Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

Arginine and Endothelial and Vascular Health[1,2]

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ABSTRACT   The vascular endothelium is a crucial regulator of vascular function and homeostasis. Nitric oxide (NO) is an important paracrine substance released by the endothelium to regulate vasomotor tone. Risk factors for atherosclerosis, as well as atherosclerosis per se, are associated with endothelial dysfunction and decreased bioavailablilty of NO. Indeed, endothelial dysfunction is integral to the pathogenesis of atherosclerosis and other cardiovascular diseases. Moreover, endothelial dysfunction relates to an increased risk of adverse cardiovascular outcomes. L-Arginine is an essential amino acid required by the constitutive enzyme, endothelial NO oxide synthase (eNOS), to produce NO. Administration of L-arginine improves endothelial function in animal models and in humans with hypercholesterolemia and with atherosclerosis. Clinical trials to date support potential clinical applications of L-arginine in the treatment of coronary artery disease and peripheral arterial disease, as well as in the prevention of in-stent restenosis. The mechanism of benefit of L-arginine on endothelial function is unclear, because intracellular concentrations of L-arginine far exceed that required by eNOS. One potential explanation of this "arginine paradox" is that L-arginine restores endothelial function in atherosclerotic patients, in whom there are elevated levels of asymmetric dimethylarginine, an endogenous inhibitor of eNOS. Given the promising findings of early studies of L-arginine as a potential therapy for cardiovascular disorders, large-scale clinical trials are warranted.   J. Nutr. 134: 2880S–2887S, 2004.

KEY WORDS: • *endothelium* • *arginine* • *nitric oxide* • *cardiovascular disease*

Vascular function and nitric oxide

The vascular endothelium is a crucial regulator of vascular function and homeostasis. In addition to regulation of vascular tone and blood pressure, the vascular endothelium also has antithrombotic properties, modulates interactions between the blood vessel wall and circulating leukocytes and platelets, and acts as a paracrine organ by secretion of vasoactive substances that mitigate these varied functions (1,2).

Nitric oxide (NO), previously known as *endothelium-derived relaxing factor,* is perhaps the most critical of the substances produced by the vascular endothelium for regulation of vasomotor tone (3,4). NO is generated in the endothelium by the conversion of the essential amino acid L-arginine to L-citrulline by the constitutive enzyme, endothelial NO synthase (eNOS). Both the L- and D-enantiomers of arginine are present within the human circulation. Only L-arginine is recognized by eNOS as the substrate for production of NO. Biochemical stimuli, such as acetylcholine or bradykinin, and also shear stress, activate receptors on the endothelial cell surface and cause influx of intracellular calcium, which activates eNOS (5,6). NO generated within vascular endothelial cells diffuses into vascular smooth muscle cells and causes vasodilation by stimulation of the guanylate cyclase pathway and the consequent generation of cyclic guanosine monophosphate (cGMP). This process is termed *endothelium-dependent vasodilation* (**Fig. 1**). Nitroglycerin and sodium nitroprusside, potent vasodilators with clinical efficacy in the treatment of hypertension, congestive heat failure, and other clinical disorders, release NO directly or by cleavage from an enzymatic reaction. NO released from these donors acts via the same signaling pathways as those of endogenously produced NO to cause relaxation of vascular smooth muscle cells and subsequent vasodilation. The ability of vascular smooth muscle cells to utilize NO donated exogenously is termed *endothelium-independent vasodilation.*

Intact function and integrity of the endothelium are fundamental to cardiovascular health. Indeed, abnormalities of endothelial function are the earliest step in the pathogenesis of atherosclerosis. Abnormalities of endothelial function are present in patients with traditional atherosclerotic risk factors,
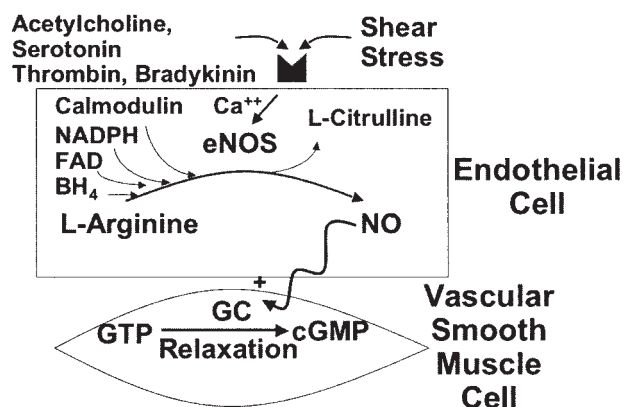
THE JOURNAL OF NUTRITION

The Endothelium and Nitric Oxide

FIGURE 1 Endothelial function and the nitric oxide pathway. A number of stimuli, including shear stress, bradykinin, and acetylcholine, ultimately active eNOS by release of intracellular calcium. L-Arginine is converted to L-citrulline by eNOS with subsequent production of NO. eNOS requires a number of cofactors, including NADPH, FAD, calcium, calmodulin, and BH_4. NO diffuses into vascular smooth muscle cells and stimulates guanylate cyclase, ultimately resulting in cGMP production and vasodilation. BH_4, tetrahydrobiopterin; FAD, flavin adenine dinucleotide; NADPH, nicotiamide adenosine diphosphate.

including hypercholesterolemia, tobacco smoking, diabetes, and hypertension, prior to any clinical manifestations of atherosclerosis (2,7,8). Moreover, endothelial dysfunction is evident in individuals with atherosclerosis, and in patients with a variety of other cardiovascular disorders, including septic shock, congestive heart failure, pulmonary hypertension, and preeclampsia. Endothelial dysfunction also has prognostic implications and is associated with an increased risk of future cardiovascular events (9).

Assessment of endothelial function in humans

During the past 3 decades, research tools have been developed to assess vascular function in humans in vivo. Measurement of substrates and products of the NO pathway, including plasma and urinary nitrite and nitrate, can provide a surrogate marker of NO synthesis, but these are not direct measures of vascular function.

The earliest direct measurement of endothelium-dependent vasodilator function in humans was performed in the coronary circulation by documenting the response to intracoronary acetylcholine infusion by angiography (10). Patients with coronary atherosclerosis had a vasoconstrictor response to acetylcholine, compared to a vasodilator response in healthy control patients. Several techniques also have been developed to assess endothelial function in peripheral blood vessels in humans without subjecting them to the potential risks incurred with cardiac catheterization. Flow-mediated endothelium-dependent vasodilation is a technique that uses high-frequency ultrasound images of the brachial artery to measure the response of this conduit vessel to a flow stimulus (11). Endothelium-dependent vasodilation is assessed by measuring the change in blood vessel diameter in response to an increase in flow and shear stress in the brachial artery. The augmentation in flow and shear stress occurs during postocclusive reactive hyperemia following release of a blood pressure cuff that is inflated around the arm to suprasystolic pressure for 5 min. Endothelium-independent vasodilation is assessed by measur-

ing the brachial artery diameter before and after administration of an NO donor, typically sublingual nitroglycerin. Flow-mediated endothelium-dependent vasodilation correlates with the coronary vasodilator response to acetylcholine (12).

Venous occlusion plethysmography is a technique that measures blood flow to a limb and enables assessment of vascular function in the resistance vessels of the upper or lower extremities (13). Endothelium-dependent vasodilation is measured as the increase in blood flow in response to intrarterial administration of drugs, such as acetylcholine, methacholine, serotonin, or other substances that affect the production of NO. Endothelium-independent vasodilation is assessed by measurement of changes in blood flow in response to either an NO donor, such as nitroglycerin or sodium nitroprusside, or a direct vasodilator, such as verapamil.

L-Arginine and vascular health in animal models

Given the knowledge that L-arginine is a substrate for eNOS, and that NO is crucial to endothelial health, a number of early experiments using animal models investigated the potential efficacy of L-arginine supplementation in reversing endothelial dysfunction in pathologic states. The promising data derived from many of these investigations form the basis for clinical studies in humans.

Hypercholesterolemia. Organ-chamber experiments performed in our laboratory and in others using vessels harvested from hypercholesterolemic rabbits demonstrated reversal of abnormalities in endothelium-dependent vasodilation following both acute intravenous infusion and prolonged oral administration of L-arginine prior to killing (14,15). One ex vivo study, however, found that 1 h exposure of thoracic aorta endothelial cells to L-arginine did not affect endothelium-dependent vasodilation (16). In an in vivo experiment performed in our laboratory, the hindlimb blood-flow responses to acetylcholine and sodium nitroprusside were measured in hypercholesterolemic and control rabbits before and after acute infusion of intravenous L-arginine ($10 \ mg \cdot kg^{-1} \cdot min^{-1}$) (17). Hypercholesterolemic rabbits had a blunted endothelium-dependent vasodilator response to acetylcholine that improved during coinfusion of L-arginine (**Fig. 2**). L-Arginine did not affect the vasodilator response to acetylcholine in the control rabbits. Endothelium-independent vasodilation was
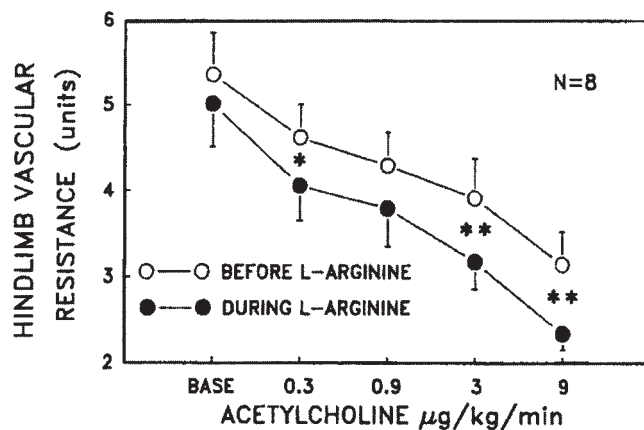
FIGURE 2 L-Arginine restores endothelium-dependent vasodilation in hypercholesterolemic rabbits. Hindlimb vasodilator response to acetylcholine in 8 hypercholesterolemic rabbits before and during infusion of L-arginine at $10 \ mg \cdot kg^{-1} \cdot min^{-1}$. *Value of $P < 0.05$; **value of $P < 0.01$. Reproduced from Girerd et al. (17) with permission.

not impaired in the hypercholesterolemic rabbits and did not change in response to the L-arginine infusion. D-Arginine infusion did not affect acetylcholine or sodium nitroprusside-mediated vasodilation in either hypercholesterolemic or control rabbits. Using similar techniques to measure hindlimb blood flow and response to acetylcholine, Jeremy et al. (18) treated hypercholesterolemic rabbits with standard high-cholesterol feed with and without L-arginine supplements over a prolonged period of time. The hindlimb vasodilator response to acetylcholine in the hypercholesterolemic rabbits fed L-arginine was equivalent to that in rabbits fed a normal diet after 7 wk of treatment. Interestingly, this protective effect of L-arginine supplementation was no longer present after 14 wk of treatment, at which point all hypercholesterolemic rabbits had a diminished vasodilator response to acetylcholine, regardless of whether they were treated with L-arginine.

In addition to effects on vascular function in hypercholesterolemic animals, several experimental studies demonstrated a reduction in the surface area of atherosclerotic plaque in the thoracic aorta of hypercholesterolemic animals treated with oral L-arginine (15,19,20). In a series of related animal experiments, oral and intralesional administration of L-arginine attenuated myointimal hyperplasia associated with balloon-induced vascular injury in hypercholesterolemic animals (21,22). One potential mechanism of these effects of L-arginine was hypothesized to be enhanced local NO synthesis at the site of the lesion, resulting in reduced monocyte binding, fewer macrophages, and increased apoptosis of vascular smooth muscle cells and macrophages within the lesions (23). These reports raise the possibility of using local L-arginine as an adjunctive therapy for percutaneous coronary intervention to prevent in-stent restenosis.

In contrast to these findings, a recent study of oral L-arginine supplementation in apolipoprotein E (apoE) knock-out mice found no beneficial effect of L-arginine on atherosclerotic lesion area (24). In fact, L-arginine supplementation negated the beneficial effect of deficiency of the inducible NO synthase (iNOS) gene in ApoE/iNOS double-knockout mice, perhaps due to an increase in production of free radicals with resultant oxidant stress (25).

Other disease states. In addition to salutary effects on endothelial function in animal models of hypercholesterolemia, L-arginine supplementation has been studied in other animal models of human risk factors and disease. Chronic administration of oral L-arginine prevented endothelial dysfunction associated with secondhand smoke in thoracic aortas of both normocholesterolemic and hypercholesterolemic rabbits in vitro (26,27). Incubation of aortic endothelial cells with L-arginine improved the vasodilator response to acetylcholine in thoracic aorta specimens from diabetic rats (28). Low-dose, but not high-dose, oral L-arginine improved endothelial dysfunction in a rat model of ischemic cardiomyopathy, although there was no effect on hemodynamics or cardiac function (29). Oral L-arginine prevented the development of hypertension in a rat model of salt-sensitive hypertension (30).

L-Arginine and vascular function in humans

Observations regarding the effect of L-arginine on endothelial function in humans are inconsistent, but the different findings may reflect the dose of L-arginine studied. Very high doses of L-arginine, administered intraarterially or intravenously, cause vasodilation in relatively young, healthy subjects (31). Endothelium-dependent vasodilation declines with age (32,33). Oral L-arginine improved flow-mediated, endothe-

lium dependent vasodilation of the brachial artery in a study of healthy elderly individuals, but in these, pretreatment endothelium-dependent vasodilation was abnormal (34). We and others found no effect of intravenous or oral L-arginine on endothelial function in healthy patients using doses that improve endothelial function in diseased states (35–37).

In the purest illustration of the effect of L-arginine on endothelial dysfunction in humans, Kamada et al. (38) studied a patient with lysinuric protein intolerance (LPI), an extraordinarily rare genetic metabolic disorder causing L-arginine deficiency. The 37-y-old Japanese man with LPI had markedly reduced serum L-arginine levels compared to control patients. Endothelium-dependent vasodilation, as measured by flow-mediated dilation of the brachial artery, was markedly impaired compared to 10 age-matched control patients. A 30-min infusion of intravenous L-arginine dramatically improved endothelium-dependent vasodilation (**Fig. 3**). Endothelium-independent vasodilation was not impaired in the LPI patient and was not affected by administration of L-arginine.
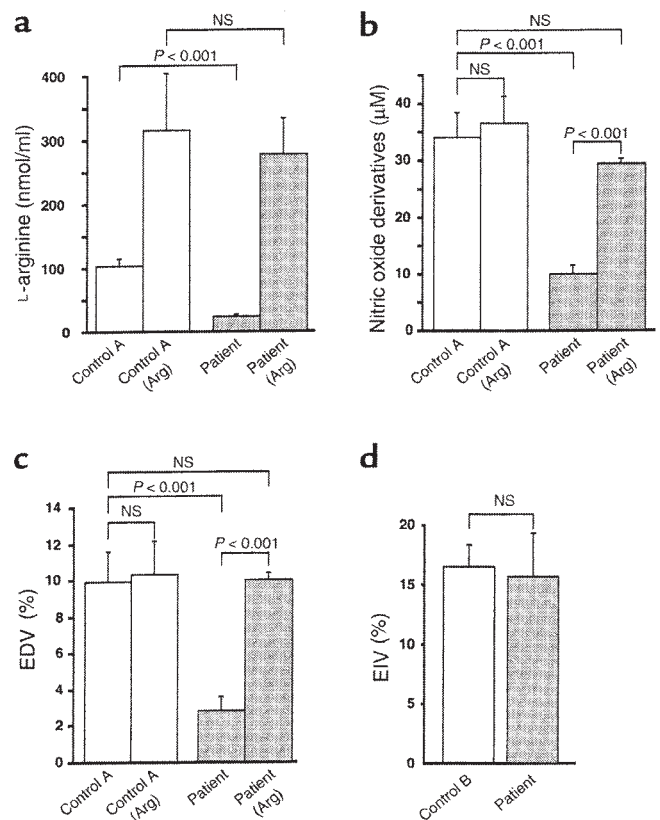
FIGURE 3 L-Arginine corrects the endothelial dysfunction of LPI, an inherited deficiency of L-arginine. (*Panel A*) Serum L-arginine levels in LPI patient (3 independent measurements) and in 10 healthy control subjects (control A) before and after intravenous infusion of L-arginine. (*Panel B*) Serum NO derivatives in the LPI patient (3 independent measurements) and 10 healthy control subjects (control A) before and after intravenous infusion of L-arginine. (*Panel C*) Flow-mediated vasodilation of the brachial artery in LPI patient (3 independent measurements) and in 10 healthy control subjects (control A) after intravenous infusion of L-arginine. L-Arginine significantly improved endothelium-dependent vasodilation (EDV) in the LPI patient. (*Panel D*) Vasodilator response of the brachial artery to nitroglycerin (EIV) did not differ in the LPI patient (5 independent measurements) and 10 healthy control subjects. (control B).Arg, L-arginine; EDV, endothelium-dependent vasodilation; EIV, endothelium-independent vasodilation; NS, not significant. Reproduced from Kameda et al. (38) with permission.

Hypercholesterolemia. L-Arginine improves endothelial function in hypercholesterolemic patients. Our laboratory studied the effect of acute administration of intravenous L-arginine (10 mg · kg^{-1} · min^{-1} × 20 min) on forearm blood flow in hypercholesterolemic and healthy subjects (36). L-Arginine infusion substantially improved the vasodilator response to methacholine in the hypercholesterolemic patients (**Fig. 4**), but did not affect the response to methacholine in the healthy subjects. L-Arginine did not affect the response to sodium nitroprusside in either group of patients. These findings were extended in a study of hypercholesterolemic patients in which 21g/d of oral L-arginine over 4 wk improved flow-mediated vasodilation of the brachial artery (39). Also, nutritional supplement bars containing L-arginine (total 6.6 g) improved flow-mediated dilation of the brachial artery over 1 wk in subjects with hypercholesterolemia, compared with placebo (40). Intracoronary L-arginine infusion also improved the coronary blood-flow response to acetylcholine in a study of 8 hypercholesterolemic patients (41). In addition to salutary effects on endothelium-dependent vasodilation, studies demonstrate benefits of oral L-arginine on other aspects of vascular and platelet function in hypercholesterolemic patients, including decreased platelet aggregation and mononuclear cell adhesion, paralleling observations in hypercholesterolemic rabbits (42–44)

Coronary artery disease. Endothelial dysfunction association with coronary artery disease is typically manifested as a vasoconstrictor response to intracoronary acetylcholine infusion (10). Dubois-Rande et al. (45) administered intracoronary L-arginine into the left anterior descending artery of 13 patients with epicardial coronary disease, 10 of whom had significant stenoses (i.e., >70%) in other vessels. L-Arginine coinfusion attenuated the vasoconstrictive response to intracoronary acetylcholine and increased coronary blood flow. In a study of men with premature coronary artery disease (mean age 41 y), oral administration of 21g/d of L-arginine for 3 d significantly improved flow-mediated vasodilation of the brachial artery, but placebo had no effect (43). In addition, L-arginine treatment decreased monocyte adhesiveness. A study of patients with coronary artery disease using lower-dose

oral L-arginine therapy (9 g/d) found no improvement in flow-mediated vasodilation of the brachial artery, suggesting that higher doses of L-arginine therapy are necessary to affect endothelial function in these patients (46). Similarly, high-dose intraarterial L-arginine infusion, but not oral L-arginine therapy (15 g/d), improved the forearm vasodilator response to acetylcholine in patients with stable angina (47). Lerman et al. (48) studied the effect of long-term administration of L-arginine (9 g/d) on 26 patients with angina pectoris and mild, nonobstructive coronary atherosclerosis (no lesion >40%). After 6 mo of treatment, patients randomized to L-arginine, but not to placebo, had a markedly improved coronary vasodilator response to acetylcholine (**Fig. 5**). L-Arginine treatment did not affect endothelium-independent vasodilation, as measured by the coronary vasodilator response to adenosine; however, it markedly improved anginal symptoms. Although these preliminary studies suggest salutary effects of L-arginine on vascular function and anginal symptoms in patients with coronary artery disease, a longitudinal cohort study of 806 elderly Dutch men found no association between dietary arginine consumption and cardiac mortality (49).

Studies of the effect of L-arginine on endothelial function in patients with the coronary syndrome X, who often present with evidence of ischemia on noninvasive testing and angiographically normal coronary arteries, report inconsistent results (50,51).

Other disorders. The effect of L-arginine therapy on endothelial dysfunction has been studied in other disease states. One single 6-g dose of L-arginine improved flow-mediated vasodilation of the brachial artery in a small randomized double-blind trial of 35 patients with essential hypertension (52). A single 30-g intravenous infusion of L-arginine improved basal femoral artery blood flow in patients with peripheral arterial disease and critical limb ischemia (53). Twice daily adminstration of intravenous L-arginine (16 g total) for 3 wk improved flow-mediated vasodilation of the superficial femoral artery in patients with intermittent claudication (54). Intravenous L-arginine infusion improved myocardial blood flow, as measured by PET scan, in smokers (55). In a human model of reperfusion injury, intraarterial administration of L-arginine attenuated abnormal endothelium-induced vasodilation during the reperfusion period following 20 min of upper extremity ischemia in 16 healthy male subjects (56). Studies of L-arginine therapy in the treatment of the endothelial dysfunction associated with congestive heart failure report inconsistent results (57–60). Limited data suggest that acute intravenous L-arginine administration may improve endothelial dysfunction, as measured by the venodilator response to acetylcholine, in dialysis patients (61).

The arginine paradox and the ADMA hypothesis

Arginine is produced via the urea cycle by degradation of dietary or endogenous proteins or from recycling of the citrulline generated by NO synthase (NOS) via the citrulline/NO cycle (62). In one series, the average plasma arginine concentration of an adult human receiving enteral nutrition was 210 μmol/L (63). Plasma L-arginine concentrations are typically stable in humans and are not decreased in the disease states associated with endothelial dysfunction (36,62,64). Arginine is concentrated into endothelial and other cells by specific transporters, many of which have been identified (62). Intracellular concentrations of L-arginine are as high as 0.1 to 1.0 mmol/L (62). Because the K_m of eNOS for L-arginine is 2.9 μmol/L, it is surprising that the administration of high doses of arginine affects endothelial function (62). This finding is
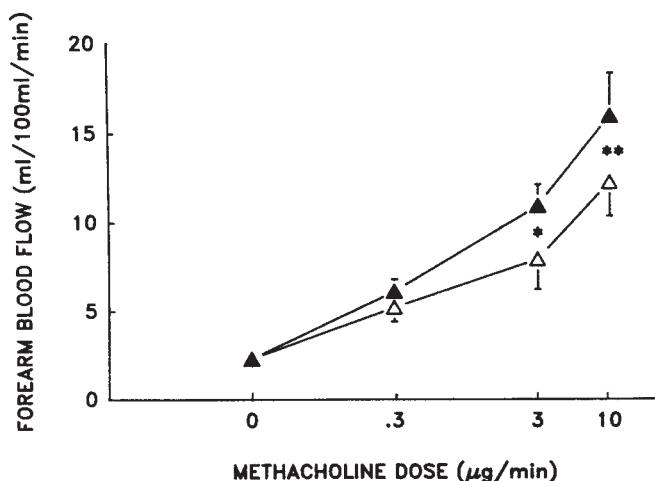
FIGURE 4 Intravenous L-arginine infusion improves endothelial function in patients with hypercholesterolemia. Forearm blood flow response to intraarterial methacholine chloride in 10 hypercholesterolemic humans before (*open triangles*) and after (*solid triangles*) coinfusion with intravenous L-arginine at 10 mg · kg^{-1} · min^{-1}. *Value of $P < 0.05$; **value of $P < 0.01$. Reproduced from Creager et al. (36) with permission.
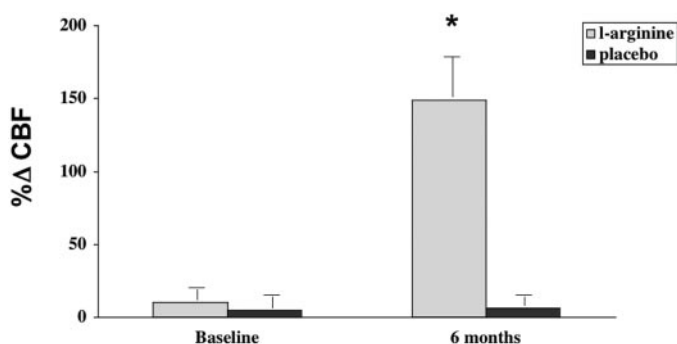
FIGURE 5   Oral L-arginine improves coronary vasodilator response to L-arginine in patients with nonobstructive coronary artery disease. Patients ($n = 26$) were randomly assigned to treatment with 9 g/d of L-arginine or placebo. Coronary endothelial function was studied at baseline and after 6 mo of treatment. CBF, coronary blood flow. *Value of $P < 0.05$. Reproduced from Lerman et al. (48) with permission.

termed the *arginine paradox*. One potential mechanism for this phenomenon is an alteration in the affinity of eNOS for its substrate, L-arginine, to a level that exceeds endothelial cell concentration in pathologic disease states associated with endothelial dysfunction (65).

Recent studies suggest an alternative hypothesis. N^G-monomethyl-L-arginine and asymmetric dimethylarginine (ADMA) are similar in structure to L-arginine and are competitive antagonists of eNOS (66,67). N-monomethyl L-arginine causes endothelial dysfunction in both animal and human models, and recognition of this action was an important step in the identification of NOS (5). Plasma concentrations of ADMA are 10 times greater than those of N^G-monomethyl L-arginine in humans (67). Plasma levels of ADMA are elevated in patients with hypercholesterolemia, hypertriglyceridemia, hyperhomocysteinemia, insulin resistance, renal failure, type 2 diabetes mellitus, and coronary syndrome X (64,68–72). Elevated plasma levels of ADMA, as well a decreased L-arginine:ADMA ratio, are associated with abnormalities of flow-mediated vasodilation of the brachial artery in patients with hypercholesterolemia, hypertriglyceridemia, and hyperhomocysteinemia (64,69,70). However, in one study of male patients with stable angina, the L-arginine:ADMA ratio did not correlate with endothelial function, as measured by forearm blood-flow response to acetylcholine (47).

ADMA is ultimately degraded to citrulline by the enzyme dimethylaminohydrolase (DDAH) (73). Rapidly evolving experimental evidence suggests that DDAH activity is critical in ADMA regulation and NO production in animal models (74,75). Recent data also suggest that DDAH plays a key role in the modulation of ADMA levels and endothelial function in humans (75–77).

In one study by Böger et al. (64) of hypercholesterolemic and normocholesterolemic subjects, plasma ADMA levels were markedly elevated in the hypercholesterolemic patients. L-Arginine levels did not differ within the 2 groups. ADMA levels were inversely correlated with endothelial function as measured by flow-mediated vasodilation of the brachial artery in a subset of 16 patients who underwent vascular function testing. Intravenous infusion of high-dose L-arginine (14 g over 20 min), but not intravenous placebo, improved endothelial function in these hypercholesterolemic patients. This improvement occurred without a change in ADMA levels, although there was an increase in the L-arginine:ADMA ratio. Urinary nitrate excretion increased after L-arginine infusion.

Whether ADMA will ultimately be proven as the link between cardiac risk factors and endothelial dysfunction, and whether the arginine paradox can be explained fully by ADMA antagonism, will need to be substantiated by further investigation.

Clinical outcome trials

Given the promising findings of the ability of L-arginine to improve endothelial function in both experimental animal models and in human disease states, clinical trials were undertaken to investigate the potential for the therapeutic use of L-arginine supplementation in clinical practice.

Angina pectoris.   The use of L-arginine has been studied in several small clinical trials as a treatment for stable angina pectoris. In an uncontrolled pilot study of men with severe (class IV) angina pectoris despite aggressive medical therapy, the administration of oral L-arginine supplements (9 g/d) over a period of 3 mo was associated with marked clinical improvement (to class II) in 7 of 10 patients (78). Interestingly, in the patients with a dramatic clinical response to L-arginine supplements, there was an associated reduction in serum levels of cell adhesion markers and inflammatory cytokines, including P-selectin, ICAM, IL 1-β, and IL-6. Levels of these inflammatory markers did not change in patients who did not have a clinical response. In all patients, anginal symptoms markedly worsened, returning to functional class IV, after cessation of L-arginine therapy.

In a randomized, controlled trial of nondiabetic patients with stable angina pectoris and ischemia on exercise treadmill testing, Ceremuzynski et al. (79) randomly assigned 22 patients to treatment with L-arginine tablets (total 6 g daily) or placebo. After 3 d of therapy, there was a marked increase in the time to maximal ST segment depression on treadmill exercise testing in patients treated with L-arginine. There was also a marked increase in the maximal workload achieved in terms of metabolic equivalent units (MET) in patients randomly assigned to L-arginine treatment (from 6.4 ± 2 to 7.4 ± 3 MET, $P < 0.006$).

In a subsequent study with a longer duration of therapy, Maxwell and colleagues (80) randomly assigned 36 patients with functional class II or III angina pectoris to treatment with L-arginine rich nutritional supplement bars (3.3 g L-arginine/bar plus antioxidants and B-complex vitamins) or placebo bars containing minimal L-arginine (0.59 g L-arginine/bar, with negligible amounts of vitamin E and folic acid). Each patient received 2 L-arginine–rich nutritional bars or 2 placebo bars for 2 wk followed by a 1-mo washout period before crossover to the other study arm. In addition to standard outcome measures, including treadmill exercise testing and ambulatory ST segment monitoring, patients underwent assessment of endothelial function by flow-mediated vasodilation of the brachial artery. After 2 wk of treatment with the L-arginine–rich nutritional bars, there was a significant increase in total treadmill exercise time (16% increase in L-arginine group vs. 4% decrease in placebo group, $P = 0.05$); a substantial improvement in quality-of-life scores as measured by 2 standard questionnaires, and a marked improvement in flow-mediated vasodilation. There was no change in total ischemic time, as measured by ambulatory ST segment monitoring in the 2 groups, or in time to onset of ST-segment depression during treadmill exercise testing. In another placebo-controlled, crossover study of patients with stable angina pectoris, the addition of L-arginine supplements (2.8 g/d) to continuous use of a nitroglycerin patch increased pain-free walking time, perhaps by prevention of the development of nitrate tolerance (81).

Peripheral arterial disease. L-Arginine was investigated as a medical treatment for peripheral artery disease and symptomatic claudication in 2 clinical trials. Böger et al. (54) randomly assigned patients with symptomatic claudication to treatment with twice-daily intravenous infusion of L-arginine, intravenous infusion of prostaglandin E_1, or standard therapy over a period of 3 wk. L-Arginine treatment markedly increased both pain-free walking distance and maximal walking distance on a standardized treadmill protocol. Prostaglandin E_1 treatment similarly improved these exercise parameters. L-Arginine therapy also markedly improved endothelium-dependent vasodilation as assessed by flow-mediated vasodilation of the superficial femoral artery; prostaglandin infusion and the control therapy had no effect. L-Arginine infusion markedly increased urinary nitrate and cGMP excretion, as well as the plasma L-arginine:ADMA ratio. All of these markers indicate normalization of the endogenous NO pathway and increased NO formation. A more recent randomized clinical trial investigated the use of the L-arginine–rich nutritional supplement bars discussed above for treatment of stable intermittent claudication. In patients randomly assigned to treatment with 2 L-arginine supplement bars/d (6.6 g L-arginine/d), pain-free walking distance on a standardized treadmill protocol increased 66% compared to patients treated with 1 L-arginine supplement bar/d plus 1 placebo bar (3.3 g L-arginine/d) or 2 placebo supplement bars (82). In addition, maximal walking distance increased 23% and scores on a general health questionnaire increased 10% among patients in the 2- bar group. Additional clinical trials of L-arginine supplementation in patients with symptomatic claudication are ongoing.

Prevention of in-stent restenosis. Given data demonstrating that L-arginine administration inhibits myointimal hyperplasia in animal models of balloon vascular injury, Suzuki et al. (83) studied L-arginine as a potential treatment to prevent in-stent restenosis after coronary stenting. Fifty patients who received Palmaz-Schatz stents during routine percutaneous coronary intervention were randomly assigned to treatment with intramural L-arginine (administered by a drug delivery balloon) or saline placebo immediately after stent deployment. Patients received standard postprocedural care, including aggressive antiplatelet therapy. Coronary angiography and intravascular ultrasound were performed 6 mo after the original procedure. L-Arginine treatment decreased neointimal volume in the stents by 35% compared to saline treatment, as measured by intravascular ultrasound. However, there was no difference in total luminal volume. This small study was underpowered to detect any difference in clinical endpoints such as death, myocardial infarction, or the need for repeat catheterization.

Conclusions

The vascular endothelium is a key mediator of homeostasis and cardiovascular health. NO is synthesized by the endothelium to regulate its function. L-Arginine is the substrate utilized by eNOS to generate NO. Multiple cardiovascular diseases share endothelial dysfunction as a common pathway, including atherosclerosis of both the coronary and peripheral circulations. Endothelial dysfunction is associated with atherosclerosis, as well as most of the major risk factors associated with its development. The administration of L-arginine improves endothelial function in many animal models and in patients with cardiovascular disease. The ability of L-arginine to antagonize the endogenous NO inhibitor asymmetric dimethylarginine (ADMA) may explain its salutary effects on vascular function.

L-Arginine also prevents myointimal hyperplasia in models of endothelial injury. Although several small clinical outcome trials indicate that L-arginine supplementation is effective in the treatment of symptomatic angina pectoris and lower extremity claudication and in the prevention of in-stent restenosis, large phase III clinical trials are warranted.

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