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Preventing Coronary Heart Disease
B Vitamins and Homocysteine

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The list of preventable and reversible risk factors for atherosclerotic cardiovascular disease continues to grow. Cigarette smoking, high blood pressure, physical inactivity, elevated cholesterol, underlying lipoprotein abnormalities, lipoprotein(a), diabetes, overweight, male gender, and age are well-established risk factors. During the 1990s, there have been many reports associating elevated plasma homocysteine levels with arteriosclerotic cardiovascular disease and consistent evidence that dietary and supplemental folic acid can reduce homocysteine levels.[1,2]

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The article by Robinson and colleagues[3] in this issue of *Circulation* presents further evidence of the importance of homocysteine and suggestive evidence that plasma folate and plasma pyrixodal-L-phosphate (vitamin B6) are protective factors. Their study is part of the European Concerted Action Project,[4] which examined 750 patients younger than age 60 with diagnoses within the previous 12 months of coronary, cerebrovascular, or peripheral vascular disease and 800 healthy control subjects. The patient groups were young (47 years for cases and 44 years for control subjects) and heterogeneous, with nonfatal clinical events or symptoms of arteriosclerotic cardiovascular disease supported by ECG, angiographic, or Doppler evidence; the study involved 19 centers in nine European countries. Men in the highest quintile for fasting total homocysteine (tHcy), compared with the remainder of the population, had an estimated relative risk of 2.2 (95% confidence interval [CI], 1.6 to 2.9), with a striking dose-response relationship and a more-than-multiplicative interaction with cigarette smoking and high blood pressure on vascular disease risk[4]; the corresponding estimated relative risk for coronary heart disease was similar (2.0; 95% CI 1.6 to 2.8). (tHcy is the sum of homocysteine and homocysteinyl moieties of oxidized disulfides, homocystine, and cysteine-homocysteine.)

Robinson and colleagues[3] examined three B vitamins in detail to determine their effects on fasting and post–methionine-loading tHcy levels and any independent effects on cardiovascular disease risk. The results should be considered preliminary. Low folate and low vitamin B6 levels were statistically significantly more frequent among patients than among control subjects; a similar tendency for plasma B12 was not statistically significant. The inverse association with disease for folate was in part accounted for by increased tHcy levels, but the association for vitamin B6 was not. Relative risks for the top quintile of fasting tHcy and for high postload tHcy were 1.69 (CI, 1.26 to 2.26) and 1.62 (CI, 1.22 to 2.16), respectively, compared with all other quintiles, after adjustment for the vitamin effects. These relative risks are similar to the weighted means of other studies; it should be noted that one must scrutinize reported results for the choice of comparison groups and the units of change in homocysteine when comparing estimated relative risks between studies.[2]

The findings regarding folate and B6 by Robinson et al[3] would be stronger if they better matched the metabolic roles of the vitamins in homocysteine metabolism. Homocysteine is formed from the sulfur-containing essential amino acid methionine. Homocysteine can be transsulfurated to cysteine via two B6-dependent reactions or remethylated to methionine via B12- and folate-dependent reactions. Because fasting levels are more influenced by remethylation and post–methionine-load levels are more influenced by transsulfuration, one would expect folate (and B12) to be acting primarily on fasting levels and B6 to be primarily acting on postload levels. Both measures of tHcy were investigated in the 1550 study participants, but no such differential effects of folate and B6 were found.

In one of several reports since the meta-analyses by our group,[2,5] Verhoef et al[6] found relative risks of 1.3 (CI, 1.0 to 1.6) for each 1 SD increase (5 μmol/L) in fasting tHcy in a comparison of 131 patients with 88 less severely affected patients with coronary artery disease and 101 population control subjects. Within patients and within control subjects, there was the expected inverse relationship between each of the three B vitamins and tHcy levels; but, contrary to expectations, pyridoxal-5-phosphate and folate levels were not lower in patients compared with the combined control groups. Among men who had received routine examinations in London, tHcy was strongly associated with death from ischemic heart disease (estimated relative risk, 2.9; CI, 2.0 to 4.1, after adjustment for apolipoprotein B and blood pressure).[7] Nygard et al[8] reported mortality results for 587 patients with angiographically confirmed coronary artery disease. After a median follow-up of 4.6 years, 64 had died (50 from cardiovascular causes). There was a striking graded relationship between plasma tHcy and overall mortality (eg, 25% of those with tHcy levels of ≥15 μmol/L had died compared with 4% of those with levels of <9 μmol/L). tHcy levels were strongly related to history of myocardial infarction, left ventricular

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ejection fraction, and serum creatinine level but much less related to extent of coronary artery disease on angiography.

Not all new studies are consistent regarding the risk of tHcy. An updated analysis of Physicians' Health Study data yielded a relative risk for elevated tHcy of only 1.3 (CI, 0.5 to 3.1).[9] An analysis of the MRFIT cohort showed no effect of tHcy after adjustments for other variables (relative risk, 0.94; CI, 0.56 to 1.56).[10] The ARIC study showed no association between the incidence of coronary heart disease and tHcy (A Folsom, unpublished data, 1997).

Thus, many questions remain regarding the relationship of folate, vitamin B12, and vitamin B6 to levels of tHcy; the relationship of homocysteine to cardiovascular risk; and the best ways to demonstrate and recommend risk reduction for individual patients and for populations.

What Is the Best Estimate for the Increased Risk of Coronary Heart Disease Mortality Associated With Elevated tHcy Levels?

On balance, based on the references above and the meta-analysis,[5] we believe the best estimate is a relative risk of 1.4 for the difference between tHcy levels of >15 μmol/L compared with levels of <10 μmol/L after adjustment for other cardiovascular risk factors. This effect is similar to the impact expected from a reduction in total serum cholesterol from 7.1 to 4.9 μmol/L (275 versus 189 mg/dL).[4]

How Much Supplementation With Folate (or Other B Vitamins) Is Desirable and Safe?

We believe it is desirable to bring tHcy levels down to the range of 9 to 10 μmol/L. Diet alone is unlikely to be sufficient to increase circulating folate levels and decrease tHcy levels.[2] Also, the bioavailability of folic acid from typical conjugated folates in the diet is one half that from supplements. Feeding studies are needed; they must incorporate information on methylenetetrahydrofolate reductase (MTHFR) genotypes (see below) to account for marked variation in response.

Fourteen intervention studies[5] showed substantial decreases in average tHcy levels after the administration of 650 to 10 000 μg of supplemental folic acid. tHcy concentrations do not appear to reach a plateau until folate intakes approach 400 μg/d and serum folate reaches ≥15 μmol/L.[11] Other studies have shown significantly lower tHcy levels in persons taking supplements containing folic acid than in those relying on diet. Thus, a supplement containing 400 μg of folic acid is expected to produce an average reduction of 5 μmol/L tHcy in nonusers of supplements.[2,5] A higher dose of folic acid might be necessary in some persons (see below).

Clinical trials with folic acid in at least four countries have shown that the incidence of children born with neural tube closure defects (spina bifida, meningomyelocoele, anencephaly) to mothers with a prior neural tube defect–affected pregnancy can be reduced by 50% to 75%, leading to an official Centers for Disease Control and Prevention recommendation that all women of childbearing age consume ≥400 μg/d folate.[12] This public health intervention may be one of the most important steps to prevent serious birth defects. Because surveys showed that only 7% to 12% of women were taking folic acid supplements at the time they became pregnant, in March 1996 the Food and Drug Administration responded to advice and petitions by requiring that cereal grain products be fortified at the level of 140 μg of folic acid/100 g of product, beginning in January 1998.

Fortification, of course, leads to exposure for the total population, which stimulated urgent consideration of potential health risks, especially for older adults and any persons with a B12 deficiency. One of us recalls that Dr William B. Castle informed the first-year Harvard Medical School class that one should "never give folic acid without giving B12 first" to avoid potential exacerbation of possibly irreversible neurological impairments. It is hard to find documentation for serious neurological complications in previously undiagnosed persons.[13] Nevertheless, to prevent such adverse outcomes even in individuals with lack of intrinsic factor for B12 absorption, 200 to 1000 μg of cobalamin (B12) could be included in supplements that contain 400 μg of folic acid.[2,13]

Is There Evidence of Genetic Variation in Folate and Homocysteine Levels?

The original proposal that hyperhomocysteinemia might be important in atherosclerosis came from work by McCully[14] on the vascular pathology of the inborn error of metabolism, homocystinuria. However, carriers for homocystinuria (1 in 400 population), who have half-deficiency of cystathionine β-synthase, rarely account for the homocysteine elevations observed in vascular disease.[15] A common thermolabile variant of the enzyme MTHFR is homozygous in 10% to 13% of the Caucasian population (*TT* genotype). Such individuals, particularly in the presence of suboptimal folate nutrition, tend to have slightly elevated homocysteine levels.[16] In two different European studies, 35% of working men in the top decile of tHcy levels had the *TT* genotype,[17] as did almost everyone with tHcy levels of >20 μmol/L.[18] Despite several reports that the *TT* genotype is increased among patients with premature vascular disease,[16] no such association could be demonstrated in 2029 patients with coronary heart disease when compared with 1639 control subjects across seven different independent studies (A.G. Motulsky, unpublished data, 1997). It is noteworthy that homozygotes for the *TT* variant had a 21% reduction in tHcy levels after supplementation with 1000 μg of folic acid compared with lesser reductions among heterozygotes (13%) and the more common homozygotes for the *CC* variant (7%).[16] It is likely, therefore, that genetic variants in MTHFR and other enzymes related to folic acid metabolism (eg, methionine synthase) will require individuals to have different nutritional and supplement needs.[19] Nutritional needs and intervention dosages must be tailored to the underlying pathophysiology, a general challenge we still face in national guidelines for screening and treatment of elevated serum cholesterol values.

What Kinds of Prevention Trials Are Needed and May Be Feasible?

Cardiovascular researchers have led the way with large-scale randomized trials of interventions for patients with specific clinical conditions (secondary prevention) and for healthy populations with risk factors for developing cardiovascular end points (primary prevention trials). The homocysteine hypoth-

esis should be well suited to a direct test with folic acid as the intervention. However, as with all trials, the choice of the study population, choice of the agent or combination of agents, determination of an adequate and safe dose, and parameters of the design (incidence rates for the end points, size of effect expected, duration of intervention and follow-up, and allowance for nonadherence and for competing causes of death) must all be taken into account. Potential study populations include cardiac patients and healthy populations, and genotyped and high tHcy subgroups of each. Many investigators around the world are considering such trials; pilot trials will be needed. The lesson learned from the randomized trials in Finland and the United States that tested the seemingly compelling hypothesis that β-carotene would reduce lung cancer and coronary heart disease incidence and found that this vitamin/chemical instead increased lung cancer incidence and cardiovascular mortality[20] is that statistical associations do not prove cause-and-effect relationships and do not rule out adverse effects. Associations should not be described as "effects."

Potential trials are complicated by the introduction of folate fortification of grains and by increasing recommendations for the use of folic acid supplements in the general population and in cardiac patients. The Beta-Carotene and Retinol Efficacy Trial (CARET) faced a similar dilemma when β-carotene was being added to cereals and multivitamins and was highly promoted before the trial results were obtained. It may prove impossible to mount a sufficiently powerful trial. In that case, the stronger the biochemical, pathophysiological, nutritional, and genetic information about the cascade from dietary intake and genetic variation to circulating levels of folate and tHcy, the more persuasive will be the current inference of benefit. In CARET, we are analyzing the full cascade, from food frequency questionnaire estimates of folate intake and polymerase chain reaction analyses of genetic variation in the MTHFR enzyme to serum folate and B12 levels, tHcy concentrations, and observed fatal cardiovascular end points (G.S. Omenn, M.R. Malinow et al, unpublished data).

The Vitamins in Stroke Prevention (VISP) trial is recruiting 3600 patients with nondisabling strokes to receive a multivitamin combination containing 2.5 mg of folic acid, 25 mg of B6, and 0.4 mg of B12 versus a multivitamin with 20 mg of folic acid, 0.2 mg of B6, and 6 mg of B12, with a primary end point of recurrent stroke and secondary end points of death from cardiovascular disease or myocardial infarction. The trial is based on a pilot study of homocysteine lowering in patients with acute stroke.[21] In addition, a protocol has been announced for a nested case-control study among 30 000 patients receiving drugs for heart disease or high blood pressure in general practices in Norway.[22]

What Should Clinicians Do With Present Knowledge?

Because we interpret the totality of the current evidence linking folic acid, homocysteine, and cardiovascular disease risk as remaining strong with respect to the potential benefits of increasing folic acid intake on a population-wide basis, we recommend that everyone consume ≥400 μg of folic acid/d. Potentially pregnant women should take more to maximize the protective effect against neural tube closure defects. Screening for tHcy levels would be useful for individual risk profiles and for targeting efforts at adherence or recommendations for higher doses. Common multivitamins contain 2 to 3 mg of B6 and 6 to 9 μg of B12. We have no recommendation on B6 because definitive evidence of an inverse association with tHcy levels and of an optimal dose does not exist. As noted above, we recommended[2] and urged the Food and Drug Administration to mandate inclusion of sufficient B12 in folic acid capsules (200 to 1000 μg) to ensure adequate absorption by passive mechanisms even in the absence of intrinsic factor. Inclusion of B12 in the fortified grains deserves consideration, as well; if it is not included, B12 should be prescribed, especially to protect older individuals with various degrees of B12 deficiency.[13]

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