"Vitamin B or L-arginine supplementation in hyperhomocysteinaemia: think twice!"

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Abstract
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Vitamin B or L-arginine supplementation in hyperhomocysteinaemia: think twice!

EXPERT’S PERSPECTIVE

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Solid epidemiological data have established the increased risk of cardiovascular disease with elevated plasma homocysteine levels. Yet, interventional studies with proven efficacy to reduce homocysteine have deceptively failed to reduce cardiovascular events. While the reason for such a paradox had long been debated, the study by Sydow et al. both provided a likely explanation and indicated a potential therapeutic solution. Most importantly, the demonstration was based on human data from a study in patients with peripheral vascular disease.

The authors’ reasoning was grounded on previous knowledge of the mechanism of homocysteine, especially metabolic pathways for its disposition in liver and other tissues. There, transulfuration involving vitamin B6-dependent cystathionine β-synthase orients homocysteine towards cystathionine and subsequent formation of sulfated metabolites, including antioxidants and signalling molecules such as hydrogen sulfide. Alternatively, homocysteine can be re-methylated through folate- and vitamin B12-dependent reactions to methionine, itself involved in subsequent methylation reactions involving adenosyl intermediates, eventually to re-form homocysteine. Of note, one of the methylation reactions involves the formation of asymmetric dimethyl-arginine (ADMA), a known inhibitor of nitric oxide synthase (NOS), as had previously been demonstrated by the same authors.

The inhibitory effect of ADMA can be competed away with excess L-arginine, the natural substrate of NOS. Therefore, the authors compared the efficacy of L-arginine supplementation with that of a mixture of folic acid and vitamins B6 and B12 (vs. placebo) on flow-mediated endothelial function in patients with hyperhomocysteinaemia and endothelial dysfunction. The functional readout was coupled with measurements of plasma homocysteine, the L-arginine/ADMA ratio, and urinary 8-iso-PGF2α, as an index of oxidant stress.

Based on previous demonstrations and prediction from metabolism, as outlined above, the vitamin B mixture was expected to reduce plasma homocysteine, and it did so. However, this failed to improve endothelial function, nor did it attenuate the prevailing oxidant stress in these vascular patients. Conversely, L-arginine supplementation did not lower homocysteine levels, but improved both endothelial function and oxidant stress. Notably, this was associated with an increased plasma L-arginine/ADMA ratio.

As elaborated in the paper’s discussion, these data could reasonably be interpreted on the basis of the differential effects of the two interventions on metabolism. Vitamin B plus folate supplementation promoted homocysteine disposition through both the transulfuration and re-methylation pathways, effectively reducing plasma homocysteine levels. But, as predicted by previous studies, it also promoted subsequent methylation reactions, including ADMA formation, that in turn could inhibit NOS and obliterate any improvement of endothelial function. Importantly, the same effect could explain the lack of improvement of oxidant stress, resulting from NOS inhibition.

In addition to resolving the paradox, the study pointed to a new mechanism of oxidant stress associated with hyperhomocysteinaemia, i.e. NOS inhibition by ADMA resulting from methylation reactions. Moreover, it proposed a likely cure for it, by supplementing L-arginine to compete ADMA’s inhibitory effect. Indeed, L-arginine supplements reduced markers of oxidant stress and improved flow-mediated endothelial function (known to be NOS dependent). Expectedly, it was paralleled with an increased L-arginine/ADMA ratio.

The data have been supported to some extent by subsequent experimental and clinical evidence. First, a meta-analysis of clinical trials with folic acid and vitamin B supplementation has confirmed the failure of such strategy to reduce mortality and has even highlighted its potential harm by promoting the incidence of new cancers, with the exception of vitamin B6 alone. Folic acid, when used alone, has been associated with positive effects in pre-clinical models and small clinical studies, but through alternative effects: indeed, folic acid can replenish tetrahydrobiopterin, an essential cofactor for the NOS. However, recent studies convincingly show that the efficacy
of raising tetrahydrobiopterin critically depends on the ratio of its reduced to oxidized forms, which may be compromised under prevailing oxidant stress. L-Arginine supplementation was also shown to produce beneficial effects in some experimental settings, in part through competition of ADMA. However, the clinical efficacy of l-arginine supplementation was again a disappointment. One reason may be that feeding the endothelial NOS with excess substrate under conditions of concurrent depletion of reduced tetrahydrobiopterin (due to prevailing oxidant stress in vascular disease) may do more harm than good, since it was shown to increase the production of superoxide anions by the uncoupled enzyme (i.e. a status where NOS production of superoxide anions offsets that of nitric oxide), thereby aggravating oxidant stress. Such uncoupling usually follows dissociation of the dimeric enzyme as a result of L-arginine depletion (itself favoured by l-arginine catabolism by arginase), oxidation of tetrahydrobiopterin, or direct glutathionylation of endothelial NOS. Dual endothelial cell replenishment with L-arginine and reduced tetrahydrobiopterin could possibly do better, but an efficient formulation remains to be tested clinically.

As usual, this teaches us a lesson of humility and brings us back to the bench to gain more understanding of the sophisticated regulation of NOS. Nevertheless, we will always learn from insightful clinical studies such as this one by Sydow et al. that offered new interpretations of the pathophysiology of NOS-dependent endothelial dysfunction and of the efficacy (or lack thereof) of therapeutic strategies for the associated cardiovascular disease.

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