REVIEW ARTICLE

The Pharmacodynamics of L-Arginine

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ABSTRACT

L-Arginine is a precursor for nitric oxide (NO) synthesis. NO is a ubiquitous mediator that is formed by a family of enzymes called NO synthases (NOSs). In the brain, NO acts as a neurotransmitter; in the immune system, it acts as a mediator of host defense; and in the cardiovascular system, it mediates the protective effects of the intact endothelium, acting as a vasodilator and endogenous, antiatherogenic molecule. About 5 g of L-arginine are ingested each day in a normal Western diet. Plasma levels of L-arginine are not significantly reduced in most diseases, except in end-stage renal failure during hemodialysis treatment. Nonetheless, intravenous or dietary (oral) administration of relatively large doses of L-arginine has been shown to result in enhanced NO formation in individuals with impaired endothelial function at baseline. In several controlled clinical trials, long-term administration of L-arginine has been shown to improve the symptoms of cardiovascular disease. However, in other trials, L-arginine was not beneficial, and in a recent study, the authors reported higher mortality for participants receiving L-arginine than for those receiving placebo. Recently, it became clear that endogenous levels of asymmetric dimethylarginine (ADMA), a competitive inhibitor of L-Arginine metabolism by NOS, may determine an individual's response to L-arginine supplementation. L-Arginine appears to exert no effect in individuals with low ADMA levels, whereas in those with high levels, L-arginine restores the L-arginine/ADMA ratio to normal and, thereby, normalizes endothelial function. In conclusion, the effects of L-arginine supplementation on human physiology appear to be multicausal and dose-related. Doses of 3-8 g/d appear to be safe and not to cause acute pharmacologic effects in humans. (*Altern Ther Health Med.* 2014;20(3):48-54.)

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ROLE IN PHYSIOLOGY AND PATHOPHYSIOLOGY

L-Arginine—2-amino-5-guanidino-pentanoic acid—is a conditionally essential, proteinogenic amino acid that is also a natural constituent of dietary proteins.[1] Since the initial isolation of L-arginine in 1886,[2] researchers have assessed its potential medical uses. L-Arginine may be beneficial in the treatment of MELAS syndrome,[3] pre-eclampsia,[4] hypertension,[5] and many other diseases and conditions.[2] Therefore, careful consideration of its potential benefits is warranted, particularly in the field of integrative medicine.

In addition to its role in protein metabolism, L-arginine is involved in various metabolic pathways, such as synthesis of creatine, L-ornithine, L-glutamate, and polyamines.[6] Decarboxylation of L-arginine can produce agmatine, a biogenic amine metabolite. L-Arginine is also involved in protein degradation by the ubiquitin-proteasome pathway.[6] A biologically important pathway involves L-arginine as the

substrate of a family of enzymes named nitric oxide synthases (NOSs).[7] Three different isoforms of NOS have been characterized that are named according to the cell type from which they were first isolated: (1) neuronal NOS—nNOS, NOS I; (2) inducible NOS—iNOS, NOS II; and (3) endothelial NOS—eNOS, NOS III.[8] Both nNOS and eNOS are expressed constitutively; their activity is regulated by calcium/calmodulin; and they produce NO at low rates. Induced in inflammatory cell types on cytokine stimulation, iNOS shows activity that is independent of calcium because of tight binding of calmodulin to the enzyme, and it produces NO at high rates. Recently, expressional regulation of eNOS has been observed,[9] indicating that the simple discrimination between constitutively and inducibly expressed enzymes is no longer correct; however, this nomenclature is still broadly used.

The reaction mechanism of NOSs involves a 2-electron transfer from molecular oxygen via a number of cofactors to L-arginine, resulting in the release of NO and L-citrulline. N^{ω}-hydroxy-L-arginine is formed as a relatively stable intermediate product of this reaction.[10]

NO possesses a range of critical roles in the regulation of the function of diverse organs throughout the body, depending on the cell type and tissue and the NOS isoform responsible. NO plays an important role as a mediator in nonadrenergic, noncholinergic neurotransmission; learning and memory; synaptic plasticity; and neuroprotection.[11,12] In the cardiovascular system, NO produced by eNOS in response to stimulation of mechanoreceptors by the shear stress of the flowing blood is critically important for the homeostasis of vascular tone; for interactions between the vascular wall and circulating blood cells, mainly thrombocytes and leukocytes; and for vascular structure. These functions exceed the scope of the present article; they have been reviewed extensively in recent years.[13-17] Impaired formation or function of NO in the vasculature is an important pathogenic factor in the development of vascular diseases, such as atherosclerosis, hypertension, and diabetic angiopathy.[13] Overproduction of NO by iNOS, on the other hand, has been shown to be a major cause of loss of arterial resistance in septic shock.[18] Another study concludes that endotoxin exposure causes the aorta to release a substance that vasodilates resistance arterioles by up-regulating iNOS.[19] Therefore, the plasma concentration of L-arginine is tightly regulated, and metabolic pathways that are L-arginine-dependent are critical determinants of several pathophysiological conditions.

PRECURSOR FOR NO: NUTRACEUTICAL ASPECTS

The relative amounts of L-arginine in various proteins range from 3% to 15%.[20] Soy protein, peanuts, walnuts, and fish are relatively rich in L-arginine, with approximately 7% of the amino acids being L-arginine in fish and approximately 15% in walnuts.[21] In contrast, cereals are protein sources that are comparatively devoid of L-arginine, with only 3% to 4% of their low-protein content being L-arginine. Therefore, differing dietary habits between populations may account for differences in plasma levels of L-arginine in various parts of the world. The usual range of its plasma levels has been determined as 81.6 ± 7.3 mmol/L in young men[22] and 113.7 ± 19.8 μmol/L in senior men compared with 72.4 ± 6.7 μmol/L in young women and 88.0 ± 7.8 μmol/L in senior women.[23]

Although levels of intracellular L-arginine have been demonstrated to be considerably higher than levels in the extracellular fluid or plasma,[24,25] evidence has indicated that extracellular L-arginine can be rapidly taken up by endothelial cells and contribute to NO production.[26] Furthermore, dietary L-arginine is absorbed in the small intestine and transported to the liver, where the major portion is taken up and used in the hepatic urea cycle; however, a small part of dietary L-arginine passes through the liver and is used as a substrate for NO production, as evidenced by animal and human studies that used[15] *N*-labeled L-arginine as a precursor.[27,28]

SUPPLEMENTAL L-ARGININE: MECHANISMS OF ACTION

L-Arginine has been studied extensively as a precursor for NO synthesis in humans. One peculiar aspect of these studies was that the early studies were performed with high intravenous doses, and low doses have only recently been adopted in oral-supplementation studies. The early, high doses stem from reports about the ability of L-arginine to stimulate secretion of pituitary growth hormone.[29] A single dose as high as 30 g of L-arginine, administered intravenously during a 30-minute period, was shown to induce vasodilation in humans.[30-32] This vasodilation appeared rapidly after initiation of the infusion for healthy participants,[30] and it was reproducible in patients with arterial disease[31] and coronary artery disease but not in patients with primary pulmonary hypertension.[33] L-Arginine-induced vasodilation was associated with increased release of NO metabolites, nitrite and nitrate, into urine.

These data suggested that the reaction was NO-dependent; however, subsequent studies demonstrated that hormone release induced by such high doses of L-arginine also contributed to the vasodilator effect. In one study, intravenous L-arginine resulted in a significant increase in the plasma concentration of growth hormone and insulin, and this endocrine effect of L-arginine was blocked by somatostatin coinfusion, and the vasodilator effect was partly abolished.[34] Another study in healthy individuals also showed release of growth hormone after intravenous L-arginine,[35] and this effect was antagonized by octreotide pretreatment and restored by coadministration of recombinant growth hormone with L-arginine. A more recent animal study demonstrated that an arginine-vasopressin injection (0.3 nmol/kg) caused a significant increase in secretions of adrenocorticotropic hormone and growth hormone, but insulin and glucagon were unaffected.[36]

Other mechanisms have been shown to contribute their parts to vasodilation induced by extremely high doses of

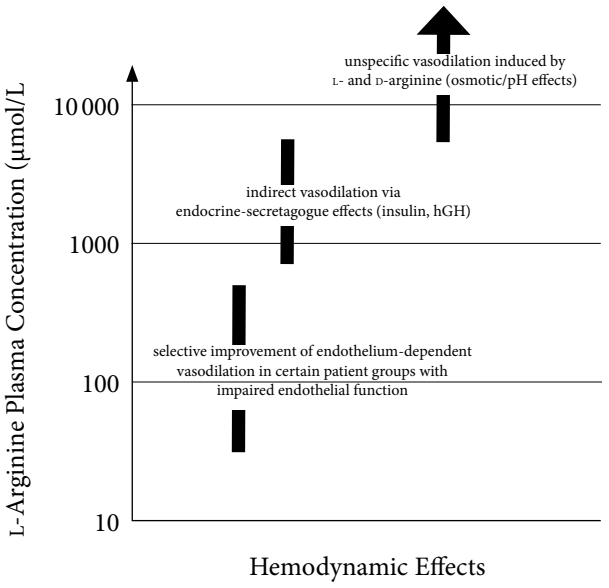
parenterally administered L-arginine. Calver et al[37] infused arginine locally into participants' dorsal hand veins, either using L-arginine or D-arginine, which is not a substrate for NOS, with both given as their free-base form or hydrochloride salts, respectively.[37] The researchers found that both the L- and the D-forms of arginine induced vasodilation at local plasma concentrations estimated to be in the range of 4 to 13 mmol/L, suggesting that this vasodilator effect was nonspecific, possibly related to osmolality or pH effects, and certainly unrelated to enhanced endothelial NO formation. All of these effects have been observed only at plasma concentrations of L-arginine that were in the very-high micromolar to millimolar range. None of these mechanisms has been demonstrated to play a role at the lower plasma concentrations of L-arginine likely to be achieved by oral supplementation with relatively low doses. In contrast, one study reported that levels of plasma growth hormone and insulin-like growth factor-1 were unchanged by oral supplementation with 8 g of L-arginine twice daily in seniors.[38]

From the different doses and routes of administration that have been used in these studies, it can be concluded that the effects of L-arginine and the underlying mechanisms vary according to the range of plasma concentration that is reached (Figure 1). No indication exists to date of acute, pharmacologic effects for oral L-arginine in the dose range below 15 g/d. An acute vasodilator effect has been shown only in studies in which L-arginine was administered via a parenteral route (ie, either intravenously or intra-arterially). Acute hemodynamic effects of L-arginine at higher intravenous or intra-arterial doses can be related to endocrine secretagogue and unspecific vasodilator actions, which have been shown to be absent in the low-dose range. These data do not explain how L-arginine modulates NO-dependent biological effects in a range of plasma concentrations that closely resembles its physiological range or provide an explanation for the variable effects of oral supplementation with L-arginine in different patient populations.

Although plasma levels of L-arginine have been reported to be unchanged in vascular disease in all studies except one,[40] it is possible that the local availability of L-arginine as a substrate for NOS may nonetheless be reduced by the activity of arginase. Arginase uses L-arginine for the production of urea and ornithine and, thus, competes with NOS for substrate availability.[41] Several studies have demonstrated that induction or activation of arginase I or II can lead to impaired NO production and endothelial dysfunction.[42-46]

Evidence has emerged that accumulation of asymmetric dimethylarginine (ADMA) impairs NO formation in certain pathophysiological conditions.[47] The relationship of elevated ADMA levels to cardiovascular disease has been reviewed recently.[48] ADMA competes with L-arginine for binding to NOS and, thus, competitively antagonizes the enzyme's catalytic activity, giving rise to the hypothesis that L-arginine may be beneficial in patients with elevated ADMA but have no effects on NO-dependent mechanisms in individuals with low or normal ADMA levels (Figure 2).[49]

Figure 1. Association between L-arginine plasma concentration range and vascular effects during L-arginine administration via different routes. The black vertical bars indicate the plasma concentration range of L-arginine for which the types of hemodynamic effects are indicated in the figure. Note that the y-axis displays a logarithmic scale. Adapted from Böger and Bode-Böger.[39]



Abbreviation: hGH = human growth hormone.

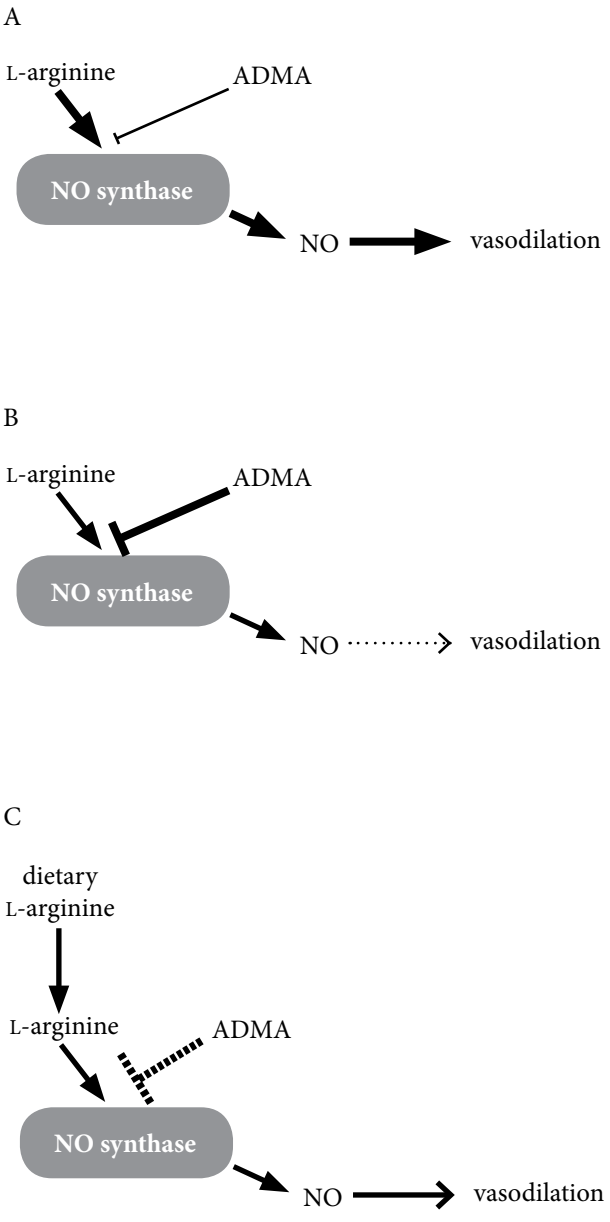
CLINICAL TRIALS

Based on observations from experimental clinical studies like those cited above, which showed vasodilation and enhanced NO production after administration of L-arginine, a series of clinical trials have been performed to investigate the potential of this amino acid to improve the symptoms of cardiovascular disease.

The first clinical application of L-arginine used in an attempt to improve vascular function in patients with cardiovascular disease was published in 1991 by Drexler et al.[50] The researchers infused L-arginine into the coronary arteries of patients with coronary artery disease during a cardiac catheterization and measured the coronary flow response to acetylcholine before and after the L-arginine. The investigators showed that L-arginine enhanced the blood-flow response to acetylcholine in coronary artery disease, but controls did not have an enhanced response. Since then, many studies have occurred in which healthy participants or patients with various cardiovascular conditions received L-arginine.

Although it is beyond the scope of this article to give a complete overview of all published clinical studies with L-arginine, it becomes clear even from studying recent studies that use of L-arginine has led to discrepant findings. For example, Ceremuzynski et al[51] reported a significant improvement in exercise capacity in 22 patients with

Figure 2. The "L-arginine paradox." L-Arginine is the substrate of NO synthase. The enzyme kinetics of endothelial NO synthase have been determined biochemically in vitro. Data show that physiological plasma L-arginine concentrations are in a range that enables full activity of the enzyme in the presence of physiological, low ADMA levels (A). However, in the presence of elevated levels of ADMA, a competitive inhibitor of NO synthase, the conversion of L-arginine to NO is impaired, resulting in decreased biological actions of NO (B). Under such circumstances, even small changes in L-arginine concentration secondary to dietary supplementation with L-arginine may result in restoring NO production to near-normal levels (C). Adapted from Böger.[48]



coronary artery disease who received 6 g/d of oral L-arginine for 3 days in a double-blind, placebo-controlled design.[51] Bednarz et al[52] later confirmed these findings in a study with a virtually identical design in which 25 patients with stable coronary artery disease underwent exercise testing before and after 3 days of treatment with oral L-arginine (6 g/d) or a placebo. L-Arginine significantly improved exercise duration but did not affect QT-segment depression in an exercise electrocardiogram.

Rector et al[53] performed a study in 15 patients with moderate to severe heart failure who received, in random sequence, 5.6 to 12.6 g/d of L-arginine or a matching placebo for 6 weeks. Compared with placebo, supplemental oral L-arginine significantly increased the forearm blood flow during forearm exercise, the 6-min walking distance, and arterial compliance as well as improved subjective well-being as assessed by the Living with Heart Failure Questionnaire. In another study,[54] 21 patients with stable heart failure were given sequential exercise tests before and after L-arginine or placebo in a double-blind crossover study that compared 9 g/d of L-arginine or placebo for 7 days. This study confirmed a significant improvement in exercise duration time for oral L-arginine compared with placebo.

In contrast, several relatively small clinical studies with experimental endpoints failed to show beneficial effects of L-arginine on vascular function. In a study including 30 patients with stable coronary heart disease who received optimized medical treatment according to current guidelines, Blum et al[55] found no significant improvement of endothelium-dependent vasodilation, blood flow, or serum levels of inflammatory markers through use of dietary L-arginine arginine at a dose of 9 g/d, given for a period of 1 month, compared with placebo. In another study, 40 patients with coronary heart disease and angiographically proven stenosis of >50% received 15 g/d of L-arginine or placebo for 2 weeks.[56] L-Arginine supplementation had no significant effect on endothelial function, blood flow, markers of oxidative stress, or exercise performance. For 60 patients with stable angina pectoris and angiographically proven stenosis of >50%, supplementation with 6 g/d of L-arginine versus a placebo for 2 weeks after coronary-stent implantation resulted in no significant change in coronary neointima formation or in in-stent restenosis.[57]

In patients without cardiovascular disease, studies show similar findings of no beneficial effects for L-arginine on vascular function or exercise capacity. For example, Trussardi Fayh et al[58] conducted a trial to investigate the effects of L-arginine supplementation on blood flow, oxidative stress, and exercise response in young adults with uncomplicated type 1 diabetes. Doses of 7 g/d were administered to participants. The authors found that L-arginine supplementation restored basal blood flow to normal levels (2.66 ± 0.3 to 4.74 ± 0.86 mL 100 mL^{-1} min^{-1}) but did not affect vascular or oxidative stress responses to exercise.[58] Furthermore, a study by Alvarez et al[59] concluded that 6-g

doses of L-arginine increased blood muscle performance, but not strength performance, which indicates NO production remained unaffected. Another study by Alvares et al[60] involved 15 healthy runners, and the researchers concluded that 6-g doses of L-arginine did not benefit metabolic or hormonal parameters after 4 weeks of daily supplementation.

Taken together, these clinical studies with experimental designs suggest that subgroups may exist of patients whose vascular function or exercise capacity is improved by L-arginine supplementation, while other patients or subgroups of patients do not profit from such a dietary intervention. Diagnostic markers are needed that allow prospective identification of individuals who have a high probability of showing a positive response to dietary intervention with L-arginine. To this end, participants' characteristics in different studies need to be analyzed carefully to define differences between studies that may account for such apparently conflicting results.

Daily doses of L-arginine below 2 to 3 g/d appear to be without beneficial effect. In addition to the dose of L-arginine, selection of participants appears to be a major factor affecting a study's outcome. Patients on optimized medical treatment may be less responsive, and patients with advanced coronary stenoses also showed a lesser effect. By contrast, L-arginine was more effective when received early, when functional changes of vascular function were chosen as endpoints, and when vascular disease may have been less advanced. Furthermore, it is possible that ADMA, the endogenous inhibitor of NOS, may be related to the effectiveness of L-arginine in treating cardiovascular disease. In patients with low ADMA levels, L-arginine appears to have no effect; however, patients with high ADMA levels see a restoration in their L-arginine/ADMA ratio and a normalized endothelial function.[61] Yet further studies need to be conducted to determine with greater specificity which patients will benefit from L-arginine supplementation.

In addition to the relatively small experimental trials, 2 recent, comparatively large clinical trials investigated the effects of oral supplementation with L-arginine for patients with coronary heart disease. In one study,[62] 792 patients with the disease were included within 24 hours of the onset of acute myocardial infarction. More than 85% of the patients received thrombolytic therapy for the acute myocardial infarction. Patients were randomized to receive 3 g of oral L-arginine 3 times daily or a matching placebo, for 1 month. The composite clinical endpoint—cardiovascular death, reinfarction, recurrent myocardial ischemia, successful resuscitation, or shock/pulmonary edema—was not significantly different between the 2 groups, but a strong trend existed in favor of L-arginine (OR = 0.63; 95% CI, 0.39 - 1.02; $P = .06$). The endpoint was significantly reduced by L-arginine in a predefined subgroup of hypercholesterolemic patients—19 versus 31 events ($P < .05$)—and a reduced incidence of events was observed in each of the components of the composite clinical endpoint. Adverse events were rare and not significantly different between the L-arginine and placebo groups, with gastrointestinal disorders, mostly loose stools, being the most frequently observed side effect.

The second study included 153 patients with stable coronary artery disease at 3 to 21 days after their first ST-segment elevation infarction.[63] Patients were randomized to 3 g of L-arginine or placebo 3 times daily for a period of 6 months. The primary endpoint was left ventricular ejection fraction, with several measures of vascular stiffness and clinical events being secondary endpoints. Almost 90% of the participants in this trial had received acute percutaneous coronary intervention for the acute myocardial infarction. In this study, the ejection fractions were not significantly different between the 2 groups, and there were differences between the 2 groups reported for any of the secondary endpoints. However, a strong trend can be seen in the data reported for L-arginine to decrease pulse wave velocity, a measure of arterial elasticity and endothelial function,[64] compared with placebo. Concern was raised because this study was stopped prematurely after 6 deaths had occurred in the L-arginine group versus none in the placebo group. A close analysis of the deaths reveals that 4 of the deaths were most probably unrelated to treatment—1 myocardial rupture at reinfarction, 2 from presumed sepsis, and 1 sudden death at 3 weeks after the study's treatment had ended—and a causal relationship could neither be confirmed nor excluded for 2 participants who were found dead at their homes in the course of the study. The study has been criticized because the authors failed to show elevation of levels of plasma L-arginine during supplementation with this amino acid, and a causal relationship between the dietary intervention and any of the deaths could not be ascertained.[65,66] Both aspects make it hard to determine the risk-benefit relationship of dietary L-arginine in this trial.

CONCLUSION

Given its natural properties, integrative practitioners and clinicians should carefully assess the potential benefits and uses of L-arginine in their practices. It is important for integrative clinicians to follow trends and recent findings in L-arginine research to understand the trajectory of knowledge in the field. It is also important to understand the potential consequences of improper L-arginine use.

Currently available data point to the fact that oral supplementation with L-arginine can affect endothelium-mediated vascular functions, such as enhancing vasodilation, decreasing platelet aggregation, and reducing endothelial monocyte adhesion. These effects occur when plasma concentrations of L-arginine are elevated minimally above the physiological range of concentration. At higher plasma concentrations of L-arginine, such as those reached during intravenous or intra-arterial infusion, other effects that are not directly linked to NO production can be observed, such as hormone release and nonspecific vasodilation.

Beneficial—endothelium-dependent, NO-mediated—vascular effects of dietary L-arginine are more likely to be reached when the following conditions are fulfilled: (1) the

patient's endothelial L-arginine-NO metabolism is impaired in a fashion that is reversible by L-arginine; (2) treated patients are those who are not maximally treated with pharmacologic agents; and (3) L-arginine is given in the early stages of disease.

Impaired Endothelial L-Arginine-NO Metabolism

Among possible causes for such impairment are increased arginine losses, such as during hemodialysis treatment; increased metabolic use of L-arginine by NO-independent pathways, such as induction of arginases[67,68]; or the presence of elevated levels of ADMA, the endogenous inhibitor of NOS that displaces L-arginine from the substrate binding site of this enzyme[48,49] and is a common cause of relative arginine deficiency in vascular pathologies.

Patients Not Maximally Treated With Pharmacologic Agents

Such optimized medical management probably does not allow any room for improvement of vascular function by L-arginine. In addition, several pharmacologic agents used in secondary prevention of cardiovascular disease have been shown not only to improve vascular function but also to reduce ADMA levels[69]; they may thereby diminish the ability of L-arginine to improve vascular function.

L-Arginine Is Given in the Early Stages of Disease

L-Arginine appears to affect pathophysiological mechanisms that contribute to the progression of atherosclerosis. Such mechanisms may be more strongly affected by dietary L-arginine in relatively early stages of the disease when functional changes are still reversible, whereas structural atherosclerotic changes of the vascular wall may be less responsive to L-arginine.

Therefore, L-arginine has a place as a nutraceutical agent in the modification of functional impairment and in the prevention of vascular disease but not as a therapy to reverse manifest atherosclerosis. Future studies should be planned after carefully considering these influencing factors, and patients should be preselected by a marker that allows prediction of a higher-than-average probability of them responding to L-arginine supplementation, such as arginase induction or elevated ADMA concentration. Diagnostic tools to determine ADMA levels easily and rapidly have been made available recently[70-72] and, therefore, should diminish the obstacles for such studies. Long-term studies are needed to determine whether a difference in the availability of dietary L-arginine exists when it is given for short or long periods. A recent study by Velickovic et al[73] suggests that long-term dietary supplementation with L-arginine causes an increase in eNOS and vasoactive intestinal peptide immunoexpression in the small intestines of rats, but more human studies of this nature are needed.

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