Acute renal response to LPS: impaired arginine production and inducible nitric oxide synthase activity

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Munger, Karen A., Roland C. Blantz, and Mark J. Lortie. Acute renal response to LPS: impaired arginine production and inducible nitric oxide synthase activity. Am J Physiol Regul Integr Comp Physiol 291: R684–R691, 2006. First published April 13, 2006; doi:10.1152/ajpregu.00873.2005.—We have previously shown in rats that lipopolysaccharide (LPS) causes both decreased renal perfusion and kidney arginine production before nitric oxide (NO) synthesis, resulting in a >30% reduction in plasma arginine. To clarify the early phase effects of LPS, we asked the following two questions: 1) is the rapid change in renal arginine production after LPS simply the result of decreased substrate (i.e., citrulline) delivery to the kidney or due to impaired uptake and conversion and 2) is the systemic production of NO limited by plasma arginine availability after LPS? Arterial and renal vein plasma was sampled at 30-min intervals from anesthetized rats with or without citrulline or arginine (2 mol·min⁻¹·kg⁻¹ iv) a dose with no effect on MAP, renal function, or NO production. Exogenous citrulline was quickly converted to arginine by the kidney, resulting in plasma levels similar to equimolar arginine infusion. Also, the increase in citrulline uptake resulted primarily from increased filtered load and reabsorption. In a separate series, citrulline was infused after LPS administration, verifying that citrulline uptake and conversion persists during impaired kidney function. Last, in rats given LPS, the elevation of plasma arginine had no discernable impact on mean arterial pressure, kidney function, or systemic NO production. This work demonstrates how arginine synthesis is normally “substrate limited” and explains how impaired kidney perfusion quickly results in decreased plasma arginine. However, contrary to in vitro studies, the significant reduction in extracellular arginine during the early phase response to LPS in vivo is not functionally rate limiting for NO production.

citrulline; arginase; argininosuccinate; high-performance liquid chromatography

ARGININE ABSORBED BY THE GUT is largely converted to citrulline before entering the circulation or is taken up by the liver from the portal circulation, generating ornithine and urea (13, 28, 35). Extracellular arginine primarily originates from the kidney, which, in turn, depends on the uptake and conversion of citrulline from plasma (7, 8, 12–14, 32). Estimates based on renal production and plasma concentration indicate that the turnover rate for extracellular arginine is ~40 min (23). Arginine is the precursor to nitric oxide (NO) via the action of at least three isoforms of nitric oxide synthase (NOS), and expression of the inducible isoform (iNOS) results in large-scale NO release (1). Experimentally, iNOS can be induced in vitro with cytokines and in vivo with lipopolysaccharide (LPS) of bacterial origin. Large-scale production of antiseptic NO is part of the innate immune response; however, overproduction may exhaust tonic maintenance of vascular tone, inhibit mitochondrial respiration, and directly modify regulatory proteins (1, 16, 20, 25, 27, 31, 36).

In previous studies, we showed that the LPS-induced changes in kidney arginine production occurred at a time and were of a sufficient magnitude to fully account for decreased plasma content (23). In fact, the production of arginine decreased before the expression of iNOS and therefore was clearly not mediated by NO. However, reduction in plasma arginine coincides with decreased kidney perfusion and citrulline uptake (22, 23), although we could not establish the causal relation between the two. Oddly, arginine production and plasma concentration decrease just as the systems for generating high levels of NO are activated. Therefore it is conceivable that a reduction in arginine production might play a regulatory role in limiting NO synthesis. A body of mostly in vitro work (2, 17, 24, 34) has characterized the dependence of iNOS activity on uptake of extracellular arginine despite seemingly ample intracellular content exceeding the $K_m$ for NO, the “arginine paradox.” Indeed, the concurrent induction of arginine transporters [e.g., cationic amino acid transporter (CAT)-2B] with iNOS suggests not only a dependence on extracellular arginine but also that iNOS activity may be substrate limited (15, 29).

In the present study, we initially conducted experiments aimed at determining arginine infusion protocols that could alter circulating plasma concentrations in a physiologically relevant fashion. We then sought to further clarify early-phase effects of LPS on factors influencing arginine metabolism by addressing the following two questions: 1) is the rapid change in renal arginine production after LPS simply the result of decreased substrate (i.e., citrulline) delivery or due to impaired uptake and conversion and 2) is the systemic production of NO limited by reduced plasma arginine after LPS? The first question is addressed by infusing citrulline in normal rats to establish a time course of changes in arginine production and to determine how substrate delivery is rate limiting. Also, we tested the possibility that LPS could downregulate the cellular uptake or enzymatic conversion of citrulline by administering citrulline after impaired arginine synthesis was established.

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The question of whether circulating arginine availability might limit systemic NO synthesis was assessed by infusing a physiologically relevant quantity of arginine to compensate for reduced kidney production and thus maintain normal plasma levels during LPS induction of iNOS. The results of these studies help elucidate just how the disposition of arginine and related compounds is regulated by renal function.

METHODS

Animal preparation. Male Wistar rats (250–280 g) were anesthetized (100 mg/kg ip thiobutabarbitol; BYK, Konstanz, Germany), and a tracheal tube was inserted to allow easy free breathing. Animals were then prepared for acute terminal studies on a thermoregulated platform by catheterizing the jugular vein, left femoral artery (to place the catheter at the level of the renal artery), the left renal vein, and left ureter. The femoral artery line was used for blood sampling and blood pressure [mean arterial pressure (MAP)] monitoring, while the jugular line was used for volume replacement, [3H]inulin infusion (1 μCi/ml; New England Nuclear, Boston, MA), amino acid infusion, and LPS administration. All solutions were made with sterile PBS (Invitrogen).

The infusion rate of the volume replacement pump was adjusted to maintain the total PBS infusion rate of 1.5 ml/h. Animals were allowed to stabilize for 45 min before the first of two 30-min control urine collection periods were initiated. Great care was taken to reduce the invasiveness of the laparotomy, minimize blood loss resulting from surgery and sampling, provide adequate volume replacement, and maintain core temperature. In control studies (data supplement available online), no significant changes in renal functional parameters occurred over comparable time, although MAP did minimally decline ~ 10 mmHg over 4 h. Also, no significant changes in renal disposition of arginine and NOX were observed under control conditions.

Plasma and urine sampling. Urine was collected in preweighed polyethylene tubes, and the volume was calculated from weight differences. Blood samples (80 μl) were collected from the arterial and renal vein catheters at 30-min intervals in heparinized capillary tubes. The maximum cumulative volume of blood drawn during the longest experiments (300 min) was within 10% of total blood volume in accordance with Veterans Administration San Diego Healthcare System Institutional Animal Care and Use Committee guidelines. For each blood sample, the plasma fraction was separated rapidly by centrifugation. Plasma and urine were promptly ultrafiltered (40 kDa mol mass cutoff; Millipore). All samples were stored at −20°C until further processing.

Experimental protocols. In a pilot study, we determined the dose-response effects of exogenous arginine infusion on plasma concentration. Vehicle or arginine (free base form, n = 3; Sigma) was infused at doses ranging from 0 to 20 μmol·min⁻¹·kg⁻¹ for 30 min at 0.5 ml/h before taking an arterial plasma sample and initiating a new infusion (see Fig. 1 for details). In a separate series of experiments, the effects of continuous exogenous arginine or citrulline infusion (2 μmol·min⁻¹·kg⁻¹, both n = 6; Sigma) were assessed over 90 min to determine steady-state changes in circulating amino acids and renal function. Also, the effect of LPS on citrulline uptake and conversion was assessed by the infusion of citrulline (2 μmol·min⁻¹·kg⁻¹, n = 6) 60 min after administration of LPS (infused iv in 30 s, 1.0 mg/kg in 0.1 ml PBS, Escherichia coli 0111:B4; List Biological Laboratories). A final experiment was conducted in which the effects of exogenous arginine supplement on NO generation and amino acid disposition by the kidney was assessed. After a 60-min arginine infusion (2 μmol·min⁻¹·kg⁻¹), animals received a bolus infusion of LPS, and 30-min sampling periods ensued for 240 min while arginine infusion was maintained. Upon termination of all experiments, animals were killed in accordance with National Institutes of Health guidelines as approved by VASDHS IACUC.

Sample analysis. Aliquots of plasma and urine (10 and 2 μl, respectively) were mixed with 5 ml of scintillation fluid to determine [3H]inulin content. Plasma concentration of NOx was established using the colorimetric Griess reaction in an automated HPLC system (22). A 10-μl aliquot of plasma and urine and appropriate standards were derivatized (Accqtag; Waters) for fluorescence detection of amino acids, as previously described (22). HPLC assays were performed in duplicate (2 separate derivatizations for amino acid analyses and repeated measurement for NOx), and mean values obtained for the same sample were treated as a single value.

Calculations and statistics. The urine excretion rate (UVx) of a substance was derived from the product of urine concentration (U) and rate of urine production (V). Glomerular filtration rate (GFR) was calculated from the clearance of inulin ([U]inulinV/[P]inulin), and renal plasma flow (RPF) was derived from the renal extraction of inulin ([U]inulinV/ΔAV[P]inulin). Factoring renal arterio-venous differences ([ΔAV/P] for arginine, citrulline, ornithine, and NOx with RPF results in a net rate of production or consumption by the kidney (i.e., net renal disposition). The filtered load of a substance was calculated as the product of arterial concentration and GFR. The fraction of renal citrulline uptake that is not accounted for by the complete reabsorption of filtrate is calculated as the difference between filtered load and net renal uptake. Fractional reabsorption for substances excreted in the urine was calculated from the difference between the filtered load and excretion rate and expressed as a percent of the filtered load. Data for each animal were normalized to whole body (2-kidney) renal function expressed per kilogram body weight. Parameters that integrate functional and composition measurements such as renal disposition, filtered load, and excretion were calculated for each individual animal. A one-way ANOVA for repeated measures was used to determine significant changes in repeated measures over time. The degree of significant changes from control (P ≤ 0.05 and 0.01) was determined from post hoc analysis using the Bonferroni test. Paired sample t-test was used to compare tissue concentration with that of control rats (n = 4).

RESULTS

Arginine infusion dose response. Multiple arginine infusion rates were tested in three rats to determine plasma levels achieved in 30 min. As seen in Fig. 1, the infusion of 2 μmol·min⁻¹·kg⁻¹ increased plasma arginine from baseline values near 135 to ~220 μM (P ≤ 0.05), whereas a 20 μmol·min⁻¹·kg⁻¹ infusion rate dose achieved a value of >880 μM (P ≤ 0.01). Interestingly, cessation of exogenous

![Fig. 1. Effect of varying arginine infusion rates on plasma composition. The concentration (conc) in arterial plasma of citrulline (cit), arginine (arg), and ornithine (orn) in anesthetized rats after successive 30-min periods at varying infusion rates (2.0–20.0 μmol·min⁻¹·kg⁻¹). Data points are mean values ± SE; n = 3 experiments. *P ≤ 0.05 and #P ≤ 0.01 vs. values in the first sampling period)](http://ajpregu.physiology.org/)

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arginine infusion saw a rapid return to near-normal values. In addition, an increase in plasma ornithine, but not citrulline, was observed at the higher arginine infusion rates. No arginine was detected in urine at any time point, and no change in MAP or plasma NOx was observed.

Arginine and citrulline infusion. A 90-min infusion of either arginine or citrulline (2 μmol·min⁻¹·kg⁻¹) had no effect on MAP, RPF, or GFR. As in previous studies, no urinary excretion of amino acids was detected, indicating complete reabsorption of infusion substances filtered by the kidney. Infusion of arginine resulted in a significant increase in arterial plasma arginine from 119 ± 8 to 160 ± 6 μM (P ≤ 0.01; Fig. 2A). Increased plasma arginine was maintained at a steady state without altering the circulating levels of endogenous ornithine or citrulline. As seen in Fig. 2B, the concentration of venous arginine was greater, and that of citrulline less, than corresponding arterial values. Arginine infusion had no impact on the baseline values of net renal arginine or citrulline disposition (1.1 ± 0.06 and 0.91 ± 0.09 μmol·min⁻¹·kg⁻¹, respectively; Fig. 2C). The infusion of citrulline resulted in the rapid increase within 30 min of both arterial citrulline and arginine from baseline values of 62 ± 10 and 158 ± 19 μM to 216 ± 63 and 215 ± 17 μM, respectively (both P ≤ 0.01; Fig. 3A).

Venous citrulline concentration also increased significantly from baseline (Fig. 3B) but was always less than corresponding arterial values. Renal disposal under these conditions showed a high reserve capacity for citrulline uptake from 0.78 ± 0.07 to 1.98 ± 0.36 μmol·min⁻¹·kg⁻¹ and arginine output from 0.96 ± 0.12 to 2.45 ± 0.34 μmol·min⁻¹·kg⁻¹ (both P ≤ 0.01) within 30 min and in the ensuing sampling periods (Fig. 3C). The liver and kidney cortex content of arginine and ornithine was evaluated and compared with control values in age-matched controls (Fig. 4). Kidney arginine levels increased modestly in the arginine-infused animals (P < 0.05), whereas ornithine content of liver tissue increased substantially in both citrulline- and arginine-infused rats (P ≤ 0.01 and 0.05, respectively). Citrulline content in either tissue was very low and unchanged by infusion.

Citrulline infusion after LPS. Intravenous LPS was administered over 30 s such that no change in MAP occurred in the first minutes, and a stable blood pressure was maintained throughout the experiment (Fig. 5A). As we have previously observed (23), LPS had a transient anti-diuretic effect whereby urine output decreased by 50% in the first 60 min (P ≤ 0.01) and then gradually returned to baseline values by 120 min (Fig. 5B). Urine samples were also analyzed for amino acids and
were essentially devoid of arginine, ornithine, or citrulline, indicating that reabsorption from filtrate was not compromised.

A progressive decrease in RPF and GFR was significant by 30 min after LPS and remained below 50% of control values ($P \leq 0.01$) during citrulline infusion in the final 60 min (Fig. 5, C and D). As a technical note, we should point out that RPF and GFR are mean values over a 30-min urine collection period; thus, the 0- to 30-min period reflects the transition from normal to impaired renal function. As we have previously reported, LPS caused reduction in the uptake of citrulline from 1.17 ± 0.11 to 0.80 ± 0.26 and the production of arginine from 1.30 ± 0.12 to 0.72 ± 0.08 μmol·min$^{-1}$·kg$^{-1}$ (both $P \leq 0.01$) by the kidney within 60 min (Fig. 6C). After citrulline infusion at 60 min post-LPS, arterial concentration was elevated fourfold from 75 ± 10 to 315 ± 40 μM ($P \leq 0.01$; Fig. 6A). Concurrently, citrulline infusion caused the concentration of renal venous arginine to increase twofold (Fig. 6B). Calculation of the net renal disposition clearly shows that the uptake of citrulline and the release of arginine (1.73 ± 0.50 and 2.65 ± 0.94 μmol·min$^{-1}$·kg$^{-1}$; $P \leq 0.05$ and 0.01, respectively) were significantly enhanced within 30 min of initiating citrulline infusion (Fig. 6C). The filtered load of citrulline decreased from a baseline value of 0.81 ± 0.05 to 0.31 ± 0.07 μmol·min$^{-1}$·kg$^{-1}$ at 60 min post-LPS ($P \leq 0.01$) and then increased almost threefold to 1.91 ± 0.18 μmol·min$^{-1}$·kg$^{-1}$ as a result of citrulline infusion ($P \leq 0.01$). However, the corresponding fraction of renal citrulline uptake from the basolateral aspect, calculated as the difference between filtered load and net uptake, did not change significantly from a baseline value of 0.35 ± 0.08 μmol·min$^{-1}$·kg$^{-1}$ (0.36 ± 0.17 and 0.28 ± 0.19 μmol·min$^{-1}$·kg$^{-1}$ at 60 and 120 min, respectively).

Arginine supplement and iNOS activity. As seen in Fig. 7A, significant reduction in MAP only occurred 180 min post-LPS, and a transient decrease in urine output at 60 and 90 min post-LPS was observed (Fig. 7B). RPF and GFR decreased by 40 and 55% within 60 min of LPS infusion that persisted over the subsequent collection periods (both $P \leq 0.01$; Fig. 7, C and D). A steady state of elevated plasma arginine was achieved (242 ± 15 μM) in arterial plasma (Fig. 8A), and a normal arginine production by the kidney persisted (1.03 ± 0.37 μmol·min$^{-1}$·kg$^{-1}$). As seen in Fig. 8B, the concentration of arginine in venous plasma decreases after LPS but remains higher than corresponding arterial concentrations such that net production of arginine by the kidney persisted, although at a significantly attenuated rate (Fig. 8C). Arterial arginine concentration after LPS decreased to a stable value (165 ± 40 μM at 30 min) equivalent to baseline values seen in normal rats. Measurement of NOx concentration in plasma and the corresponding renal parameters are shown in Fig. 9 together with values from previously reported experiments conducted in parallel without arginine supplement (23). As seen in Fig. 9A, LPS administered to rats with high plasma arginine resulted in an increase in arterial plasma NOx from baseline (36 ± 4 μM).

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Fig. 4. Tissue composition after infusion of arginine or citrulline. A comparative histogram of liver and kidney arginine and ornithine content from anesthetized rats given citrulline and arginine (2.0 μmol·min$^{-1}$·kg$^{-1}$ for 90 min) vs. control. Data points are mean values ± SE; $n = 6$. *$P \leq 0.05$ and #P ≤ 0.01 vs. control.

Fig. 5. Effect of lipopolysaccharide (LPS) before and after citrulline infusion. Temporal changes in mean arterial blood pressure (BP), urine flow (UV), renal plasma flow (RPF), and glomerular filtration rate (GFR). Anesthetized rats received a bolus of LPS (1 mg/kg) at $t = 0$ min and a continuous infusion of 2.0 μmol·min$^{-1}$·kg$^{-1}$ citrulline starting at $t = 60$ min. Data points are mean values ± SE; $n = 6$. *$P \leq 0.05$ and #P ≤ 0.01 vs. control ($t = 0$ min).
that was significant after 90 min and fourfold higher by 240 min ($52 \pm 4$ and $189 \pm 19$ μM, $P < 0.05$ and 0.01, respectively; Fig. 9A). However, the concentration of NOx achieved in the plasma of arginine-infused rats appears no different than levels observed in nonsupplemented animals. In addition, we verified that the renal handling of NOx was not altered by arginine infusion (Fig. 9, B and C). The filtered load of NOx increased in proportion to increasing plasma concentration despite a reduction in filtration rate. Last, only a minor fraction of filtered NOx appeared in urine, since >90% of the filtered load was reabsorbed.

**DISCUSSION**

There is little doubt that a significant component of septic and endotoxic shock involves untoward effects of iNOS activity. Also, it is clear that renal perfusion is paradoxically constricted at a time when systemic vascular tone is depressed. We and others (6, 19, 21, 26, 33) have used various approaches to examine the possible relation between these phenomena at time points after iNOS expression. However, time course studies in which we assessed changes in function preceding de novo iNOS expression and activity obviated effects that were independent of enhanced arginine consumption and NO production. Therefore, in the current studies, we investigated the very early events after LPS in terms of arginine production and the potential impact on the activity of newly expressed iNOS. The results of these studies cause us to reassess our understanding of how renal function responds to endotoxin and provide a better understanding of the functional impact in vivo of arginine availability for NO production.

The results of experiments in which we infused arginine either at varying or constant rates (Figs. 1, 2, and 8) provide some interesting observations. Considering the quantity of arginine infused ($2–20$ μmol·min$^{-1}$·kg$^{-1}$) in relation to the extracellular content ($<40$ μmol/kg), plasma levels rose modestly and normalized rapidly during the washout periods. These phenomena attest to the very high capacity of low-affinity arginine exchangers able to rapidly normalize extracellular...
concentration (i.e., 100 –150 μM). The continuous infusion of arginine (2.0 μmol·min⁻¹·kg⁻¹; ~2 times the normal kidney production rate) resulted in a steady-state elevation of plasma content by only ~25–35% within 30 min without altering renal function, systemic blood pressure, or plasma NOx. This indicates that NO production in normal animals is not readily enhanced by providing excess substrate. It should be noted that a broad range of physiological effects have been documented after experimental and therapeutic administration of arginine (4, 28). However, the doses administered to elicit such effects are as much as two to four orders of magnitude greater than those used in the present study.

Supplementing exogenous arginine had no effect on the net renal production of arginine or on the uptake of citrulline despite the fact that kidney tissue levels decreased as a result of the reabsorption of filtered amino acids (Fig. 4B). This is unexpected, as both argininosuccinate synthase and lyase are subject to product inhibition by arginine and suggest the possibility of intracellular arginine compartmentalization in proximal tubules. Infusion of arginine at 2 μmol·min⁻¹·kg⁻¹ did not alter plasma ornithine concentration in control animals, whereas at higher infusion rates (Fig. 1), a significant increase was observed, suggesting some functional reserve in arginase activity. Indeed, arginine infusion resulted in a twofold increase in liver ornithine but no change in arginine content (Fig. 4A). These data serve to demonstrate the dynamic nature of arginine production and degradation, as well as the reserve capacity of amino acid transporters able to rapidly normalize plasma arginine concentration.

As seen in Fig. 3, increasing the plasma concentration of citrulline delivered to the normal kidney resulted in enhanced arginine production within 30 min. This confirms the results of others demonstrating that normal arginine output by the kidney is limited by the availability of delivered citrulline (14) and furthermore that a change in RPF can quickly alter kidney production. Reduced arginine synthesis by the kidney after LPS was reversed by augmenting substrate delivery, indicating that cellular uptake and conversion were not impaired in this model of sepsis. The net molar uptake and conversion of citrulline by the kidney in these studies account for ~60–70% of the arginine and ornithine produced by the kidney and are in agreement with recently published data in mice (5).

The results of these studies demonstrate how uptake of citrulline exceeds the filtered load by 20–40% under normal
conditions, indicating inward transport from the basolateral aspect of tubule epithelia. Interestingly, in the experiments in which citrulline was infused after changes in GFR, we noted that the difference between total citrulline uptake and the filtered load remains constant. Because filtered citrulline is fully reabsorbed, this demonstrates that basolateral uptake capacity is limited and that enhanced arginine production is primarily dependent on GFR, as postulated by Brosnan and Brosnan (9) and Dhanakoti et al. (14). As detailed in RESULTS, this was most evident when citrulline was administered to rats 60 min post-LPS and filtered load increased 500%. The basolateral component of renal uptake did not change significantly from 0.35 ± 0.08 μmol·min⁻¹·kg⁻¹, whereas substrate delivery to the kidney decreased by one-half, due to reduced RPF, and then increased threefold after the infusion of citrulline. A better characterization of basolateral citrulline transport would be of interest since it may play an important role in the synthesis of arginine by remnant or nonfiltering kidneys.

Simply infusing arginine per se had no impact on plasma NOx concentration or its clearance by the kidney in normal rats, indicating that constitutively expressed NOS is not limited by extracellular arginine availability. After LPS administration, changes in MAP (Fig. 7) and the progressive accumulation of NO metabolites in plasma (Fig. 9A) provide clear evidence of newly expressed iNOS activity. However, no difference in the plasma concentration of NOx was observed between arginine-infused and nonsupplemented rats within 4 h of LPS administration. This also holds true with respect to renal functional parameters where one might have suspected that a decrease in arginine production could play a role in increasing renal vascular tone, yet addition of exogenous arginine had no effect on decreased RPF or changes in MAP.

The fact that maintaining normal extracellular arginine concentration did not enhance NO production is perhaps counterintuitive as much has been made of the dependency on extracellular arginine for iNOS activity. Cell uptake of arginine occurs via multiple facilitated transporters or exchangers of varying selectivity (11). The CAT-2B transporter has been studied extensively, since it is also induced by LPS and/or cytokines (18) and has been shown in ex vivo studies to be rate limiting for iNOS activity in certain cell types (3, 24, 30). Indeed, normal plasma arginine is maintained at or just below the half-saturation constant for uptake (Kₘ) of the CAT-2B transporter (10, 11) such that a 30% reduction in extracellular concentration should have a significant impact on the rate of uptake. Thus, although an extracellular source of arginine may be required for NO production and the rate of uptake is likely to be reduced after LPS, iNOS activity (with a Kₘ below that of CAT-2B: 10–20 μM) does not appear to be substrate limited under these conditions.

This study has demonstrated that rapid changes in arginine production by the kidney in the minutes after LPS results from decreased citrulline delivery secondary to a reduction in renal perfusion. More specifically, a decrease in the filtered load of citrulline resulting from low GFR was the primary limiting factor in the production of arginine. In addition, because restoration of normal circulating arginine levels had no discernable effect on the generation of NO, it does not appear that the early effects of LPS on kidney arginine production limit iNOS activity. Questions remain as to what mechanisms triggered by LPS cause a reduction in kidney perfusion before the onset of de novo iNOS activity. Also, it will be important to determine whether and when plasma arginine availability becomes limiting for NO production in the course of septicemia or endotoxic shock.

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