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REVIEW ARTICLE

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Effect of L-arginine supplementation on NO production in man

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Abstract L-arginine is the substrate for the enzyme nitric oxide synthase (NOS), which is responsible for the production of nitric oxide (NO), an endogenous messenger molecule involved in many of the processes associated with the development of atherosclerosis. Acute and chronic administration of L-arginine has been shown to improve endothelial function in animal models of hypercholesterolemia and atherosclerosis. Therefore, numerous studies have been conducted to elucidate whether dietary L-arginine supplementation can augment NO production in humans and thereby improve vascular health. In this review, the results of studies of intravenous and oral L-arginine supplementation with a wide spectrum of doses, study duration, and surrogate parameters of endothelial function are summarized. The pharmacokinetics of L-arginine have been investigated; side effects are rare and mostly mild and dose-dependent. Several possible mechanisms of action of L-arginine are discussed. An evaluation of L-arginine as a therapeutic agent from the point of view of a clinical pharmacologist is given.

Keywords Oral L-arginine · L-arginine paradox · Asymmetric dimethylarginine (ADMA)

Introduction

The initial description in 1980 by Furchgott and Zawadzki [33] of endothelium-derived relaxing factor (EDRF) has stimulated more than two decades of intense research into the elucidation of its basic biology and importance in the clinical setting, such as in human coronary atherosclerosis. The identification of the endothelium-derived vasodilator as nitric oxide (NO) with smooth-muscle relaxing effects led to the recognition that nitrovasodilators act by providing an exogenous source of NO to the diseased blood vessel. NO is formed in the endothelium from the amino acid L-arginine by endothelial isoform of NO synthase (eNOS), which is the product of the NOS 3 gene. In addition to producing NO constitutively, the enzyme may be stimulated to increase NO synthesis by a variety of physiological agonists, shear stress, and pharmacological agents including acetylcholine. NO mediates a number of protective functions of the endothelium: It inhibits platelet aggregation and adhesion, it inhibits smooth muscle proliferation, and it limits vascular recruitment of leukocytes by inhibiting the expression of proinflammatory cytokines, chemokines, and leukocyte adhesion molecules. In the presence of known atherogenic risk factors, the normal endothelium can become dysfunctional. In the setting of many vascular disorders, including essential hypertension, several forms of dyslipidemia, diabetes mellitus, cigarette smoking, aging, and hyperhomocysteinemia, endothelium-dependent dilation occurs. The endothelium is a direct, sensitive target for the damaging effects of atherogenic risk factors, as evidenced from the experimental introduction of risk factors into healthy subjects; for example, eating a high-fat meal leads to endothelial vasodilator dysfunction in a time span of just a few hours [32].

Central to the development of endothelial dysfunction, regardless of its cause, is a loss of bioactive endothelial NO. There are two fundamental mechanisms for the loss of NO bioactivity: reduced synthesis and increased oxidative inactivation by reactive oxygen intermediates. Reactive oxygen species (ROS) are produced in abundance in the dysfunctional endothelium, and limitation of ROS generation increases the availability of NO. For this reason, antioxidative therapy with vitamin C [36] and cholesterol-lowering therapy with HMG-CoA reductase inhibitors improves endothelial function [75]. An alternative approach to increase levels of bioactive NO and to improve endothelial function is to increase the synthesis of NO. Enhanced synthesis of NO can be achieved by increased

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availability of agonists that stimulate release of NO from the endothelial cells, such as bradykinin.

Another straightforward approach to increase NO synthesis is to provide additional substrate to the endothelial cell. The semi-essential amino acid L-arginine serves as the substrate for the enzyme eNOS. First isolated from lupin seedlings in 1886 by Schulze and Steiger [64], L-arginine was shown to be a product of protein hydrolysis by Hedin 9 years later [40], but its structure was not proven until Sorensen in 1910 [65]. While much is known about the intermediary metabolism of L-arginine in several metabolic pathways, such as the urea cycle, the importance of this amino acid was heightened by the recognition in the 1980s that L-arginine is a precursor of NO synthesis: NO synthase catalyzes the 5-electron oxidation of L-arginine to L-citrulline and produces stoichiometric amounts of NO in the process. Providing supplementation substrate to individuals with inadequate NO, therefore, has been suggested as a rational approach to increase NO production by the NO synthase, and this therapeutic paradigm has been met with some success in recent years.

The first evidence for the effect of L-arginine came from animal studies showing that acute or chronic administration of L-arginine in vivo improves vascular responsiveness, probably via enhanced NO elaboration [27]. Long-term oral administration of L-arginine has been associated with a significant improvement in NO-dependent vasodilation in cholesterol-fed rabbits and in slowing the development of intimal plaques in the carotid arteries [14]. In 1991, Drexler and colleagues [31] demonstrated that local intracoronary infusion of L-arginine normalized coronary vasomotor responses to acetylcholine in hypercholesterolemic humans. A similar observation was also made upon intravenous infusion of L-arginine in hypercholesterolemic subjects in whom endothelium-dependent forearm vasodilation was improved [28]. Since that time, numerous studies have confirmed that both the acute administration of L-arginine and its chronic administration improve vascular function in hypercholesterolemia [26], in exercising patients with stable angina pectoris [22], in patients with heart failure [62], at sites of coronary artery stenosis [70], and in small-vessel disease [48]. In hypertensive patients, however, the majority of studies on L-arginine revealed a lack of effect of this amino acid on endothelial function [59]. Long-term effects of L-arginine in patients with hypertension require further investigation.

So, the use of L-arginine as a tool to examine the NO pathway grew into a potential role for L-arginine as a therapeutic tool in cardiovascular disease. In a series of clinical studies, we investigated the clinical pharmacology of L-arginine. First, we showed that an intravenous infusion of 30 g of L-arginine significantly increased arterial blood flow in the femoral artery of healthy subjects [8]. Plasma L-arginine concentration increased to 6.0±0.4 μmol/l. A lower dose of L-arginine (6 g) administered by either the intravenous or the oral route increased plasma levels of L-arginine (822±59 μmol/l and 310±152 μmol/l, respectively) but failed to produce acute vasodilation. In a study in patients suffering from severe peripheral arterial 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. The vasodilator effect of L-arginine was due to increased blood flow velocity because the femoral artery diameter remained unchanged [9]. It then became interesting to know whether the action of L-arginine increased muscle capillary blood flow. This was addressed with a further clinical study in which we performed serial measurements of muscle capillary blood flow by using positron emission tomography. A single systemic infusion with 30 g of L-arginine significantly increased nutritive muscle blood flow, whereas 8 g of L-arginine had no significant effect [63].

Clinical pharmacokinetics and pharmacodynamics

We determined the pharmacokinetics of single intravenous doses of L-arginine (30 and 6 g) with those of oral L-arginine (6 g). After an intravenous infusion, peak plasma L-arginine levels are achieved within 20-30 min, and after oral administration within 60 min. Orally administered L-arginine is rapidly and almost completely absorbed via active uptake by the intestinal y^+-transporter system for cationic amino acids. Data for oral bioavailability vary between 21±4% and 68±9%. The half-life of L-arginine was 1.5-2 h after an oral dose of 6 g [10]. These data and the 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 convinced us to give patients with peripheral arterial disease a therapy with intravenous L-arginine (three doses for 8 g/day) for 3 weeks. Claudication distance was significantly increased and absolute and pain-free walking distances were improved, whereas the control group showed no significant changes [16]. Other investigators have also shown that oral L-arginine supplementation improves clinical symptoms of vascular disease: Ceremuzynski et al. [22] showed beneficial effects of oral administration of 6 g L-arginine for 3 days in patients with stable angina. Lerman et al. [48] showed that 6 months of oral L-arginine (3 g TID) resulted in a significantly improved angina symptom score and improved coronary blood flow response to acetylcholine in 26 patients with small-vessel coronary artery disease. In contrast, Blum et al. [6] administered oral L-arginine chronically to individuals with established coronary artery disease. In this randomized, double-blind crossover study, the investigators failed to find an effect of 9 g of daily L-arginine for 1 month on flow-mediated brachial artery dilation or cell adhesion molecule expression. However, patients were on an optimized medical treatment including cholesterol-lowering, and flow-mediated vasodilation was normal at baseline.

Side effects

L-arginine has generally been well tolerated by healthy volunteers and patients when administered via the intrave-

nous or oral route in doses <30 g. When high doses of L-arginine are given intravenously, local irritation and phlebitis may occur because of the high osmolality of the solution [44]. The vasodilator action of L-arginine may lead to hypotension [55], but usually the blood pressure-lowering effect of L-arginine is relatively low. Because the L-arginine hydrochloride solution is acidic, a sudden drop in blood pH may cause metabolic acidosis, which has been associated with arrhythmias [57]. Flushing and other dermal side effects have been reported [54]. After oral L-arginine, which has a bitter taste in higher doses, nausea and vomiting were reported in about 3% of the patients [18].

Mechanisms of action of L-arginine effects on NO production in humans

Both acute and chronic administration of L-arginine evoke benefits in improving endothelial function, but what may be the underlying mechanism? The precise molecular mechanisms by which L-arginine improves endothelial function remain puzzling. Providing the enzyme NOS with substrate because of lowered availability of L-arginine does not appear to be rate limiting: The intracellular levels of the amino acid are in the millimolar range [37], whereas the enzyme's K_M for substrate is in the micromolar range (2.9 μmol/l [19]. This biochemical discrepancy was termed the "arginine paradox." For this reason, other possible explanations have been proposed to explain the effects of L-arginine.

Arginase (the enzyme that converts arginine to ornithine and urea) activity may alter the cellular levels of L-arginine, leading to decreased NO production. Wei et al. [73] have shown in animal models that the upregulation of arginase may contribute to smooth muscle proliferation, resulting in endothelial dysfunction. L-arginine is first converted to N^G-hydroxy-L-arginine by eNOS, which inhibits arginase and may thereby increase intracellular steady-state levels of L-arginine [20]. Another interesting possible mechanism is that oxidized LDL and lysophosphatidycholine decrease L-arginine transport into endothelial cells [43, 45]. This may be the reason for the beneficial effects of L-arginine in patients with hypercholesterolemia. L-arginine competes with other cationic amino acids for transport into cells, especially L-glutamine, and increased L-arginine may increase intracellular substrate concentration by competitively enhancing cellular uptake in this setting.

Another possibility could be the compartmentalization of L-arginine in the cytoplasm so that local concentrations in the vicinity of endothelial NOS may be lower than expected from L-arginine levels in whole cell homogenates [51]. Extracellular L-arginine may be preferentially utilized by NOS within this microenvironment.

Another explanation for the L-arginine paradox may be the presence of endogenously occurring analogs of L-arginine, asymmetric dimethylarginine (ADMA) and N^G-monomethyl-L-arginine (L-NMMA), which exert biological activity by competitively inhibiting NO synthase activity. Today we know that an elevation in plasma ADMA occurs in hypercholesterolemia and that the elevation in ADMA correlates with endothelial dysfunction [15]. Elevations in plasma ADMA may play a role in hyperhomocyst(e)inemia, hypertension, diabetes mellitus, insulin resistance, and chronic heart failure, resulting in diminished NOS activity. Inhibition of NOS activity may be overcome by excess substrate and could explain how L-arginine improves endothelial function in patients with vascular disease [17]. Several studies have provided convincing data to support the hypothesis that ADMA could be a potential novel cardiovascular risk factor [53, 71, 76]. This implicates further therapeutic options for L-arginine supplementation.

Very recently, Suschek et al. [66] discussed the critical role of L-arginine in endothelial cell survival during oxidative stress and showed that arginine concentrations corresponding to physiological serum levels do not allow for optimal endothelial iNOS activity and would therefore impair the endothelial iNOS-mediated stress response, increasing the risk of endothelial dysfunction. Even small concentration changes within the physiological range will lead to increased iNOS activity and thus improve the antioxidative effects of L-arginine.

Other experiments show that L-arginine has direct antioxidant effects and that this is due to the alpha-amino group, a chemical moiety different from that necessary for NO biosynthesis [46, 72]. By simply acting as an antioxidant, L-arginine may scavenge superoxide or restore the crucial cofactor tetrahydrobiopterin (BH_4) and thereby prevent eNOS-mediated superoxide production in an "uncoupled" status. We could show in patients with peripheral arterial occlusive disease (PAOD) and hyperhomocyst(e)inemia that L-arginine significantly improved flow-dependent vasodilation and reduced oxidative stress as reflected by decreased urinary levels of the established biomarker of oxidative stress 8-*iso*-prostaglandin $F_{2\alpha}$ [67].

Proposed indirect mechanisms by which L-arginine increases bioactive NO in the vasculature are equally diverse. L-arginine increases insulin and growth hormone (GH) secretion. We were able to show that 30 g of L-arginine induced a rapid release of insulin and a delayed release of GH [11]. 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. We conclude that GH contributes to the prolonged NO-dependent vasodilation with high doses of L-arginine via IGF-1. This was not found during oral L-arginine supplementation in human subjects.

In addition, L-arginine stimulates histamine release from mast cells [35], which also evokes a vasodilator response. Lastly, L-arginine can undergo decarboxylation to L-agmatine, which induces clonidine-like effects and which has also been shown to inhibit NO synthesis at millimolar concentrations [34].

Evidence for therapeutic use of L-arginine?

NO plays an integral role in endothelial function, and the biochemical pathways associated with its production are well understood. Most of the evidence to enhance the

production of NO in endothelial tissue by dietary supplementation came from studies with oral L-arginine. Oral L-arginine feeding in animal studies has especially shown beneficial effects. In animals with hypercholesterolemia, L-arginine appears to inhibit the progression of atherosclerotic plaques and preserve endothelial function. In addition, L-arginine affects other mediators of atherosclerosis, including inflammatory cells and platelets. Although animal model data are positive, results from human studies have been inconclusive. Table 1 summarizes 20 studies of intravenous L-arginine use in humans. Four of the 20 human studies showed no vascular health benefit from intravenous application. The selection of the studies was not complete because studies with intravenous L-arginine represent a wide spectrum. Without exception, studies were positive in hypercholesterolemia and coronary artery disease. Hypercholesterolemia is known to increase ADMA, a condition that may be overcome with supplemental L-arginine by increasing the L-arginine/ADMA molar ratio. Dosing also represents a heterogenous spectrum.

Table 1 Effects of intravenous L-arginine in humans (not complete; *HC* hypercholesterolemia, *Con* control, *IV* intravenous, *FMD* flow-mediated dilation, *CAD* coronary artery disease, *TPR* total peripheral resistance, *CO* cardiac output, *MBP* mean blood pressure, *PET* positron emission tomography, *RF* renal failure, *RPF* renal plasma flow, *PPH* primary pulmonary hypertension, *PAOD* peripheral arterial disease, *PVR* peripheral vascular resistance, *PAP* pulmonary arterial pressure, *ESRF* end-stage renal failure, *HD* hemodialysis)

Event	Subjects	Study design	Results	Author
Hypercholesterolemia	8 HC, 7 Con	160 μmol/min intracoronary	+ACh-induced vasodilation in HC ↑	Drexler et al. 1991 [31]
	14 HC, 11 Con	14 g IV/20 min	+MCh-induced forearm blood flow in HC ↑	Creager et al. 1992 [28]
	8 HC, 8 Con	14 g IV/20 min	+FMD ↑ in brachial artery, urinary nitrate ↑	Böger et al. 1998 [15]
	9 HC, 9 Con	7 g IV	+FMD in HC	Thorne et al. 1998 [69]
CAD	16 CAD	30 g	+CAD: TPR ↓, CO ↑, cGMP ↑	Böger et al. 1996 [13]
	21 CAD with different progression of artherosclerosis		+ACh-induced vasodilation intracoronary in relation to vessel wall morphology	Otsuji et al. 1995 [58]
Hypertension	32 patients, 30 Con, 7 patients RF	35 g	+MBP ↓ in all groups, nitrate ↑, renal vascular resistance ↓, Co > patients, no effect in hypertensive patients with RF	Higashi et al. 1999 [41]
	14 patients, 12 Con	40 μmol/min/20 min	−forearm blood flow in patients, +in healthy controls	Panza et al. 1993 [59]
Smoking	9 smoker, 9 Con	7 g IV	+FMD in smoker	Thorne et al. 1998 [69]
	11 long-term smoker, 12 Con	30 g IV	+MBF with PET normalized by L-arginine	Campisi et al. 1999 [21]
Diabetes	9 diabetes mellitus, 9 Con	7 g IV	−FMD	Thorne et al. 1998 [69]
	23 diabetes mellitus II, 20 Con	7 g IV	+RPF ↑	Delles et al. 2003 [30]
Aging	34 patients, 27–73 years old	160 μmol/min/20 min	+coronary blood flow to ACH ↑ after L-arginine	Chauhan et al. 1996 [24]
PAOD	10 patients PAOD, 6 patients placebo	30 g IV single	+↑ femoral blood flow	Bode-Böger et al. 1996 [9]
	10 patients with PAOD, 10 patients placebo	2×8 g/day IV for 3 weeks	+↑ walking distance	Böger et al. 1998 [16]
PPH	10 PPH	35 g	+PVR ↓ +- PAP	Mehta et al. 1995 [52]
	5 PPH, 16 CAD	30 g	−TPR, CO and cGMP not changed	Böger et al. 1996 [13]

Table 1 continued

Event	Subjects	Study design	Results	Author
Cardiac syndrome	9 patients, 14 Con	15 g/120 min	+forearm blood flow ↑, NOx ↑, cGMP ↑, endothelin-1 ↓	Piatti et al. 2003 [61]
Chronic RF	12 ESRF, 8 Con	100 nmol/min	+reversal of ACH-induced vasodilation	Hand et al. 1998 [39]
	8 predialysis patients, 18 HD	50 μmol/min i.a.10 g IV	−FMD of radial artery FMD of brachial artery	Cross et al. 2001 [29]

Table 2 Effects of oral L-arginine in humans (*HC* hypercholesterolemia, *Con* control, *FMD* flow-mediated dilation, *CAD* coronary artery disease, *TPR* total peripheral resistance, *CO* cardiac output, *MBP* mean blood pressure, *PET* positron emission tomography, *RF* renal failure, *RPF* renal plasma flow, *PPH* primary pulmonary hypertension, *PAOD* peripheral arterial disease, *PVR* peripheral vascular resistance, *PAP* pulmonary arterial pressure, *ESRF* end-stage renal failure, *HD* hemodialysis, *MI* myocardial infarction, *CHF* chronic heart failure, *BiD* twice a day, QD once a day, *TiD* three times daily, *PAOD* peripheral arterial occlusive disease, *HRQL* health-related quality life)

Event	Subjects	Study design	Results	Author
Hypercholesterolemia	27 HC	7 g TiD ×30 days	+brachial artery dilation (BAD) ↑	Clarkson et al. 1996 [26]
	24 HC, 18 Con	14-21g/day ×3 months	+ADMA and L-arginine, mononuclear and T-cell adhesiveness	Chan et al. 2000 [23]
	20 HC, 12 Con	8.4 g/day ×2 weeks	+mononuclear cell adhesiveness	Theilmeier et al. 1997 [68]
	23 HC, 14 Con	8.4 g/day ×2 weeks	+platelet aggregation	Wolf et al. 1997 [74]
	43 HC	2 HeartBars/day 1 week	+flow-mediated dilation	Maxwell et al. 2000 [50]
	47 HC	3.3 g HeartBar or placebo TiD ×2 weeks	−FMD, platelet aggregation, endothelial platelet adhesion molecules	Abdelhamed et al. 2003 [1]
CAD	10 men	7 g TiD ×3 days	+BAD, monocyte adhesion	Adams et al. 1997 [4]
	29 men, 1 woman	9 g daily ×1 month	−BAD; ICAM-1, VCAM-1, E-selectin	Blum et al. 2000 [6]
	22 CAD, stable angina pectoris post-MI	2 g TiD ×3 days	+exercise capacity on a treadmill	Ceremuzynski et al. 1997 [22]
CHF	40 CHF	8 g/day ×4 weeks +exercise	+BAD	Hambrecht et al. 2000 [38]
	20 CHF, 7 Con	20 g/day ×28 days	−forearm blood flow	Chin-Dusting et al. 1996 [25]
	15 CHF	2.8 g BiD ×6 weeks, 4.2 g TiD ×6 weeks	+forearm blood flow, CHF questionnaire, 6-min walking test	Rector et al. 1996 [62]
Hypertension	35 patients, 17 Con	6 g acute	+flow-mediated BAD	Lekakis et al. 2002 [47]
Diabetes	6 patients, type 2 diabetes	3 g/h for 10 h	+sBP and dBP ↓	Huynh and Tayek 2002 [42]
	12 patients, 10 Con	3 g TiD for 1 month, double-blind, placebo-controlled	+GMP ↑, forearm blood flow ↑, sBP ↓, endogenous glucose production ↓ → improves peripheral and hepatic insulin sensitivity	Piatti et al. 2001 [60]

Table 2 continued

Event	Subjects	Study design	Results	Author
Smoking	8 smokers, 8 Con	One-time dose 7 g	+monocyte/ endothelial cell adhesion, ICAM-1, VCAM-1, E-selectin	Adams et al. 1997 [3]
Aging	806 elderly men	Observational study intake based on dietary history, calculated as approx. 4 g/day	−CAD morbidity	Oomen et al. 2000 [56]
	12 healthy elderly people	Placebo randomized cross-over 8 g BiD	+BAD ↑	Bode-Böger 2003 [12]
Hyperhomocyst(e)inemia	27 patients PAOD +- hyperhomocyst(e)inemia	Placebo-controlled 24 g/day ×8 weeks	+flow-dependent dilation (FDD) ↑radial artery	Sydow et al. 2003 [67]
PAOD	41 patients with claudication	Oral L-arginine HeartBar 3.3 g BiD ×2 weeks 2 bars/ 1 bar+1 placebo/ 2 placebo	+pain-free walking distance HRQL scores	Maxwell et al. 2000 [49]
Healthy controls	12 healthy men 10 postmenopausal women	7 g TiD ×3 days 9 g daily ×1 month	+platelet aggregation, BAD −flow-mediated BAD, cell adhesion, NO	Adams et al. 1995 [2] Blum et al. 2000 [7]

Table 2 summarizes published studies on oral L-arginine. These are of higher impact with respect to L-arginine as a therapeutic agent because the oral application enables us to get the drug into the patient in a more comfortable way. In hypercholesterolemia we had five positive studies and one negative with the HeartBar, an arginine-enriched compound (Unither Pharma, Silver Spring, MD, USA). For coronary artery disease we had one negative: Blum et al. [6] conducted a randomized, double-blind crossover study with 9 g daily for 1 month and assessed flow-mediated brachial artery dilation and cell adhesion molecule expression. In this study it was unclear whether NO production was increased. The study subjects were on full cardiovascular medication with statins and ACE inhibitors, both of which have been shown to improve endothelial function. In addition, β-blockers and aspirin as an

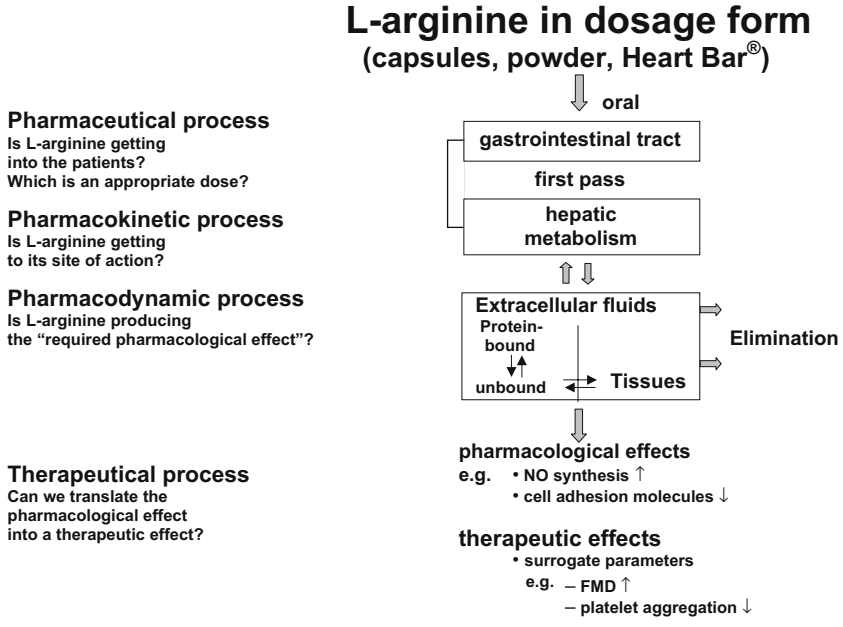

Fig. 1 The fate of L-arginine in humans

antioxidant were used. This may have confounded possible effects of L-arginine itself. In chronic heart failure, Chin-Dusting et al. [25] described a negative result with 20 g/day L-arginine for 28 days. In contrast to intravenous application in hypertension, oral L-arginine was given by Lekakis et al. [47] with a positive result, but only 6 g were given acutely. Studies in patients with diabetes, hyperhomocyst(e)inemia, and peripheral arterial disease and in healthy elderly people and smokers were without exception positive. Oomen et al. [56] conducted an observational study based on dietary history calculated as L-arginine intake of 4 g/day, which is within the normal average intake of 5 g/day of L-arginine. In healthy controls, Adams et al. [2] showed a positive effect on platelet aggregation and brachial artery dilation. Blum et al. [5] observed a lack of effect on major endocrine hormones and lipid profile; this finding supports the safety of oral L-arginine administration, but the study failed to show a beneficial effect on brachial artery dilation, cell adhesion molecules, or NO synthesis in 10 healthy postmenopausal women [7]. The dosing of oral L-arginine represents a wide spectrum, from two HeartBars per day for 1 week [2] to 24 g/day of L-arginine for 8 weeks [11]. The longest application was given by Chan et al. [23], who gave 14–21 g/day of L-arginine for 3 months.

So where do we stand with clinical testing regarding L-arginine as a therapeutic agent?

To look at the four processes of drug therapy (Fig. 1), we have the first problem: Is the drug L-arginine getting into the patient? L-arginine in oral form could be given in capsules, which, for example, means 42 huge capsules of 500 mg three times a day for a total amount of 21 g. Another possibility is L-arginine intake as a powder in water or in the form of HeartBar.

As to the pharmacokinetic process that involves absorption, distribution, and elimination, we have data from about 18 healthy subjects. This could be considered as phase 1, in which usually 20–80 healthy volunteers participate. Data on the bioavailability vary between 20% and 60%. So the second problem is, how much of the L-arginine dose, which varies between 5 g (this is within the range of average arginine intake with food) and 24 g, reaches the systemic circulation and is available for distribution to the site of action?

To address the question of whether the *drug* L-arginine produces the required pharmacological effect and to shed light on the underlying mechanisms, we have to admit that we have no clear explanation for the benefit in prior studies and that we need future studies to address underlying mechanisms. Today, we have reached phase 2 of human testing: About 548 subjects and patients received oral L-arginine, and we have results about the effectiveness and safety aspects. The problem is the heterogeneity of the published studies concerning dose and outcome parameters. What should we consider to be a pharmacodynamic effect, and what do we mean by the therapeutic effect? Is an increase in NO synthesis measured by a variety of methods (for example, GC-MS as the gold standard, Griess assay, cGMP as the second messenger, and so on) the appropriate surrogate parameter? Is the improvement of flow-mediated dilation a real clinical benefit? How long should the effect of L-arginine last? We are standing only at the beginning of clinical trial design, and we need larger, randomized clinical trials with an appropriate L-arginine dose of sufficiently long duration to fully elucidate the effects of dietary L-arginine supplementation on vascular health.

References

1. Abdelhamed AI, Reis SE, Sane DC, Brosnihan KB, Preli RB, Herrington DM (2003) No effect of an L-arginine-enriched medical food (HeartBars) on endothelial function and platelet aggregation in subjects with hypercholesterolemia. Am Heart J 145:E15
2. Adams MR, Forsyth CJ, Jessup W, Robinson J, Celermajer DS (1995) Oral L-arginine inhibits platelet aggregation but does not enhance endothelium-dependent dilation in healthy young men. J Am Coll Cardiol 26:1054–1061
3. Adams MR, Jessup W, Celermajer DS (1997) Cigarette smoking is associated with increased human monocyte adhesion to endothelial cells: reversibility with oral L-arginine but not vitamin C. J Am Coll Cardiol 29:491–497
4. Adams MR, McCredie R, Jessup W, Robinson J, Sullivan D, Celermajer DS (1997) Oral L-arginine improves endothelium-dependent dilatation and reduces monocyte adhesion to endothelial cells in young men with coronary artery disease. Atherosclerosis 129:261–269
5. Blum A, Cannon RO III, Costello R, Schenke WH, Csako G (2000) Endocrine and lipid effects of oral L-arginine treatment in healthy postmenopausal women. J Lab Clin Med 135:231–237
6. Blum A, Hathaway L, Mincemoyer R, Schenke WH, Kirby M, Csako G, Waclawiw MA, Panza JA, Cannon RO III (2000) Oral L-arginine in patients with coronary artery disease on medical management. Circulation 101:2160–2164
7. Blum A, Hathaway L, Mincemoyer R, Schenke WH, Kirby M, Csako G, Waclawiw MA, Panza JA, Cannon RO III(2000) Effects of oral L-arginine on endothelium-dependent vasodilation and markers of inflammation in healthy postmenopausal women. J Am Coll Cardiol 35:271–276
8. Bode-Böger SM, Böger RH, Creutzig A, Tsikas D, Gutzki FM, Alexander K, Frölich JC (1994) L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. Clin Sci 87:303–310
9. Bode-Böger SM, Böger RH, Alfke H, Heinzel D, Tsikas D, Creutzig A, Alexander K, Frölich JC (1996) L-arginine induces nitric oxide-dependent vasodilation in patients with critical limb ischemia. A randomized, controlled study. Circulation 93:85–90
10. Bode-Böger SM, Böger RH, Galland A, Tsikas D, Frölich JC (1998) L-arginine-induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. Br J Clin Pharmacol 46:489–497
11. Bode-Böger SM, Böger RH, Löffler M, Tsikas D, Brabant G, Frölich JC (1999) L-arginine stimulates NO-dependent vasodilation in healthy humans—effect of somatostatin pretreatment. J Investig Med 47:43–50
12. Bode-Böger SM, Muke J, Surdacki A, Brabant G, Böger RH, Frölich JC (2003) Oral L-arginine improves endothelial function in healthy individuals older than 70 years. Vasc Med 8:77–81
13. Böger RH, Mugge A, Bode-Böger SM, Heinzel D, Hoper MM, Frölich JC (1996) Differential systemic and pulmonary hemodynamic effects of L-arginine in patients with coronary artery disease or primary pulmonary hypertension. Int J Clin Pharmacol Ther 34:323–328

14. Böger RH, Bode-Böger SM, Brandes RP, Phivthong-Ngam L, Böhme M, Nafe R, Mugge A, Frölich JC (1997) Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits: comparison with lovastatin. Circulation 96:1282–1290
15. Böger RH, Bode-Böger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP (1998) Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 98:1842–1847
16. Böger RH, Bode-Böger SM, Thiele W, Creutzig A, Alexander K, Frölich JC (1998) Restoring vascular nitric oxide formation by L-arginine improves the symptoms of intermittent claudication in patients with peripheral arterial occlusive disease. J Am Coll Cardiol 32:1336–1344
17. Böger RH, Bode-Böger SM (2001) The clinical pharmacology of L-arginine. Annu Rev Pharmacol Toxicol 41:79–99
18. Boyd JR, Olin BR (1984) Drug facts and comparisons. Lippincott, St. Louis
19. Bredt DS, Snyder SH (1990) Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc Natl Acad Sci USA 87:682–685
20. Buga GM, Singh R, Pervin S, Rogers NE, Schmitz DA, Jenkinson CP, Cederbaum SD, Ignarro LJ (1996) Arginase activity in endothelial cells: inhibition by N^G-hydroxy-L-arginine during high-output NO production. Am J Physiol 271:H1988–1998
21. Campisi R, Czernin J, Schoder H, Sayre JW, Schelbert HR (1999) L-arginine normalizes coronary vasomotion in long-term smokers. Circulation 99:491–497
22. Ceremuzynski L, Chamiec T, Herbaczynska-Cedro K (1997) Effect of supplemental oral L-arginine on exercise capacity in patients with stable angina pectoris. Am J Cardiol 80:331–333
23. Chan JR, Böger RH, Bode-Böger SM, Tangphao O, Tsao PS, Blaschke TF, Cooke JP (2000) Asymmetric dimethylarginine increases mononuclear cell adhesiveness in hypercholesterolemic humans. Arterioscler Thromb Vasc Biol 20:1040–1046
24. Chauhan A, More RS, Mullins PA, Taylor G, Petch C, Schofield PM (1996) Aging-associated endothelial dysfunction in humans is reversed by L-arginine. J Am Coll Cardiol 28:1796–1804
25. Chin-Dusting JP, Kaye DM, Lefkovits J, Wong J, Bergin P, Jennings GL (1996) Dietary supplementation with L-arginine fails to restore endothelial function in forearm resistance arteries of patients with severe heart failure. J Am Coll Cardiol 27:1207–1213
26. Clarkson P, Adams MR, Powe AJ, Donald AE, McCredie R, Robinson J, McCarthy SN, Keech A, Celermajer DS, Deanfield JE (1996) Oral L-arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. J Clin Invest 97:1989–1994
27. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME (1992) Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. J Clin Invest 90:1168–1172
28. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, Cooke JP (1992) L-arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. J Clin Invest 90:1248–1253
29. Cross JM, Donald AE, Kharbanda R, Deanfield JE, Woolfson RG, MacAllister RJ (2001) Acute administration of L-arginine does not improve arterial endothelial function in chronic renal failure. Kidney Int 60:2318–2323
30. Delles C, Schneider MP, Oehmer S, Fleischmann EH, Schmieder RE (2003) L-arginine-induced vasodilation of the renal vasculature is not altered in hypertensive patients with type 2 diabetes. Diabetes Care 26:1836–1840
31. Drexler H, Zeiher AM, Meinzer K, Just H (1991) Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. Lancet 338:1546–1550
32. Esposito K, Nappo F, Giugliano F, Giugliano G, Marfella R, Giugliano D (2003) Effect of dietary antioxidants on postprandial endothelial dysfunction induced by a high-fat meal in healthy subjects. Am J Clin Nutr 77:139–143
33. Furchgott RF, Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373–376
34. Galea E, Regunathan S, Eliopoulos V, Feinstein DL, Reis DJ (1995) Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem J 316:247–249
35. Giraldelo CM, Zappellini A, Muscara MN, De Luca IM, Hyslop S, Cirino G, Zatz R, De Nucci G, Antunes E (1994) Effect of arginine analogues on rat hind paw oedema and mast cell activation in vitro. Eur J Pharmacol 257:87–93
36. Gokce N, Keaney JF Jr, Frei B, Holbrook M, Olesiak M, Zachariah BJ, Leeuwenburgh C, Heinecke JW, Vita JA (1999) Long-term ascorbic acid administration reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 99:3234–3240
37. Gold ME, Bush PA, Ignarro LJ (1989) Depletion of arterial L-arginine causes reversible tolerance to endothelium-dependent relaxation. Biochem Biophys Res Commun 164:714–721
38. Hambrecht R, Hilbrich L, Erbs S, Gielen S, Fiehn E, Schoene N, Schuler G (2000) Correction of endothelial dysfunction in chronic heart failure: additional effects of exercise training and oral L-arginine supplementation. J Am Coll Cardiol 35:706–713
39. Hand MF, Haynes WG, Webb DJ (1998) Hemodialysis and L-arginine, but not D-arginine, correct renal failure-associated endothelial dysfunction. Kidney Int 53:1068–1077
40. Hedin SG (1895) Über die Bildung von Arginin aus Proteinkörper. Z Physiol Chem 21:155–168
41. Higashi Y, Oshima T, Ozono R, Matsuura H, Kambe M, Kajiyama G (1999) Effect of L-arginine infusion on systemic and renal hemodynamics in hypertensive patients. Am J Hypertens 12:8–15
42. Huynh NT, Tayek JA (2002) Oral arginine reduces systemic blood pressure in type 2 diabetes: its potential role in nitric oxide generation. J Am Coll Nutr 21:422–427
43. Jay MT, Chirico S, Siow RC, Bruckdorfer KR, Jacobs M, Leake DS, Pearson JD, Mann GE (1997) Modulation of vascular tone by low density lipoproteins: effects on L-arginine transport and nitric oxide synthesis. Exp Physiol 82:349–360
44. Kastrup EK, Olin BR (1987) Drug facts and comparisons. Lippincott, St. Louis, pp 310–311
45. Kikuta K, Sawamura T, Miwa S, Hashimoto N, Masaki T (1998) High-affinity arginine transport of bovine aortic endothelial cells is impaired by lysophosphatidylcholine. Circ Res 83:1088–1096
46. Lass A, Suessenbacher A, Wolkart G, Mayer B, Brunner F (2002) Functional and analytical evidence for scavenging of oxygen radicals by L-arginine. Mol Pharmacol 61:1081–1088
47. Lekakis JP, Papathanassiou S, Papaioannou TG, Papamichael CM, Zakopoulos N, Kotsis V, Dagre AG, Stamatelopoulos K, Protogerou A, Stamatelopoulos SF (2002) Oral L-arginine improves endothelial dysfunction in patients with essential hypertension. Int J Cardiol 86:317–323
48. Lerman A, Burnett JC Jr, Higano ST, McKinley LJ, Holmes DR Jr (1998) Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. Circulation 97:2123–2128
49. Maxwell AJ, Anderson BE, Cooke JP (2000) Nutritional therapy for peripheral arterial disease: a double-blind, placebo-controlled, randomized trial of HeartBar. Vasc Med 5:11–19
50. Maxwell AJ, Anderson BE, Zapien MP, Cooke JP (2000) Endothelial dysfunction in hypercholesterolemia is reversed by a nutritional product designed to enhance nitric oxide activity. Cardiovasc Drugs Ther 14:309–316

51. McDonald KK, Zharikov S, Block ER, Kilberg MS (1997) A caveolar complex between the cationic amino acid transporter 1 and endothelial nitric-oxide synthase may explain the "arginine paradox". J Biol Chem 272:31213–31216
52. Mehta S, Stewart DJ, Langleben D, Levy RD (1995) Short-term pulmonary vasodilation with L-arginine in pulmonary hypertension. Circulation 92:1539–1545
53. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T (1999) Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation 99:1141–1146
54. Mudge GH (1980) Agents affecting volume and composition of body fluids. In: Goodman LS, Gilman AG (eds) The pharmacologic basis of therapeutics, 6th edn. Macmillan, New York, pp 892–915
55. Nakaki T, Hishikawa K, Suzuki H, Saruta T, Kato R (1990) L-arginine-induced hypotension. Lancet 336:696
56. Oomen CM, van Erk MJ, Feskens EJ, Kok FJ, Kromhout D (2000) Arginine intake and risk of coronary heart disease mortality in elderly men. Arterioscler Thromb Vasc Biol 20:2134–2139
57. Orchard CH, Cingolani HE (1994) Acidosis and arrhythmias in cardiac muscle. Cardiovasc Res 28:1312–1319
58. Otsuji S, Nakajima O, Waku S, Kojima S, Hosokawa H, Kinoshita I, Okubo T, Tamoto S, Takada K, Ishihara T (1995) Attenuation of acetylcholine-induced vasoconstriction by L-arginine is related to the progression of atherosclerosis. Am Heart J 129:1094–1100
59. Panza JA, Casino PR, Badar DM, Quyyumi AA (1993) Effect of increased availability of endothelium-derived nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and in patients with essential hypertension. Circulation 87:1475–1481
60. Piatti PM, Monti LD, Valsecchi G, Magni F, Setola E, Marchesi F, Galli-Kienle M, Pozza G, Alberti KG (2001) Long-term oral L-arginine administration improves peripheral and hepatic insulin sensitivity in type 2 diabetic patients. Diabetes Care 24:875–880
61. Piatti P, Fragasso G, Monti LD, Setola E, Lucotti P, Fermo I, Paroni R, Galluccio E, Pozza G, Chierchia S, Margonato A (2003) Acute intravenous L-arginine infusion decreases endothelin-1 levels and improves endothelial function in patients with angina pectoris and normal coronary arteriograms: correlation with asymmetric dimethylarginine levels. Circulation 107:429–436
62. Rector TS, Bank AJ, Mullen KA, Tschumperlin LK, Sih R, Pillai K, Kubo SH (1996) Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. Circulation 93:2135–2141
63. Schellong SM, Böger RH, Burchert W, Bode-Böger SM, Galland A, Frölich JC, Hundeshagen H, Alexander K (1997) Dose-related effect of intravenous L-arginine on muscular blood flow of the calf in patients with peripheral vascular disease: a $H_2{}^{15}O$ positron emission tomography study. Clin Sci 93:159–165
64. Schulze E, Steiger E (1886) Über das Arginin. Z Physiol Chem 11:43–46
65. Sorensen SPL (1910) Über die Synthese des dl-Arginins (α-amino-δ-guanidino-n-Valeriansäure) und der Isomeren (δ-guanidino-α-amino-*n*-Valeriansäure). Chem Ber 43:643–651
66. Suschek CV, Schnorr O, Hemmrich K, Aust O, Klotz LO, Sies H, Kolb-Bachofen V (2003) Critical role of L-arginine in endothelial cell survival during oxidative stress. Circulation 107:2607–2614
67. Sydow K, Schwedhelm E, Arakawa N, Bode-Böger SM, Tsikas D, Hornig B, Frölich JC, Böger RH (2003) ADMA and oxidative stress are responsible for endothelial dysfunction in hyperhomocyst(e)inemia: effects of L-arginine and B vitamins. Cardiovasc Res 57:244–252
68. Theilmeier G, Chan JR, Zalpour C, Anderson B, Wang BY, Wolf A, Tsao PS, Cooke JP (1997) Adhesiveness of mononuclear cells in hypercholesterolemic humans is normalized by dietary L-arginine. Arterioscler Thromb Vasc Biol 17:3557–3564
69. Thorne S, Mullen MJ, Clarkson P, Donald AE, Deanfield JE (1998) Early endothelial dysfunction in adults at risk from atherosclerosis: different responses to L-arginine. J Am Coll Cardiol 32:110–116
70. Tousoulis D, Davies G, Tentolouris C, Crake T, Toutouzas P (1997) Coronary stenosis dilatation induced by L-arginine. Lancet 349:1812–1813
71. Valkonen VP, Paiva H, Salonen JT, Lakka TA, Lehtimaki T, Laakso J, Laaksonen R (2001) Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 358:2127–2128
72. Wallner S, Hermetter A, Mayer B, Wascher TC (2001) The alpha-amino group of L-arginine mediates its antioxidant effect. Eur J Clin Invest 31:98–102
73. Wei LH, Jacobs AT, Morris SM Jr, Ignarro LJ (2000) IL-4 and IL-13 upregulate arginase I expression by cAMP and JAK/STAT6 pathways in vascular smooth muscle cells. Am J Physiol Cell Physiol 279:C248–256
74. Wolf A, Zalpour C, Theilmeier G, Wang BY, Ma A, Anderson B, Tsao PS, Cooke JP (1997) Dietary L-arginine supplementation normalizes platelet aggregation in hypercholesterolemic humans. J Am Coll Cardiol 29:479–485
75. Yamamoto A, Hoshi K, Ichihara K (1998) Fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, scavenges free radicals and inhibits lipid peroxidation in rat liver microsomes. Eur J Pharmacol 361:143–149
76. Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frölich JC, Böger RH (2001) Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet 358:2127–2128