Are we losing homocysteine as a cardiovascular risk factor?

About 12 y ago, some pioneering publications reported an association between hyperhomocysteinemia and atherosclerosis [1]. This association was extraordinarily appealing because this new cardiovascular disease risk factor depended on folate and vitamin B12 nutrition. Therefore, reductions in homocysteine concentrations could be easily achieved through supplementation with folate and vitamin B12. Soon, new studies in adults showed a close association between homocysteine concentrations and mortality caused by cardiovascular diseases [2].

As always happens, however, it was not long before doubts were raised about the real value of this easily managed cardiovascular disease risk factor. The first obvious doubt was whether homocysteine was the real risk factor or merely a marker of vitamin B6, vitamin B12, and folate deficiencies and whether deficiencies of these vitamins were the real arterial-damaging agents. The second doubt was the lack of demonstration, in prospective trials, that lowering homocysteine is associated with a decrease in cardiovascular events or mortality.

With respect to the real toxicity of homocysteine, some have postulated that the modification by homocysteine of low-density lipoproteins could enhance the endothelial-damaging effects of these particles [3]. In this issue of *Nutrition*, Su et al. [4] report a series of in vitro studies in which they show that homocysteine, when incubated with human monocytes, induces mRNA and protein expressions of inflammatory cytokines, such as tumor necrosis factor-α and several interleukins, and reduces the expression of the migration inhibitory factor. Although the experiments were carefully done and show the effects of homocysteine at the transcription, translation, and protein expression levels, the reader experiences some concern about the translation of these results to clinical practice. Most of the effects were shown with homocysteine concentrations of at least 25 μg/mL. This corresponds to 185 μM, whereas the range of homocysteine concentrations that we normally observe in adult patients with hyperhomocysteinemia rarely, if ever, exceeds 40 μM. Even in patients with homocystinuria, mean homocysteine concentrations range from 130 to 140 μM [5]. Therefore, the results of Su et al. are difficult to extrapolate to clinical situations.

If we take into account the concentration problem, the possibility that homocysteine per se generates an inflammatory response could be an interesting pathogenic mechanism for endothelial dysfunction. Clinically, there are reports showing that hyperhomocysteinemia is associated with abnormal vascular reactivity, but these effects cannot be separated from those of folate or vitamin B12 deficiency. However, we have not been able to show that hyperhomocysteinemia is associated with altered endothelium-dependent vascular reactivity in young subjects or that endothelial function is modified by folate supplementation [6]. In the elderly, we have observed that homocysteine alters vascular reactivity only when there is a concomitant folate depletion [7,8]. Other reports have shown that tetrahydrofolate infusions acutely modify vascular reactivity without modifying homocysteine concentrations [9]. A recent clinical trial showed that 6 mo of supplementation with folic acid, vitamin B12, and pyridoxine had no effects on markers of endothelial inflammation, endothelial dysfunction, or hypercoagulability despite significant reductions in homocysteine concentrations [10].

In another line of thought, several in vitro studies have shown that homocysteine causes oxidative stress and alters the vasoactive properties of cultured endothelial cells, thereby impairing the production or bioavailability of vasoactive mediators such as endothelin-1, nitric oxide, and prostacyclin [11–13]. However, those results were obtained with homocysteine concentrations higher than 200 μM in the culture medium, and such high concentrations may be deleterious to the endothelial cell. In contrast, other investigators support the hypothesis that high concentrations of homocysteine cause reductive rather than oxidative stress, because homocysteine inhibits the expression of antioxidant enzymes [14]. We showed that homocysteine at concentrations lower than 100 μmol/L has no effect on thiobarbituric acid–reactive substances produced in the endothelial cell system. In contrast, when copper ions were used to initiate oxidation, homocysteine in concentrations of 15 and 100 μmol/L reduced the generation of lipid peroxides after 60 to 120 min of incubation. The antioxidant properties of homocysteine may be explained by the free radical–scavenging properties of thiol groups [15].

The results of the long-awaited clinical trials measuring the effects of folate supplementation on cardiovascular events are even more disappointing. A large, randomized, controlled trial performed in 3680 adults to study the effects

of folate, vitamin B12, and pyridoxine supplementation on the risk of recurrent stroke found no positive clinical effect, despite a mean reduction of 2 μmol/L in homocysteine concentrations. A dim light of hope remains, however, because even though the supplementation had no effect, basal homocysteine concentrations were predictive of recurrent strokes [16].

The results of another supplementation study of patients who underwent angioplasty with stent placement were even more disappointing. That study enrolled 636 patients to receive supplementation with folate, vitamin B12, and pyridoxine in a double-blind fashion for 6 mo [17]. At the end of the observation period, the degree of restenosis was greater in the supplemented patients than in those who received placebo. The sad conclusion is that the two most recent prospective trials have not shown positive effects of vitamin supplementation on cardiovascular events or, even worse, have shown negative effects.

The hope for the future is that high homocysteine concentrations, more than a pathologic condition per se, are a good marker of deficiency of those vitamins involved in one-carbon transfer and that this defect may have negative effects on many targets. Currently, there is great interest in the consequences of acquired methylation defects in DNA and the resulting changes in gene expression [18]. A good deal of evidence suggests that demethylation of promoter regions of DNA could unmask oncogenes and increase the incidence of certain types of cancer [19]. Other lines of investigation are exploring the effects of methylation defects on cognitive function [20] or alcoholic liver disease [21]. Methylation may also play a role in vascular reactivity and vascular remodeling, and this may explain the association between hyperhomocysteinemia and atherosclerosis.

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