vascular disease, presumably because of increased blood folate and decreased blood homocysteine in the population. *Am J Clin Nutr* 2007; 86(suppl):1563S–8S.

KEY WORDS Arteriosclerosis, cobalamin, folate, homocysteine, processed foods, vitamin B-6

INTRODUCTION

In mid-20th century United States in 1955, deaths from heart attack and vascular disease reached a crescendo, becoming the leading cause of death and affecting many men and some women in the prime of their lives. Many physicians were appalled at the death toll from this epidemic and were baffled by the inability of medical science to understand the underlying cause of this disease. Scientists at the Framingham Heart Study were beginning to determine that smoking, high blood pressure, and blood cholesterol concentrations in middle-aged men were somehow related to this great increase in vascular disease. During this period, cholesterol chemistry and biosynthesis were studied by Louis Fieser and Konrad Bloch and their colleagues at Harvard. Also during this period, Frederick Stare and his colleagues produced vascular disease by cholesterol feeding in monkeys at the Harvard School of Public Health. Nevertheless, the way in which all of these "risk factors" contributed to the vascular disease problem seemed very difficult for physicians of the period to understand.

The American biochemist Vincent DuVigneaud won the Nobel Prize in Chemistry in 1955 for his pioneering studies of sulfur amino acid chemistry and for synthesizing a biologically active peptide hormone, oxytocin, from its constituent amino acids. In 1932 DuVigneaud discovered a new amino acid by treating methionine with sulfuric acid (1). Because this amino acid was similar in structure to cysteine and contained an extra carbon atom, he named it homocysteine. DuVigneaud investigated the role of homocysteine in metabolism and the ability of homocysteine and choline to replace methionine as an essential nutrient for growth of animals (2). However, little else was known about the importance of homocysteine in medicine or vascular disease in the 1950s. In 1953 Frederick Stare and his colleagues found that cholesterol concentrations and experimental atherogenesis in monkeys were inhibited by dietary methionine (3), which suggested a relation between arteriosclerosis and sulfur amino acid metabolism.

THE ORIGIN OF VASCULAR DISEASE: LESSONS FROM INHERITED DISEASES

In 1962 children with mental retardation, dislocated ocular lenses, accelerated growth, osteoporosis, and a tendency to thrombosis of arteries and veins were discovered to excrete the amino acid homocystine in their urine (4–6). These children had a rare inherited enzymatic defect in homocysteine metabolism that was caused by deficiency of cystathionine synthase, an enzyme requiring pyridoxal phosphate (vitamin B-6) for normal activity (7).

Through some remarkable medical sleuth work, pediatricians at Massachusetts General Hospital discovered an archival case of homocystinuria published as a case report in 1933 (8). This 8-y-old boy was the uncle of a patient who was diagnosed with homocystinuria in 1965 (9). The boy was mentally retarded and

[1] From the Pathology and Laboratory Medicine Service, Department of Veterans Affairs Medical Center, West Roxbury, MA.

[2] Presented at the Harvard College 50th Reunion, held in Cambridge, MA, June 6–9, 2005.

[3] Address reprint requests to KS McCully, Pathology & Laboratory Service, Veterans Affairs Medical Center, 1400 Veterans of Foreign Wars Parkway, West Roxbury, MA 02132. E-mail: kilmer.mccully@med.va.gov.

had dislocated lenses and skeletal abnormalities. He expired with symptoms of a stroke in 1932. In discussing the pathological findings in this case, the pathologist Tracey Mallory found that the cause of death was thrombosis of the carotid artery with cerebral infarct and stroke. He remarked that the carotid arteries were narrowed by arteriosclerotic plaques caused by "a simple sclerotic process such as one sees in elderly people."

Because of my interest in amino acid metabolism and my experience in the laboratory of Giulio Cantoni and Harvey Mudd at the National Institutes of Health, I decided to restudy this interesting case of homocystinuria and arteriosclerosis. In 1968, the original protocol, 6 original slides, and several fragments of tissue imbedded in paraffin had survived since 1933 in the Pathology Department at Massachusetts General Hospital. In my review of this case, I found that arteriosclerotic plaques were scattered throughout the arteries in many organs. It was difficult to prove, however, that homocysteine was connected to the arteriosclerotic plaques and thrombosis that had caused the death of this child.

Later in 1968 I was fortunate to learn of another case of homocystinuria that had been studied at Massachusetts General Hospital, the National Institutes of Health, and Brandeis University. A 2-mo-old baby boy with growth failure and pneumonia was discovered to excrete homocysteine, cystathionine, and methylmalonic acid in the urine. Biochemical study disclosed deficiency of methionine synthase, an enzyme dependent on cobalamin (vitamin B-12) and methyltetrahydrofolate, and the case was reported as the index case of cobalamin C disease in the medical literature (10). In restudying the autopsy findings of this case, I discovered astonishingly advanced arteriosclerotic plaques scattered throughout the arteries in the major organs of the body. Because the accumulation of homocysteine was caused by a different enzyme abnormality from the earlier 1933 case, I concluded that homocysteine causes arteriosclerotic plaques by a direct effect of the amino acid on the cells and tissues of the arteries (11). In 1976, a child with the third major cause of homocystinuria, deficiency of methylenetetrahydrofolate reductase, was found to have similar arteriosclerotic plaques throughout the body, which independently corroborated my earlier conclusion (12).

HOMOCYSTEINE THEORY OF ARTERIOSCLEROSIS

In his classic monograph of 1923 entitled *Inborn Errors of Metabolism*, Sir Archibald Garrod pointed out that experiments of nature, consisting of inherited diseases of metabolism, help to illuminate the causes of disease processes (13). Investigation of cases of homocystinuria showed that 3 different inherited enzyme abnormalities cause elevation of blood homocysteine, producing arteriosclerotic changes in the arteries. This discovery suggested that elevation of blood homocysteine is likely to be a factor in the pathogenesis of arteriosclerosis in the general population (11, 14). Thus, dietary, genetic, metabolic, hormonal, or toxic factors cause arteriosclerotic plaques and thrombosis because of elevation of blood homocysteine, which affects the cells and tissues of the arteries (15).

This interpretation suggests that elevations in blood homocysteine may explain the experimental atherogenesis in animals caused by deficiency of vitamin B-6 in monkeys (16), deficiency of choline and other methyl donors in rats (17), methionine deficiency produced by feeding of cholic acid with thiouracil in rats (18, 19), and methionine deficiency produced by feeding soy protein and the goitrogenic isoflavones and saponins of soy in monkeys (3). Methionine deficiency elevates blood homocysteine concentrations because of decreased synthesis of adenosyl methionine and dysregulation of sulfur amino acid metabolism (20).

According to current concepts, homocysteine damages cells and tissues of arteries by inciting the release of cytokines, cyclins, and other mediators of inflammation and cell division (15). By affecting smooth muscle cells, homocysteine produces the connective tissue changes of arteriosclerotic plaques, causing fibrosis, calcification, proteoglycan deposition, and damage to elastic tissue layers. Homocysteine is a potent procoagulant that promotes the deposition of fibrin and mural thrombosis in artery walls. Homocysteine thiolactone is the reactive anhydride of homocysteine that interacts with LDL, causing aggregation, increased density, and uptake by vascular macrophages to form foam cells (21). Degradation of these aggregates leads to deposition of cholesterol and other fats in developing plaques. In addition, reaction of homocysteine thiolactone with serum proteins leads to the production of new protein antigens and autoimmune antibodies, facilitating the inflammatory response (22). Homocysteine causes oxidant stress by effects on cellular respiration, leading to oxidation of LDL and other constituents of plaques (23). Homocysteine also antagonizes the vasodilator properties of nitric oxide by the formation of *S*-nitrosohomocysteine, leading to endothelial dysfunction, the earliest stage in atherogenesis (24).

In the decades since the discovery and development of the homocysteine theory of arteriosclerosis, numerous clinical and epidemiologic studies have established elevation of blood homocysteine as a potent, independent risk factor for vascular disease (25). The results of the Physicians' Health Study, the Nurses' Health Study, the European Concerted Action Study, and the Hordaland Homocysteine Study all support the validity of the homocysteine theory of arteriosclerosis (26). Meta-analysis of published studies suggests that elevation of homocysteine is a causal factor in atherogenesis; such studies predicted that lowering homocysteine concentrations would be estimated to benefit ≈15–40% of the population by preventing vascular disease (27). This estimate is conservative, because it is based on an arbitrary definition of "normal" blood homocysteine concentrations in the population. In fact, many studies have shown that vascular disease risk is directly correlated with blood homocysteine over a wide range of values, which suggests that lowering blood homocysteine may benefit a large fraction of the population. Current trials have been designed to test this possibility.

The landmark report by the Framingham Heart Study in 1993 showed in a group of 1160 elderly participants between the ages of 67 and 96 y that blood homocysteine becomes elevated because of dietary deficiencies of vitamin B-6 and folic acid and decreased absorption of vitamin B-12 (28). Elevation of blood homocysteine is associated with increased prevalence of heart attack, as shown by the third National Health and Nutrition Examination Survey (29). These findings show that dietary deficiencies of vitamins B-6 and folic acid, and absorptive deficiency of vitamin B-12, lead to elevation in blood homocysteine concentrations, which produces vascular disease in the population. In addition, genetic factors are involved. A genetic variant of methylenetetrahydrofolate reductase, *677TT*, that affects ≈12%

of the population, leads to increased risk of vascular disease if dietary folic acid is marginal (27).

The amounts of dietary B vitamins needed to prevent elevations in blood homocysteine are 3 mg vitamin B-6 and 400 μg folic acid, as shown by the Framingham Heart Study (28). These figures agree well with the findings of the Nurses' Health Study, which showed that these amounts of dietary vitamin B-6 and folic acid are needed to prevent mortality and morbidity from heart disease (26). Before fortification of grain products with folic acid in 1998, intakes of vitamin B-6 and folic acid were well below these figures, amounting to ≈1.5 mg vitamin B-6 and 250 μg folic acid per day (30).

Vitamin B-12 intakes are generally adequate, except in vegans, who consume no meat, fish, or dairy foods. Vitamin B-12 is present only in foods of animal origin, so strict vegans may have insufficient intakes to prevent elevations in blood homocysteine (31). Vegans may obtain small amounts of vitamin B-12 from commercially baked goods, some of which contain lard or milk products. Vitamin B-12 absorption is inadequate in ≈15% of the elderly population aged >65 y because of lack of gastric acidity; decreased intrinsic factor synthesis by gastric mucosal cells, as originally discovered by William Castle at Harvard; and infection by *Helicobacter pylori*, the etiologic agent for gastric and duodenal peptic ulcers, as discovered by Barry Marshall and Robin Warren of Australia (32). For this reason, some elderly persons are susceptible to the subtle mental symptoms, neurological changes, weakness, and fatigue that are associated with deficiency of vitamin B-12.

In addition to vitamin B-6, folic acid, and vitamin B-12, vitamin B-2 (riboflavin) was recently shown to be a determinant of blood homocysteine (33–35). The requirement for riboflavin in preventing elevations in blood homocysteine is primarily found in persons with the common genetic variant of methylenetetrahydrofolate reductase, *677TT*. These persons require adequate dietary folate and riboflavin for normal enzyme activity of methylenetetrahydrofolate reductase to prevent elevations in blood homocysteine. Other conditions that predispose to vascular disease, such as renal failure, hypothyroidism, and estrogen deficiency, are also characterized by elevated blood homocysteine concentrations (36). The hyperhomocysteinemia in hypothyroidism is likely related to the diminished conversion of dietary riboflavin to its coenzyme derivatives, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FAD is the coenzyme required by methylenetetrahydrofolate reductase. Hypothyroidism in rodents depresses this conversion, which results in decreased hepatic concentrations of FMN and FAD (37, 38). These results have been confirmed and extended in human hypothyroidism. The metabolic defects of riboflavin metabolism in hypothyroid adults are completely corrected by treatment with thyroid hormones without increasing dietary riboflavin intake (39).

In many individuals, dietary vitamin B-6 and folic acid intakes are marginal because traditional methods of food processing partially destroy these sensitive B vitamins (40). Thus, milling of grains, canning, extraction of sugars and oils, and the addition of bleaching agents and other chemical additives account for losses of these B vitamins of ≥85% in highly processed foods. Countries such as Japan, France, and Spain with higher intakes of vitamin B-6 and folic acid have lower homocysteine concentrations, averaging 6–8 μmol/L, than do countries such as Finland, Scotland, and Northern Germany with lower B vitamin intakes and correspondingly higher homocysteine concentrations of 10–12 μmol/L. Because of these differences in vitamin B consumption, the mortality rates from coronary heart disease are related to homocysteine concentrations in a group of 30 countries on the basis of stored plasma samples collected in the 1970s (41).

PREVENTION OF VASCULAR DISEASE

Any "young elderly" person should have his or her blood homocysteine concentration monitored while in a fasting state every year. If the homocysteine concentration is in the range of 4–8 μmol/L, the risk of vascular disease from this etiology is low, and a healthful, nutritious diet, such as the Heart Revolution Diet, should be continued (42). Many authorities have advocated similar diets with abundant vitamin B-6, folic acid, and vitamin B-12 from fruit, vegetables, whole grains, fresh meats, and seafood. If the homocysteine concentration is in the range of 8–12 μmol/L, an effort should be made to improve the quality of the diet, providing sufficient vitamin B-6, folic acid, and vitamin B-12 to keep homocysteine concentrations low and to minimize disease risk. The aging process is associated with decreased ability to absorb these B vitamins, which results in a gradually rising homocysteine concentration with age, ≈1 μmol/L per decade. Over the age of 60 y, consideration should be given to consuming 3 mg vitamin B-6, 400 μg folic acid, and 100 μg vitamin B-12 as dietary supplements, most conveniently in a daily multivitamin pill, in addition to consuming the Heart Revolution Diet.

The following guidelines are personal recommendations based on clinical experience. If the elderly person is sedentary, obese, and a smoker consuming a poor diet, the homocysteine concentration may be in the range of 10–14 μmol/L. In addition to consuming an improved diet, supplements of 10 mg vitamin B-6, 1000 μg folic acid, and 100 μg vitamin B-12 should be considered to decrease disease risk. If there is a family history of heart disease, hypertension, and a low HDL concentration, the disease risk is high and the homocysteine concentration is likely to be in the range of 12–20 μmol/L. An improved diet and supplements of 50 mg vitamin B-6, 2000 μg folic acid, and 500 μg vitamin B-12 should be considered. If there is a history of angina, ischemic attacks, kidney failure, or diabetes and homocysteine concentrations are in the range of 16–30 μmol/L, disease risk is very high, and an improved diet with 100 mg vitamin B-6, 5000 μg folic acid, and 1000 μg vitamin B-12 should be considered. Another advisable supplement is fish oil, which decreases homocysteine concentrations when taken in doses of 12 g/d (43). Fish oil contains n−3 fatty acids that have a beneficial antiinflammatory effect.

The Heart Revolution Diet consists of fresh vegetables, fresh fruit, fresh meats and seafood, whole-grain foods, nuts, fresh eggs, yogurt, milk or cream, and occasional liver or liver pâté (42). Highly processed foods should be minimized, because they are partially depleted of vitamin B-6 and folic acid. Canned vegetables, fruit, meats, and seafoods contain only one-half or less the vitamin B-6 and folic acid that fresh foods do. Foods containing sugar, white flour, or white rice are seriously depleted of vitamin B-6 and folic acid, because these methods of food processing destroy ≥90% of these nutrients. Processed and packaged foods that are made with powdered eggs, powdered

milk, and partially hydrogenated oils contain potentially damaging oxidized cholesterol and *trans* fats. Following the Heart Revolution Diet, combined with smoking cessation and moderate regular exercise, will help to control blood homocysteine concentrations and prevent vascular disease from this cause (42).

The Centers for Disease Control and Prevention issued a report on mortality from vascular disease in the 20th century (44). This report shows that mortality from vascular disease, in particular diseases of the heart, increased dramatically from 1900 to 1950, becoming the leading cause of death and reaching a peak in the late 1950s and early 1960s. The report stated, "Since 1950, age-adjusted death rates from cardiovascular disease have declined 60%, representing one of the most important public health achievements of the 20th century."

In 1978, almost 20 y after the dramatic decline in heart disease mortality became apparent, a nationwide conference at the National Institutes of Health concluded that none of the traditional risk factors, such as changes in dietary fats, blood cholesterol concentrations, smoking, hypertension, exercise, or coronary care units could explain this dramatic decline (45). In the 1950s and 1960s, synthetic vitamin B-6 was added to the US food supply in the form of fortification of cereals and supplements (15). In the 1960s, synthetic folic acid was also added to the food supply, and in 1998, the US Food and Drug Administration mandated the addition of folic acid to enriched flours and other refined-grain foods. Lowering of blood homocysteine concentrations by the addition of vitamin B-6 and folic acid to the US diet may explain in part the dramatic decline in vascular disease mortality in the United States to less than one-half the peak incidence. In recent years, additional factors such as smoking cessation; treatment of hypertension, hyperlipidemia, and diabetes; use of low-dose aspirin; and improved medical and surgical treatments (acute management of myocardial infarction, angioplasty, stenting, coronary bypass, etc) have also contributed to the decline in mortality.

Since 1998 folic acid fortification of refined grain foods has lowered the incidence of neural tube defects and other serious birth defects by as much as 78% in Newfoundland by lowering maternal blood homocysteine concentrations (46). A recent study by the Centers for Disease Control and Prevention found that the decline in stroke mortality in the United States and Canada from 1990 to 2002 accelerated from a 0.3% annual decline from 1990 to 1998 to a 2.9% annual decline beginning in 1998, accounting for 16 700 fewer deaths from stroke per year over a 6-y period (47). The accelerated rate of decline was attributed in part to lowering of blood homocysteine concentrations by folic acid fortification of refined grain foods, because other factors that might have accounted for this dramatic decline were unchanged. No change in stroke mortality was found during the same period in England and Wales, countries where there is no fortification of foods with folic acid. This study (47) and the Framingham Heart Study (48) showed that blood folate concentrations almost doubled and homocysteine concentrations declined 15% after folate fortification of enriched grains in the United States in 1998.

Current efforts to demonstrate reduced mortality and morbidity from vascular disease through interventional studies with dietary improvement and supplemental B vitamins to lower blood homocysteine are complicated by the large number of participants needed to power the studies, the length of the trials required, and the fortification of the North American food supply with folic acid (49). Recently, 3 large prospective trials of supplementation with B vitamins in patients with advanced vascular disease (VISP, NORVIT, and HOPE2) concluded that moderate doses of folic acid and vitamins B-6 and B-12 over a 3–5-y period have little effect on risk of recurrent heart attack or stroke (50-52). In the VISP trial of stroke survivors (50), a subgroup analysis concluded that those participants without renal impairment, without malabsorption of vitamin B-12, or who were not taking nonstudy vitamin B-12 supplements had a significant 21% reduction in adverse vascular events from B vitamin therapy (53). In the HOPE2 trial of patients with advanced vascular disease, there was a significant 24% reduction in stroke from B vitamin therapy, but the slight reductions in all-cause mortality, myocardial infarction, and cardiovascular death were not significant (52). Homocysteine concentrations were measured in only 19% of the HOPE2 participants after 5 y, and the lowering of homocysteine concentrations was not statistically significant (54). In the NORVIT trial of heart attack survivors (51), the placebo group had a significantly higher percentage of patients who were treated with cardiac bypass grafts or angioplasty (447/943 = 47.4%) than did the B vitamin group (395/937 = 42.2%), which may explain the decreased rate of late adverse vascular events in the placebo group (54).

In all of these trials, the participants had advanced disease that had been progressing for several decades, and the intervention with supplemental B vitamins was only for a 2–5-y period. Longer periods of intervention may be required. In addition, most of the participants were taking multiple drugs, including aspirin, statins, beta blockers, and other medications that may have obscured the potential beneficial effect of the B vitamin intervention. A review of 43 earlier studies of blood homocysteine concentrations and risk of cardiovascular disease concluded that most cross-sectional and case-control studies, with a few exceptions, supported elevation of blood homocysteine as a risk factor for coronary heart disease (55). Most of the prospective studies, however, did not support a relation between blood homocysteine and coronary heart disease, and the authors questioned whether blood homocysteine concentrations are a marker rather than a cause of the disease.

These findings, along with the generally negative results of the recent secondary prevention trials with B vitamin supplements (50-52), suggest that blood homocysteine, as measured by the present methods, likely reflects an underlying metabolic abnormality in the chronic disease process (25). In theory, the metabolic abnormality in advanced vascular disease is considered to involve depletion of the homocysteine derivative, thioretinaco ozonide, from cellular membranes (56). Thioretinaco, which is synthesized from homocysteine thiolactone, vitamin A, and vitamin B-12, prevents homocysteine-induced vascular disease in rats (57) and is anti-carcinogenic and anti-neoplastic in mice (58). Use of this compound in future human studies may be found to benefit advanced vascular disease by correcting this theoretical abnormality of homocysteine metabolism.

The most important role of B vitamin supplementation appears to be in primary prevention, as suggested by the reduction in stroke mortality after the institution of folic acid fortification (47). A recent study showed that folic acid supplementation suppresses the autoimmune response to homocysteinylated albumin and hemoglobin in hyperhomocysteinemic subjects without coronary artery disease but had no effect on the autoimmune response in subjects who already have coronary artery disease

(59). A recent trial of B vitamin supplements in elderly patients with vascular disease showed no improvement in cognitive function despite a lowering of blood homocysteine concentrations (60). These results and the negative results from the secondary prevention trials of advanced vascular disease (50-52) do not support the use of B vitamin supplements to reverse the effects of advanced vascular disease.

My advice to keep the young elderly healthy is to eat an improved diet that is rich in nutrients, including vitamins, minerals, antioxidants, and phytochemicals (42). This simple strategy should help to prevent the life-long progression of vascular disease attributable to elevated blood homocysteine, which leads to life-threatening heart attack, stroke, amputations, and kidney failure. Additional helpful preventive measures are smoking cessation, stress reduction, moderate exercise, weight control, and treatment of malignant hypertension, dyslipidemia, and diabetes. Furthermore, in the young elderly who do not have advanced vascular disease, homocysteine reduction may have a role in disease prevention.

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