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THE CLINICAL PHARMACOLOGY OF L-ARGININE

Rainer H Böger[1,2] and Stefanie M Bode-Böger[1]
[1]*Institute of Clinical Pharmacology, Hannover Medical School, D-30623 Hannover, Germany*
[2]*Clinical Pharmacology, Institute of Experimental and Clinical Pharmacology and Toxicology, University Clinic Eppendorf, D-20246 Hamburg, Germany*

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■ **Abstract** L-Arginine (2-amino-5-guanidinovaleric acid) is the precursor of nitric oxide, an endogenous messenger molecule involved in a variety of endothelium-mediated physiological effects in the vascular system. Acute and chronic administration of L-arginine has been shown to improve endothelial function in animal models of hypercholesterolemia and atherosclerosis. L-Arginine also improves endothelium-dependent vasodilation in humans with hypercholesterolemia and atherosclerosis. The responsiveness to L-arginine depends on the specific cardiovascular disease studied, the vessel segment, and morphology of the artery. The pharmacokinetics of L-arginine have recently been investigated. Side effects are rare and mostly mild and dose dependent. The mechanism of action of L-arginine may involve nitric oxide synthase substrate provision, especially in patients with elevated levels of the endogenous NO synthase inhibitor asymmetric dimethylarginine. Endocrine effects and unspecific reactions may contribute to L-arginine-induced vasodilation after higher doses. Several long-term studies have been performed that show that chronic oral administration of L-arginine or intermittent infusion therapy with L-arginine can improve clinical symptoms of cardiovascular disease in man.

BIOCHEMISTRY AND PHYSIOLOGICAL ROLES OF L-ARGININE

L-Arginine (2-amino-5-guanidinovaleric acid) is a basic, semiessential amino acid. Its occurrence in mammalian protein was discovered by Hedin in 1895 (1). At that time, the existence of L-arginine as a naturally occurring molecule had already been known since 1886, when it was first isolated from lupin seedlings (2). Our present knowledge about the involvement of L-arginine in several different metabolic pathways is the result of discoveries that were made during the 20th century (Figure 1). One was that synthesis of L-arginine and its subsequent disintegration into L-ornithine and urea, catalyzed by the activity of arginase, is a

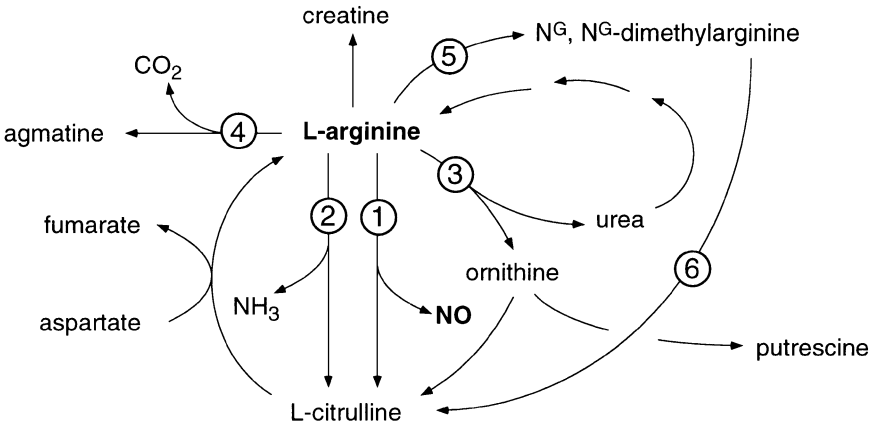

Figure 1 Schematic representation of the metabolism of L-arginine. Enzymatic pathways indicated by numbers: 1, biosynthesis of nitric oxide (NO) from L-arginine by nitric oxide synthase; 2, synthesis of L-citrulline from L-arginine by arginine deaminase; 3, conversion of L-arginine to urea and L-ornithine by arginase (part of the urea cycle); 4, decarboxylation of L-arginine to agmatine by arginine decarboxylase; 5, methylation of L-arginine by protein arginine *N*-methyltransferases; 6, metabolism of dimethylarginine to L-citrulline by dimethylarginine dimethylaminohydrolase.

ubiquitous pathway that serves to eliminate nonessential nitrogen-containing substances from the body. In 1932, Krebs & Henseleit reported their finding that L-arginine is an essential component in this cyclic metabolic pathway, the urea cycle (3). This is the only pathway in mammals that allows elimination of continuously generated toxic ammonia from the body. Furthermore, the "byproduct" of this reaction, L-ornithine, is a precursor for synthesis of polyamines, molecules essential for cell proliferation and differentiation (4). In 1939, Foster et al (5) discovered that L-arginine is also required for the synthesis of creatine. In its phosphorylated form (creatine phosphate), creatine is an essential energy source for muscle contraction. Its degradation product, creatinine, is eliminated by glomerular filtration in the kidney and is used as a surrogate measure of glomerular filtration rate. In the 1980s it was discovered that L-arginine is a precursor of nitric oxide (NO) (6–8), the chemical entity shown to be identical with endothelium-derived relaxing factor (9). The enzyme that synthesizes NO from the terminal guanidino nitrogen group of L-arginine was isolated, cloned, and characterized in 1991 from macrophages (10), endothelial cells (11), and neuronal cells (12). In the 1990s, the enzyme arginine decarboxylase was discovered in mammalian cells (13). This enzyme converts L-arginine into agmatine, a molecule whose physiological functions are still under investigation. Agmatine has been shown to bind to α_2-adrenoceptors and imidazoline receptors, potentially evoking clonidinelike effects on blood pressure (13). Agmatine is also a weak inhibitor of NO synthase (NOS) isoenzymes, suggesting a possible role for it as an endogenous modulator of NO production if local concentrations are sufficiently high (14).

In early studies L-arginine was characterized as a nonessential amino acid in the healthy adult human (15) but as an essential amino acid for young, growing animals (16). Homeostasis of plasma L-arginine concentrations is regulated by dietary arginine intake, protein turnover, arginine synthesis, and metabolism. This may explain why, under certain disease conditions, L-arginine may become an essential dietary component. The main tissue in which endogenous L-arginine synthesis occurs is the kidney, where L-arginine is formed from L-citrulline, which is released mainly by the small intestine (17). The liver is also capable of synthesizing considerable amounts of L-arginine; however, this is completely reutilized in the urea cycle so that the liver contributes little or not at all to plasma L-arginine flux (18). The nearly complete separation between hepatic and systemic L-arginine pools has also been partly attributed to the fact that the active L-arginine uptake system, the y^+ transporter (19), has a very low activity in hepatocytes (20). Cell types containing NOS have been demonstrated to be able to reutilize L-citrulline, the byproduct of NO synthesis, to L-arginine via the so-called arginine-citrulline cycle (21, 22). This pathway is mediated by enzymes also involved in the hepatic urea cycle; however, the fact that L-citrulline accumulates in the medium of NO-producing cells indicates that the arginine-citrulline cycle is far less efficient than the urea cycle (23). In macrophages and other cell types, induction of inducible NOS is accompanied by induction of argininosuccinate synthase, the rate-limiting enzyme of L-arginine biosynthesis (24), suggesting that NOS substrate availability is tightly regulated and may be rate limiting for NO production under certain conditions.

Approximately 5.4 g of L-arginine is absorbed each day in adults who ingest an average US diet (25). Thus, each gram of dietary protein supplies about 54 mg of L-arginine. Walser (26) estimated that a 70-kg adult person eating about 50 g of protein per day consumes about 0.2 mmol (34.8 mg) of L-arginine per kg of body weight per day or a total of 2.4 g of L-arginine per day. The difference between the two studies is because of differences in the estimates of average protein intake in usual Western diets. Average L-arginine intake can therefore be assumed to be on the order of 4–5 g/day.

ROLE OF L-ARGININE AS A PRECURSOR OF NITRIC OXIDE: Preclinical Pharmacology

L-arginine is the precursor for the endogenous synthesis of NO due to the activity of NOS, which releases L-citrulline as a byproduct (6–8). Although only a minor portion of L-arginine is metabolized via this pathway in vivo, it has attracted much interest in recent years because of the prominent role that NO plays in vascular physiology and pathophysiology (for reviews, cf 27, 28). NO generated from L-arginine is a highly reactive radical gas and an important messenger molecule that is involved in functions as diverse as neurotransmission, vasodilation, inflammation, and regulation of gene expression. At low concentrations like those produced by constitutive endothelial NOS (ecNOS) in the vasculature in vivo,

NO acts as a paracrine-signaling molecule, mediating vasodilation (29), inhibition of platelet activation (30), inhibition of monocyte and leukocyte adhesion (31), and inhibition of smooth muscle cell proliferation (32) and controlling vascular oxidative stress and the expression of redox-regulated genes (33).

In certain animal models and in human diseases (see below), the biological functions of endothelium-derived NO are impaired, leading to dysregulation of endothelial control of vascular tone and blood flow. Such models include hypercholesterolemic rabbits (34–36), rat models of hypertension (37), and hyperlipidemic monkeys (38). The mechanisms behind this phenomenon are probably multifactorial, including reduced NO elaboration by NOS, increased oxidative inactivation of NO, and enhanced formation of vasoconstrictor mediators like endothelin-1 and thromboxane A_2 (28, 39).

What is the role of L-arginine in this setting? NOS is inhibited by L-arginine analogs that are substituted at the guanidino nitrogen atom, like N^G-monomethyl-L-arginine or N^G-nitro-L-arginine (40). Inhibitory action of these molecules is overcome by excess L-arginine (40), indicating that there is competition for enzyme binding between L-arginine and its inhibitory analogs. Reduced activity of endothelial cell NOS was also shown to occur in the presence of low-density-lipoprotein cholesterol; again, this effect can be overcome by excess L-arginine (41). Although the mechanism behind this latter phenomenon has not yet been fully elucidated, these data demonstrate that, under certain conditions, L-arginine availability regulates endothelial cell NOS activity.

On the other hand, depletion of L-arginine in endothelial cells is hardly possible, due to high intracellular L-arginine concentrations (42) and the ability of endothelial cells to synthesize L-arginine from L-citrulline (21). Incubation of isolated blood vessels with high concentrations of L-arginine does not directly affect vascular tone, nor does it modulate endothelium-dependent relaxation (43). However, a plethora of reports from animal studies have shown that acute or chronic administration of L-arginine in vivo improves vascular responsiveness, probably via enhanced NO elaboration. Acute administration of L-arginine augments endothelium-dependent vasodilation in cholesterol-fed rabbits (34, 44) and transiently increases urinary excretion of nitrate (the metabolite of NO) in rats (45). Apart from these acute effects, long-term oral administration of L-arginine has been associated with a significant improvement in NO-dependent vasodilation in cholesterol-fed rabbits (35, 36, 46, 47) and in low-density-lipoprotein receptor knockout mice (48). In these animal models, other NO-dependent vascular functions are also modulated by chronic supplementation with L-arginine: Endothelial leukocyte adhesion is reduced (49), platelet aggregation is inhibited (50, 51), and vascular smooth muscle cell proliferation in vivo is attenuated (52). The latter effect may prominently contribute to reduced restenosis after experimental angioplasty (53–55) and to reduced intimal thickening in vein grafts (56).

L-arginine treatment influences the progression of the atherosclerotic disease process in animal models: When administered via the oral route to rabbits fed a cholesterol-enriched diet, development of intimal plaques in the carotid arteries

is slowed (35, 36), the intima/media ratio in the thoracic and abdominal aorta is reduced (35, 36, 46), and intimal thickening in the coronary arteries is inhibited (57). Enhanced NO elaboration after L-arginine supplementation also contributes to improved perfusion of collaterals after arterial occlusion in rabbit ear tissue (58). There is controversy about whether L-arginine can induce regression of preexisting lesions. Candipan and coworkers (47) demonstrated that, after 10 weeks on a high-cholesterol diet, supplementation with L-arginine for an additional 13 weeks led to significant reduction in aortic lesion size. We (36) found that addition of L-arginine to the diet for 8 weeks after 4 weeks of preceding hypercholesterolemia completely halted the progression of vascular lesions in the aorta and carotid artery of rabbits, but did not induce regression. Inhibition of smooth-muscle-cell proliferation by L-arginine (52) and induction of apoptotic cell death in vascular lesions (59) may both contribute to the beneficial effects of L-arginine on vascular structure in this animal model.

FROM BENCH TO BEDSIDE: Cardiovascular Effects of L-Arginine in Humans

Very soon after the first animal experiments had proven a beneficial effect of L-arginine on endothelial function, it was shown that local intracoronary infusion of L-arginine normalized coronary vasomotor responses to acetylcholine in hypercholesterolemic humans (60). A similar observation was also made upon systemic (intravenous) infusion of L-arginine in hypercholesterolemic subjects, in whom endothelium-dependent forearm vasodilation was improved (61). These were important findings, because endothelial dysfunction precedes angiographically visible atherosclerotic lesions in large coronary arteries (62). Recent evidence from prospective clinical trials suggests that endothelial dysfunction is a predictor of future coronary events (63, 64). Therefore, reversal of endothelial dysfunction by L-arginine in vivo may suggest that this amino acid exerts antiatherosclerotic effects in humans.

The responsiveness of endothelial dysfunction to L-arginine is not a ubiquitous phenomenon. It depends on factors such as the arterial segment studied, the presence or absence of morphological atherosclerotic lesions, the underlying cardiovascular disease, and the L-arginine concentration reached. Egashira et al (65) showed that coronary vasodilator response to acetylcholine was significantly improved after intracoronary L-arginine in patients with microvascular angina and normal coronary angiograms. Drexler et al (66) demonstrated that, in cardiac transplant recipients, improvement of coronary endothelial function with L-arginine is more likely in vessels with normal wall morphology. By contrast, in another study a more prominent vasodilator effect of L-arginine was found in stenosed coronary arteries than in healthy vessel segments (67).

Although there is a bulk of evidence that supplementation with L-arginine—via the intraarterial, intravenous, or oral route—improves endothelial dysfunction in

hypercholesterolemia and atherosclerosis, endothelial dysfunction in other cardiovascular diseases was not consistently improved by L-arginine administration. The majority of studies with L-arginine in hypertensive patients revealed a lack of effect of this amino acid on endothelial function (68, 69). In a comparative trial between patients with coronary artery disease and patients with primary pulmonary hypertension, we also found no vasodilator effect of L-arginine in pulmonary hypertension (70). Reduced NO elaboration in certain types of hypertension may be caused by reduced expression of endothelial NOS (71). This may explain why increased substrate availability has no beneficial effect in this condition.

In a series of clinical studies, we investigated direct hemodynamic effects of L-arginine in the peripheral vasculature. We found that intravenous infusion of 30 g of L-arginine significantly increased arterial blood flow in the femoral artery of healthy subjects by a mean 44% (72). In a subsequent study, the peripheral vasodilator action of 30 g of L-arginine was reproduced by using impedance cardiography to assess total peripheral resistance (73). Plasma L-arginine concentrations increased to 6.0 $\pm$ 0.4 mM. A lower dose of L-arginine (6 g), administered by either the intravenous or the oral route, failed to produce acute vasodilation. Plasma L-arginine concentrations rose to 822 $\pm$ 59 μM and 310 $\pm$ 152 μM after intravenous and oral administration, respectively, of 6 g of L-arginine.

In a study in patients suffering from severe peripheral arterial occlusive disease, we demonstrated that an acute intravenous infusion of 30 g of L-arginine increased femoral arterial blood flow in the more severely affected leg by a mean 43% (74). In this study the plasma L-arginine concentration increased to a mean 3.8 $\pm$ 0.4 mM. This vasodilator effect of L-arginine, which was measured by duplex ultrasonography in the femoral artery, was due to increased blood flow velocity, whereas femoral artery diameter remained unchanged. Although this observation pointed to a peripheral vasodilator action of L-arginine, we had no evidence from that study about whether that action involved opening of arteriovenous shunts (which, in the long run, might further decrease nutritive muscle blood flow in the diseased limb) or increasing muscle capillary blood flow (which might prove to be therapeutically favorable for peripheral arterial occlusive disease patients). We addressed this question in a further clinical study in which we performed serial measurements of muscle capillary blood flow by using positron emission tomography with isotope-labeled water as the flow tracer. We found that a single systemic infusion of 30 g of L-arginine increased nutritive muscle blood flow by a mean 43%, whereas a lower dose of 8 g of L-arginine had no significant effect (75).

Increased nutritive tissue blood flow, as well as our observation that blood flow remained elevated for 1–2 h after the end of L-arginine infusion (72, 74), made us confident that, on a medium- to long-term scale, L-arginine might improve the symptoms of occlusive arterial disease. In the first study to address this question, we found that, after 3 weeks of intermittent intravenous L-arginine therapy (3 doses of 8 g/day), claudication distance was significantly increased (76). Absolute and pain-free walking distances were improved by 230 $\pm$ 63% and 155 $\pm$ 48%,

respectively, whereas in the control group there were no significant changes. Six weeks after the end of the infusion therapy, walking distance was still significantly prolonged, which may be a result of persistent improvement of endothelium-dependent vasodilation in response to increased peripheral blood flow (e.g. during walking exercise).

Other investigators also showed that oral L-arginine supplementation improves clinical symptoms of vascular disease (see Table 1, p. 91): Ceremuzynski et al (77) showed that exercise capacity was improved as compared to placebo in patients with stable angina pectoris after 3 days of 6 g of L-arginine daily. Six months of oral treatment with L-arginine (3 doses of 3 g/day) resulted in a significantly improved angina symptom score and improved coronary blood flow response to acetylcholine in another placebo-controlled study that included 26 patients with small-vessel coronary artery disease (78). By contrast, Blum et al (79) observed no improvement of NO elaboration, flow-mediated vasodilation of the brachial artery, or serum adhesion molecule levels after 1 month of oral L-arginine (9 g/d) in patients with coronary artery disease. However, in that study, patients were on an optimized medical treatment including cholesterol-lowering and vasoactive medication before and during the study, and flow-mediated vasodilation was normal at baseline, which may have left too little room for improvement by L-arginine.

Acute intravenous infusion of 20 g of L-arginine resulted in significantly reduced peripheral resistance, increased stroke volume, and cardiac output without a change in heart rate in 12 patients with congestive heart failure (80). In a placebo-controlled study with oral L-arginine (3.6–12.6 g/d over 6 weeks), Rector et al (81) found significant improvements in forearm blood flow, increased walking distance in a 6-min walk test, and improved arterial compliance and quality of life in patients with heart failure.

In patients with Raynaud's phenomenon and scleroderma, vasospastic attacks are significantly reduced by L-arginine, suggesting that NO deficiency may be involved in the pathogenesis of vasospasms in Raynaud's phenomenon (82).

MECHANISM(S) OF ACTION OF L-ARGININE'S CARDIOVASCULAR EFFECTS IN HUMANS

The first assumption of the mechanism behind the vascular effects of L-arginine in vivo was that it acts via substrate provision for NOS (34). However, this assumption was controversial because of the obvious discrepancy between the half-saturating L-arginine concentration (K_m value) for isolated, purified endothelial NOS [2.9 μM (11)] and plasma L-arginine concentration (60–100 μM). From an enzyme-biochemical point of view, it was argued that additional L-arginine could not have any effect on NOS activity, because this enzyme should be saturated with substrate at physiological levels and not be dependent on extracellular supply. However, L-arginine did have a beneficial effect on endothelium-dependent vasodilation in vivo. This phenomenon was called the "L-arginine paradox." Several

explanations have been brought forward to resolve this paradox. First, L-arginine may be compartmentalized in the cytoplasm, and local concentrations in the vicinity of endothelial NOS may be lower than expected from L-arginine levels in whole-cell homogenates. Indeed, there is recent evidence that endothelial NOS is colocalized in caveolae formed by the cytoplasmic membrane with the y^+ transporter (83). Extracellular L-arginine may be preferentially utilized by NOS within this microenvironment. This may explain earlier findings showing rapid conversion of extracellular L-[guanidino-$^{15}N_2$]-arginine into ^{15}N-nitrate by cultured endothelial cells (8).

Another explanation for the L-arginine paradox may be the presence of endogenous NOS inhibitors in certain diseases. Presence of asymmetric dimethylarginine (ADMA), an endogenous molecule that exerts NOS-inhibitory effects, has been demonstrated in human plasma and urine (84). Elevated concentrations of ADMA are present in patients with vascular diseases, resulting in diminished NOS activity (see below). As discussed above for synthetic L-arginine analogs, inhibition of NOS activity may be overcome by excess substrate and could explain how L-arginine improves endothelial function in patients with vascular disease. However, this mechanism would not explain L-arginine-induced vasodilation in healthy humans in whom ADMA levels are low.

Endocrine mechanisms may contribute to vasodilation induced by L-arginine in healthy humans and in patients. High intravenous doses of L-arginine (30 g) have been used since the 1960s to stimulate growth hormone (GH) secretion (85). In addition, L-arginine stimulates the release of pancreatic insulin (86) and glucagon (87) and pituitary prolactin (88). Of these, GH and insulin can induce vasodilation by mechanisms that have long remained unclear. Giugliano and coworkers (89) recently demonstrated that an intravenous infusion of 30 g of L-arginine induced vasodilation and insulin release in healthy humans. When insulin secretion was blocked by octreotide coinfusion, no vasodilation occurred. However, vasodilation was restored by insulin coadministration. Unfortunately, these investigators did not measure GH release, which is also blocked by octreotide. We were able to show that L-arginine (30 g, intravenously) induced a rapid release of insulin and a delayed release of GH (90). During coinfusion of somatostatin, release of both hormones was blocked; however, somatostatin inhibited only the late response but not the early increase in NO production. Our conclusion therefore was that GH contributes to the prolonged NO-dependent vasodilation to high doses of L-arginine. Other studies corroborate this hypothesis. GH exerts many of its effects via insulinlike growth factor-1 (91). Insulinlike growth factor-1 activates ecNOS in vitro (92, 93), and it induces NO-dependent vasodilation in human forearm tissue in vivo (94). We have further shown that chronic administration of recombinant GH increases NO production in GH-deficient patients (95) and in patients with dilated cardiomyopathy (96). Oral administration of L-arginine in combination with L-lysine has also been found to stimulate GH release, but L-arginine alone given by the oral route has no such effect (97). Therefore, whether this mechanism contributes significantly to the beneficial effect of oral L-arginine

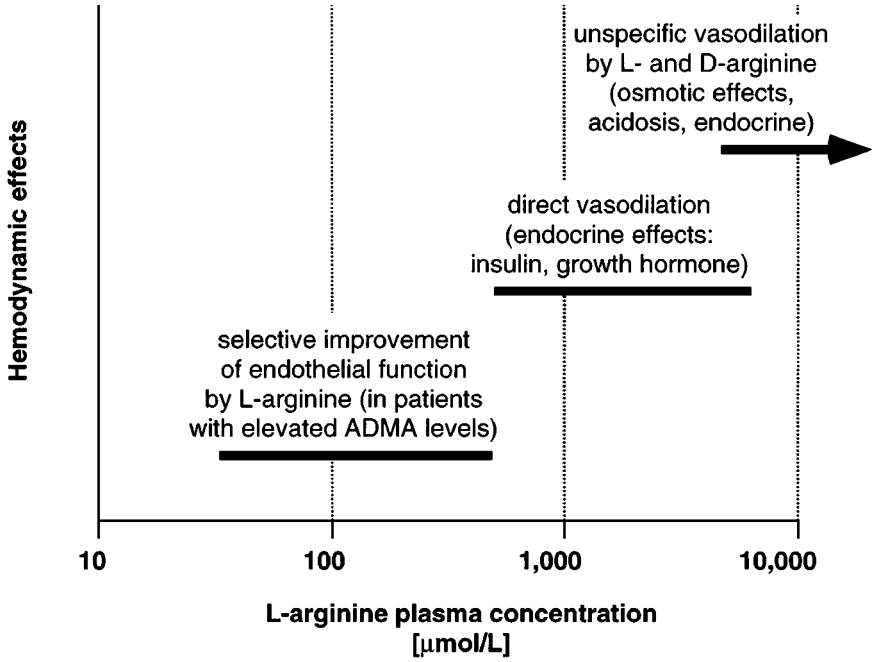

Figure 2 Concentration-dependent clinical effects of L-arginine as observed in studies in human subjects.

on endothelium-dependent vasodilation in humans is not known. We did not find evidence for increased GH secretion during oral L-arginine supplementation (2 doses of 8 g/day) in human subjects (S Bode-Böger & RH Böger, unpublished observation) (Figure 2).

Agmatine is another metabolite that may be involved in the vasodilatory action of L-arginine. Agmatine is a ligand at central α_2-and imidazoline receptors (13), where it induces clonidine-like effects (97) and—by lowering peripheral sympathetic tone—may lower blood pressure and induce vasodilation. No data are available to date showing whether this mechanism contributes to L-arginine-induced vasodilation in humans in vivo.

Unspecific effects have been suggested to be involved in vasodilation induced by high intravenous doses of L-arginine. MacAllister et al (88) reported that, after intravenous infusion resulting in concentrations in the millimolar range, neither L-arginine nor D-arginine had an effect on systemic hemodynamics in healthy humans or in patients with insulin-dependent diabetes or hypertension. Increases in plasma insulin, prolactin, glucagon, and growth hormone were seen with both enantiomers. However, as outlined above, the vasodilatory effect of GH is mediated by NO, which may explain why, in some experimental settings, D-arginine may have effects on endothelium-dependent vasodilation similar to the effects of L-arginine.

Antioxidant effects have been reported for L-arginine that may contribute to increased biophase availability of NO. We found that superoxide radical release by isolated aortic rings from cholesterol-fed rabbits was significantly diminished after L-arginine supplementation (35). We later confirmed our findings using a different approach, that is, by showing reduced urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$, a marker for lipid peroxidation in vivo (99). Reduced superoxide elaboration by endothelial cells has been shown to be specific for L-arginine but not D-arginine (100), suggesting that it may be related to NOS activity. Indeed, NOS catalytic activity may be decoupled under certain pathophysiologic conditions, resulting in a shift of catalytic activity from NO elaboration to O_2^- production (101); this effect can be reversed by L-arginine (102). In vivo, L-arginine treatment decreases NOS-derived superoxide while increasing NO accumulation during ischemia/reperfusion injury in skeletal muscle (103). We have evidence from our laboratory showing that isoprostane excretion is reduced in humans with vascular disease during L-arginine treatment (RH Böger & D Tsikas, unpublished observation).

SIDE EFFECTS

L-arginine has generally been well tolerated when administered via the intraarterial, intravenous, or oral route, in doses $\leq$30 g. When high doses of L-arginine are given intravenously, local irritation and phlebitis may occur due to the high osmolality of the solution (104, 105). Dilution to a 10% solution is recommended but not always feasible because high doses may cause fluid overload. Accidental extravasation of L-arginine solutions may lead to local tissue injury and necrosis (106). The vasodilator action of L-arginine may lead to hypotension (107), but usually, the blood pressure-lowering effect of L-arginine is relatively limited. Allergic reactions including anaphylaxis are possible in response to L-arginine (108). It is therefore contraindicated in individuals with high allergic tendencies. Antihistamines and epinephrine should be available for treatment of such reactions. Infusion solutions of L-arginine hydrochloride have a high chloride content; this may be hazardous in patients with electrolyte imbalance. Because the L-arginine hydrochloride solution is acidic (pH 5–6.5), a sudden drop in blood pH may cause metabolic acidosis, which has been associated with arrhythmias in a variety of clinical settings (109). Hyperkalemia, including one fatality, has been reported after L-arginine infusion in patients with severe liver disease and/or renal insufficiency (110–113). Massara et al (114) reported hyperkalemia and hypophosphatemia in diabetic patients receiving L-arginine infusions. It was suggested that hyperkalemia is the result of displacement of intracellular potassium by L-arginine, a cationic amino acid (115). Potassium clearance from plasma is enhanced by the action of insulin stimulated by L-arginine. In diabetics, lack of insulin may cause hyperkalemia. Stimulation of insulin release may cause hypoglycemia in patients with intact pancreatic function. Release of histamine from skin (116) may be a cause of flushing and other dermal side effects (117). A major part of an L-arginine

dose is metabolized to ornithine and urea. Increases in blood nitrogen urea and urea may occur in patients with renal function impairment owing to their limited capacity to eliminate urea. Oral L-arginine may cause nausea and vomiting; the frequency was given as ~3% of patients (118). Abdominal cramps and bloating have been observed in patients with cystic fibrosis receiving oral L-arginine (119). One fatal case due to an acute overdose of L-arginine was recently reported (120). A 21-month-old girl inadvertently received eightfold the dose of L-arginine that is routinely given to stimulate growth hormone release, and she died from cardiac arrest and myelinolysis. Patients receiving L-arginine infusions should be monitored closely for cardiac arrhythmias and electrolyte disturbances.

CLINICAL PHARMACOKINETICS OF L-ARGININE

There are only a few studies that have specifically addressed the pharmacokinetics of L-arginine in humans. In two older studies, arginine levels were measured by photometric methods, and owing to various limitations related to the route of administration, low assay sensitivity, short follow-up (15 and 60 min after the end of a 30-min infusion period), and data analysis, pharmacokinetic parameters obtained were unreliable (121, 122). In another study, Matera et al (123) tested the relative bioavailability and bioequivalence of two oral poly-amino acid formulations in association with vitamin B_{12} used as parenteral nutrition supplements. The dosage of L-arginine in these formulations was only 100 mg per day, and no data are available from that study on absolute bioavailability of L-arginine. More recently, Bode-Böger et al (72) compared the pharmacokinetics of single intravenous doses of L-arginine (30 and 6 g) with that of oral L-arginine (6 g), and Tangphao et al (124) studied the pharmacokinetics after administration of 30 g of L-arginine via the intravenous route and after oral administration of 10 g of L-arginine. The latter two studies, in combination with data from animal studies and metabolism studies, provide much of the pharmacokinetic data on L-arginine that are known to date.

After an intravenous infusion, peak plasma L-arginine levels are achieved within 20–30 min. Peak plasma levels range from 0.8 mM after 6 g of L-arginine (72) to 4.8 mM after 14 g of L-arginine (125) and to 6.2 mM (72), 6.5 mM (90), and 8.0 mM (124) after 30 g of L-arginine. The peak L-arginine plasma concentration after oral administration of 6 g is 0.31 mM at $T_{max} = 90$ min (72). Oral administration of 10 g of L-arginine leads to a peak plasma concentration of 0.29 mM at 60 min after dosing (124). These data suggest a less than proportional increase in plasma L-arginine after high doses, and indeed there is substantial urinary excretion of L-arginine when the renal threshold for reabsorption is exceeded (124).

Orally administered L-arginine is rapidly and almost completely absorbed by the intestinal brush border membrane via active uptake by the intestinal y^+ transporter system for cationic amino acids (19); thereafter, it is extensively metabolized by enterocytes (126). Data given for oral bioavailability vary between 21 ± 4% (5–50%) (124) and 68 ± 9% (51–87%) (72). Splanchnic uptake of isotope-labeled

L-arginine after oral administration was 31–38% in another study (127). Although the reason for these differences is not clear, it is obvious that a considerable fraction of an oral L-arginine dose is being metabolized presystemically or excreted from the gut. The liver may not contribute significantly to this first-pass metabolism, because the y^+ transporter in hepatocytes has a very low activity, almost completely separating hepatic arginine metabolism from the systemic L-arginine pool (128).

The half life of L-arginine was 1.5–2 h after an oral dose of 6 g (72). This value corresponds to the half life determined in an earlier study for two different poly-amino acid formulations, in which half life of L-arginine was reported to be 1.2–1.9 h (123). In the latter study, no change in L-arginine's half life was found after repeated intake. After higher intravenous doses of L-arginine, its apparent half life is shorter, which may be caused by a "spillover" into urine from the extremely high plasma concentrations reached (124).

Frondoza et al (129) assessed tissue distribution of [^{14}C]L-arginine after intraperitoneal injection of the tracer in rats and found the largest portions of radioactive label in the skin, liver, small intestine, and stomach. The disappearance of radioactivity from tissues gave semilog decay curves, suggesting that there are several labeled products with different turnover rates (129). The bulk of radioarginine was eliminated through the urine, probably after conversion of arginine to urea. In the same study, a similar observation was also made in humans with multiple myeloma, in whom 22–26% of injected [^{14}C]L-arginine was found in urine during the first 24 h. Cumulative radioactivity excreted until 8 days after injection was 70% of injected dose (129). In another study it was found that L-arginine levels increase to three- to sixfold of baseline levels in NO-generating tissues like the heart and aorta of rats (130). Beaumier et al (131) reported that the rate of conversion of arginine to ornithine significantly increased in humans with a high dietary L-arginine supplementation (36 g/day), with no apparent change in total body conversion of L-arginine to NO_3^-. Only a very small fraction of exogenous L-arginine is converted via NO into nitrate. Leaf et al (132) estimated this fraction as 0.07% of an intravenous dose of ^{15}N-labeled L-arginine; Castillo et al (133) calculated that 0.4% of ^{15}N-labeled L-arginine applied via the intragastric route was converted into ^{15}N-NO_3^-. These findings are in line with our recent observation that $<$1% of oral L-[guanidino-$^{15}N_2$]-arginine is converted into ^{15}N-NO_3^- in rabbits (RH Böger, S Bode-Böger, & D Tsikas, unpublished observation).

Under physiological conditions, excretion via the kidneys plays no role for the elimination of L-arginine. L-arginine is filtered in the renal glomerulus and almost completely [$>$99% (134)] reabsorbed in the proximal renal tubules (135) and in the thin ascending limb of Henle's loop (136). Reabsorption is accomplished by the y^+ transporter and—in contrast to other amino acids—displays saturation kinetics. In dogs, the renal tubular transport maximum for L-arginine is reached at a glomerular filtration of 3.5–4 mg/min/100 ml of glomerular filtrate (137). No such data are available for humans. However, the finding of L-arginine spillover into urine in human subjects after high intravenous doses of the amino acid (124) is consistent with saturable tubular reabsorption.

TABLE 1 Diseases in which L-arginine has been demonstrated to improve clinical end points of cardiovascular disease

Disease	L-arginine dose[a]	Effect[b]	Reference
Peripheral arterial disease	3 × 8 g/d i.v.	↑ Walking distance	76
	30 g i.v.	↑ Nutritive muscle blood flow	75
Coronary artery disease	3 × 3 g/d p.o.	↓ Angina symptom score	78
	3 × 2 g/d p.o.	↑ Exercise capacity	77
Congestive heart failure	5.6−12.6 g/d p.o.	↑ Exercise capacity	81
Raynaud syndrome	8.5 mg/min i.a.	↓ Vasospasm attacks	82

[a]Routes of administration: i.v., intravenously; p.o., orally; i.a., intra-arterially.

[b]↑ , Increased; ↓ , decreased.

SOLVING THE "L-ARGININE PARADOX"?

While many of the effects of high intravenous doses of L-arginine observed in clinical trials can be explained by endocrine actions of this amino acid, the improvement of endothelial function brought about by relatively low daily oral doses of L-arginine has long remained unclear. The in vivo actions of L-arginine were in contrast to the absence of similar effects in vitro and were not predictable based on the huge excess of physiological L-arginine concentrations over the apparent K_m value of NOS isoenzymes as determined in vitro. The presence of an endogenous inhibitor of NOS may account for L-arginine's effects in many cardiovascular and other diseases. The endogenous NOS inhibitors, N^{ω}-monomethyl-L-arginine and ADMA have been detected in human plasma and urine (84); ADMA is present in concentrations $\leq$10-fold higher than those of N^{ω}-monomethyl-L-arginine. Elevated concentrations of ADMA have been found in patients with peripheral arterial occlusive disease (138), hypercholesterolemia (125), chronic heart failure (139), end-stage renal disease (140), hyperhomocysteinemia (141), and hypertension (142). Elevated ADMA levels account for endothelial dysfunction in hypercholesterolemia and hyperhomocysteinemia (125, 143). This is consistent with data from several experimental studies suggesting that ADMA concentrations in a pathophysiologically high range (3–15 μmol/liter) significantly inhibit vascular NO elaboration (144–146). Recent data from a prospective clinical trial suggest that ADMA is a prognostic marker of cardiovascular and of all-cause mortality in patients with end-stage renal disease (RH Böger & C Zoccali unpublished observation). Future investigations will help to clarify the role of ADMA in pathophysiology and in the therapeutic effects of L-arginine.

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