Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT
- L-Arginine is a semiessential amino acid that is converted to nitric oxide (NO) by NO synthase (NOS).
- NO improves endothelial function by elevating cyclic guanosine monophosphate.
- However, oral L-arginine treatment in humans is hampered by extensive metabolism.

WHAT THIS STUDY ADDS
- Oral L-citrulline supplementation raises plasma L-arginine concentration and augments NO-dependent signalling in a dose-dependent manner.
- L-Citrulline may thus be an alternative to L-arginine in patients with impaired NOS activity.

AIMS
Oral L-arginine supplementation has been used in several studies to improve endothelium-dependent, nitric oxide (NO)-mediated vasodilation. L-Arginine treatment is hampered by extensive presystemic elimination due to intestinal arginase activity. In contrast, L-citrulline is readily absorbed and at least in part converted to L-arginine. The aim of our study was to assess this metabolic conversion and its subsequent pharmacodynamic effects.

METHODS
In a double-blind, randomized, placebo-controlled cross-over study, 20 healthy volunteers received six different dosing regimes of placebo, citrulline, and arginine. Pharmacokinetic parameters (C_{max}, T_{max}, C_{min}, AUC) were calculated after 1 week of oral supplementation. The ratio of plasma L-arginine over asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase (arginine/ADMA ratio), urinary cyclic guanosine monophosphate (cGMP) and nitrate excretion rates, and flow-mediated vasodilation (FMD) was measured to assess pharmacodynamic effects.

RESULTS
L-Citrulline dose-dependently increased AUC and C_{max} of plasma L-arginine concentration more effectively than L-arginine ($P < 0.01$). The highest dose of citrulline (3 g bid) increased the C_{min} of plasma L-arginine and improved the L-arginine/ADMA ratio from 186 ± 8 (baseline) to 278 ± 14 [$P < 0.01$, 95% confidence interval (CI) 66, 121]. Moreover, urinary nitrate and cGMP were increased from 92 ± 10 to 125 ± 15 μmol mmol^{-1} creatinine ($P = 0.01$, 95% CI 8, 58) and from 38 ± 3.3 to 50 ± 6.7 nmol mmol^{-1} creatinine ($P = 0.04$, 95% CI 0.4, 24), respectively. No treatment improved FMD over baseline. However, pooled analysis of all FMD data revealed a correlation between the increase of arginine/ADMA ratio and improvement of FMD.

CONCLUSION
Our data show for the first time that oral L-citrulline supplementation raises plasma L-arginine concentration and augments NO-dependent signalling in a dose-dependent manner.

Introduction

The three isoforms of nitric oxide synthase (NOS), neuronal NOS (nNOS, NOS 1), inducible NOS (iNOS, NOS 2) and endothelial (eNOS, NOS 3), convert L-arginine to nitric oxide (NO) and L-citrulline [1]. NO is a vasoactive compound that induces vasodilation of arterial and venous blood vessels. In endothelial cells, L-arginine is transported via the cell membrane by cationic amino acid transporters that are colocalized with eNOS [2]. The Michaelis–Menten constant (K_m) for eNOS is ~3 μM L-arginine [3]. This is at least one order of magnitude lower than the normal plasma concentrations of L-arginine, which are usually in the range 60–140 μM [4]. Nevertheless, oral supplementation with L-arginine has been shown to enhance NO-mediated vasodilation in several clinical studies [5, 6], but not in all [7, 8]. One possible explanation for this 'arginine paradox' is the presence of an endogenous inhibitor of NOS, which may shift the steep part of the substrate–activity curve of NOS towards higher L-arginine levels [9]. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of all three isoforms of NOS and it is circulating at low μM concentrations in humans [9]. The ratio of L-arginine over ADMA (arginine/ADMA ratio) is one determinant of NO production by NOS [10]. Once produced, NO activates soluble guanylyl cyclase (sGC) in smooth muscle cells, which leads to elevated intracellular cyclic guanosine monophosphate (cGMP). In human blood vessels this mechanism results in vasodilation [11]. This process is essential for endothelial function, and disturbed NO production in the human endothelium contributes to endothelial dysfunction [1, 9, 12].

The semiessential amino acid L-arginine is part of the human diet and only 5–15% of plasma arginine originate from *de novo* synthesis [4, 13]. After oral administration, L-arginine is subject to extensive presystemic and systemic elimination, i.e. by bacteria in the gut and arginases in the gut and liver, respectively [14]. The non-essential amino acid L-citrulline is not subject to presystemic elimination but to systemic metabolism. L-Citrulline is converted to L-argininosuccinate by argininosuccinate synthase and subsequently to L-arginine by argininosuccinate lyase [15]. It may therefore serve as an L-arginine precursor [16].

The aim of this study was to investigate the pharmacokinetic (PK) and pharmacodynamic (PD) effects of different oral doses of L-arginine and L-citrulline in humans subjects with impaired NO elaboration secondary to elevated ADMA concentrations.

Methods

Subjects

Twenty healthy, non-obese volunteers (13 male, 7 female) were included in this study. They were recruited from a group of 168 cl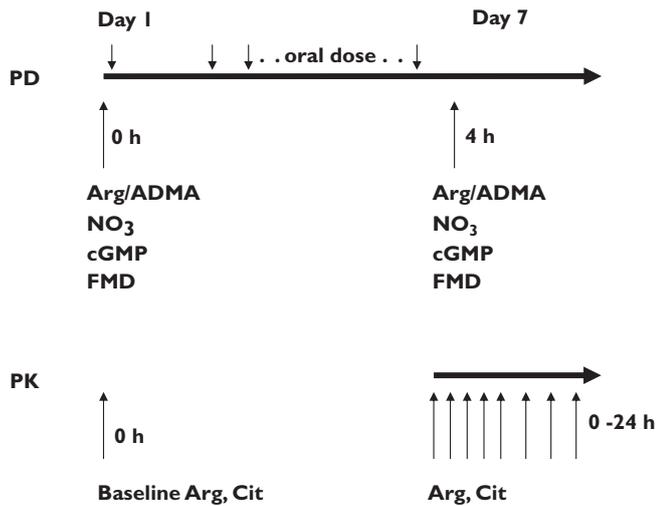inically healthy humans screened for fasting plasma ADMA concentration. Subjects were eligible if they had ADMA concentrations within the highest quartile of the distribution of the screened population. All participants had normal clinical history and physical examination, 12-lead electrocardiogram, haematological and biochemical screen. Diabetes, obesity, hypertension, cardiovascular disease, liver or kidney disease, current infections or smoking were exclusion criteria. None of the volunteers received any drugs that might alter amino acid or vitamin status, and dietary habits were kept constant during the study. A history of hormone replacement therapy (HRT) was known in two female participants. HRT was stopped 21 days prior to receiving the first dose of study drug. Written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of the Hamburg Board of Physicians, and the investigation was conducted in accordance with the Declaration of Helsinki.

Study design

In a randomized, double-blind, placebo-controlled crossover design participants received either L-citrulline 0.75 g twice daily, L-citrulline 1.5 g twice daily, L-citrulline 3 g twice daily, L-arginine immediate-release (IR) 1.0 g tid, L-arginine sustained-release (SR) 1.6 g twice daily, or placebo for 7 days each. The study periods were separated by washout phases of 1 week, and the sequence of the medications was randomly chosen in each participant. On day 7 of each medication phase, venous blood samples were drawn from an antecubital vein for PK analyses at 0, 0.5, 1, 2, 4, 6, 8, 12, 16 and 24 h. On day 7 only a single dose, equivalent to half of the total daily dose, was administered. The twice daily or three times daily dosing was administered on days 1 through 6. At baseline and on day 7 (at 4 h after dosing) additional blood and urine samples were collected for ADMA plasma concentrations and urinary nitrate and cGMP excretion rates, respectively (Figure 1). Finally, at baseline and at 4 h after dosing on day 7, endothelial function was assessed by flow-mediated vasodilation (FMD) testing of the brachial artery as detailed below.

Biochemical analyses

Plasma L-arginine and L-citrulline concentrations were determined by liquid chromatography (LC)-tandem mass spectrometry (MS) analysis as described previously [17]. Briefly, a 50-μl aliquot of plasma was spiked with stable isotope-labelled L-citrulline and L-arginine, which served as internal standards. Protein was precipitated with 100 μl of methanol, filtrated through a 0.22-μm hydrophilic membrane (Multiscreen HTS™; Millipore, Molsheim, France), derivatized with butanolic HCl (1 N, 65°C, 17 min) and analysed by LC-tandem MS. Quantification was performed by selected reaction monitoring of the respective daughter ions of analytes and internal standards (Waters, Eschborn, Germany). Plasma ADMA was analysed by enzyme-linked immunosorbent assay (ELISA), as previ-

Figure 1
Study design

Table 1
Groups' baseline characteristics

	Subjects investigated ($n = 20$)	
	Mean	95% CI
Age (years)	57	52, 61
Gender (n)	7 females (35%)	
Smoker (n)	0 (0%)	
Body mass index (kg m^{-2})	25.6	24, 27
Blood pressure (mmHg)		
Systolic	130	125, 135
Diastolic	83	78, 87
Blood lipids (mg dl^{-1})		
Total cholesterol	226	211, 242
LDL-cholesterol	137	122, 154
HDL-cholesterol	65	58, 73
Triglycerides	116	84, 146
Asymmetric dimethylarginine (µmol l^{-1})	0.60	0.56, 0.63
Blood glucose (mg dl^{-1})	85	79, 92
Blood urea nitrogen (mg dl^{-1})	17	15, 19
Serum creatinine (mg dl^{-1})	0.92	0.84, 0.99

LDL, Low-density lipoprotein; HDL, high-density lipoprotein.

ously described [18]. Urinary nitrate levels were determined by GC-MS as described elsewere [10]. Urinary cGMP was analysed by ELISA [11]. Urinary excretion rates of nitrate and cGMP were corrected for creatinine excretion.

Pharmacokinetic analyses
PK parameters (C_{max}, T_{max}, C_{min}, AUC) were calculated for each dose of L-arginine and L-citrulline after 1 week of oral supplementation. After L-citrulline supplementation, PK parameters were calculated for L-arginine and L-citrulline plasma concentrations, whereas PK parameters were calculated only for L-arginine concentrations after L-arginine supplementation. Areas under the plasma concentration–time curve (AUC) were calculated for up to 24 h. To account for the circadian rhythms of endogenous L-arginine and L-citrulline concentrations, plasma concentrations following L-arginine and L-citrulline administration at each time point were corrected for individual baseline and placebo data prior to calculation of C_{max}, T_{max}, C_{min} and AUC values. Even for corrected data, calculation of half-life was still not possible. All PK calculations were performed using Win-Nonlin (v. 5.0; Pharsight Corp., Mountain View, CA, USA).

Vascular function testing
Methods of assessing endothelium-dependent vasodilation followed the principles set by the International Brachial Artery Reactivity Task Force [19]. Endothelial function was assessed in the volunteers' right arm in a quiet, temperature-controlled room (22°C) by high-resolution ultrasound (12 MHz linear array transducer; Siena, Siemens, Germany). Longitudinal scans of the brachial artery were obtained approximately 5 cm proximal of the antecubital fossa. The transmit focus zone was set at the depth of the anterior wall. Anatomical landmarks and snapshot images

were used to assess FMD in the same vessel section on each study day and at each time point. A view of a 5-cm longitudinal section of the brachial artery was recorded for time periods of 30 s at baseline and during peak reactive hyperaemia (60 s after deflation of a blood pressure cuff previously inflated to 50 mmHg above the volunteer's systolic blood pressure for 5 min). Each 30-s recording was digitalized (Vascular Imager 4.1.3; Medical Imaging Applications LLC, IA, USA) at a rate of 10 high-resolution frames per second (= 300 frames per recording), by using specialized software (Brachial Analyser 4.1.3; Medical Imaging Applications LLC). FMD was calculated as the percent change in diameter 1 min after cuff release relative to the baseline diameter before cuff release. Ultrasound studies and image analysis were performed separately by independent investigators in an observer-blinded fashion. The mean intraindividual coefficient of variation of the arterial diameter at the baseline measurements obtained on the six separate study days was 4.65%.

Statistical analyses
All data are given as mean ± SEM, together with 95% confidence intervals for the mean differences (CI). Statistical comparisons were made by Student's t-test (two-tailed) for paired data. Statistical analysis was performed with SPSS (release 10 for Windows; Chicago, IL, USA).

Results

Baseline characteristics of subjects investigated are given in Table 1. All participants were apparently healthy White nonsmokers. Oral L-citrulline supplementation increased

the plasma concentrations of L-citrulline (Table 2) and L-arginine in a dose-dependent manner (Figure 2). Oral L-arginine did not alter the plasma concentrations of L-citrulline (data not shown), but increased plasma L-arginine concentrations (Table 2). The change in L-arginine AUC was about as pronounced after oral L-citrulline administration at a dose of 0.75 g twice daily as after a twofold higher dose of oral L-arginine SR (1.6 g bid) and a twofold higher total daily dose of L-arginine IR (1.0 g tid). The higher doses of oral L-citrulline induced dose-dependent elevations of L-arginine C_{max} and AUC (Table 2). The peak plasma arginine concentration was significantly increased for L-citrulline

Table 2a

Kinetic parameters of arginine in human plasma after 1 week of oral supplementation with either citrulline or arginine‡

Compound	Dose (mg)	C_{max} (µmol l^{-1})	T_{max} (h)	C_{min} (µmol l^{-1})	AUC (µmol h l^{-1})
Citrulline	750 bid	54 ± 5	2.3 ± 0.7	19 ± 4	271 ± 38
Citrulline	1500 bid	79 ± 8*	1.6 ± 0.3	21 ± 4	421 ± 65*
Citrulline	3000 bid	149 ± 42*†	1.4 ± 0.1	45 ± 5*†	898 ± 67*†
Arginine SR	1600 bid	49 ± 6	3.7 ± 1.3§	19 ± 4	289 ± 50
Arginine IR	1000 tid	84 ± 9	0.7 ± 0.1	10 ± 3	283 ± 51

*$P < 0.01$ vs. arginine sustained-release (SR). †$P < 0.01$ vs. arginine immediate-release (IR). §$P = 0.03$ vs. arginine IR. ‡Kinetic parameters are calculated for baseline-placebo corrected data. Data are given as mean ± SEM. bid, twice daily.

Table 2b

Kinetic parameters of citrulline in human plasma after 1 week of oral supplementation with either citrulline or arginine‡¶

Compound	Dose (mg)	C_{max} (µmol l^{-1})	T_{max} (h)	C_{min} (µmol l^{-1})	AUC (µmol h l^{-1})
Citrulline	750 bid	163 ± 14	0.7 ± 0.1	9 ± 2	288 ± 35
Citrulline	1500 bid	350 ± 38*	0.8 ± 0.1	6 ± 1	566 ± 47*
Citrulline	3000 bid	864 ± 45*†	0.7 ± 0.1	9 ± 2	1486 ± 78*†

*$P < 0.01$ vs. citrulline 750 bid. †$P < 0.01$ vs. citrulline 1500 bid. ‡Kinetic parameters are calculated for baseline–placebo corrected data. Data are given as mean ± SEM. ¶Kinetic parameters of citrulline in human plasma after arginine supplementation were not available (no increase of citrulline in human plasma over baseline). C_{max}, Maximal plasma concentration; T_{max}, time of reach C_{max}; C_{min}, minimal plasma concentration.
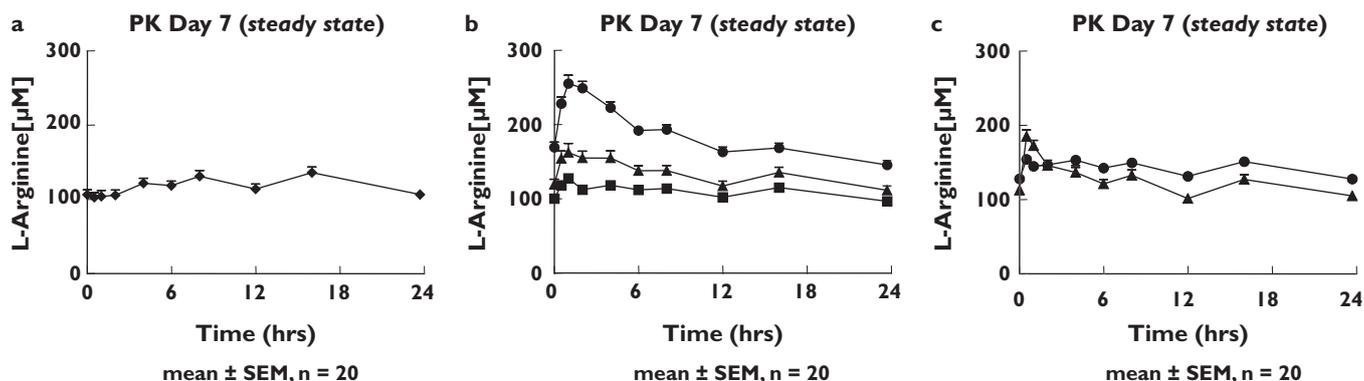
Figure 2

Plasma concentrations of L-arginine at steady state (mean ± SEM, $n = 17$ for arginine immediate-release (IR) and $n = 20$ for all others). (A) Placebo (◆) curve. (B) After 1 week of 0.75 (■), 1.5 (▲) and 3 g (●) twice-daily citrulline supplementation. (C) After 1 week of 1.0 g (▲) tid arginine IR and 1.6 g (●) bid arginine sustained-release supplementation

administration at a dose of 1.5 g twice daily compared with L-arginine SR ($P < 0.01$, 95% CI for difference between mean values 17, 43 µmol l^{-1}) and at a dose of 3 g twice daily compared with L-arginine SR ($P < 0.01$, 95% CI 84, 116 µmol l^{-1}) and with L-arginine IR ($P < 0.01$, 95% CI 43, 84 µmol l^{-1}). The L-arginine AUC was significantly increased after L-citrulline administration at a dose of 1.5 g twice daily compared with L-arginine SR ($P < 0.01$, 95% CI 49, 214 µmol h l^{-1}) and at a dose of 3 g twice daily compared with L-arginine SR ($P < 0.01$, 95% CI 497, 721 µmol h l^{-1}) and with L-arginine IR ($P < 0.01$, 95% CI 475, 723 µmol h l^{-1}). [Correction added after online publication 13 September 2007: Units of measurement corrected]

Both 1.6 g L-arginine SR and 3 g L-citrulline improved the plasma L-arginine/ADMA ratio from 171 ± 7 to 232 ± 14 ($P < 0.01$, 95% CI 36, 91) and from 186 ± 8 to 278 ± 14 ($P < 0.01$, 95% CI 66, 121), respectively (Figure 3a). Other treatments were ineffective. Only the highest dose of L-citrulline significantly increased urinary excretion of nitrate and cGMP from 92 ± 10 to 125 ± 15 µmol mmol^{-1} creatinine ($P = 0.01$, 95% CI 8, 58; Figure 3b) and from 38 ± 3.3 to 50 ± 6.7 nmol mmol^{-1} creatinine ($P = 0.04$, 95% CI 0.4, 24; Figure 3c), respectively. Neither blood urea nitrogen nor serum creatinine was altered by active treatment. Baseline arterial diameter in the first treatment period was 4.8 ± 0.1 mm. None of the treatments was associated with a significant change in baseline arterial diameter (all $P > 0.05$). Baseline FMD in the first treatment period was 6.9 ± 1.0%. None of the treatments significantly improved FMD (Figure 3d). However, analysis of pooled data over all treatments revealed a correlation between mean changes of FMD and mean changes of plasma L-arginine/ADMA ratio (Pearson's correlation, $r = 0.92$, $P = 0.01$, Figure 4).

Discussion

The major finding of our study is that oral administration of L-citrulline efficiently increases L-arginine plasma concentrations in healthy human. After 1 week of oral supplementation, L-citrulline 0.75 g twice daily increased C_{max} for plasma L-arginine and AUC for plasma L-arginine to the same extent as did L-arginine SR 1.6 twice daily and L-arginine IR 1.0 g tid (Table 2). Moreover, higher doses of L-citrulline dose-dependently elevated C_{max} and AUC for plasma L-arginine. Trough plasma concentrations of L-arginine were also dose-dependently elevated by L-citrulline. They were significantly higher after L-citrulline 3 g twice daily than after L-arginine IR and L-arginine SR ($P < 0.01$, Table 2). These findings strongly suggest that oral L-citrulline is at least as efficient in improving plasma L-arginine concentrations in man as is oral administration of L-arginine.

Oral supplementation with L-arginine has been used in a variety of clinical conditions, including hypercholesterolaemia, coronary artery disease, congestive heart failure, peripheral arterial disease, sickle cell disease, and in elderly humans [5–9, 20], in attempts to improve NO-mediated vascular function. Metabolic data from experimental and human studies suggest that after oral administration, L-arginine is extensively metabolized by arginase in the gut wall and liver [14, 21]. This may limit its bioavailability as a substrate for NOS and subsequent effect on vascular function. L-Citrulline has been suggested as a precursor of L-arginine [16, 22], because it can be converted in a two-step enzymatic reaction into L-arginine. A recent small clinical study has suggested that oral L-citrulline may actually lead to higher elevations of plasma L-arginine concentrations than administration of L-arginine itself [23]. Our present data add further evidence by showing that one-half the dosage strength of L-citrulline results in similar plasma L-arginine AUCs compared with oral L-arginine SR and IS (Table 2). Our observation that L-arginine concentrations were increased in peripheral venous blood suggests that L-arginine derived from orally administered L-citrulline is systemically converted to L-arginine, presumably by the kidney and other tissues, including the vasculature [24].

In an experimental study using stable isotope-labelled L-arginine and MS analysis, we were able to show that only a minute proportion of oral L-arginine (approximately 1% of the dose) was being utilized as a substrate of NOS [25]. Metabolic studies using the same technology in man have demonstrated that extensive metabolism of L-arginine occurs in the intestinal tract [21]. This, in combination with a very short half-life of about 1 h [11], may have contributed to the negligible effect of IR L-arginine on any of the PK and PD parameters measured in the present study. Besides using a SR formulation of L-arginine, i.e. L-arginine SR [26], L-citrulline administration may thus be an elegant way of prolonging the exposure of the vasculature to elevated concentrations of plasma L-arginine. In our healthy study population, L-citrulline supplementation was well tolerated and no related side-effects were observed. Nevertheless, in patients with elevated L-citrulline concentrations, e.g. renal failure [27], the efficacy and side-effects of this supplementation should be investigated.

The second aim of our study was to investigate whether these PK findings translate into PD effects. Plasma L-arginine is one important source of L-arginine substrate for NOS [13], because L-arginine is readily taken up from plasma into endothelial cells by the y+ transport system for cationic amino acids [2], which is colocalized with eNOS in caveolae [28]. The ratio of L-arginine over the endogenous NOS inhibitor ADMA is one predictor for the substrate availability for NOS [10, 29]. Thus, treatment-induced elevation of plasma L-arginine concentrations can be expected to increase the L-arginine/ADMA ratio. On the other hand, high concentrations of L-arginine are known to inhibit dimethylarginine dimethylaminohydrolase (DOAH), the enzyme responsible for ADMA catabolism, which would increase ADMA concentrations [30]. However, the relatively low dose of L-arginine investigated in our study,
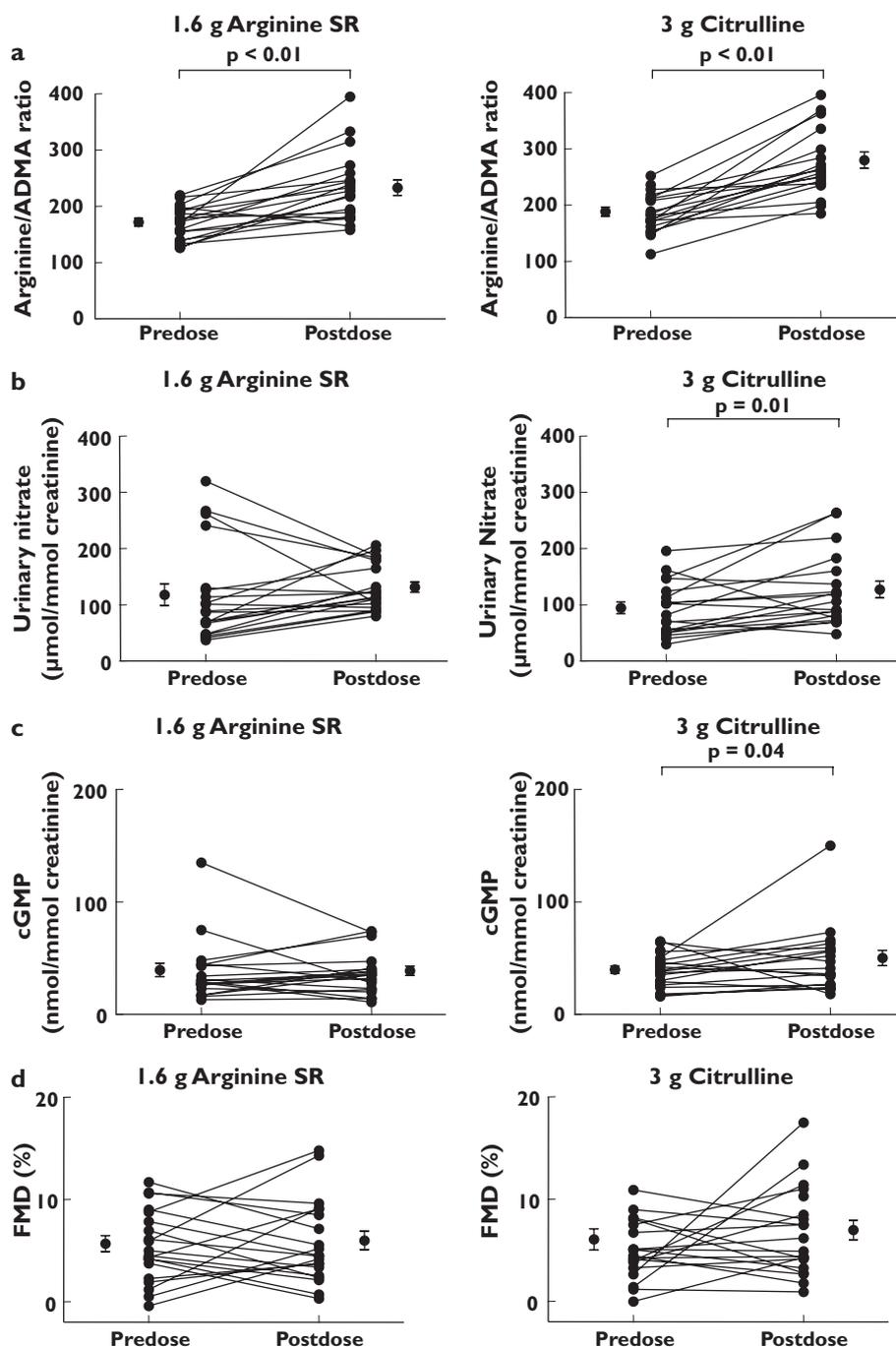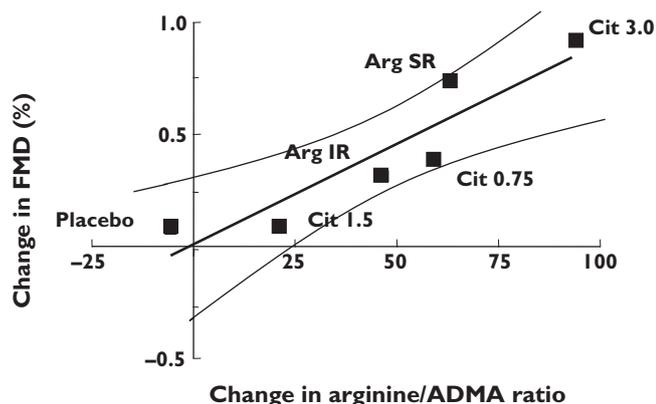
Figure 3

Change in pharmacodynamic parameters after 1 week of 1.6 g bid arginine sustained-release (SR) and 3 g bid citrulline in 20 healthy subjects. Change in (**A**) L-arginine/asymmetric dimethylarginine ratio, (**B**) urinary nitrate, (**C**) urinary cyclic guanosine monophosphate and (**D**) flow-mediated vasorelaxation (FMD). Individual changes and changes of the mean $\pm$ SEM are illustrated. Only statistically significant P-values are given

i.e. a maximum of 3.2 g day^{-1}, did not increase ADMA plasma concentrations in participants.

An improved arginine/ADMA ratio would enhance the conversion of L-arginine to NO and subsequently increase urinary excretion of the major urinary metabolite of NO, nitrate. In fact, the L-arginine/ADMA ratio was elevated after 1 week of oral supplementation with L-arginine SR or L-citrulline 3 g (Figure 3a). Other treatments, including L-arginine IR or lower doses of L-citrulline, did not elicit significant changes in the ratio. L-Citrulline 3 g was more efficient in increasing C_{max}, C_{min} and AUC for plasma L-arginine over baseline placebo than L-arginine SR or IR.

Figure 4

Cumulative data over all treatments. Mean change of flow-mediated vasorelaxation (FMD) is correlated with the mean change of L-arginine/asymmetric dimethylarginine ratio (Pearson's correlation, $r = 0.92$, $P = 0.01$, 95% confidence intervals indicated)

This could explain why L-citrulline 3 g was the only treatment to enhance urinary nitrate excretion significantly (Figure 3b). Urinary nitrate excretion as a measure of systemic NO production is highly confounded by other, i.e. dietary, sources, which serves as another explanation why only the highest dose of L-citrulline appeared effective [10, 25]. Also, only L-citrulline 3 g increased urinary excretion of cGMP (Figure 3c). cGMP is the product of sGC in vascular smooth muscle and other cells, which is activated upon stimulation by NO. The increased NO production observed after the highest dose of L-citrulline resulted in increased urinary excretion of cGMP. Both urinary nitrate and cGMP have previously been used as markers of systemic NO production and bioactivity [6, 11, 25]. Thus, NO was not only produced, but also bioactive, in the present study.

The third question this study set out to answer was whether increased bioavailability of L-arginine resulted in an improvement of endothelium-dependent vasodilation as a surrogate marker of NO-mediated physiological effects. There was no significant improvement of endothelium-dependent vasodilation by any of the treatments after 1 week of administration (Figure 3d). Although disappointing, this was only a secondary goal of the present study, and there are several possible explanations for this negative result. First, in a previous study in patients with severe intermittent claudication in which intravenous infusions of L-arginine (8 g bid) were used for 3 weeks [31], FMD was enhanced in a time-dependent manner. After 1 week, there was only a slight tendency for improvement which was in the range of 1–2% absolute change in FMD; this response to L-arginine treatment built up to a significant improvement after 3 weeks, which was also associated with a significantly prolonged claudication distance. Thus, the absolute difference in FMD in the present study corresponds well to the change observed in the previous study after the same treatment period, and one can

therefore speculate that a significant improvement of endothelial function may occur if treatment with L-arginine or L-citrulline is prolonged.

In previous studies, L-citrulline and L-arginine administration has resulted in improved endothelium-dependent vasodilation [5, 6, 31], and has not [7, 8]. In one study, oral administration of 5 g tid L-arginine for 2 weeks failed to increase further acetylcholine-induced forearm blood flow [7]. Importantly, a recent placebo-controlled study has suggested possible harm in 153 post-myocardial infarction (MI) patients treated with oral L-arginine (3 g tid) vs. placebo [32]. However, those results are discordant with another randomized, placebo-controlled study, in which 792 post-MI patients clearly benefited from L-arginine supplementation (3 g tid) [33], and the causal relationship between the excess number of deaths observed in the former study and the L-arginine administration has been questioned [34]. One reason for these contradictory findings of L-arginine supplementation studies could be that unselected patients have been included. Selection of patients in whom L-arginine-derived NO production is impaired secondary to elevated ADMA concentrations may increase the chance of identifying patients that respond positively to L-arginine or L-citrulline administration [35]. Indeed, restoration of plasma L-arginine concentrations was found to reverse the endothelial dysfunction attributable to high ADMA in some clinical and experimental studies [5, 31, 36, 37]. This suggestion is further supported by our finding of a correlation between the mean change in L-arginine/ADMA ratio and the mean change in FMD when data from all treatments were pooled (Figure 4). This supports the assumption that the change of FMD, at least in part, depends on the L-arginine/ADMA ratio, and therefore corroborates previous findings by our group [36, 38] and others [6, 39].

Finally, although the subjects included in the present study had been selected according to their comparatively high ADMA concentrations, the mean ADMA concentration in study participants was 0.6 ± 0.1 μM, which is still within the normal range [40]. High ADMA concentrations have been associated with endothelial dysfunction and vascular disease [20, 41, 42], and competition with high ADMA has been suggested to explain the so-called 'L-arginine paradox' [9]. Therefore, it is not surprising that endothelial function was normal in our apparently healthy study population. Baseline FMD was $6.9 \pm 1.0\%$, a value which is at the lower margin, but still within the range previously reported for healthy individuals [31]. This may be another explanation why neither L-citrulline nor L-arginine treatment resulted in significant improvement of endothelial function within the short treatment period of 1 week.

In conclusion, our results provide a rationale for larger, prospective clinical studies with longer treatment periods to investigate the effects of oral L-citrulline supplementation on endothelial function in patients with endothelial dysfunction and vascular disease.

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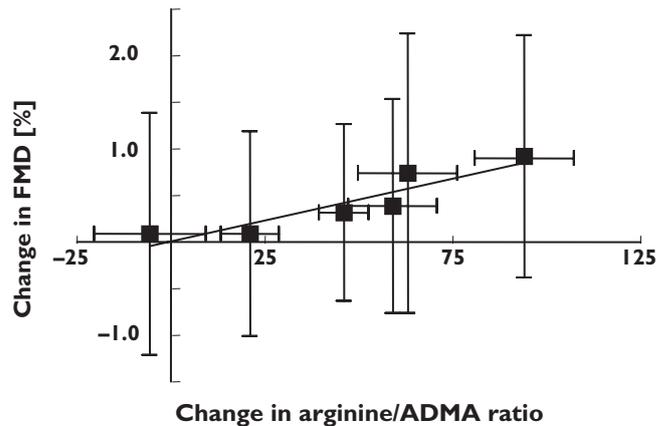
Supplemental Figure

Alternative presentation of data from Figure 4. Changes of flow-mediated vasorelaxation (FMD) *vs.* L-arginine/ asymmetric dimethylarginine ratio (mean $\pm$ SEM)