Adverse Effects of Supplemental L-Arginine in Atherosclerosis
Consequences of Methylation Stress in a Complex Catabolism?
Joseph Loscalzo

Atherosclerosis and its risk factors are associated with endothelial dysfunction,¹ one manifestation of which is inadequate production of bioactive endothelial NO. Two basic mechanisms account for the loss of endothelial NO: decreased synthesis and increased oxidative inactivation. In the atherothrombotic vessel, both mechanisms appear to be active. Vascular production of reactive oxygen species creates an environment in which NO can be oxidatively inactivated to peroxynitrite and other derivatives. The sources of one of these reactive oxygen species—superoxide anion—include NAD(P)H oxidase elaborated on the plasma membrane of leukocytes, endothelial cells, and vascular smooth muscle cells; mitochondria; and the NO synthases. Both endothelial NO synthase (eNOS) and inducible NO synthase (iNOS) isoforms are expressed in the atherothrombotic vasculature and, owing to a loss of substrate or reducing cofactors required for NO synthesis, undergo enzymatic “uncoupling” leading to both a loss of NO production and an increase in superoxide anion generation.

The semiessential amino acid L-arginine is the principal substrate of the NO synthases, which catalyze its five-electron oxidation to L-citrulline and NO. As early as 1992, Creager and colleagues² showed that supplemental dietary L-arginine improved endothelial vasodilator response in hypercholesterolemic subjects, and Dubois-Rande and coworkers³ showed that intravenous L-arginine improved endothelial vasodilator response in patients with atheromatous left anterior descending coronary arteries. These initial observations were followed by numerous studies supporting the benefits of acute and chronic L-arginine supplementation on endothelial NO production in animal models and human subjects.

These studies were all predicated on the view that L-arginine will enhance the production of NO by eNOS, and that eNOS-derived NO is antiatherogenic. In established atherothrombosis, however, iNOS is upregulated, and its expression and activity can promote atherogenesis. The high flux of NO from iNOS in an environment rich in reactive oxygen species, especially superoxide anion, leads to the generation of peroxynitrite. The formation of this oxidant consumes NO, leads to 3-nitration of protein tyrosyl side chains, promotes oxidant stress, and oxidizes tetrahydrobiopterin; this last effect causes uncoupling of the NO synthases, which leads to their serving as additional sources of superoxide anion generation. In support of this mechanism, recent studies of mice with a genetic deficiency of iNOS that were also hyperlipidemic from apolipoprotein E deficiency [iNOS(−/−)/apoE(−/−)] revealed that the absence of iNOS expression was associated with a decrease in markers of oxidant stress and attenuated atherogenesis.⁴,⁵

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Chen and colleagues⁶ continue to explore the relationship between iNOS and atherogenesis by examining the effects of chronic L-arginine treatment on atherosclerotic burden in apoE(−/−) and in iNOS(−/−)/apoE(−/−) mice fed a Western diet. These authors hypothesized that L-arginine would reduce atherosclerotic plaque burden by providing substrate for eNOS. Unexpectedly, however, they observed that L-arginine had no effect on lesion area after 16 weeks in the apoE(−/−) mice, and, in fact, eliminated the protective effect of iNOS deficiency in the iNOS(−/−)/apoE(−/−) mice.

These results may at first appear surprising, but are consistent with several other studies in animals and human subjects that failed to demonstrate a benefit of L-arginine supplementation on vascular function or lesion burden.⁷⁻¹² Taken together, these negative studies suggest that the effect of supplemental L-arginine differs as a function of the species studied, the anatomic location of the lesions analyzed, and the presence or absence of established atherothrombotic disease. This conclusion, however, is not very satisfying in that it fails to address the possible molecular mechanisms by which to explain the inconsistent results.

One simple explanation for the adverse effect of supplemental L-arginine in these atherosclerotic mouse models is that L-arginine does, indeed, increase the production of vascular NO by eNOS in the iNOS(−/−)/apoE(−/−) mice and by iNOS in the apoE(−/−) mice; however, in the setting of established atherothrombotic disease with enhanced vascular superoxide production, this increase in NO leads to an increase in the production of peroxynitrite with a resulting increase in local vascular oxidant stress. This argument is supported by the results of immunostaining for 3-nitrotyrosine⁶ but weakened by results showing that a deficiency of iNOS is associated with a decrease in lipid peroxides.⁶

To explore other possible explanations, it is first necessary to review the complex metabolism of L-arginine (please see Figure 1). This semiessential amino acid engages in a variety of catabolic reactions that differ by physiological compartment and can be modulated by diet, hormones, and cyto-

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from plasma L-arginine is also low, representing only 1.2% of adenosine. The ingestion of 25 g/L L-arginine in the drinking water of the mice used in the experiments described by Chen and colleagues6 (without knowing precisely the amount consumed each day) may, therefore, have led to a significant increase in L-homocysteine generation. Owing to the sizeable methylation stress created by supplemental L-arginine in these animals (creatine synthesis accounts for nearly 10 times the flux of plasma L-arginine yielding NO), one might predict that dietary creatine supplementation would reduce de novo creatine synthesis by suppressing L-arginine:glycine amidinotransferase expression,22 thereby attenuating methylation stress and homocysteine production.22

In addition to the generation of L-homocysteine, L-arginine metabolism to guanidinoacetate can directly lead to increased generation of reactive oxygen species,23 thereby offering another potentially deleterious mechanism by which to explain the L-arginine effects in these animals. In the setting of hypercholesterolemia, the plasma and intracellular concentrations of the naturally occurring NOS inhibitor asymmetric dimethylarginine (ADMA) are increased,24 and hyperhomocysteinemia is also associated with an even greater increase in ADMA owing to the ability of homocysteine to inhibit dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that metabolizes ADMA to L-citrulline.25 As one final metabolic derangement to consider, L-arginine can undergo decarboxylation to L-agmatine, which has also been shown to inhibit NO synthases.26,27

The results of the study by Chen and colleagues6 together with several other prior studies7–12 suggest that we must temper our enthusiasm for the simple administration of L-arginine to increase the synthesis of NO by eNOS as a means to improve endothelial function and inhibit atherogenesis. In the setting of established atherothrombotic disease, the data for the benefits of L-arginine supplementation are at best inconsistent, and from the theoretical perspective, one can hypothesize rational mechanisms by which L-arginine can worsen existing vascular dysfunction and disease. Clearly, more experimental work will be required before we can confidently identify those individuals in whom L-arginine therapy will be consistently beneficial, and those in whom it should be used with caution, if at all.

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References


Figure 1. Principal catabolic fates of L-arginine. Dashed arrows indicate inhibition of enzyme activity. SAH hydrolase indicates S-adenosylhomocysteine hydrolase.


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