Assessment of Endothelial Function of the Renal Vasculature in Human Subjects

Christian Delles, Johannes Jacobi, Markus P. Schlaich, Stefan John, and Roland E. Schmieder

Background: \( \text{L-arginine, the substrate of nitric oxide (NO) synthase, and } N^\text{G}-\text{monomethyl-L-arginine (L-NMMA), a competitive inhibitor of endothelial NO synthase, are used to analyze endothelial function of the renal vasculature. However, little is known about the appropriate dose of L-arginine to be used and the duration of action of L-arginine and L-NMMA.} \)

Methods: Twenty-nine healthy male subjects (age, 27 \( \pm \) 1 years) were examined. In protocol 1 (\( N = 17 \)), L-arginine at low (100 mg/kg) and high dose (250 mg/kg), and high-dose L-arginine combined either with L-NMMA (total dose, 4.25 mg/kg; \( N = 9 \)) or placebo (\( N = 8 \)) were given. In protocol 2 (\( N = 12 \)), L-NMMA was given before L-arginine infusion (100 mg/kg). Glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured at rest and at the end of each infusion step.

Results: In protocol 1, L-arginine dose dependently increased RPF and GFR (RPF: 599 \( \pm \) 19 \( \pm \) 630 \( \pm \) 18 \( \pm \) 690 \( \pm \) 24 mL/min, \( P < .05 \); GFR: 111 \( \pm \) 3 \( \pm \) 115 \( \pm \) 3 \( \pm \) 121 \( \pm \) 3 mL/min, \( P < .01 \); for baseline, L-arginine 100 mg/kg and 250 mg/kg, respectively). However, these changes could not be antagonized by coinfusion of L-NMMA to L-arginine 250 mg/kg: RPF and GFR remained unchanged in both the placebo and the L-NMMA group. In protocol 2, L-NMMA decreased RPF (492 \( \pm \) 18 \( \pm \) 567 \( \pm \) 27 mL/min, \( P < .01 \)) and increased GFR (122 \( \pm \) 4 \( \pm \) 118 \( \pm \) 3 mL/min, \( P < .05 \)). These changes could only be partially reversed by subsequent infusion of L-arginine (RPF: 533 \( \pm \) 15 mL/min; GFR: 121 \( \pm \) 4 mL/min; both parameters \( P = \text{NS} \) both L-NMMA and \( v \) baseline).

Conclusions: L-arginine at a dose of 100 mg/kg is sufficient to analyze endothelial function of the renal vasculature. The prolonged effect of L-NMMA and L-arginine must be taken into account in study protocols using both substances. Thus, stimulation and blockade of NO synthase cannot be examined in the same protocol. Am J Hypertens 2002;15:3–9 © 2002 American Journal of Hypertension, Ltd.

Key Words: Kidney, endothelium, nitric oxide, L-arginine, L-NMMA.
Methods
Study Participants
A total of 29 young, male, white, healthy volunteers were included in the study. The study protocol was approved by the Clinical Investigation Ethics Committee of the University of Erlangen-Nürnberg. All subjects gave written consent before study inclusion. All participants underwent a clinical workup including 12-lead electrocardiography and routine laboratory studies to exclude any significant cardiac, hepatic, or renal disease.

Measurement of Hemodynamic Parameters
Renal hemodynamic parameters were determined by constant infusion input clearance technique with inulin (Inutest; Fresenius, Linz, Austria) and sodium para-aminohippurate (Nephrotest; Merck, Sharp & Dohme, Hertfordshire, UK) for GFR and RPF, respectively. These procedures have been described previously. Briefly, the examination was performed in a quiet laboratory from 8 AM to 12 AM with the subject in the supine position. The participants fasted overnight and drank 15 mL/kg of mineral water during the examination in addition to infusion of 500 mL of normal saline. After bolus infusion of inulin and sodium para-aminohippurate over 15 min and a subsequent constant infusion over 105 min, a steady state between input and renal excretion of the tracer substances was reached, and the administration of experimental substances was started. Systemic hemodynamic parameters (ie, blood pressure and heart rate) were monitored by means of an oscillometric device (Dinamap 1846 SX; Criticon, Norderstedt, Germany).

Experimental Protocols
Study participants were assigned to one of the following study protocols. In protocol 1 (N = 17), L-arginine (1 M-L-Argininhydrochlorid-Lösung pfriemer; Pharmacia, Erlangen, Germany) was administered at a dose of 100 mg/kg and then at 250 mg/kg over 30 min, respectively. In the following 30 min, either L-NMMA (Clinalfa, Bad Soden, Germany); 3 mg/kg as a bolus over 5 min followed by infusion of L-arginine at a dose of 250 mg/kg/h over the remaining 25 min, ie, a total dose of 4.25 mg/kg; (N = 9) or placebo (0.9% NaCl over 30 min, N = 8) was infused to determine L-arginine at a dose of 250 mg/kg. Assignment to L-NMMA was double-blind and randomized. Blood samples to determine inulin and para-aminohippurate concentration were drawn at 0, 120, 150, and 180 min. During the last 5 min of each infusion step, blood pressure was monitored every minute, and the mean of these measurements was taken.

In protocol 2 (N = 12), the sequence of administration of L-arginine and L-NMMA was reversed: L-NMMA (same dose as above) was administered followed by the infusion of L-arginine (100 mg/kg over 30 min). Blood samples to determine inulin and para-aminohippurate concentration were drawn at 0, 120, 150, and 180 min. During the last 5 min of each infusion step, blood pressure was monitored every minute, and the mean of these measurements was taken.

Table 1. Baseline characteristics of the study cohort

<table>
<thead>
<tr>
<th>Baseline Characteristics (N = 29)</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76 ± 1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>182 ± 1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.1 ± 0.3</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.97 ± 0.02</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>125 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>82 ± 1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>54 ± 1</td>
</tr>
<tr>
<td>Renal plasma flow (mL/min)</td>
<td>585 ± 16</td>
</tr>
<tr>
<td>Glomerular filtration rate (mL/min)</td>
<td>114 ± 2</td>
</tr>
</tbody>
</table>

Statistical Analysis
All statistical analysis was carried out with SPSS software (release 6.1; SPSS Inc., Chicago, IL). In protocol 1, the unpaired Student t test was used for comparison between the L-NMMA and placebo group. To compare changes in hemodynamic parameters evoked by the experimental substances, paired Student t test analysis was performed in protocol 1 and protocol 2. All values are expressed as means ± standard error of the mean (SEM). A two-tailed P value of <.05 was considered to be significant.

Results
Baseline Data
Baseline characteristics of the study participants are depicted in Table 1. Baseline clinical and hemodynamic data were not significantly different between the subjects assigned to protocol 1 (N = 17) and those assigned to protocol 2 (N = 12). Also, in protocol 1, subjects receiving L-NMMA (N = 9) did not differ from subjects receiving placebo (N = 8) (data not shown).

L-Arginine and Renal Hemodynamics
In protocol 1 (N = 17), L-arginine significantly influenced renal hemodynamics already at a dose of 100 mg/kg; an increase of RPF by 39 ± 13 mL/min (P < .05) and in GFR by 3.9 ± 1.0 mL/min (P < .01) was observed. A further increase of both RPF (P < .01) and GFR (P < .001) was found with L-arginine at higher dose (Table 2, Fig. 1). Mean arterial pressure decreased by 3.0 ± 1.3 mm Hg (P < .05) with L-arginine 250 mg/kg, but not with L-arginine 100 mg/kg. Heart rate increased with L-arginine 100 mg/kg by 2.8 ± 1.0 beats/min (P < .05), but no further increase was found with the higher dose of L-arginine (Table 2, Fig. 1).
Table 2. Effect of L-arginine (subsequent infusions of 100 mg/kg and 250 mg/kg) on hemodynamic parameters (protocol 1, \(N = 17\))

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>L-Arginine (100 mg/kg)</th>
<th>L-Arginine (250 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>89 ± 2</td>
<td>88 ± 2*</td>
<td>86 ± 2*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>55 ± 2</td>
<td>58 ± 2*</td>
<td>58 ± 2†</td>
</tr>
<tr>
<td>Renal plasma flow (mL/min)</td>
<td>599 ± 19</td>
<td>630 ± 18*</td>
<td>690 ± 24‡§</td>
</tr>
<tr>
<td>Glomerular filtration rate (mL/min)</td>
<td>111 ± 3</td>
<td>115 ± 3†</td>
<td>121 ± 3‡§</td>
</tr>
</tbody>
</table>

Significant changes from baseline: * \(P < .05\); † \(P < .01\); ‡ \(P < .001\).
Significant difference between L-arginine 100 and 250 mg/kg: § \(P < .001\).

**L-NMMA and Renal Hemodynamics**

In protocol 2 (\(N = 12\)), L-NMMA (total dose, 4.25 mg/kg) significantly influenced renal and systemic hemodynamics; RPF decreased by 75 ± 18 mL/min (\(P < .01\)) and GFR increased by 4.0 ± 1.6 mL/min (\(P < .05\)). Mean arterial pressure increased with L-NMMA by 4.5 ± 1.2 mm Hg (\(P < .01\)) and heart rate decreased by 5.6 ± 0.8 beats/min (\(P < .001\)) (Table 3, Fig. 2).

**Interaction Between L-NMMA and L-Arginine**

In protocol 1, simultaneous administration of L-NMMA (total dose, 4.25 mg/kg) together with L-arginine 250 mg/kg did not change the hemodynamic parameters compared with L-arginine 250 mg/kg given alone (mean arterial pressure, 83 ± 2 \(v\) 85 ± 3 mm Hg; heart rate, 57 ± 2 \(v\) 57 ± 2 beats/min; RPF, 665 ± 31 \(v\) 669 ± 32 mL/min;
Table 3. Effect of subsequent infusions of \(N^\text{G}\)-monomethyl-L-arginine (L-NMMA; total dose, 4.25 mg/kg) and L-arginine (100 mg/kg) on hemodynamic parameters (protocol 2, \(N = 12\))

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>L-NMMA (3 mg/kg)</th>
<th>L-Arginine (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>86 ± 1</td>
<td>90 ± 2(^\dagger)</td>
<td>84 ± 2(^|$)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>53 ± 2</td>
<td>48 ± 2(^\ddagger)</td>
<td>53 ± 2(^|$)</td>
</tr>
<tr>
<td>Renal plasma flow (mL/min)</td>
<td>567 ± 27</td>
<td>492 ± 18(^\dagger)</td>
<td>533 ± 15</td>
</tr>
<tr>
<td>Glomerular filtration rate (mL/min)</td>
<td>118 ± 3</td>
<td>122 ± 3(^*)</td>
<td>121 ± 4</td>
</tr>
</tbody>
</table>

Significant changes from baseline: \(* P < .05; \dagger P < .01; \ddagger P < .001.\)
Significant difference between L-NMMA and L-arginine: \(\|$ P < .01; \| P < .001.\)

GFR, 120 ± 5 vs 118 ± 4 mL/min, for L-NMMA and L-arginine vs L-arginine alone; NS for all comparisons). Similarly, coadministration of placebo to L-arginine 250 mg/kg caused no changes in hemodynamics as compared with L-arginine 250 mg/kg alone (mean arterial pressure, 87 ± 3 vs 89 ± 3 mm Hg; heart rate, 61 ± 3 vs 59 ± 3 beats/min; RPF, 699 ± 27 vs 712 ± 36 mL/min; GFR, 126 ± 5 vs 124 ± 4 mL/min, for L-arginine with placebo vs L-arginine alone; NS for all comparisons). The effect of L-NMMA vs placebo on hemodynamic parameters is depicted in Fig. 3.

In protocol 2, the changes in hemodynamic parameters induced by L-NMMA were partially restored by subsequent infusion of L-arginine 100 mg/kg. The RPF tended

![Figure 2](image-url)
to increase by $41 \pm 20$ mL/min ($P = .063$), but this change did not reach our predetermined level of significance. The GFR did not change (increase by $1.0 \pm 4$ mL/min, NS); mean arterial pressure decreased by $5.6 \pm 1.5$ mm Hg ($P < .01$); and heart rate decreased by $5.1 \pm 1.0$ beats/min ($P < .001$). Compared with baseline values, no significant changes in hemodynamic parameters induced by l-arginine 100 mg/kg were observed if l-arginine was infused after the administration of L-NMMA (Table 3, Fig. 2).

**Discussion**

Infusions of l-arginine and L-NMMA have been widely used to analyze endothelial function of the renal vasculature.$^{3,5,8-15}$ L-arginine is the substrate for NO synthesis; thus, l-arginine infusion permits the assessment of stimulated NO-dependent vasodilation. L-NMMA is a competitive inhibitor of endothelial NO synthase. Therefore, L-NMMA infusion permits the assessment of basal NO-dependent vasodilation, ie, the contribution of NO to the balance between vasodilating and vasoconstricting agents. It was the aim of the present study to find out an appropriate dose of l-arginine to analyze endothelial function of the renal vasculature. Furthermore, interactions of l-arginine and L-NMMA were studied.

**Which Dose of l-Arginine Is Appropriate?**

Like any other amino acid, l-arginine infusion causes unspecific changes in GFR by osmotic effects.$^7$ Therefore, it appears reasonable to use doses of l-arginine as low as possible to limit the contribution of nonspecific effects to the vasodilating action. In our present study, significant changes in renal hemodynamics were already found with l-arginine at a low dose (100 mg/kg). These data are in accordance with our previous findings and with those from other investigators who used l-arginine at doses of 50 to 150 mg/kg and found similar effects on renal hemodynamics.$^{11-13}$ Because the changes in RPF and GFR are relatively small with low-dose l-arginine infusion, one is compelled to use higher doses of l-arginine to observe more marked results and thus to require smaller sample sizes for clinical studies. Previously, we and other investigators who used l-arginine at doses up to 500 mg/kg and found an increase in RPF by 10% to 15%.$^8-10,12$ However, there are two arguments against the use of l-arginine at such high doses. First, in contrast to low-dose l-arginine, high-dose l-arginine markedly influences systemic hemodynamics. In
a previous study, we found a decrease in mean arterial pressure by 6.8 ± 4.2 mm Hg with \( L \)-arginine 500 mg/kg, and similar results have been reported by other research groups. The interaction of reduced renal perfusion pressure and the NO-dependent effects of \( L \)-arginine are then hardly able to be separated. Second, as mentioned above, nonspecific effects of amino acids on renal hemodynamics must be taken into account. By comparing the effects of \( L \)-arginine with \( D \)-arginine, which is not a substrate for stereospecific NO synthase, we have already shown specific effects of \( L \)-arginine 100 mg/kg on NO-mediated renal vasodilation. In the same study, we have demonstrated the nonspecific effect of \( L \)-arginine 500 mg/kg, too, as this dose caused an increase in RPF as well when \( D \)-arginine was used instead of \( L \)-arginine.

In the present study, we also tested an intermediate dose of \( L \)-arginine (250 mg/kg). In fact, increases in both RPF and GFR were greater with this dose of \( L \)-arginine compared with 100 mg/kg. However, also with this intermediate dose, we observed a significant decrease in mean arterial pressure compared with baseline values, suggesting a pronounced effect of \( L \)-arginine 250 mg/kg on systemic hemodynamics. Similar to \( L \)-arginine 500 mg/kg, it might be difficult to separate effects of \( L \)-arginine 250 mg/kg on renal endothelial function from effects evoked by changes in renal perfusion pressure.

Interactions Between \( L \)-Arginine and \( L \)-NMMA

Subsequent administration of \( L \)-NMMA and \( L \)-arginine is of great interest in study protocols focusing on both basal and stimulated NO production of the renal vasculature. The effect of \( L \)-NMMA on renal hemodynamics has already been examined by others. \( L \)-NMMA was found to decrease RPF and GFR, and, depending on the study cohort, to decrease GFR or not to affect it. However, little is known about the duration of action of \( L \)-NMMA and \( L \)-arginine in the human renal vasculature.

In protocol 1, we administered \( L \)-NMMA on top of the vasodilation due to previous and simultaneous \( L \)-arginine infusion. No effect of \( L \)-NMMA on renal and systemic hemodynamics was found. This might simply be the result of an inappropriate dose of \( L \)-NMMA to counteract the vasodilating effects of \( L \)-arginine. However, for several reasons this explanation appears improbable. First, the dose of \( L \)-NMMA used in the current study was chosen according to data from other investigators who have reported a decrease in RPF by approximately 15% to 30% with the dose applied in our experiments. It should be adequate to counteract the expected 10% to 15% increase in RPF due to \( L \)-arginine 250 mg/kg. Second, in a reverse experiment, Wolzt et al found a reversal of \( L \)-NMMA (3 mg/kg) effects with \( L \)-arginine 510 mg/kg, supporting the dosage chosen in our experiment. Third, administration of \( L \)-NMMA in parallel to the infusion of \( L \)-arginine at a dose of 250 mg/kg on a 1:1 base to the \( L \)-arginine concentration would have been nearly 100-fold the dose of \( L \)-NMMA so far given in human subjects. We wanted to keep the potential risks of \( L \)-NMMA, such as an increase of blood pressure as low as possible for the participants. To our knowledge, only one group of investigators recently has used \( L \)-NMMA at doses markedly greater than 3 mg/kg (50 mg/kg) in human subjects. However, when we planned the present study, such data were not available.

Because of these factors, we favor two other explanations for the lack of \( L \)-NMMA effects on top of \( L \)-arginine infusion. First, nonspecific effects of \( L \)-arginine independent of an increased renal NO production are at least in part responsible for the effects of \( L \)-arginine on renal hemodynamics, even at a dose of 250 mg/kg. Previously we found a significant increase in serum osmolality by approximately 8 mosm/L with \( L \)-arginine infusion at a dose of 500 mg/kg, but not at 100 mg/kg. Second, vasodilation of the renal vasculature after \( L \)-arginine infusion has been observed for at least 30 min (M.P. Schlaich, unpublished data). Over such a long time, regulatory and counterregulatory processes independent of the \( L \)-arginine–NO pathway are expected to play a role and might be the reason for the lack of \( L \)-NMMA effects.

In our protocol 2, the sequence was reversed: first \( L \)-NMMA, and then \( L \)-arginine infusion. Renal hemodynamics at the end of \( L \)-arginine infusion were not significantly different from baseline values, suggesting a partial reversal of the vasoconstrictive effects of \( L \)-NMMA by \( L \)-arginine. Most importantly, however, no increase in RPF beyond the baseline value was observed when \( L \)-arginine was infused after \( L \)-NMMA. An increase in RPF and GFR due to \( L \)-arginine was seen in protocol 1 when \( L \)-arginine was administered first. Of note, the lack of an increase in RPF and GFR beyond the baseline values indicates a prolonged effect of \( L \)-NMMA infusion, despite the great difference in \( L \)-NMMA (3 mg/kg) and \( L \)-arginine (100 mg/kg) concentration given intravenously. This prolonged effect is in accordance with observations by other investigators. In a smaller study cohort it has also been reported that the effects of \( L \)-NMMA can be reversed only with excess doses of \( L \)-arginine (510 mg/kg). Our study demonstrates that a dose of \( L \)-arginine that is sufficient to change renal hemodynamic parameters compared with baseline (100 mg/kg) has no effect compared with baseline when it is administered after \( L \)-NMMA.

We do certainly not rule out an effect of \( L \)-arginine after preinfusion with \( L \)-NMMA, as a clear increase in RPF was observed in protocol 2. Preinfusion of \( L \)-NMMA has changed the conditions under which \( L \)-arginine stimulates NO synthesis. However, the clear demonstration of a prolonged effect of \( L \)-NMMA makes it difficult to interpret the results of \( L \)-arginine on a still partially blocked endothelial NO synthase and is therefore not recommended.

In summary, our data suggest that \( L \)-arginine at a dose of 100 mg/kg is more appropriate than 250 mg/kg to examine endothelial function of the renal vasculature. Systemic administration of \( L \)-NMMA appears to be safe in
healthy human subjects, but potentially hazardous effects due to its prolonged effect on renal hemodynamics must be taken into account if patients with severely impaired renal function are examined. Both L-NMMA and L-arginine have profound effects on renal hemodynamics. In studies with the aim of examining both the baseline and stimulated NO synthase activity of the renal vasculature, L-NMMA and L-arginine should therefore be given on separate days to rule out completely any prolonged effect of either substance. Despite our current study findings, other details concerning L-arginine and L-NMMA infusion (in particular, dose-response curves in human subjects) remain to be resolved.

Acknowledgments
This study was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 423/TP 5B). The assistance of Ingrid Fleischmann, RN, throughout this study is gratefully acknowledged.

References