Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 39, No. 1, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(01)01693-X

EDITORIAL COMMENT

Oral L-Arginine (and Other Active Ingredients) for Ischemic Heart Disease?*

Richard O. Cannon III, MD, FACC
Bethesda, Maryland

The endothelium maintains a nonthrombotic surface for blood flow, prevents platelet and leukocyte activation and adhesion, modulates cellular composition of the arterial wall, and promotes dilator tone of arteries and veins, homeostatic properties regulated in part by the endothelial synthesis of nitric oxide (NO). Endothelial release of NO is reduced or absent in patients with coronary artery disease (CAD) or its risk factors (1) and may contribute to myocardial ischemia by limiting appropriate blood flow during stress (2). Furthermore, reduced NO bioactivity—as evidenced by abnormal dilator responsiveness to acetylcholine, cold pressor testing or shear stress during hyperemia—has been reported by several groups to indicate increased risk of serious cardiovascular events in the few years that follow (3,4). Strategies for increasing endothelial NO synthase activity that may be amenable to pharmacologic intervention include enhanced transcription of the gene for this enzyme, stabilization of messenger RNA (mRNA) for greater enzyme synthesis, provision of cofactors for the enzyme and administration of the substrate, L-arginine.

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This last approach, in particular, has generated considerable interest and controversy in recent years. Normally, L-arginine is not rate-limiting in the enzymatic conversion of this semi-essential amino acid to L-citrulline and NO: the cytosolic concentration of L-arginine within endothelial cells exceeds by a factor of 1,000 the maximum substrate utilization rate by NO synthase. However, under specific conditions, administration of L-arginine might be expected to enhance NO synthesis. First reported by Vallance et al. (5) a decade ago, methylated arginines such as asymmetric dimethylarginine (ADMA), enzymatically generated from proteins that regulate RNA processing and transcriptional control, may compete with L-arginine for the active sustrate binding site on NO synthase. Because ADMA cannot be converted to NO, NO synthesis could be reduced if sufficient L-arginine were displaced. Elevated levels of ADMA have been measured in serum samples from patients with hypercholesterolemia and peripheral arterial disease, possibly a consequence of reduced catabolism by the enzyme dimethylarginine dimethylaminohydrolase (6–8). The relevance of endogenous NO synthase inhibitors to human atherosclerosis was suggested by a strong association between ADMA levels and carotid artery intimal-medial thickness measured by ultrasound in a Japanese population (9). Additional proposed mechanisms by which L-arginine bioavailability may be reduced include excess enzymatic conversion to ornithine via enhanced activity of arginase in plasma or within endothelial cells (10), and inhibition of L-arginine transport into endothelial cells (11).

Several groups have reported that intravascular infusion of L-arginine in patients with CAD and hypercholesterolemic subjects improves coronary and systemic endothelial function, often evidenced by prevention of constriction or enhanced dilation in response to intra-arterial acetylcholine (12–15). Although improved endothelial function in these studies is consistent with enhanced substrate availability for NO synthase, other effects of L-arginine may account for augmented NO synthesis. In this regard, intravenous infusion of L-arginine stimulates the release of insulin, which in turn activates endothelial NO synthase and promotes NO release (16). Furthermore, Nagase et al. (17) reported that L-arginine or D-arginine reacts nonenzymatically with H_2O_2—likely present at least transiently in the high-oxidant environment of the atherosclerotic arterial wall—to form NO. These mechanisms of arginine-mediated increase in NO that are independent of L-arginine substrate availability for NO synthase may explain why intra-brachial arterial infusion of D-arginine, which is not a substrate for this enzyme, improved forearm blood flow responses to acetylcholine to the same degree as L-arginine in patients with CAD (18). Regardless of the mechanism of L-arginine effect on endothelial function, two groups reported that intravenous infusion of L-arginine failed to improve exercise tolerance or ST segment responses in patients with CAD (18,19).

It is possible that longer duration of treatment with L-arginine is required to improve endothelial function enough that coronary blood flow increases during stress, preventing myocardial ischemia. Oral administration of L-arginine has been reported to improve brachial artery endothelial function in young hypercholesterolemic subjects and young patients with CAD, and coronary microvascular endothelial function in patients without angiographically significant CAD (20–22). Furthermore, studies including small numbers of patients with CAD reported improved exercise performance with delayed appearance of ST-segment depression and maximum ST-depression after three days of oral L-arginine (6 g daily) compared with exercise stress testing on placebo (23), and reduced angina after three months of oral L-arginine (24).

In the current issue of the *Journal*, Maxwell et al. (25) provide a new angle to the L-arginine story by testing the

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the Cardiology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland.

possible anti-ischemic effects of a "medical food bar" containing not only L-arginine (3.2 g per bar) but also folate, vitamins (C, E, B_6, B_{12}), niacin and soy isoflavones in a randomized, double-blind, single crossover trial of 36 patients with CAD maintained on a regimen of conventional medication. They report that these study participants had better brachial artery endothelial function (flow-mediated dilation), greater exercise duration on treadmill testing and higher scores on quality of life testing when two medical food bars were taken daily for two weeks than when two placebo bars were taken daily for two weeks. However, there were no significant differences between medical food bar and placebo periods in the ST segment response or onset of angina during exercise testing after two weeks of treatment, or in angina frequency or episodes and duration of ischemia as assessed by ambulatory monitoring during the two weeks of treatment. Nevertheless, the authors conclude that this medical food bar might be useful adjunctive therapy in the management of symptomatic patients with CAD.

This novel approach to managing ischemic heart disease is likely to be embraced by many patients eager for alternative or non-drug treatments for their disease, which already requires many medications, with a mechanism of action that is intuitively appealing, free of apparent toxicity and possibly tastes good as well (at 360 calories a day for two bars). However, the benefit of oral L-arginine to endothelial function and exercise performance in patients with CAD on medical management reported by this group is at variance with the published experience of two other groups that also used randomized, double-blind protocols. Blum et al. (26) administered L-arginine to 30 patients with CAD at a higher daily dosage (9 g) than, and for twice the duration (one month) of, the Maxwell et al. study (25). Compared with the placebo treatment period, no effect of L-arginine could be demonstrated on flow-mediated brachial artery dilation (as a bioassay for NO release into the arterial wall), on levels of nitrogen oxides in serum (as an index of NO released into the bloodstream), or on levels of markers of inflammation transcriptionally inhibited by NO in experimental preparations. Walker et al. (27) reported recently that L-arginine, 15 g daily for two weeks, did not reduce levels of ADMA or 8-epi-prostaglandin $F_{2\alpha}$ (as a measure of vascular oxidant stress), improve the forearm blood flow response to intra-brachial acetylcholine infusion or improve exercise duration in 20 men with CAD and stable angina differently than in 20 patients randomized to placebo. In both studies, significant increases in plasma arginine levels during L-arginine treatment periods were reported. It is possible that had medical management been discontinued (including statins, which improve endothelial function) in these studies, a robust anti-ischemic effect of L-arginine on endothelial function and inducible ischemia might have been detected. However, appropriate medical management was continued, testing the potential of L-arginine as adjunctive therapy to further benefit endothelial NO bioactivity and improve coronary blood flow during stress in patients who continue to have myocardial ischemia.

The benefit of the medical food bar of Maxwell et al. (25) to endothelial function, which is in contrast to the negative findings of studies cited above that used even higher daily doses of L-arginine, may be due to the ingredients in the bar other than L-arginine—folic acid, vitamins and micronutrients—that might enhance NO synthesis. Thus, folate regenerates tetrahydrobiopterin, a critical cofactor for NO synthase, from its inactive oxidized form. Levels of this cofactor may be reduced in atherosclerosis, possibly by increased oxidation to dihydrobiopterin, and contribute to impaired endothelial function. In this regard, in experimental preparations, endothelial NO synthase generates superoxide anions in the absence of tetrahydrobiopterin (28). Consistent with increased tetrahydrobiopterin generation, long-term folate administration improves endothelial function of patients with CAD (29). Administration of vitamin C also improves endothelial function of patients with CAD (30), possibly by increasing levels of tetrahydrobiopterin from its oxidized form (31). Thus, consistent with the design of the medical food bar, the biological effect of its component ingredients on endothelial function could be greater than any given alone.

So, if this medical food bar actually improves endothelial function, consistent with enhanced NO bioactivity and with the potential of improving coronary blood flow, why wasn't there objective evidence of an anti-ischemic effect? The authors believe that the medical therapy taken by patients may have obscured the anti-ischemic benefit of the medical food bar as assessed by treadmill exercise testing, although there was no effect of the medical food bar on ambulatory ischemia or angina frequency during the treatment period. However, the improved quality of life scores (especially "bodily pain" and "vitality" for the SF-36, and "treatment satisfaction" and "disease perception" for the Seattle Angina Questionnaire) during the medical food bar treatment period suggests an interesting line of investigation: the possible central nervous system effects of enhanced NO from neuronal NO synthase. Clearly, more placebo-controlled clinical trials will be necessary to resolve the issue of L-arginine (and other active ingredients) as adjunctive therapy for ischemic heart disease and the mechanism of benefit, if real.

Reprint requests and correspondence: Dr. Richard O. Cannon III, National Institutes of Health, Bldg. 10, Room 7B-15, 10 Center Drive, MSC 1650, Bethesda, Maryland 20892-1650. E-mail: cannonr@nih.gov.

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