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Arginine Nutrition and Cardiovascular Function[1,2]

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ABSTRACT ʟ-Arginine (Arg) is the substrate for the synthesis of nitric oxide (NO), the endothelium-derived relaxing factor essential for regulating vascular tone and hemodynamics. NO stimulates angiogenesis, but inhibits endothelin-1 release, leukocyte adhesion, platelet aggregation, superoxide generation, the expression of vascular cell adhesion molecules and monocyte chemotactic peptides, and smooth muscle cell proliferation. Arg exerts its vascular actions also through NO-independent effects, including membrane depolarization, syntheses of creatine, proline and polyamines, secretion of insulin, growth hormone, glucagon and prolactin, plasmin generation and fibrinogenolysis, superoxide scavenging and inhibition of leukocyte adhesion to nonendothelial matrix. Compelling evidence shows that enteral or parenteral administration of Arg reverses endothelial dysfunction associated with major cardiovascular risk factors (hypercholesterolemia, smoking, hypertension, diabetes, obesity/insulin resistance and aging) and ameliorates many common cardiovascular disorders (coronary and peripheral arterial disease, ischemia/reperfusion injury, and heart failure). Dietary Arg supplementation may represent a potentially novel nutritional strategy for preventing and treating cardiovascular disease. J. Nutr. 130: 2626–2629, 2000.

KEY WORDS: • arginine • cardiovascular disease

As a precursor for syntheses of nitric oxide (NO),[4] ornithine, proline, polyamines, creatine, agmatine and protein, and as an allosteric activator of *N*-acetylglutamate synthase, ʟ-arginine (Arg) plays vital roles in nutrition and metabolism (1). On the basis of growth or nitrogen balance, Arg is classified as an essential amino acid for birds, carnivores and young mammals, and as a conditionally essential amino acid for adults particularly at times of trauma or disease (1,2). Because NO is the endothelium-derived relaxing factor essential for regulating vascular tone and hemodynamics (3), there has been growing interest in the last 10 years in using Arg to prevent and treat cardiovascular disorders (4), the leading cause of death in developed nations. The major objective of this article is to review the recent studies concerning the role of Arg on cardiovascular function and therapy.

Arginine Availability and Vascular Effects. *Sources of Arg for endothelial cells (EC).* Endothelial cells line blood vessels and are in direct contact with the circulation. Endothelial Arg is derived from plasma, intracellular synthesis from citrulline and the net degradation of intracellular proteins (1). The diet, however, is the ultimate source of Arg in the body. Dietary Arg intake by the average American adult has been estimated to be 5.4 g/d (2). Because of a relatively high arginase activity in the small intestinal mucosa, ~40% of dietary Arg is degraded during absorption and the remainder enters the portal vein (1). Because the transport system y^+ (a high-affinity, Na^+-independent transporter of basic amino acids) is virtually absent from hepatocytes, >85% of the Arg delivered to liver is not taken up by this organ (1). Thus, assuming the digestibility of protein-bound Arg to be 90%, only ~50% of the dietary Arg enters the systemic circulation.

Normal plasma Arg concentrations in humans and animals range from 95 to 250 μmol/L, depending on developmental stage and nutritional status (1). Although extracellular Arg is the major source of the Arg for endothelial NO synthesis, intracellular protein degradation or the Arg-citrulline cycle may provide Arg for supporting short-term basal NO production by EC when extracellular Arg is limited (1).

The Arg paradox. Intracellular Arg concentrations are ~1 to 2 mmol/L in freshly isolated EC or EC cultured in the presence of 0.2–0.4 mmol/L Arg, but the K_m value of purified endothelial NO synthase (eNOS) for Arg is only 2.9 μmol/L (5). These observations imply that eNOS may be saturated with intracellular Arg and that endothelial NO synthesis may not respond to alterations in extracellular Arg concentrations. However, increasing extracellular Arg concentrations from 0.1 to 10 mmol/L in a dose-dependent manner increases NO production by cultured EC (5), and elevating plasma Arg levels enhances systemic and vascular NO production in vivo (4,6). A number of theories have been proposed to explain this Arg paradox, including colocalization of Arg transporter (CAT-1) and eNOS in membrane-associated caveolae, intracellular compartmentation of Arg, interaction between Arg and glutamine, alterations in eNOS dimerization and competitive inhibition of eNOS by endogenous inhibitors [e.g., asymmetric dimethylarginine (ADMA)] (4,5). Nevertheless, compelling evidence indicates that increasing extracellular Arg drives endothelial NO production (1,4).

NO-dependent and independent effects of Arg. Arg, a substrate for eNOS, is essential for maintaining the enzyme in the active dimerization state (5). In blood vessels, endothelium-derived NO activates guanylyl cyclase to generate cGMP from GTP in smooth muscle cells, elevates cellular cGMP concentrations and causes smooth muscle relaxation (3). Thus, NO plays an essential role in regulating vascular tone

TABLE 1

Nitric oxide (NO)-dependent and independent vascular actions of L-arginine[1]

NO-dependent vascular actions	NO-independent vascular actions
↑ Smooth muscle cell relaxation	↑ Polarization of EC membranes
↑ EC proliferation and angiogenesis	↑ Extracellular and intracellular pH
↓ Endothelin-1 release	↑ Release of insulin, GH, glucagon and prolactin
↓ Leukocyte adhesion	↑ Synthesis of urea, creatine, PRO and PA
↓ Platelet aggregation	↑ Plasmin generation and fibrinogenolysis
↓ Superoxide production	↓ Leukocyte adhesion to non-EC matrix
↓ Expression of cell adhesion molecules	↓ Blood viscosity
↓ Expression of monocyte chemotactic peptides	↓ Angiotensin-converting enzyme activity
↓ Proliferation of smooth muscle cells	↓ O_2^- release & lipid peroxidation
↓ EC apoptosis	↓ Formation of TXB_2, fibrin and platelet-fibrin

[1] Abbreviations: EC, endothelial cells; GH, growth hormone; PA, polyamines; PRO, proline; TXB_2, thromboxane B_2. The symbols ↑ and ↓ denote increase and decrease, respectively.

and hemodynamics. NO stimulates EC proliferation and angiogenesis, thereby playing an important role in wound healing and microcirculation (7). In addition, NO inhibits the release of endothelin-1 (a vasoconstrictor) by EC, leukocyte adhesion to the endothelium, platelet aggregation, superoxide generation by NADPH oxidase, the expression of vascular cell adhesion molecules and monocyte chemotactic peptides, and the proliferation of smooth muscle cells (3,4). NO also inhibits apoptosis in EC possibly through two mechanisms: *1*) increasing cGMP generation, which interrupts apoptotic signaling, and *2*) directly inhibiting cysteine protease (caspase) activity (8). Thus, in addition to its effect on vasorelaxation and angiogenesis, NO is a novel antiatherogenic, antiproliferative and antithrombotic factor.

Although the vascular effects of Arg are mediated primarily by NO production (4), Arg also exerts NO-independent hemodynamic effects (**Table 1**). As a basic amino acid, Arg may contribute to the depolarization of EC membranes and regulate blood and intracellular pH. As an antioxidant, Arg (0.5–1 mmol/L) can scavenge O_2^-, reduce copper-induced lipid peroxidation and inhibit O_2^- release by EC (9). As a possible regulator of the binding of macromolecules to RBC, high Arg concentrations (≥ 2.5 mmol/L) decrease blood viscosity (10). As a precursor for the synthesis of protein, urea, creatine, polyamines, proline, glutamate and agmatine, Arg plays vital roles in nutrition and physiology (1). For example, creatine participates in energy metabolism in muscle and nerves, polyamines are crucial to cell proliferation and differentiation, and proline is critical to collagen synthesis and thus extracellular matrix formation and vessel remodeling. As an allosteric activator of *N*-acetylglutamate synthase to synthesize *N*-acetylglutamate (an essential cofactor for carbamoylphosphate synthase I), Arg maintains the urea cycle in the active state for ammonia detoxification (1). As a stimulator of the secretion of insulin, growth hormone, glucagon and prolactin, Arg regulates the metabolism of glucose, protein and lipids, factors that are closely linked to atherogenesis (4). As an inhibitor of

angiotensin-converting enzyme, Arg reduces plasma angiotensin II levels and thus amplifies its hypotensive effect (11). Through inhibiting the formation of thromboxane B_2 and the platelet-fibrin complex while enhancing plasmin generation and fibrin degradation, Arg stimulates fibrinogenolysis (12). Finally, Arg may directly inhibit leukocyte adhesion to non-endothelial matrix independently of NO production (13), thereby inhibiting the development of atherosclerosis.

L-Arginine Reversal of Endothelial Dysfunction in Patients with Cardiovascular Risk Factors. Epidemiologic and clinical studies over the last 50 years have established hypercholesterolemia, smoking, hypertension, diabetes, obesity/insulin resistance and advanced age as major cardiovascular risk factors, all of which are associated with impaired endothelium-dependent relaxation (Table 1). Although extensive cardiovascular research has focused historically on dietary fat and cholesterol due to the recognition of their roles in atherosclerosis, studies over the last 10 years have shown the promise of using Arg to reverse endothelial dysfunction associated with these major cardiovascular risk factors (4).

Hypercholesterolemia. Plasma Arg concentrations are not altered in hypercholesterolemic subjects, but those of ADMA increase as a result of its impaired catabolism (14). Using the rabbit model of hypercholesterolemia, many studies have consistently shown that oral Arg administration (22.5 g/L in drinking water for 10–12 wk) alleviates or completely reverses endothelial dysfunction in cerebral and coronary arteries, hind-limb microvasculature and thoracic aorta, and inhibits the progression of atherosclerosis (4). Human studies have also consistently demonstrated the beneficial effect of Arg on improving endothelium-dependent relaxation in hypercholesterolemic patients (4). For example, intravenous Arg infusion (0.2 g/kg body over 20 min) or oral Arg supplementation (3 × 7 g/d for 4 wk) to these patients increases endothelium-dependent forearm blood flow and dilation of the conduit arteries (15,16). These findings have led to the recent development of Arg-enriched HeartBar (6.6 g Arg/d; Cooke Pharma, Belmont, CA) to reverse endothelial dysfunction in hypercholesterolemic humans (17).

Smoking. Cigarette smoke contains a large number of oxygen-derived radicals and prooxidants, and causes endothelial dysfunction in coronary and peripheral conductance and resistance vessels, as well as in veins (4). The discovery that cigarette smoke extract decreases endothelial NO synthesis has provided a metabolic basis for using Arg to reverse smoking-induced endothelial dysfunction in humans and animal models. Oral Arg (22.5 g/L in drinking water) prevents endothelial dysfunction associated with environmental tobacco smoke in normocholesterolemic and hypercholesterolemic rabbit models (18). Remarkably, intravenous Arg infusion (30 g over 45 min) normalizes coronary vasomotion in long-term smokers (19).

Hypertension. NO plays a crucial role in regulating blood pressure; thus, Arg or NO deficiency results in hypertension in animals and humans (3). Much research has shown that intravenous or oral Arg administration increases NO synthesis and prevents endothelial dysfunction in animal models of pulmonary hypertension or salt-induced hypertension (4). The same beneficial effect of Arg has been observed in most studies involving hypertensive patients, who exhibit elevated plasma levels of ADMA and reduced NO synthesis (4). For example, intravenous Arg infusion (0.5 g/kg body over 30 min) to infants with persistent pulmonary hypertension increases partial pressure of oxygen and systemic oxygenation within 90 min of the administration (20). Similarly, intravenous Arg infusion (0.525 g/kg body over 35 min) to infants with pul-

monary hypertension reduces pulmonary vascular resistance and enhances cardiac output (21). In contrast to some earlier reports (see Ref. 4 for review), recent studies have also demonstrated beneficial effects of Arg on essential hypertensive patients. For example, intravenous Arg infusion (20–30 g over 30 min) improves systemic and renal hemodynamics in salt-sensitive patients with essential hypertension (4), and intravenous Arg infusion (0.5 g/kg body over 30 min) reduces blood pressure and renal vascular resistance in essential hypertensive patients with normal or insufficient renal function (22).

Diabetes. Diabetes is associated with reduced plasma Arg concentrations (23), and thus Arg administration may become a promising solution to improve endothelial function in diabetic subjects. In support of this proposition, oral Arg administration (12.5 g/L in drinking water) to diabetic rats for 3 d reverses endothelial dysfunction, and an intravenous bolus of 3–5 g Arg reduces mean blood pressure and platelet aggregation in patients with noninsulin-dependent diabetes (23). A more recent study has shown that 4 wk of oral Arg supplementation (1.25 g/L in drinking water) to diabetic rats lowers blood pressure, restores the defective endothelium-dependent relaxation and decreases plasma levels of malondialdehyde (an indicator of oxidative stress) (24). Similarly, intravenous Arg infusion (30 g over 30 min) to newly diagnosed noninsulin-dependent diabetic patients reduces blood pressure and improves hemodynamic function (25).

Obesity/insulin resistance. Obesity/insulin resistance is associated with endothelial dysfunction, and recent studies have identified an important role for NO in the pathogenesis of insulin resistance. For example, insulin resistance at the level of the liver and peripheral tissues occurs in eNOS knock-out mice (26). Interestingly, intravenous Arg infusion (94 mg/kg body over 3 h) improves insulin sensitivity and insulin-mediated vasodilation in obese patients and in patients with non-insulin-dependent diabetes (27).

Advanced age. Aging is associated with decreases in plasma Arg concentrations and NO synthesis, and intravenous Arg infusion (0.56 g over 20 min) reverses the aging-associated endothelial dysfunction in humans (28). In a more recent study involving 1-y-old spontaneously hypertensive rats, Susic et al. (29) showed that 6 mo of oral Arg supplementation (1.2 g/L in drinking water) reduces arterial pressure and total peripheral resistance, diminishes left ventricular mass and improves coronary hemodynamics.

L-Arginine Therapy for Cardiovascular Disorders.
Through enhancing NO production, restoring vasodilation and inhibiting the progression of atherosclerosis, Arg benefits patients with cardiovascular disorders. These disorders include coronary and peripheral arterial disease, ischemia/reperfusion injury and heart failure, all of which are associated with impaired NO synthesis and endothelial dysfunction (4).

Coronary artery disease (CAD). This disorder affects the arteries that supply blood to the heart muscle and is the major cause of heart attack. Systemic or oral Arg administration has been shown to improve cardiovascular function, increase exercise capacity and reduce myocardial ischemia in CAD patients (4). For example, an intravenous bolus of 30 g Arg to CAD patients normalizes coronary blood flow response to acetylcholine, and intracoronary infusion of 26 g Arg over 8 min induces coronary stenosis dilatation in CAD patients with chronic stable angina (4). In addition, oral Arg enhances brachial artery flow-mediated vasodilation and inhibits monocyte adhesion to EC in young men with CAD (30). Strikingly, 6 mo of oral Arg supplementation (9 g/d) decreases plasma endothelin-1 levels by 30% and increases coronary blood flow response to acetylcholine by 150% in nonobstructive CAD patients (31).

Peripheral arterial disease (PAD). This disorder results from the narrowing or blocking of peripheral arterial vessels in the legs and other parts of the body. Because damage to leg tissues is so severe in some cases as to result in gangrene and amputation, improving peripheral circulation will have tremendous impact in PAD patients. Interestingly, daily intravenous Arg infusion (12.6 g/d) for 7 d increases calf blood flow and enhances walking distance (32). Intravenous Arg infusion (30 g over 60 min) to PAD patients also increases NO synthesis and femoral artery blood flow (4). For hypercholesterolemic humans, oral daily consumption of Arg-enriched HeartBar (6.6 g Arg/d) for 2 wk increases pain-free and total walking distance by 66 and 23%, respectively, as well as quality of life in PAD patients (33).

Ischemia/reperfusion. NO plays an important role in regulating cerebral vascular tone and circulation, and thus NO deficiency contributes to large cerebral infarct size (34). Previous studies with animal models have shown that Arg administration improves tissue preservation during reperfusion and increases regional blood flow in focal cerebral ischemia (4). Addition of 3 mmol/L Arg to the perfusate of isolated rat hearts during hypoxia and reperfusion protects the myocardium against reoxygenation injury (35), suggesting that Arg supplementation to the cardioplegic solution may exert a cardioprotective effect. There is also evidence indicating that exogenous Arg protects hepatic, intestinal and lung microcirculation from ischemia/reperfusion injury (4). These findings may have important implications for cardioplegic arrest, organ transplantation and other surgeries requiring periods of ischemia or reperfusion.

Heart failure. Heart failure results from the heart's inability to pump sufficient blood to maintain normal circulation. This often leads to congestive heart failure (CHF), in which blood and fluids accumulate in the lungs and elsewhere, causing congestion in the abdomen or legs. Heart failure is associated with decreased plasma Arg levels, increased plasma ADMA levels and reduced NO synthesis (4). There is increasing evidence indicating that systemic or oral Arg administration enhances forearm blood flow response to acetylcholine, decreases systemic vascular resistance and mean arterial pressure, and increases ventricular stroke volume and cardiac output (4). For example, 6 wk of oral Arg (5.6–12.6 g/d) reduces plasma levels of endothelin-1, increases forearm blood flow in response to exercise, and improves arterial compliance and overall functional status (36). Similarly, 4 wk of oral Arg (8 g/d) improves endothelium-dependent vasodilation in patients with heart failure, and this beneficial effect is additive with exercise training (37). In contrast to some earlier reports (see Ref. 4 for review), recent studies have demonstrated the beneficial effect of Arg on CHF patients. For example, 5 d of oral Arg (15 g/d) improves renal hemodynamics in CHF patients (38), and iv Arg infusion (30 g over 30 min) enhances cardiac performance in patients with severe CHF (39).

Problems and Areas for Future Research.
Although intravenous Arg infusion (up to 0.5 g Arg · HCl/kg body for infants or 30 g Arg · HCl for adults over 30–60 min) or oral Arg (9 g Arg · HCl/d for adults) has little or no adverse effect on humans (20–22), higher oral doses of Arg · HCl are occasionally associated with nausea, stomach cramps and diarrhea (16,31,40). Possible causes may be excess NO production by the gastrointestinal tract and impaired intestinal absorption of other dietary basic amino acids (lysine and histidine). A solution to this potential problem may be the

alternative use of L-citrulline, an effective precursor for Arg synthesis (1). As a neutral amino acid, L-citrulline does not compete with basic amino acids for transport by cells, its conversion to Arg consumes 1 mol of ammonia in the form of aspartate and its administration does not require equimolar HCl. Thus, enteral or parenteral L-citrulline may be particularly useful for patients with elevated ammonia concentrations, impaired Arg transport or enhanced intestinal Arg catabolism. Second, recent puzzling findings from some CAD patients reveal that elevating plasma Arg levels through oral Arg provision does not always increase vascular NO synthesis or availability (40). This underscores the need to understand the complexity of the mechanisms for the regulation of Arg metabolism and NO synthesis. Such work is necessary to fully explain the Arg paradox and some conflicting findings regarding the effects of Arg on the vascular system (4,40). Third, because of the lack of published reports, much research will be required to evaluate the role for oral Arg or L-citrulline in reversing endothelial dysfunction associated with smoking, hypertension, diabetes, obesity/insulin resistance, aging and pre-eclampsia in humans. Collectively, these studies will help establish dietary Arg or L-citrulline supplementation as a potentially novel nutritional strategy for preventing and treating cardiovascular disorders.

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