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

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Atherothrombosis and its risk factors are associated with endothelial dysfunction, 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 colleagues showed that supplemental dietary L-arginine improved endothelial vasodilator response in hypercholesterolemic subjects, and Dubois-Rande and coworkers 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.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Chen and colleagues 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. 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-nitrotyrosine but weakened by results showing that a deficiency of iNOS is associated with a decrease in lipid peroxides.

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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kines.13 While there is as yet no complex systems model of L-arginine catabolism in mammals that can be used to determine the kinetic and stoichiometric fates of ingested L-arginine, some basic facts have been well substantiated by studies using stable isotopic tracers. First, the plasma pool of L-arginine appears to be rather strictly segregated from the hepatic pool as only 5% of urea production is derived from plasma L-arginine.14 Second, the relative rate of NO synthesis from plasma L-arginine is also low, representing only 1.2% of total plasma L-arginine flux.14 Third, and most important, creatine synthesis represents a major fraction of total-body arginine utilization accounting for approximately 10% of the total plasma L-arginine flux,14 ie, nearly 10 times the flux of plasma L-arginine yielding NO.

If we take this metabolic argument a bit further, we find that the genesis of creatine from L-arginine creates a methylation demand that may have adverse consequences for the atherothrombotic vasculature. L-Arginine reacts with L-glutamate to yield L-ornithine and guanidinoacetate via the enzyme L-arginine:glutamate amidotransferase, principally localized to the kidney and pancreas. Guanidinoacetate is, in turn, taken up by the liver where it undergoes methylation to creatine via S-adenosyl-L-methionine: guanidinoacetate N-methyltransferase, which converts S-adenosylmethionine into S-adenosylhomocysteine in the process. S-adenosylhomocysteine is next reversibly hydrolyzed by S-adenosylhomocysteine hydrolase into homocysteine and 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 creatine generation15 and, in the process, a considerable increase in L-homocysteine generation. Owing to the sizeable methylation stress created by supplemental L-arginine in these animals (creatine synthesis accounts for 70% of the total utilization of labile methyl groups in mammals under normal circumstances16), the remethylation of homocysteine to methionine would be limited. Thus, transsulfuration, which is localized principally to the liver, is likely to have been the principal metabolic mechanism for eliminating the increase in homocysteine. As hepatic homocysteine will also enter the plasma and vascular cells are not capable of transsulfuration,17 the resulting increase in vascular homocysteine concentration would be expected to contribute to progressive endothelial dysfunction18,19 and injury, and, ultimately, to promote athereothrombosis in these hypercholesterolemic apoE(−/−) mice.20,21 If this hypothesis were correct, one would expect that the L-arginine-fed animals should have increased plasma concentrations of guanidinoacetate, creatine, and homocysteine, as well as an increase in markers of oxidant stress associated with the hyperhomocysteinemic state.19 Similarly, one might predict that dietary creatine supplementation would reduce de novo creatine synthesis by suppressing L-arginine:glutamate amidotransferase 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 dimethylaminoimidohydrolase (DDAH), the enzyme that metabolizes ADMA to L-citrulline.25 As one final metabolic deregament 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

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