

# Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

## L-Arginine and Atherothrombosis<sup>1,2</sup>

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**ABSTRACT** L-arginine, the principal substrate for endothelial nitric oxide synthase, is oxidized to L-citrulline and nitric oxide. Endothelial dysfunction is associated with decreased bioactive nitric oxide production, an abnormality observed in atherothrombosis. Acute or chronic administration of supplemental L-arginine enhances endothelial nitric oxide production and improves endothelial function in the setting of atherothrombosis. The mechanisms by which L-arginine improves endothelial nitric oxide bioactivity include increased intracellular uptake via the high-affinity cationic transporter; substrate competition with asymmetric dimethylarginine, a naturally occurring inhibitor of nitric oxide synthase; direct antioxidant activity; stimulated release of histamine from mast cells, which produces a vasodilator response; decreased activity of norepinephrine, which promotes the effect of endogenous vasodilators including nitric oxide; and increased insulin secretion, which causes vasodilation. By virtue of its link to methyl group metabolism, supplemental L-arginine can, however, also increase the production of S-adenosylhomocysteine from S-adenosylmethionine through the methylation-dependent generation of creatine from guanidinoacetate. This reaction can theoretically lead to increased homocysteine synthesis from its S-adenosyl derivative, which itself can have adverse effects on endothelial function. The interrelationships among these effects of L-arginine are reviewed here, and the potential benefits and risks of L-arginine supplementation are discussed. J. Nutr. 134: 2798S–2800S, 2004.

**KEY WORDS:** • arginine • atherothrombosis • vasodilation • nitric oxide

Endothelial dysfunction is both an early manifestation of atherothrombosis and a consequence of the established disease. Known risk factors for atherothrombosis can induce endothelial dysfunction in the absence of frank vascular pathology. The normally functioning endothelium maintains the blood vessel in the relaxed state, impairs platelet activation and thrombosis, prevents smooth muscle proliferation, inhibits leukocyte adhesion and diapedesis, and maintains a permeability barrier to blood cells and plasma proteins. With the development of a dysfunctional phenotype, these properties are attenuated or reversed: vascular tone increases, a prothrombotic state ensues, inflammatory leukocytes attach to the endothelial cell and enter the vessel wall, smooth muscle cells migrate and proliferate, and vascular permeability increases. This dysfunctional endothelial phenotype is critical for the initiation of the atherothrombotic process and its perpetuation.

One essential molecular mediator of the normally functioning endothelium is nitric oxide. This simple heterodiatomic

molecule is synthesized by the family of oxidoreductase enzymes, the nitric oxide synthases. The nitric oxide synthases utilize L-arginine as their principal substrate, oxidizing it to L-citrulline and nitric oxide, and require the cofactors reduced nicotinamide adenine dinucleotide phosphate, flavin adenine dinucleotide and mononucleotide, tetrahydrobiopterin, and calcium-calmodulin. The endothelial isoform of this enzyme, endothelial nitric oxide synthase (eNOS),<sup>4</sup> is expressed constitutively in endothelial cells where it is primarily localized in the Golgi apparatus, as well as in caveolae; it is responsible for the basal release of nitric oxide from the endothelium, and for the rapid change in flux of nitric oxide in response to physical (e.g., shear stress) and molecular (e.g., bradykinin) agonists.

Virtually all of the phenotypic properties of a healthy, normally functioning endothelial cell are, at least in part, a consequence of the bioactivity of nitric oxide. Thus, a central feature of endothelial dysfunction is a loss of bioactive nitric oxide. Nitric oxide insufficiency can occur as a result of decreased synthesis; increased oxidative inactivation to nitrite, nitrate, and peroxynitrites; or both. Impaired synthesis is the result of decreased expression of eNOS or decreased availability of substrate or cofactors. Enhanced oxidative inactivation is caused by an excess of reactive oxygen species (ROS)

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<sup>4</sup> Abbreviations used: ADMA, asymmetric dimethylarginine; apoE, apolipoprotein E; eNOS, endothelial nitric oxide synthase; iNOS: inducible nitric oxide synthase; ROS, reactive oxygen species.

generated in the vasculature. These ROS include superoxide anion, hydrogen peroxide and derivative hydroxyl radical and hydroxide, and lipid peroxides and derivatives peroxy radicals. Under normal circumstances, abundant antioxidant enzymes (e.g., superoxide dismutases and glutathione peroxidases) and low-molecular-weight antioxidants (e.g.,  $\alpha$ -tocopherol and ascorbate) metabolize these highly reactive derivatives of normal oxidative metabolism; however, risk factors for atherosclerosis or frank atherothrombotic disease leads to an increase in the flux of ROS in the vasculature. The source of the ROS include mitochondrial metabolism, NAD(P)H oxidases, xanthine/xanthine oxidase, glucose/glucose oxidase, and nitric oxide synthases. The nitric oxide synthases produce superoxide anion by reducing oxygen, and do so when the enzymes become "uncoupled" by limited availability of the cofactor tetrahydrobiopterin or limited availability of L-arginine.

With this background, investigators have posited that one method by which to increase the flux of bioactive nitric oxide from the endothelial cell is to provide supplemental substrate L-arginine. This therapeutic paradigm was first proposed in 1992 by 2 groups: Creager and colleagues (1) showed that supplemental L-arginine given to hypercholesterolemic subjects improved endothelium-dependent, nitric oxide-mediated forearm vasodilator response. Dubois-Rande and colleagues (2) demonstrated that an infusion of L-arginine into a coronary artery improved endothelial nitric oxide-induced vasomotor responses. Since these initial observations, numerous studies have shown that acute and chronic supplemental L-arginine improves endothelial nitric oxide bioactivity in individuals with risk factors for atherosclerosis (e.g., hypercholesterolemia, diabetes mellitus, hypertension), as well as individuals with established atherothrombotic disease.

The potential mechanisms by which supplemental L-arginine improves endothelial function are quite diverse (Table 1). The high-affinity cationic arginine transporter colocalizes in caveolae with eNOS (3), for which reason many investigators believe that extracellular L-arginine concentration is the principal determinant of intracellular L-arginine availability for eNOS. In that the intracellular levels of L-arginine far exceed the  $K_m$  for eNOS [2.9  $\mu$ M for the purified enzyme (4)], however, it is unlikely that substrate availability is limiting in most cases. In atherothrombotic disease, oxidized low-density lipoprotein and lysophosphatidylcholine decrease L-arginine transport into cells (5,6). As L-arginine competes with other cationic amino acids for transport via the high-affinity cationic transporter, supplemental L-arginine may increase intracellular L-arginine levels by competitive enhancement. Individuals with atherosclerosis also have increased plasma concentrations of asymmetric dimethylarginine (ADMA), an endogenous, competitive inhibitor of nitric oxide synthases (7); in this setting, supplemental L-arginine may improve nitric oxide production by eNOS.

**TABLE 1**

*Potential mechanisms for improving endothelial function with supplemental L-arginine*

- Increased intracellular transport
- Increased intracellular levels
- Competitive antagonism of ADMA
- Antioxidant effect
- Stimulation of histamine release from mast cells
- Decreased norepinephrine activity
- Increased insulin secretion
- Alteration in intracellular pH and pH-dependent signalling

Other indirect mechanisms for improvement in nitric oxide bioactivity by supplemental L-arginine are widely varied. L-arginine has direct antioxidant activity (8); stimulates release of histamine from mast cells, which produces a vasodilator response (9); decreases the activity of norepinephrine, which promotes the effect of endogenous vasodilators such as nitric oxide (10); and increases insulin secretion, which causes vasodilation (11). In addition, L-arginine, administered as the hydrochloric acid salt, can alter intracellular pH to favorably affect calcium transients and eNOS activation, as well as support the nonenzymatic reduction of nitrite to nitric oxide (12).

The majority of recent studies that have shown a benefit of supplemental L-arginine have involved animal models or human subjects with risk factors for atherosclerosis but without established disease. By contrast, studies in animals or human subjects with atherothrombotic disease have yielded results that are, at best, inconsistent. Blum and colleagues (13) administered oral L-arginine for 1 mo to individuals with coronary artery disease in a randomized, double-blind crossover trial design, and assessed flow-mediated brachial artery dilation and cell adhesion molecule expression. In this well-designed trial, they found no benefit of L-arginine compared with placebo on these indices of nitric oxide bioactivity. Several possible explanations for this lack of benefit have been proposed (14), including levels of supplemental L-arginine that were inadequate to boost endothelial nitric oxide production; limited cofactor availability, especially tetrahydrobiopterin (15); enhanced production of superoxide anion by the inducible isoform of nitric oxide synthase (iNOS), which is upregulated in atheromata (16); and maximally improved endothelial function in the study subjects owing to baseline treatment with statins, beta-blockers, and aspirin.

Notwithstanding the logic of these explanations, recent animal data have shed additional light on possible explanations for the lack of benefit of supplemental L-arginine under conditions of established atherosclerosis. To understand the basis for this argument, one must first appreciate that iNOS expression is clearly upregulated in established atherosclerosis (16), and that iNOS is a high-flux enzyme compared with eNOS. The large increase in nitric oxide production by iNOS compared with that produced by eNOS in an atheromatous environment generating abundant ROS has adverse effects: superoxide anion and lipid peroxy radicals react with nitric oxide to yield peroxynitrite and lipid peroxynitrites, respectively, which inactivate nitric oxide and are themselves potent oxidants. Peroxynitrites both oxidatively modify proteins in the vessel wall and oxidize tetrahydrobiopterin, which uncouples eNOS leading to decreased nitric oxide production as well as de novo superoxide production by this enzyme. Consistent with this description, 2 studies showed that mice genetically deficient in iNOS rendered hyperlipidemic by apolipoprotein E (apoE) deficiency [iNOS(-/-)/apoE(-/-)] fed a Western diet had less atherosclerosis and lower markers of oxidant stress than apoE(-/-) control mice fed a Western diet (17,18).

In a very recent study (19), however, unexpected results with supplemental L-arginine were observed. The authors posited that L-arginine given to the iNOS(-/-)/apoE(-/-) mice would increase eNOS-derived nitric oxide and further reduce their atheromatous burden; however, they observed just the opposite results: L-arginine supplementation increased atheromatous burden to levels observed in the apoE(-/-) mice, offsetting entirely the benefit of eliminating iNOS. While one can propose a variety of explanations for this outcome, including species differences, I proposed in an accompanying editorial (20) that L-arginine administration may have adverse

effects in this setting owing to its metabolism to creatine. Approximately 10-fold more L-arginine is metabolized to creatine than is used for nitric oxide synthesis (21), and creatine synthesis requires the methylation of guanidinoacetate by S-adenosyl-L-methionine in the liver, yielding S-adenosyl-L-homocysteine. This latter compound is then hydrolyzed to adenosine and L-homocysteine by S-adenosyl-L-homocysteine hydrolase. L-homocysteine can either be metabolized by methylation to methionine or undergo transsulfuration to cysteine. The former metabolic pathway requires adequate methylation support, which may be limited by the consumption of as much as 70% of labile methyl groups by creatine synthesis (although the source of methyl groups is in a separate, but metabolically linked pool) (22). Because vascular cells can only remethylate L-homocysteine and not transsulfurate it to cysteine (23), local concentrations of this atherogenic amino acid would be expected to increase in the vasculature. Furthermore, L-homocysteine can increase plasma concentrations of ADMA by inhibiting dimethylarginine dimethylaminohydrolase (24), the enzyme that metabolizes this nitric oxide synthase inhibitor to L-citrulline. It is important to point out that there are, as yet, no published data to support this mechanism; however, we have obtained preliminary results in collaboration with the Huang laboratory showing that L-homocysteine levels in the iNOS(-/-)/apoE(-/-) mice are significantly higher than those in the apoE(-/-) mice (unpublished results, J. Loscalzo, D. E. Handy, P. Huang).

One possible mechanism for offsetting this adverse effect of supplemental L-arginine would be to coadminister creatine. Supplemental creatine would decrease endogenous creatine synthesis by expression of L-arginine:glycine amidinotransferase (25), as well as decrease methylation stress and homocysteine synthesis. Furthermore, by decreasing the metabolic flux of L-arginine to creatine synthesis, more L-arginine would be made available for synthesis of nitric oxide by nitric oxide synthases.

Taken together, published data suggest that supplemental L-arginine does, indeed, improve endothelial function in individuals with risk factors for atherothrombosis. Whether supplemental L-arginine truly provides benefit when given chronically to individuals with established atherothrombotic disease remains to be established. Furthermore, there is at least a theoretical basis for urging caution in administering the amino acid to individuals with atherothrombotic disease, perhaps especially in the setting of hyperhomocysteinemia. Future studies will be required to determine the precise mechanisms by which and conditions under which supplemental L-arginine improves endothelial function and vascular health.

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