Homocysteine, Vitamins, and Cardiovascular Disease

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The significance of any association between cardiovascular disease and circulating homocysteine concentrations is attracting considerable attention. The normal activities of the transsulfuration and remethylation pathways maintain intracellular homocysteine levels within a narrow range, and the controlled release of homocysteine into blood results in blood measurements that provide an accurate index of homocysteine status. In the circulation, homocysteine is rapidly oxidized, and very little homocysteine remains in the reduced form. The majority of homocysteine forms a disulfide bridge with protein, and some reacts either with itself to produce homocystine or with cysteine to form the mixed disulfide cysteine-homocystine. Most analytical procedures produce homocystine or with cysteine to form the mixed reduced form. The majority of homocysteine forms a disulfide bridge with protein, and some reacts either with itself to produce homocystine or with cysteine to form the mixed disulfide cysteine-homocystine. Most analytical procedures include a reduction step and do not distinguish between the reduced and various oxidized forms of homocysteine; thus, the analyte measured is referred to as homocyst(e)ine. The normal range is unclear but may fall between 5 and 15 μmol/L.

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Analyses of homocysteine usually involve fasting samples of either serum or plasma. The concentrations are higher in serum, and increases of ≈10% have been reported in the postprandial stage. Homocysteine levels also increase with age and are higher in men than in women. A variety of disease states and medications modify homocysteine concentrations, and notably, impaired renal function may greatly increase homocysteine levels. Measurement of homocysteine should avoid blood samples that have been stored at room temperature, because red blood cells may release homocysteine, causing an artificial increase in extracellular homocysteine concentrations.

A complicating aspect of homocysteine metabolism for cardiovascular studies is that homocysteine concentrations may increase after a myocardial infarction or a stroke. Critically, data are not available for samples obtained before and after an event. However, analysis of samples obtained at the time of a myocardial infarction and up to 180 days later indicated an increase in homocysteine concentration from 12.9±0.9 to 15.3±1.1 μmol/L. Similarly, samples collected within 2 days of a stroke and up to 645 days later exhibited a rise in homocysteine concentration from 11.4 to 14.5 μmol/L.

In vitro, a very wide range of effects have been attributed to homocysteine. These include direct damage to endothelial cells, flawed platelet activity, elevated procoagulant activity, increased collagen synthesis, and enhanced proliferation of smooth muscle cells. Biochemically, it has been proposed that homocysteine modifies eicosanoid metabolism, promotes translocation of protein kinase C from cell nuclei and cytoplasm to the cell membranes, and induces c-fos and c-myb activity. Many of these observations are related to the pathogenesis of cardiovascular disease. General concerns about the results, however, involve whether the effects are specific for homocysteine and occur at concentrations found in the majority of cardiovascular patients. Cysteine, which is also a sulfur-containing amino acid, is present in blood at far higher concentrations than homocysteine. In vitro, experiments are typically performed at homocysteine concentrations of ≈100 μmol/L, levels that are found only in the rare individuals homozygous for cystathionine β-synthase deficiency. Furthermore, the results are compared with those obtained from tissue suspended in homocysteine-free buffer, a concentration never observed in vivo. In contrast, epidemiological studies showing a significant correlation between homocysteine levels and cardiovascular disease tend to report homocysteine concentrations among patients of ≈11 to 16 μmol/L, only ≈3 μmol/L higher than the corresponding control group. Although researchers are attempting to mimic experimentally in a matter of a few hours or days what may occur naturally over many years, it is questionable whether a metabolite can be raised 10-fold above normal concentrations without causing some derangement of metabolism.

In animal experiments, the feeding of an atherogenic-type diet to cynomolgus monkeys produces both hypercholesterolemia and hyperhomocysteinemia. However, the subsequent reduction of homocysteine levels by vitamin B supplementation was not associated with an improvement in endothelial function. The available mouse models of atherosclerosis may provide a better method to directly test whether moderately elevated homocysteine levels or reduced folic acid concentrations cause atherosclerotic lesions and whether the process can be reversed by supplementation with folic acid or vitamins B₆ and B₁₂.

In studies of human patients, the results of the numerous case-control studies comparing subjects with myocardial infarction, stroke, and peripheral vascular disease with control subjects are consistent with the hypothesis that higher levels of homocysteine and lower blood levels of folic acid, B₁₂, or B₆ are found more frequently in cases than in controls. There is, however, no evidence that moderately high levels of homocysteine (generally found in these case-control studies...
or in prospective human studies) are a direct cause of atherosclerosis.

The inconsistent results of genetic studies of homocysteine metabolism involving moderate elevations of homocysteine and risk of cardiovascular disease are puzzling.\textsuperscript{10} We would expect that individuals with the genetic disorders of homocysteine metabolism who were subjected to moderately elevated homocysteine levels for long periods of time (ie, from an early age) would have an increased risk of vascular disease. The results of the current Atherosclerosis Risk in Communities (ARIC) study are consistent with those of other studies that fail to document an association between mutations in homocysteine-metabolizing enzymes and risk of vascular disease.

Schwartz et al\textsuperscript{11} recently studied 79 women <45 years old with coronary heart disease and control subjects selected in a population-based study in the state of Washington. Case patients had higher mean homocysteine levels, with a 2-fold relative risk versus control subjects and lower folic acid concentration in their blood. Among control subjects, 12.7% were homozygous for the MTHFRT\textsuperscript{677} allele compared with 10% of the case patients. Those with this allele had higher plasma homocysteine levels, and the differences in homocysteine levels between those with and without the MTHFRT\textsuperscript{677} allele were similar to the differences in homocysteine levels between the cases and controls.

In a related study, deJong et al\textsuperscript{12} reported that, among 450 siblings of 167 young patients with vascular disease, they observed that \( \approx 28\% \) of the siblings had hyperhomocysteinemia as estimated by either fasting measurements or postmethionine loading. They then compared measures of subclinical atherosclerosis, either peripheral, coronary, or carotid, among the siblings with or without elevated homocysteine levels. There was no relationship between levels of homocysteine and subclinical vascular disease or with the genetic polymorphisms of the MTHFR gene. The primary determinants of high risk of subclinical vascular disease among the siblings of the probands with premature coronary disease were smoking, high blood pressure, and high cholesterol levels.

The overall results from prospective studies such as ARIC are inconclusive.\textsuperscript{13} Some show a positive relationship between coronary artery disease and homocysteine levels and an inverse correlation with folic acid, B\textsubscript{12}, or B\textsubscript{6} concentrations. Other well-designed studies with equally large numbers of cases and power show no consistent relationship between homocysteine levels and the risk of disease.

In ARIC, only plasma pyridoxal 5’-phosphate was consistently associated with a lower risk of cardiovascular disease. There was, as noted, no relation between dietary intake of either folic acid, B\textsubscript{12}, B\textsubscript{6} or vitamin supplements and risk of cardiovascular disease. The higher serum levels of the vitamins are almost certainly related to vitamin supplementation rather than strictly dietary intake. Unfortunately, the ARIC study has limited information regarding specific vitamin intake. No detailed information was provided about selection bias for vitamin supplement use. We know, however, that vitamin supplement users are healthier and better educated and have fewer cardiovascular risk factors and therefore would have lower risk of cardiovascular disease. Thus, the lower risk associated with pyridoxal 5’-phosphate may be causal or a measure of selection for lower cardiovascular risk.

What, then, are the possible associations between homocysteine, B vitamins, and vascular disease?

1. The null hypothesis is that there is no causal association between moderately elevated homocysteine levels and risk of vascular disease or atherosclerosis. The causal pathway could be that vascular disease results in an increase in homocysteine levels. This hypothesis would be consistent with the results of numerous case-control studies. The cases would have higher homocysteine levels because they have more atherosclerosis and vascular disease, not because homocysteine caused the vascular disease. How, then, could vascular disease cause an elevation of homocysteine levels? We know that vascular disease is an inflammatory process. Individuals with vascular disease have elevated levels of inflammatory markers, such as acute-phase proteins (ie, C-reactive protein),\textsuperscript{14} adhesion molecules,\textsuperscript{15} and sedimentation rate.\textsuperscript{16} It is unlikely that these markers are causal, but they probably relate to the inflammatory process of vascular disease. Folic acid is important in DNA synthesis. Inflammation is associated with increased mitotic activity. Could the inflammatory process result in an increased demand for folic acid and secondary elevation of homocysteine levels, especially in those individuals with low folic acid intake or those who have a specific genetic abnormality of homocysteine metabolism?

The results of studies of subclinical vascular disease have shown that higher homocysteine levels are associated with greater carotid artery intimal-medial wall thickness and plaque,\textsuperscript{17} and the extent of coronary artery disease on angiography would also be consistent with the null hypothesis. Individuals with more atherosclerotic burden would have more inflammation, greater demand for folic acid, and higher homocysteine levels. Thus, we would anticipate that individuals with extensive subclinical vascular disease would have both elevated levels of inflammatory markers and raised homocysteine concentrations associated with low folic acid, B\textsubscript{12}, or B\textsubscript{6} levels. The ARIC study, for example, showed a positive association between carotid artery intimal-medial thickness and homocysteine levels\textsuperscript{17} but, as noted, no consistent relationship for risk of clinical cardiovascular disease. The association of homocysteine and subclinical vascular disease appears to be stronger among participants with hypertension.\textsuperscript{18} Possibly, homocysteine has effects on vascular disease independent of atherosclerosis.

The conclusions of longitudinal studies with regard to homocysteine levels and the risk of cardiovascular disease could be influenced by the varied prevalence of subclinical atherosclerosis among the subjects. Levels of homocysteine would be higher in clinical cases, who had more extreme subclinical disease at time of blood draw, than in noncases. It is important, in prospective studies, to look at the relationship between homocysteine levels and folic acid in relation to age as well as the extent of subclinical vascular disease. There is some evidence that homocysteine levels are positively correlated with inflammatory markers.

The Cardiovascular Health Study is currently evaluating homocysteine levels and risk of cardiovascular disease. This
longitudinal study is similar to the ARIC study and also includes measures of subclinical disease and markers of inflammation. Preliminary results to be presented at the Second International Conference on Homocysteine by Schwartz et al (unpublished data, 1998) show that higher homocysteine levels were significantly related to the risk of myocardial infarction and coronary heart disease deaths but not to stroke. Again, consistent with the ARIC study, there was no relationship between the MTHFR genotype and the risk of coronary heart disease or stroke. This study includes predominantly older individuals, age ≥ 65 years, with a heavy burden of subclinical disease, inflammatory markers, and measures of clotting and thrombosis. Further analysis may provide important information regarding the association between homocysteine and disease.

Case-control studies (no matter how well designed or how large) cannot provide information with regard to this null hypothesis and probably should be abandoned in future studies of homocysteine, vitamins, and disease. The association of homocysteine levels and, perhaps, B vitamins should be investigated in other inflammation-related diseases, and the effects of various therapies that moderate the inflammatory process should be examined in relation to homocysteine blood levels.

2. A decrease in folic acid or B12 and B6 is the primary cause of the increased risk of vascular disease. Elevated levels of homocysteine may just be a marker of low vitamin levels but not be important in the causal pathway of the disease. Treatment with folic acid would decrease the risk of vascular disease and concurrently reduce homocysteine levels. However, the decline in homocysteine is not necessarily beneficial. A high sedimentation rate is associated with the risk of vascular disease. Aspirin will reduce the risk of myocardial infarction and possibly also reduce the sedimentation rate. It is unlikely that the high sedimentation rate and its reduction is the primary benefit of aspirin in terms of reducing vascular disease.

The reduction in stroke reported in the Linxian County, China, Vitamin Trial might be consistent with the benefits of folic acid, B12, or B6. A comparison of the prevalence of atherosclerosis in populations that are discordant (ie, have high or low cholesterol levels and high or low homocysteine levels) and the extent of atherosclerosis or cardiovascular disease might be useful, especially in relation to intake of B vitamins and perhaps genetic polymorphism. It will be interesting to see whether there are populations in which there are high homocysteine levels at the population level but low LDL cholesterol levels and whether, in these populations, there is evidence of extensive atherosclerosis. To date, no population data have been presented to support such an association.

4. Homocysteine levels are related to the risk of vascular disease independently of any effect on the development of atherosclerosis. There are at least 2 possibilities:

A. High homocysteine levels could be associated with an enhancement of inflammatory processes, with the stability of the atherosclerotic plaque, and with increased risk of clinical disease, given subclinical atherosclerotic disease. A high homocysteine level would be additive to other risk factors for atherosclerosis and subclinical disease. The strong association between homocysteine levels and risk of clinical disease among higher-risk populations, such as hypertensives and diabetics, would support this hypothesis. Longitudinal studies, such as ARIC and perhaps the Cardiovascular Health Study, could test the hypothesis that individuals with subclinical disease and with high homocysteine levels would have an increased risk of clinical disease independently of other inflammatory markers and risk factors. Other studies could evaluate the relation of plaque morphology and changes in plaque characteristics to homocysteine levels.

B. Higher homocysteine levels could also be related to increased risk of thrombosis and subsequent clinical disease. Experiments to characterize the effects of homocysteine include the infusion of homocysteine into animals. The results appear to be species dependent, but particularly in baboons, the infusions result in damage to the vascular endothelium and reduced platelet survival. Results of studies in homocystinuric patients, although not unanimous, suggest impairment of platelet activity and of the clotting cascade. The association of homocysteine levels and thrombosis could be related to platelet function, thrombin generation, or fibrinolysis. The investigation of such associations in human observational studies is difficult. Several of the large, ongoing longitudinal studies of cardiovascular disease, such as CHS and ARIC, have relatively high-quality measures of thrombosis and fibrinolysis and should look at the associations of these data with homocysteine and B vitamin levels.

At present, the totality of evidence does not refute or support any of the above-mentioned hypotheses. More case-control studies will be of little or no value. Essays describing the wonders of folic acid and folic acid supplementation and reduction of homocysteine levels to prevent heart attacks may be counterproductive by offering yet another false promise to the public, who may become less responsive to more proven methods of reducing coronary heart disease (such as lowering LDL cholesterol, ceasing smoking, and controlling high blood pressure).

The test of the above hypotheses requires good human clinical trials and more animal experimental studies. It is very
unfortunate that it takes so many years to move from a hypothesis based on observational case-control studies to prospective longitudinal studies and then to important clinical trials. This time process needs to be substantially shortened to bring proven therapies to public health and clinical utility and to discard promising but relatively ineffective therapies. Clearly, too much time is spent on reproducing results of observational studies that have similar limitations.

Secondary prevention trials require smaller sample sizes and probably shorter follow-up time than primary prevention. If secondary prevention trials show a direct benefit of lowering homocysteine with vitamin supplements on the risk of cardiovascular disease, then the causal hypothesis would be greatly strengthened, and the much larger and long-term primary prevention trials may not be necessary. Trials using intermediate vascular end points (such as changes in carotid artery intimal-medial thickness, plaque, coronary calcification) may also be of some value. If the secondary prevention trials are negative, it may force us either to accept surrogate subclinical end points or to wait a longer period of time for the results of the primary prevention trials.

It would be pleasant to be able to put folic acid in a hamburger bun and enjoy half a pound of a juicy high-fat hamburger without worrying about high LDL cholesterol, coronary atherosclerosis, and thrombosis. Carrots were not the panacea. Some hope that putting folic acid in bread will be the next great public health advance for cardiovascular disease.

References


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