L-arginine-induced vasodilation in healthy humans: pharmacokinetic–pharmacodynamic relationship

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Introduction

L-arginine is the physiological precursor of nitric oxide (NO), an important mediator of vasodilation and inhibition of platelet aggregation via increased formation of cyclic GMP [1]. NO is synthesized in endothelial cells from the terminal guanidino nitrogen of L-arginine by the activity of the endothelial, calcium–dependent isoform of NO synthase [2]. NO is rapidly oxidized to NO²⁻ and NO³⁻ in vivo [3], and NO³⁻ is subsequently eliminated via the kidneys [4], as is cyclic GMP [5]. Quantification of the urinary excretion of NO³⁻ and cyclic GMP has therefore been shown to be a suitable non-invasive method to assess NO formation rates in human subjects in vivo [6–8]. It has previously been shown that an intravenous infusion of 14–30 g of L-arginine induces vasodilation and inhibition of platelet aggregation [6, 8], and improves acetylcholine-induced, endothelium-dependent vasodilation [9, 10]. In atherosclerotic patients, intravenous infusion of 30 g L-arginine induces peripheral vasodilation [7], resulting in improved nutritive muscle blood flow of the calves [11]. Chronic oral administration of 5–8 g of L-arginine 2–3 times per day during 2–6 weeks has also resulted in improved endothelium-dependent vasodilation [12], inhibition of platelet aggregation [13], and in reduced monocyte...

Aims Administration of L-arginine by intravenous infusion or via oral absorption has been shown to induce peripheral vasodilation in humans, and to improve endothelium-dependent vasodilation. We investigated the pharmacokinetics and pharmacokinetic–pharmacodynamic relationship of L-arginine after a single intravenous infusion of 30 g or 6 g, or after a single oral application of 6 g, as compared with the respective placebo, in eight healthy male human subjects.

Methods L-arginine levels were determined by h.p.l.c. The vasodilator effects of L-arginine were assessed non-invasively by blood pressure monitoring and impedance cardiography. Urinary nitrate and cyclic GMP excretion rates were measured as non-invasive indicators of endogenous NO production.

Results Plasma L-arginine levels increased to (mean ± s.e.mean) 623 ± 407 (range, 5100–7680) and 822 ± 59 (527–955) μmol l⁻¹ after intravenous infusion of 30 g and 6 g L-arginine, respectively, and to 310 ± 152 (118–1219) μmol l⁻¹ after oral ingestion of 6 g L-arginine. Oral bioavailability of L-arginine was 68 ± 9 (51–87)%. Clearance was 544 ± 24 (440–620), 894 ± 164 (470–1190), and 1018 ± 230 (710–2130) ml min⁻¹, and elimination half-life was calculated as 41.6 ± 2.3 (34–55), 59.6 ± 9.1 (24–98), and 79.5 ± 9.3 (70–121) min, respectively, for 30 g i.v., 6 g i.v., and 6 g p.o. of L-arginine. Blood pressure and total peripheral resistance were significantly decreased after intravenous infusion of 30 g L-arginine by 4.4 ± 1.4% and 10.4 ± 3.6%, respectively, but were not significantly changed after oral or intravenous administration of 6 g L-arginine. L-arginine (30 g) also significantly increased urinary nitrate and cyclic GMP excretion rates by 97 ± 28 and 66 ± 20%, respectively. After infusion of 6 g L-arginine, urinary nitrate excretion also significantly increased, (nitrate by 47 ± 12% [P < 0.05], cyclic GMP by 67 ± 47% [P = ns]), although to a lesser and more variable extent than after 30 g of L-arginine. The onset and the duration of the vasodilator effect of L-arginine and its effects on endogenous NO production closely corresponded to the plasma concentration half-life of L-arginine, as indicated by an equilibration half-life of 6 ± 2 (5.7–8.4) min between plasma concentration and effect in pharmacokinetic–pharmacodynamic analysis, and the lack of hysteresis in the plasma concentration–versus-effect plot.

Conclusions The vascular effects of L-arginine are closely correlated with its plasma concentrations. These data may provide a basis for the utilization of L-arginine in cardiovascular diseases.

Keywords: nitric oxide, endothelium, peripheral resistance, impedance cardiography, non-compartmental analysis.
adhesiveness for the endothelium [14] in humans. t-arginine has also been used for other purposes like stimulation of growth hormone release from the pituitary gland [15]. Physiological uptake of t-arginine with usual diets is about 4–6 g day$^{-1}$ [16]. The daily doses of t-arginine utilized in these studies significantly exceed physiological uptake of this semi-essential amino acid by 3–8-fold.

However, few data are known on the pharmacokinetic parameters and the pharmacokinetic-pharmacodynamic relationship of t-arginine in humans. One study in healthy human subjects investigated the pharmacokinetics of a multi-amino acid formulation used for the supplementation of polytraumatic patients [17]; however, the dose of t-arginine which was administered in this study was much lower than the doses used to induce NO-dependent vasodilation or growth hormone secretion. To characterize better the pharmacokinetic behaviour of t-arginine in healthy humans after acute intravenous and oral administration, we performed a double-blind, placebo-controlled dose-comparison study in healthy human subjects. Measurements were made during the time interval during which vasodilator effects can be expected after t-arginine infusion, in order to analyze the pharmacokinetic-pharmacodynamic relationship of t-arginine.

Methods
Subjects and study design

Eight healthy male human subjects (mean age, 25.2 ± 0.2 years, height, 184.4 ± 3.7 cm, weight, 77.9 ± 2.6 kg) participated in this double-blind, placebo controlled study. All subjects underwent a physical examination and a routine laboratory screening (peripheral blood cell count, serum creatinine, sodium, potassium, chloride, ALT and AST activities) and gave their written informed consent before inclusion into the study. The protocol had previously been approved by the Institutional Review Board for Studies in Humans. The study consisted of 5 study days for each subject in randomized sequence with at least 7 days of washout between them. Before each study day, the participants were subjected to a standardized diet with a participated in this double-blind, placebo controlled study. All subjects underwent a physical examination and a routine laboratory screening (peripheral blood cell count, serum creatinine, sodium, potassium, chloride, ALT and AST activities) and gave their written informed consent before inclusion into the study. The protocol had previously been approved by the Institutional Review Board for Studies in Humans. The study consisted of 5 study days for each subject in randomized sequence with at least 7 days of washout between them. Before each study day, the participants were subjected to a standardized diet with a reduced nitrate content (~32 mg nitrate day$^{-1}$) for 24 h. On each day, they received either an intravenous infusion of t-arginine (30 g or 6 g t-arginine-hydrochloride (Freseinus, Bad Homburg, Germany) dissolved in 150 ml of physiological saline) or placebo (150 ml of physiological saline) during 30 min, or 6 g t-arginine or placebo (12 capsules of 0.5 g of t-arginine-hydrochloride or lactose). At the beginning of each study day the subjects emptied their bladder. Mild oral volume loading with herbal tea (using demineralized water) was started with 3 ml kg$^{-1}$ of body weight initially, and continued during the entire study period with 1–2 ml kg$^{-1}$ of body weight h$^{-1}$. Self-adhesive ring electrodes were fixed around the thorax and left there during the entire study period for continuous registration of the impedance cardiogram (see below). A blood pressure cuff was fixed to the right arm for blood pressure registration. Haemodynamic measurements were started after at least 60 min of adaptation, during which the subjects remained in the supine position. Baseline blood pressure was calculated as the mean of five measurements during the last 20 min before start of the t-arginine infusion.

Plasma samples were drawn into vacuum containers containing sodium EDTA at baseline and 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90, 150, and 210 min after the start of the infusion. On the days with capsule intake, time points for plasma generation were 0, 30, 60, 90, 150, and 210 min. Subjects came back to the laboratory on the next morning when additional plasma samples were drawn at 16, 20, and 24 h after drug intake. On the infusion days, urine samples were collected during 1 h immediately before the start of the infusion (baseline), at the end of the infusion (30 min), as well as 60, 120, and 180 min after the start of the infusion. On the days on which the drugs were applied orally, urine samples were collected during 1 h immediately before drug intake (baseline), as well as 60, 120, 180, and 240 min after drug intake.

Quantification of urinary NO$_3^-$ and cGMP

Urine NO$_3^-$ was determined as its pentahaloarobenzyl (PFB) derivative by gas chromatography-mass spectrometry (GC-MS) as described previously [18, 19]. Aliquots (1 ml) of urine were spiked with [15N]NO$_3^-$ (MSD Isotopes Merck Frosst, Montreal, Canada) as internal standard. Reduction of nitrate to nitrite was performed prior to derivatization under alkaline conditions (5 weight% ammonium chloride buffer adjusted to pH 8.8 by sodium borate) by incubating sample or standard with 5 mg of cadmium (30 min, 20°C). These samples (100 μl) were treated with 400 μl of acetone and 5 μl of PFB bromide, and the mixture was allowed to react for 60 min at 50°C. Acetone was then removed under nitrogen, and reactants were extracted by vortexing with 1000 μl of tolune. Aliquots (1 μl) thereof were injected into the GC-MS instrument. GC-MS was carried out on a Hewlett Packard MS Engine 5890 series II (Waldbronn, Germany). A fused-silica capillary column DB-5 MS (30 m × 0.25 mm i.d.; 0.25 μm film thickness) from J&W Scientific (Rancho Cordova, CA) was used with helium as the carrier gas (70 kPa). Negative ions were produced by chemical ionization using methane as the reactant gas at an electron energy of 230 eV and an electron current of 300 μA. Quantitation was performed by selected ion monitoring at m/z 46 for endogenous NO$_2^-$/NO$_3^-$ and m/z 47 for the internal standard. The detection limit of the method was 20 fmol nitrate. Inter- and intersubject variabilities were below 3.8%.

For the determination of cGMP, urine samples were diluted 1:500 in phosphate buffered saline and acetylated by a mixture of acetic acid anhydride/triethylamine. Cyclic GMP content was measured by radioimmunoassay using [3H]-cGMP as a tracer and globulin precipitation. The detection limit of the assay was 160 fmol ml$^{-1}$. Intra- and intersubject variabilities were 3.5% and 8.1%, respectively.

Urinary and plasma creatinine was determined spectrophotometrically with the alkaline picric acid method in an automatic analyzer (Beckman, Galway, Ireland). The urinary excretion rates of NO$_3^-$ and cGMP were corrected by urinary creatinine concentration in order to limit the
variability due to differences in renal excretory function as described previously [4].

**Determination of plasma L-arginine concentrations**

Plasma L-arginine concentrations were determined by high-performance liquid chromatography (h.p.l.c.) using pre-column derivatization with o-phthalaldehyde (OPA) as described previously [20]. Plasma samples and standards were extracted on CBA solid phase extraction cartridges (Varian, Harbor City, CA, USA). The eluates were dried under nitrogen and residues dissolved in double distilled water for h.p.l.c. analysis (Gynekotek, Munich, Germany). Samples and standards were incubated for exactly 30 s with the OPA reagent (5.4 mg ml\(^{-1}\) OPA in borate buffer, pH 8.5, containing 0.4% 2-mercaptoethanol) before automatic injection into the h.p.l.c. system. Chromatographic separation was performed on a C\(_{18}\) column (Macherey and Nagel, Düren, Germany) with the fluorescence monitor set at \(\lambda_{ex} = 340\) nm and \(\lambda_{em} = 455\) nm. Samples were isocratically eluted from the column with 0.96% citric acid/methanol (2:1 v/v) at a flow rate of 1 ml min\(^{-1}\). The coefficients of variation of the method had previously been determined as 5.2% within-assay and 5.5% between-assay; the detection limit of the assay was 0.1 nmol l\(^{-1}\).

**Haemodynamic measurements**

Systemic arterial blood pressure was measured by the standard sphygmomanometric method using an automatic device (Boso digital II, Bosch und Sohn, Jungingen, Germany). Non-invasive determination of cardiac output (CO) was performed by impedance cardiography [21]. The patients were attached to an impedance cardiograph as described by Brandes et al. [22]. Briefly, a constant sinusoidal alternating current (100 MHz, 4 mA) was applied between two thoracic electrodes placed on the level of the jugular fossa and lower chest. The associated voltage is detected by two inner electrodes positioned 5 cm apart from the outer electrode pair and parallel to the current path. This voltage is transmitted to an amplifier and a thoracic impedance signal is produced. Thoracic impedance was registered from beat to beat with an impedance cardiograph (BMT; Stuttgart, Germany) and transferred online to an IBM-compatible computer. Data analysis was performed offline using 5 min mean values. Stroke volume was automatically calculated for each heartbeat according to the equation described by Kubisz et al. [21]. There is good correlation of intraindividual changes in cardiac output obtained by impedance cardiography and echo-Doppler cardiography [23]. Total peripheral resistance was calculated as TPR = 80 × (mean blood pressure)/CO [dyn s cm\(^{-5}\)].

**Calculations and statistics**

Data are given as mean ± s.e.mean. Statistical significance was tested using analysis of variance followed by Fisher's protected least significant difference test for comparisons between the treatment groups. Linear regression curves and correlation coefficients were obtained by the least squares method. Statistical significance was accepted at the 0.05 level of probability.

**Results**

**Urinary nitrate and cyclic GMP excretion rates**

Baseline hourly urinary nitrate and cyclic GMP excretion rates were 68.7 ± 4.7 µmol mmol\(^{-1}\) creatinine and 30.1 ± 1.6 µmol mmol\(^{-1}\) creatinine, respectively. There were no significant differences at baseline between the 6 study days. Infusion of 30 g L-arginine induced a maximum increase in urinary nitrate excretion by 96.5 ± 28.4% and in cyclic GMP excretion by 65.8 ± 20.0% (each \(P<0.05\) vs baseline; Figure 1). Infusion of 6 g L-arginine resulted in maximum increases 46.8 ± 11.7% in nitrate (\(P<0.05\)) but a highly variable and not statistically significant response in cyclic GMP excretion (66.9 ± 46.6%, \(P=NS\)). These maximum increases in urinary nitrate and cyclic GMP excretion rates were noted in urines collected from 0–30 min after intravenous infusion of L-arginine. The respective maximum increases in nitrate and cyclic GMP after oral administration of 6 g L-arginine were also highly variable and not statistically significant (32.1 ± 18.0% and 31.8 ± 36.3% respectively each, \(P=NS\) vs baseline). After oral L-arginine, peak urinary nitrate excretion was observed in urines collected from 60–120 min, and peak urinary cyclic GMP excretion was observed in urines collected from 30–60 min. Urinary
arginine administration, the peak in urinary nitrate excretion after oral administration of 6 g GMP excretion rates peaked in the first urine sample after were 822 ± 407 mol l⁻¹ min⁻¹ creatinine, to a maximum plasma concentration of 6223 ± 152 µmol l⁻¹ at tₘₐₓ = 30 min. Peak plasma l-arginine concentrations were 822 ± 59 µmol l⁻¹ at tₘₐₓ = 22 min after intravenous administration of 6 g l-arginine, and 310 ± 132 µmol l⁻¹ at tₘₐₓ = 50 min after oral administration of 6 g l-arginine. Figure 4 shows the time course of mean l-arginine plasma concentrations after the three different dosages of l-arginine.

Pharmacokinetic data analysis was performed after allowing for baseline l-arginine concentrations determined at the same time points during and after placebo administration. Pharmacokinetic data for l-arginine were best described by a non-compartmental model (r=0.993); they are given in Table 1. There was evidence for nonlinear pharmacokinetics, as evidenced by a dose-dependent elimination half life (42 ± 2 min, 60 ± 9 min, and 76 ± 9 min for 30 g i.v., 6 g i.v., and 6 g p.o., respectively). Moreover, AUC (0, ∞) values showed a proportional increase with increasing doses of l-arginine (r=0.978, P<0.05). Comparison of AUC for intravenous and oral administration of 6 g l-arginine revealed that l-arginine was incompletely bioavailable after oral administration, absolute bioavailability of oral l-arginine was 68 ± 9%.

**Figure 1** Urinary nitrate excretion rates of nitrate (a) and cyclic GMP (b) in healthy human subjects before and after the infusion of 30 g l-arginine (●), 6 g l-arginine or placebo (□), or oral ingestion of 6 g l-arginine (▲) or placebo (△). Values represent mean ± s.e. mean of n=8 subjects. *P<0.05 vs baseline.

**Figure 2** Effects of intravenous l-arginine (30 mg (●), 6 mg (▲)), and placebo (□) on systolic and diastolic blood pressure in healthy human subjects. Values represent mean ± s.e. mean of n=8 subjects. The shaded area represents the duration of the infusion. *P<0.05 vs baseline.

**Pharmacokinetic parameters of intravenous and oral l-arginine**

During the intravenous infusion of 30 g l-arginine, endogenous plasma concentrations increased from 71 ± 4 µmol l⁻¹ at baseline to a maximum plasma concentration of 6223 ± 407 µmol l⁻¹ at tₘₐₓ = 30 min. Peak plasma l-arginine concentrations were 822 ± 59 µmol l⁻¹ at tₘₐₓ = 22 min after intravenous administration of 6 g l-arginine, and 310 ± 132 µmol l⁻¹ at tₘₐₓ = 50 min after oral administration of 6 g l-arginine. Figure 4 shows the time course of mean l-arginine plasma concentrations after the three different dosages of l-arginine.

**Haemodynamic effects of l-arginine**

Infusion of 30 g l-arginine significantly reduced diastolic blood pressure (maximum, −9.2 ± 1.8 mm Hg, P<0.05; Figure 2), but induced only minor changes in heart rate. 6 g l-arginine had no significant effect on diastolic or systolic blood pressure, neither after intravenous nor after oral administration. Neither intravenous nor oral placebo had any significant effects on blood pressure and heart rate.

Impedance cardiographic measurements showed a significant increase in cardiac output after 30 g l-arginine, but no significant changes after 6 g l-arginine or placebo (Figure 3a). Total peripheral resistance significantly decreased after intravenous infusion of 30 g l-arginine, but showed no significant increase in cardiac output after 30 g l-arginine, but no significant changes after 6 g l-arginine or placebo (Figure 3a).
Pharmacokinetics of L-arginine

Decrease in TPR (%)

0            50          100          150         200

250

Plasma L-arginine (µmol _1)

10000

1000

100

10

1

120

100

80

60

40

20

0

Urinary nitrate (µmol mmol _1 creatinine)

120

100

80

60

40

20

0

Figure 5 (a) Comparison of the time course of plasma L-arginine concentrations, total peripheral resistance, and urinary nitrate excretion rates in healthy human subjects after intravenous infusion of 30 g of L-arginine. (b) Plot of plasma L-arginine concentrations against e\text{Vect} (change in total peripheral resistance).

Discussion

The salient findings of our study are that L-arginine concentration-dependently induces vasodilation in healthy human subjects. This haemodynamic effect is paralleled by increased urinary excretion rates of nitrate, the final oxidative metabolite of NO, and its second messenger cyclic GMP. Pharmacokinetic analysis showed evidence for dose-related kinetics of L-arginine. Oral bioavailability of L-arginine is about 70%, and the maximum plasma concentration reached after oral ingestion of L-arginine is considerably lower than after intravenous infusion, due to the delay in absorption from the intestinal tract. In PK/PD analysis there is evidence for a direct link between the vasodilator effect of L-arginine and its plasma concentration.

We and others have previously demonstrated that L-arginine induces peripheral vaso dilation during intravenous
vasoactive effects of a single intravenous infusion of \(l\)-arginine have also been observed in patients with hypercholesterolaemia [9] and coronary [10] or generalized atherosclerosis [7]. In several studies, \(l\)-arginine has also been used in an oral dosage form in patients with cardiovascular diseases. Clarkson et al. [12] found improved endothelium-dependent brachial artery vasodilation after 4 weeks of 21 g day\(^{-1}\) of oral \(l\)-arginine in hypercholesterolemic subjects. Adams et al. [14] administered 21 g \(l\)-arginine day\(^{-1}\) for 3 days to young patients with coronary artery disease; they found improved endothelium-dependent vasodilation and reduced monocyte adhesion after \(l\)-arginine as compared with placebo. Rector et al. [28] gave 5.6 to 12.6 g day\(^{-1}\) of \(l\)-arginine during 6 weeks to patients with heart failure and found significantly improved limb blood flow and functional performance of the patients during exercise testing. Although \(l\)-arginine has been applied intravenously or orally in these and other studies in patients, the pharmacokinetic data of \(l\)-arginine are largely unknown. Investigation of the oral bioavailability of \(l\)-arginine as well as its pharmacokinetics are an important basis for further clinical studies.

In the present study, the temporal pattern of \(l\)-arginine plasma concentration closely corresponded to the temporal pattern of its vasodilator effect, i.e., the reduction in total peripheral resistance and blood pressure. Pharmacokinetic/pharmacodynamic modelling indicated that the effect (reduction in TPR) was directly linked to \(l\)-arginine plasma concentration. Alternative models using an indirect link of the effect, e.g., to a tissue compartment, or using a separate effect compartment, less closely represented the data. The presence of a direct link between \(l\)-arginine plasma levels and its haemodynamic effect was further confirmed by the lack of hysteresis in the concentration-effect plot. A counterclockwise hysteresis loop would have been expected if a delay in equilibration between plasma \(l\)-arginine concentration and its concentration at the effect site would have occurred, if a metabolite of \(l\)-arginine was responsible for the effect, or if the effect would be mediated by an indirect mechanism like protein synthesis [25]. This, taken together with the short equilibration half-life between \(l\)-arginine plasma concentration and the effect, suggests a direct vasodilator action of \(l\)-arginine within the vasculature.

\(l\)-arginine induces vasodilation may involve stimulation of endogenous, endothelial NO formation, as suggested by studies in which the urinary excretion rate of nitrate, the final oxidative metabolite of NO, was increased [6, 8]. The present study further supports this hypothesis, as we found a close linear relationship between \(l\)-arginine plasma levels and urinary nitrate excretion rates. In other studies, increased exhalation of NO was reported as an indicator for enhanced endogenous NO formation after \(l\)-arginine administration [29]. We here report that the urinary excretion rates of nitrate and cGMP, two index molecules for endogenous NO formation in vivo [4], are increased in a dose-related manner after intravenous infusion of \(l\)-arginine. The maximum elevation of urinary nitrate and cGMP excretion rates occurred within 30–60 min after the end of \(l\)-arginine infusion [6, 7]. 90 min after the end of the infusion, these index metabolites had returned to the basal range again.

The hypothesis that \(l\)-arginine induces NO elaboration by the endothelium and thereby causes vasodilation is in agreement with the present observation of a direct link between \(l\)-arginine plasma levels and vasodilation. Endothelial NO synthase is continuously stimulated by shear stress induced by the blood streaming along the endothelial surface [30]. NO is released from the endothelium almost instantaneously after stimulation, and NO itself has a very short biological half-life in the range of a few seconds [31]. It is rapidly inactivated by oxidation to nitrite and nitrate [3, 4]. These biochemical observations correspond well to the results of the biochemical measurements performed in the present study. We found that the elevation of urinary nitrate excretion corresponded closely to the time pattern of \(l\)-arginine plasma levels, as did the change in urinary excretion of the second messenger, cyclic GMP, and the haemodynamic response.

The fact that \(l\)-arginine increases NO release in vivo has been called the ‘\(l\)-arginine paradox’, because compared with the \(K_m\) value of the endothelial NO synthase (\(\sim 2.9 \mu\text{M}\) [39]) physiological fasting \(l\)-arginine plasma levels should be high enough to saturate the enzyme with substrate [16]. This paradox may be resolved by several observations: Firstly, the \(V_{\max}\) value was determined in vitro in a crude enzyme preparation [32], in vivo, however, factors like intracellular compartmentalization of \(l\)-arginine and NO synthase may differ, potentially reducing the availability of

**Table 1** Pharmacokinetic parameters of intravenous and oral \(l\)-arginine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intravenous</th>
<th>Oral</th>
</tr>
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<tbody>
<tr>
<td>(t_{\text{1/2}}) (min)</td>
<td>41.6 ± 2.3</td>
<td>59.6 ± 9.1</td>
</tr>
<tr>
<td>(C_{\text{max}}) (μmol l(^{-1}))</td>
<td>621.9 ± 380</td>
<td>822 ± 359</td>
</tr>
<tr>
<td>(t_{\text{max}}) (min)</td>
<td>30 (30–30)</td>
<td>25 (35–30)</td>
</tr>
<tr>
<td>(V_d) (l kg(^{-1}))</td>
<td>53.6 ± 2</td>
<td>58.2 ± 9</td>
</tr>
<tr>
<td>(CL_{\text{ext}}) (m l min(^{-1}))</td>
<td>544 ± 24</td>
<td>898 ± 164</td>
</tr>
<tr>
<td>AUC (mol l(^{-1}) min)</td>
<td>265,435 ± 11,861</td>
<td>38,223 ± 5,179</td>
</tr>
<tr>
<td>(F) (%)</td>
<td>—</td>
<td>24,786 ± 3,222*</td>
</tr>
</tbody>
</table>

Data are mean ± s.e.mean of \(n=8\) subjects per group.

**Abbreviations:** \(t_{\text{1/2}}\), terminal elimination half-life; \(C_{\text{max}}\), maximal plasma concentration; \(t_{\text{max}}\), time after which the maximal plasma concentration was reached; \(V_d\), volume of distribution; \(CL_{\text{ext}}\), total clearance; AUC, area under the concentration-time curve extrapolated from time 0 to \(\infty\); \(F\), systemic bioavailability. *Clearance and AUC for the oral dose are given as \(CL/F\) and AUC/F.
substrate for the enzyme. Moreover, the presence of endogenous compounds competing with L-arginine for the enzyme binding site may also cause relative substrate depletion. One such endogenous competitive inhibitor is asymmetric dimethylarginine (ADMA); it has been reported to be present in human plasma [33] and in cultured human endothelial cells [34, 35]. Whether ADMA downregulates NO elaboration in healthy humans, in whom ADMA plasma levels are low [33, 36], remains undetermined. Finally, L-arginine uptake may be a crucial step limiting intracellular L-arginine availability in vivo [37].

Besides stimulating NO production, L-arginine is known to exert other effects which may contribute to its vasodilator properties, like stimulation of growth hormone secretion [15] and insulin release [27]. However, the peak in growth hormone secretion after L-arginine infusion occurs later than the peak in vasodilation [own, unpublished observation]. Interestingly, these endocrine effects of L-arginine may also cause secondary increases in NO release. Many of the physiological effects of growth hormone have been shown to occur via local production of NO-generating organs like aorta, heart, and vena cava. In conclusion, our present study provides pharmacokinetic data for L-arginine after absorption in healthy humans, indicating that L-arginine uptake and metabolism are of endogenous compounds competing with L-arginine for the enzyme binding site. Therefore, the rate of utilization of L-arginine after absorption in the splanchnic region and the factors affecting it are of considerable interest, since L-arginine uptake and metabolism in the liver may affect systemic bioavailability of this amino acid. There is evidence that the activity of the L-arginine uptake mechanism (y^+-transporter for basic amino acids) is low in hepatocytes as compared with other cell types [46]. Accordingly, Fehlg & Wahren [47] found no concentration gradient between the portal and hepatic vein in the postabsorptive state. Taken together, these studies suggest that L-arginine metabolism in the liver is functionally separated from whole-body L-arginine metabolism. Blancher et al. [48] showed that a minor part of an oral L-arginine dose administered to healthy rats is metabolized by enterocytes. These findings may explain why the majority of an orally administered L-arginine dose was systemically available in the present study. Even outside the liver, L-arginine can be a substrate for several metabolic pathways: ingestion of L-arginine can be a source of ornithine in the intestine [49], it may serve to replenish arginine which is lost during hepatic urea synthesis [50]; it may undergo decarboxylation to agmatine in the kidney and the brain [51], or be used by the NO synthase to generate NO and citrulline [52].

Comparison of the AUC values for the oral or intravenous administration of 6 g L-arginine showed that the bioavailability of L-arginine is ~70% after oral ingestion. Using stable-isotope-labeled L-arginine, Castillo et al. [44] also found incomplete bioavailability of orally ingested L-arginine (~38%). In contrast, Matura et al. [17] reported complete bioavailability of oral L-arginine in healthy humans. They administered in a very low daily dose (100 mg day^-1), contained in a complex polyaminoacid formulation intended for use as an oral supplement in intensive care medicine, for 7 days. In the same study, these authors reported a terminal elimination half-life of ~1.2 h, which is in agreement with our results obtained with considerably higher doses of L-arginine. Half-life calculations in our study was somewhat hampered by the long time interval between the sampling times at 210 and 960 min. This was caused by logistic reasons which did not allow us to keep the subjects in the lab overnight. The significance of half life calculations was potentially reduced by this time pattern, but there is still a relatively close correspondence between our findings and those reported by others. Noch et al. [45] studied tissue distribution of L-arginine in rats after intraperitoneal application; they found close correlation between the rise in plasma arginine levels and arginine concentrations in NO-generating organs likeorta, heart, and vena cava. In their study, L-arginine half-life was ~1 h in plasma, and 1–2 h in the various tissues.
concentrations. These data may provide a basis for the utilization of L-arginine in cardiovascular diseases.

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References


303–310.


37 Griffith TM, Edwards DH, Lewis MJ, Newby AG, Henderson AH. The nature of the endothelium-derived relaxing factor synthesized from cultured and native bovine aortic


46 White MF, Christensen HN. Cationic amino acid transport into cultured animal cells. II. Transport system barely perceptible in ordinary hepatocytes, but active in hepatoma cell lines. *J Biol Chem* 1982; 258: 8028–8038.