in improved urinary control. At the same time, the trial also serves to raise the bar for the evaluation of future surgical innovations.

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Homocysteine Trials — Clear Outcomes for Complex Reasons

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In 1969, McCully first proposed that homocysteine causes atherosclerosis. His hypothesis was based on the finding of atherosclerotic plaque at autopsies of young people with homocystinuria. This hypothesis was later modified to include a broader population, positing that mild hyperhomocysteinemia caused by dietary deficiencies of the vitamin cofactors required for the metabolism of homocysteine — folic acid, vitamin B₁₂, and vitamin B₆ — is a risk factor for atherothrombosis. In developed countries, these vitamins are partially removed from foods during processing, and typical diets are rich in the precursor amino acid methionine (which is derived from animal proteins). These conditions result in elevated homocysteine concentrations.

Although at first not generally accepted, epidemiologic studies conducted over the past 25 years have provided ample support for the association of mild hyperhomocysteinemia with an elevated risk of atherothrombosis. In a meta-analysis of prospective observational studies of first events, the members of the Homocysteine Studies Collaboration concluded that a 25 percent reduction in the serum homocysteine concentration (a reduction of approximately 3 μmol per liter) is associated with an 11 percent lower risk of ischemic heart disease (odds ratio, 0.89; 95 percent confidence interval, 0.83 to 0.96) and a 19 percent lower risk of stroke (odds ratio, 0.81; 95 percent confidence interval, 0.69 to 0.95). The results of prospective studies of recurrent cardiovascular events are more consistent than those for first events; they show in general that the hazard ratio for a recurrent event increases by 16 percent with each increase of 5 μmol per liter in the serum homocysteine concentration.

The independent risk of cardiovascular events conferred by mildly elevated serum homocysteine levels and the association of elevated levels with a deficiency of folic acid and vitamin B₁₂ have offered a unique target for preventive approaches. The metabolism of homocysteine is complex. In hepatic cells, it involves transsulfuration (by means of the vitamin B₆-dependent rate-limiting enzyme cystathionine β-synthase) to cystathionine and thence to cysteine; in nonhepatic cells, the principal pathway is remethylation to methionine. Methionine synthesis is based on the folic acid–dependent and vitamin B₁₂-dependent and homocysteine methyltransferase.

Several large, prospective trials have been initiated over the past five years to study the consequences on cardiovascular events of lowering serum homocysteine concentrations with the use of folic acid, vitamin B₁₂, and vitamin B₆. The ease of administration of these inexpensive, naturally occurring cofactors has offered a straightforward...
approach to testing the homocysteine hypothesis. Data from animal models and small trials in humans involving surrogate end points — including measurements of endothelial function and markers of oxidant stress and inflammation and their responses to folic acid (and vitamin B₁₂) — have yielded reasonably consistent results. Some study results have differed depending on the dose and duration of folic acid therapy and its independent benefit with regard to vascular function. Nevertheless, this overall body of work has provided a credible basis for the design of the main trials.

Three of these prospective trials of the effects of homocysteine-lowering therapy on recurrent cardiovascular events among subjects with known cardiovascular disease have now been completed, In the Vitamin Intervention for Stroke Prevention (VISP) trial, two groups of patients with stroke (3680 patients in total) were treated with different daily doses of folic acid, vitamin B₁₂, and vitamin B₆; after two years, there was a dose-dependent reduction in homocysteine concentration but no significant difference in the rates of vascular events between the two groups.

The results of the Norwegian Vitamin (NORVIT) trial and the Heart Outcomes Prevention Evaluation (HOPE) 2 trial, both reported in this issue of the Journal, are similar. The NORVIT trial was a study of secondary prevention involving 3749 patients who had had an acute myocardial infarction and who were treated daily with folic acid, vitamin B₁₂, and vitamin B₆; folic acid and vitamin B₁₂; vitamin B₆ alone; or placebo. After a median follow-up of 40 months, despite a 27 percent lowering of the mean total homocysteine concentration from the baseline value among those treated with folic acid and vitamin B₁₂, there was no significant effect of folic acid and vitamin B₁₂ on the risk of the composite primary end point of recurrent myocardial infarction, stroke, or sudden death from coronary artery disease. There was, however, a near-significant trend toward more myocardial infarctions, as well as a marginally significant trend toward fewer strokes, among patients receiving folic acid, vitamin B₁₂, and vitamin B₆ than among those receiving placebo.

HOPE-2 was a prevention trial involving 5522 patients with vascular disease or diabetes who were treated daily with a combination of folic acid, vitamin B₁₂, and vitamin B₆ or with placebo for an average of five years. Again, vitamin treatment was associated with a substantial reduction in plasma homocysteine concentration but not with a significant reduction in the risk of the composite primary end point of myocardial infarction, stroke, or death from cardiovascular causes. In addition, this trial showed a marginally significant reduction in stroke among the patients receiving vitamins than among those receiving placebo.

The data are quite consistent among these three similar (but not identical) patient populations, including patients who had and those who did not have access to foods fortified with folic acid. Although the vitamin doses used, the consequences of folic acid fortification on the expected event rates, and the implications of the trend toward lower rates of stroke could all be debated, the consistency among the results leads to the unequivocal conclusion that there is no clinical benefit of the use of folic acid and vitamin B₁₂ (with or without the addition of vitamin B₆) in patients with established vascular disease.

The results also raise two other questions that merit consideration. First, does the failure of homocysteine-lowering therapy to reduce the rates of cardiovascular events suggest that the homocysteine hypothesis is incorrect? And if so, is homocysteine a surrogate for another, metabolically related species that is the true atherogenic culprit? Although suggested by the results, affirmative answers to these questions are inconsistent with the abundant evidence in vitro and in vivo that homocysteine is an atherogenic determinant that promotes oxidant stress, inflammation, thrombosis, endothelial dysfunction, and cell proliferation.

Second, if homocysteine is an atherogenic determinant, do the results of these trials suggest that vitamin therapy has other, potentially adverse effects that offset its homocysteine-lowering benefit? Three mechanisms might explain the potential adverse effects of this therapy.

One possible mechanism is that, through its role in the synthesis of thymidine, folic acid promotes cell proliferation (which is the basis for chemotherapies that disrupt the methylation cycle). Folic acid may do the same in the atherosclerotic plaque. This mechanism has been offered as an explanation for the worsening rates of in-stent restenosis in a recent study of patients who had undergone angioplasty and were treated with folic acid, vitamin B₁₂, and vitamin B₆.
Another possible mechanism is based on the relation of homocysteine to the methylation cycle (Fig. 1). High homocysteine concentrations lead to increased S-adenosylhomocysteine concentrations: folic acid and vitamin B\textsubscript{12} promote the remethylation of homocysteine to methionine, which in turn lowers S-adenosylhomocysteine and increases S-adenosylmethionine levels. This latter species is the sole source of methyl groups for all methylation reactions in the cell.\textsuperscript{16} As a result of their influence on the steady-state concentrations of these S-adenosyl derivatives, high homocysteine concentrations are associated with a reduced methylation potential, whereas folic acid and vitamin B\textsubscript{12} increase the methylation potential. The methylation of CpG-rich islands (short regions of DNA in which the frequency of the CG sequence is greater than in other regions) in promoter regions of DNA is an epigenetic mechanism for modulating gene expression. First recognized as a means of silencing genes during development and of inhibiting carcinogenesis, DNA methylation also appears to play a role in atherogenesis. Atherogenesis involves local hypermethylation and hypomethylation of genes, and recently, atherogenic lipoproteins have been shown to promote DNA hypermethylation in cultured human macrophages.\textsuperscript{17,18} Thus, the use of folic acid and vitamin B\textsubscript{12} in the setting of mild hyperhomocysteinemia may alter the methylation potential in vascular cells, resulting in a change in the cell phenotype that promotes the development of plaque.

As a third possible mechanism, another important methylation reaction that can promote atherogenesis, independently of changes in gene expression, is the methylation of L-arginine to asymmetric dimethylarginine. The latter, a metabolic product of protein arginine residues, inhibits the activity of nitric oxide synthase and is associated with an increased risk of vascular disease. Again, one might predict that by increasing the methylation potential, treatment with folic acid and vitamin B\textsubscript{12} might increase, or at the very least not change considerably, the concentration of asymmetric dimethylarginine.\textsuperscript{19}

What, then, can we conclude from the results of these trials? Clearly, folic acid, vitamin B\textsubscript{12}, and vitamin B\textsubscript{6} are not the therapeutic solution expected, and they do not provide a preventive benefit in patients with mild hyperhomocysteinemia. The straightforward but incorrect view that folic acid can decrease homocysteine levels and, thus, reduce the risk of atherosclerosis effectively may be an unintended consequence of oversimplifying a complicated metabolic network. Further exploration of the relations among the intermediates in this metabolic pathway and their association with atherothrombotic mediators will be needed. Meanwhile, we should consider alternative approaches to reducing homocysteine concentrations, perhaps with new methods of enhanc-
ing the conversion of homocysteine to cysteine in
the liver or enhancing the urinary excretion of the
amino acid.

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Treating Acute Asthma with Antibiotics — Not Quite Yet
Frédéric F. Little, M.D.

Although the root cause of asthma is not known, treatments have been developed that target facets of the underlying pathologic abnormalities of asthma: airway inflammation, hypersecretion of mucus, and airway hyperresponsiveness. These treatments not only have improved the site of drug delivery (e.g., inhaled corticosteroids) but have directly targeted the relevant pathways that contribute to symptoms (e.g., leukotriene modifiers and anti-IgE). Although these treatments have proved to be effective, practitioners are often humbled as the condition progresses despite their best efforts to control it; therapeutic strategies that target unexplored disease associations are needed.

Viral infection is strongly associated with both wheezing in children and exacerbations of asthma in adults, and there is evidence that acute infection with the atypical pathogens *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* is associated with acute asthma episodes. Despite the recommendations of clinical practice guidelines against the empirical use of antibiotics in acute exacerbations of asthma, the presence of clinically purulent sputum and cough occasionally leads the clinician to “play it safe” and start treatment, especially in the setting of severe disease or exacerbation.

The results of the Telithromycin, Chlamydophila, and Asthma (TELICAST) study, reported by Johnston et al. in this issue of the *Journal*, address this issue directly. In the study, patients who...