L-Arginine supplementation in pigs decreases liver protein turnover and increases hindquarter protein turnover both during and after endotoxemia[1–3]

Maaike J Bruins, Peter B Soeters, Wouter H Lamers, and Nicolaas EP Deutz

ABSTRACT

Background: Accumulating evidence suggests that L-arginine, under conditions of septicemia, not only enhances immune function but also improves protein metabolism.

Objective: Because the effect of L-arginine administration on the protein metabolism of different organs is unknown, the aim of the study was to elucidate the effects of exogenous supplementation of L-arginine during endotoxemia on the in vivo protein metabolism of individual organs and at the whole-body level.

Design: Female pigs were cannulated with catheters in the aorta and the splenic, caval, portal, hepatic, and renal veins, enabling measurements across the hindquarter, portal-drained viscera, liver, and kidneys. Endotoxemia was induced by a 24-h continuous intravenous infusion of endotoxin (3 $\mu g \cdot kg$ body $wt^{-1} \cdot h^{-1}$). At 8 h, an intravenous infusion of L-arginine was started ($n = 8$). Control pigs ($n = 6$) received L-alanine. At 24 h, blood was sampled. After cessation of the endotoxin infusion, L-arginine and L-alanine infusions were continued as a supplement in the enterally infused diet. At 48 h, blood samples were obtained during the postendotoxemic and nutritionally supported conditions. Stable isotopes were used to assess protein metabolism and phenylalanine hydroxylation.

Results: Both during and after the endotoxin challenge, L-arginine administration enhanced protein synthesis and degradation across the hindquarter and simultaneously reduced protein synthesis and degradation in the liver at equal rates. Protein turnover across the kidneys and portal-drained viscera remained unaffected. After endotoxemia, L-arginine infusion decreased whole-body protein turnover without affecting the net protein balance.

Conclusion: L-Arginine administration affects protein turnover of the muscle area and the liver oppositely. *Am J Clin Nutr* 2002;75:1031–44.

KEY WORDS Arginine supplementation, enteral nutrition, endotoxin, sepsis, disease model, intestine, gut, liver, muscle, kidney, stable isotopes, protein metabolism, acute phase protein, phenylalanine hydroxylation, pigs

INTRODUCTION

L-Arginine plays a pivotal role in many bodily functions, including protein synthesis and breakdown (reviewed in 1). In stressful situations such as injury, burn, and sepsis, L-arginine may become an indispensable amino acid (2). In animals and humans with sepsis, plasma L-arginine concentrations are markedly low, and low L-arginine concentrations are correlated with a worse prognosis in septic patients (3). In times of stress or severe injury, the endogenous supply of L-arginine may become critical for sustenance of homeostasis. Dietary L-arginine has been shown to enhance the immune system (4) and wound healing (5). Under catabolic conditions, L-arginine has anticatabolic properties because it improves nitrogen balance (5–8). Moreover, L-arginine may be involved in protein metabolism through its metabolites. L-Ornithine serves as a precursor in the synthesis of polyamines that are required for DNA synthesis and cell growth (9), and of proline, which is used in collagen synthesis (5). Dietary supplementation with L-ornithine or L-ornithine analogues was shown to exert anticatabolic or anabolic effects (10). L-Arginine–derived nitric oxide, on the other hand, seems to be involved in decreased protein synthesis in vitro (11–13).

Results of clinical studies using L-arginine–supplemented dietary formulas suggest a significant benefit for injured patients, not only through improved immune function but also through improved nitrogen balance (14–16). However, because these formulas contain many different supplements, it is not possible to discriminate the effect of L-arginine as an individual component.

It remains to be elucidated whether L-arginine supplementation in critical illness can modify protein metabolism; therefore, we assessed the effect of L-arginine supplementation on protein metabolism in the hindquarter, portal-drained viscera, liver, and kidney in this model of endotoxemia.

Because, in general, endotoxin is slowly released from the gut (17) and only low doses of endotoxin are detected in the circulation (18) in humans with sepsis, we infused a low dose of endotoxin for several hours in pigs. We hypothesized that exogenous

[1] From the Department of Surgery, Maastricht University, Maastricht, Netherlands (MJB, PBS, and NEPD), and the Department of Anatomy and Embryology, University of Amsterdam (WHL).

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[3] Address reprint requests to NEP Deutz, Department of Surgery, Fac II, Maastricht University, PO Box 616, 6200 MD Maastricht, Netherlands. E-mail: nep.deutz@ah.unimaas.nl.

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L-arginine administration during the initial phase of sepsis could induce an excessive production of nitric oxide. Therefore, L-arginine was started a few hours after initiation of the endotoxin infusion to prevent nitric oxide–mediated hypotension and shock.

The aim of the study was to investigate the effects of intravenously administered L-arginine during endotoxemia and of intragastrically administered L-arginine after endotoxemia on protein metabolism. The use of stable isotopes in a multicatheterized animal model enabled us to investigate the changes in protein synthesis and breakdown at the whole-body level and in individual organs. To our knowledge, the influence of L-arginine treatment during or after endotoxemia on protein metabolism in individual organisms has not been studied.

MATERIALS AND METHODS

Animals

Pigs were the offspring of Yorkshire and Dutch Landrace species. Three-month-old pigs weighing 20–22 kg body wt were individually housed and fed 1 kg regular pig feed (149 g crude protein/kg body wt, or 16% crude protein; Landbouwbelang, Roermond, Netherlands) daily, which supported a growth rate of ≈300 g/d. Before surgery, pigs were randomly assigned to 1 of 2 treatment groups. The Animal Ethics Committee of Maastricht University approved the study.

Surgical procedure

The night before surgery, the pigs were not allowed food. One hour after the pigs were premedicated with 10 mg azoperone/kg body wt (Stresnil; Janssen Pharmaceutica, Beersse, Belgium) intramuscularly, anesthesia was induced with a mixture of nitrous oxide:oxygen (1:2, by vol) and halothane (0.8%). After intubation, the pigs were intravenously administrated 6.25 mg Lincomycin·2 HCl/kg body wt (AUV, Cuyk, Netherlands) as a bactericidal prophylactic and 12.5 mg Spectinomycin·HCl lyophil/kg body wt (AUV) as a bacteriostatic prophylactic. Fluxinine (50 mg/kg body wt, Finadyne; Schering-Ploegh, Brussels, Belgium) was given to prevent coagulation and as a postoperative analgesic. During surgery, anesthesia was maintained with a mixture of nitrous oxide:oxygen and halothane and with intravenous Lactetrol (5.76 g NaCl/L, 0.37 g KCl/L, 0.37 g $CaCl_2$/L, 0.2 g $MgCl_2$/L, 5 g sodium lactate/L, and methyl *p*-hydroxybenzoic acid/L; Janssen Pharmaceutica). The surgical procedure was described in detail previously (19, 20). In brief, 8 vessels were cannulated after a midline incision was made. Two catheters were inserted in the abdominal aorta; one just above the bifurcation (A1) and one above the right renal vein (A2). Two catheters were inserted in the inferior caval vein (V1 and V2 catheters). Furthermore, catheters were inserted in the portal, hepatic, splenic, and renal veins. The abdominal aorta and the splenic vein catheters were used for the infusion of *p*-aminohippuric acid (PAH) to measure plasma flow, and the V2 catheter was used for the infusion of isotopes and endotoxin. Blood collected from the abdominal aorta (A2) in combination with the portal vein, hepatic vein, V1, and renal vein catheters was used to measure amino acid flux in the portal-drained viscera, liver, hindquarter, and kidney, respectively. In addition, a gastrostomy catheter was inserted in the stomach to enable infusion of a liquid diet. All catheters were tunneled through the abdominal wall and skin.

The pigs wore a canvas harness to protect the catheters and the stoma and to allow easy handling. Postoperatively, the pigs were fed 100 g on day 1, 200 g on day 2, and 1 kg on day 3 and thereafter. To prevent infections and to maintain patency, the catheters were regularly flushed with a mixture of saline (150 mmol/L), gentamycin sulfate (20 g/L, 5%; AUV), and chymotrypsin (0.225 U/L; Merck, Darmstadt, Germany).

Postoperative care

The postoperative care was standard (19, 20). The pigs were placed in a movable cage to get accustomed to their experimental condition. The postoperative care procedures were standard (19, 20). During the recovery (10–15 d) and experimental (7 d) periods, the animals remained healthy and had no signs of infection. Four weeks after the operation, the position of the catheter tips was checked under anesthesia with fluoroscopy.

Experimental protocol

From the tenth postoperative day onward, a ready-to-use liquid diet with an osmolarity of 255 mOsmol/L (63 g casein protein/L, 141 g carbohydrate/L, 11 g sugar/L, 128 g polysaccharides/L, 49 g fat/L, and unspecified amounts of minerals, trace elements, and vitamins; Nutrison Steriflo Protein-Plus; Nutricia, Zoetermeer, Netherlands) was intragastrically infused into the gastrostomy catheter via a swivel system. This diet is high in protein, has an optimal protein-energy ratio, and contains all the vitamins, minerals, and other nutritional elements to meet the requirements of clinical patients and growing pigs (21). To accustom the pigs to the diet, the pigs received 500 g of their regular diet plus the liquid diet at a rate of 2 mL·kg body $wt^{-1}\cdot h^{-1}$ on day −3, ie, 10 d postsurgery. On the following 3 d, the diet was infused at a rate of 4 mL·kg body $wt^{-1}\cdot h^{-1}$ in the absence of the regular diet, corresponding with 0.3 g protein·kg body $wt^{-1}\cdot h^{-1}$. The experimental design is depicted in **Figure 1**.

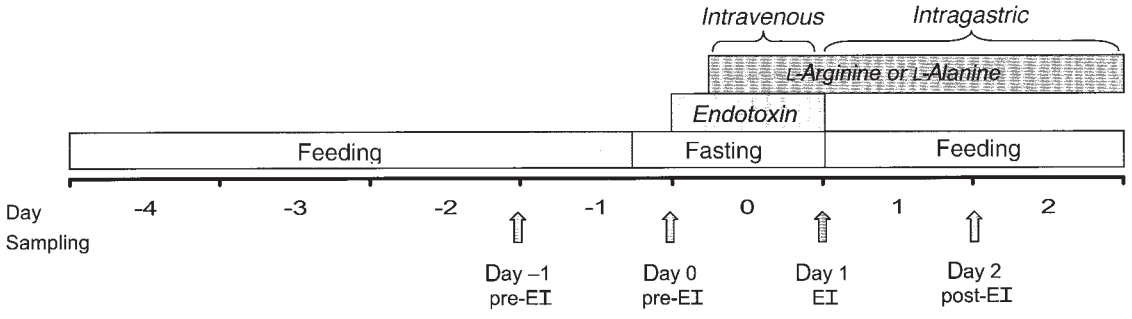

FIGURE 1. Illustration of the experimental design. EI, endotoxin infusion.

There were a total of 14 pigs in the study. On the last day (day −1) of the enteral infusion, control blood samples were obtained from all 14 pigs in the postprandial state. After an 8-h fast, control blood samples were obtained from all 14 pigs in the postabsorptive state (day 0). Immediately afterward, all 14 pigs were infused with lipopolysaccharide endotoxin from *Escherichia coli* (serotype 055:B5, Sigma Chemical Co, St Louis) dissolved in saline at a rate of 3 μg·kg body $wt^{-1}\cdot h^{-1}$ via the V2 catheter for 24 h. For the next 24 h, all pigs were infused with saline to replenish intravascular volume losses: 30 mL·kg body $wt^{-1}\cdot h^{-1}$ during the first 8 h and 20 mL·kg body $wt^{-1}\cdot h^{-1}$ during the last 16 h.

Eight pigs were intravenously infused with L-arginine (isoosmolar rate of 5.3 μmol·kg body $wt^{-1}\cdot min^{-1}$, pH 7.3), starting 8 h after initiation of the endotoxin infusion (arginine group). Six pigs were infused with alanine (isoenergetic rate of 10.6 μmol·kg body $wt^{-1}\cdot min^{-1}$, pH 7.3) to correct for the effect of giving an amino acid. The following day (day 1), 24 h after initiation of the endotoxin infusion, blood samples were collected from the arginine group and the control group and then the infusions of endotoxin, saline, and amino acids were stopped. Immediately thereafter, enteral nutrition was restarted (4 mL·kg body $wt^{-1}\cdot h^{-1}$). The intravenous infusion of L-arginine or L-alanine continued at equal infusion rates through the gastrostomy catheter as a supplement in the enteral meal. The following day (day 2), 24 h after cessation of the endotoxin infusion, blood samples were collected from the arginine group and the control group. The arterial blood pressure, weight, and temperature of the pigs were monitored throughout the experimental period.

Infusion protocol

Background blood samples were taken before the infusions began. On the morning of the trial, 1 h before the endotoxin infusion began, a primed infusion of 25 mmol PAH (A 1422; Sigma Chemical Co) was administered at a rate of ≈40 mL/h through the splenic vein and abdominal aorta (A1) catheters after an initial bolus of 5 mL (19). Directly after the primed, constant infusion of PAH, a primed, constant infusion of the stable isotopes L-[*ring*-$^2H_5$] phenylalanine ([D_5]phenylalanine; prime: 1 μmol/kg body wt; infusion: 1.9 μmol·kg body $wt^{-1}\cdot h^{-1}$), L-[*ring*-3,5-$^2H_2$] tyrosine ([D_2] tyrosine; prime: 1 μmol/kg body wt; infusion: 1.9 μmol·kg body $wt^{-1}\cdot h^{-1}$), and L-[1-^{13}C]valine ([^{13}C]valine; prime: 5.3 μmol/kg body wt; infusion: 5.3 μmol·kg body $wt^{-1}\cdot h^{-1}$) was started via the V2 catheter. In addition, a primed, constant infusion of L-[*guanidino*-$^{15}N_2$]arginine, L-[*ureido*-^{13}C; 5,5-$^2H_2$]citrulline, and [^{13}C] urea was administered for the purpose of measuring nitric oxide metabolism, the results of which will be reported elsewhere. Stable isotopes were purchased from Mass Trace, Woburn, MA. During the last 60 min of the infusion protocol, an isotopic plateau (the calculated slope of isotopic enrichment against time not different from zero) was observed (22). One hour after the start of the primed infusion of PAH, steady state conditions for PAH (data not shown) were obtained. Blood samples were collected in triplicate at 15-min intervals, during the last 60 min of the infusion of PAH and the isotopes.

Sample processing

Immediately after the blood samples were collected, the blood was distributed into heparin-containing tubes (Sarstedt, Nümbrecht, Germany) on ice. For the analysis of blood gases (arterial pH, HCO_3^-, arterial pressure of oxygen and carbon dioxide, and standard pressure of oxygen), 0.2 mL blood was sealed in 1-mL airtight heparin-containing syringes and immediately analyzed with an automatic blood gas system (Acid Base Laboratory, Radiometer, Copenhagen). Hematocrit was obtained with a microfuge. For the measurement of PAH concentrations, 300 μL whole blood was added to 600 μL of 120 g trichloroacetic acid solution/L; the solution was thoroughly mixed and centrifuged (4°C, 5 min, 8500 × *g*) followed by the collection of supernatant fluid and plasma. The plasma was kept on ice until used. For the amino acid analysis, 500 μL plasma was deproteinized by mixing it with 20 mg dry sulfosalicylic acid. All samples were stored at −80°C until analyzed.

Biochemical analysis

PAH was detected spectrophotometrically after deacetylation of the supernatant fluid at 100°C for 45 min (23). The plasma concentrations of amino acids were measured with a fully automated HPLC system (Pharmacia, Woerden, Netherlands) after precolumn derivatization with *o*-phthaldialdehyde (24). The enrichments of amino acids were calculated as the tracer-tracee ratios (TTRs) and were calculated with a fully automated online liquid chromatography–mass spectrometry (LC-MS; Thermoquest LCQ, Veenendaal, Netherlands) system connected to an HPLC system (22).

Concentrations of the acute phase proteins haptoglobin, fibrinogen, and α_1-antitrypsin and of total protein in plasma were measured with a nephelometer (model BN 100; Dade-Behring Vertriebs GmbH and Co, Schwalbach, Germany). Proteins were determined by using antihuman haptoglobin, fibrinogen, and α_1-antitrypsin antibodies from rabbits (Dade-Behring) in the same way as human plasma samples. Standard curves for haptoglobin and fibrinogen were constructed by using a purified human (Dade-Behring) and a secondary porcine standard (Sigma Chemical Co). High correlation coefficients (>0.9) were found for the 2 proteins between the standards. Because a porcine standard for fibrinogen was not available, protein concentrations were expressed in human units.

Calculations

The total of amino acids represents the sum of measurable α-amino acids (glutamine, glycine, threonine, histidine, citrulline, alanine, taurine, arginine, α-amino butyric acid, tyrosine, valine, methionine, isoleucine, phenylalanine, tryptophan, leucine, ornithine, and lysine) and the total branched-chain amino acids (BCAAs) represents the sum of valine, leucine, and isoleucine.

Organ phenylalanine and valine kinetics

The portal-drained viscera are defined as the total of all portal-drained organs, mainly the intestines. The splanchnic area includes the portal-drained viscera and liver; therefore, calculations for the liver were made by subtracting values for the portal-drained viscera from those for the splanchnic area. Substrate metabolism across the hindquarter, the portal-drained viscera, the liver, and the kidneys was calculated in a 2-compartment model as described previously (25). The plasma flow rates (mL·kg body $wt^{-1}\cdot min^{-1}$) across the organs were calculated as follows:

$$Flow_{blood} = I/([PAH]_V - [PAH]_A) \quad (1)$$

where I is the rate at which PAH is infused (nmol·kg body $wt^{-1}\cdot min^{-1}$) and $[PAH]_V$ and $[PAH]_A$ are the concentrations (μmol/L) of PAH in venous and arterial blood, respectively. Equation *1* is based on the principle of indicator dilution methods. The blood flow was converted to plasma as follows:

$$\text{Flow}_{\text{plasma}} = \text{flow}_{\text{blood}} \times [100/(100 - \text{hematocrit})] \quad (2)$$

Substrate net balance (NB; in nmol·kg body $wt^{-1}\cdot min^{-1}$) was calculated as shown below:

$$NB = \text{flow} \times ([AA_V] - [AA_A]) \quad (3)$$

where $[AA_V]$ and $[AA_A]$ are the plasma concentrations of the amino acid (μmol/L) in venous and arterial blood, respectively. Therefore, a positive NB represents net efflux and a negative NB represents net influx of substrate across the organ.

The NB of the tracer (nmol·kg body $wt^{-1}\cdot min^{-1}$) was calculated similarly, but corrected for the background (pretracer infusion) TTR values:

$$\text{Tracer NB} = \text{flow} \times \{([AA_A] \times TTR_A) - ([AA_V] \times TTR_V)\} \quad (4)$$

The disposal rate (nmol·kg body $wt^{-1}\cdot min^{-1}$) is the total rate of metabolism of the amino acid (incorporation into protein plus degradation) and was calculated as follows:

$$\text{Disposal} = \text{tracer NB}/TTR_V \quad (5)$$

The TTR_V was thought to approach best the intracellular enrichment (precursor pool) of the organ (26). Because the NB of an amino acid across an organ is the net difference between production and disposal, the production is represented as

$$\text{Production} = NB + \text{disposal} \quad (6)$$

In the muscle, gut, and kidneys, the disposal and production of phenylalanine are a reflection of protein synthesis and protein breakdown, respectively, because these organs have relatively low phenylalanine 4-monooxygenase activity (27). Valine disposed in these organs can either become transaminated or be used in protein synthesis (28). In the liver, valine is mainly used for protein synthesis because the valine degradation rate is considered to be low (28, 29). Disposal of phenylalanine by the liver is a combination of protein synthesis and hydroxylation of phenylalanine to tyrosine (27). To calculate the rate of phenylalanine hydroxylation in an organ, the conversion rate of $[D_5]$phenylalanine to $[D_4]$tyrosine (Phe → Tyr) was calculated by using Equations *7*–*10*. The arterial $[D_5]$phenylalanine TTR was thought to best reflect the precursor pool. Because of the eventual loss of tyrosine across the organ by fractional extraction, the NB of $[D_4]$tyrosine ($NB_{[D_4]Tyr}$) was corrected. This fractional extraction of tyrosine ($FE_{[D_4]Tyr}$) was estimated by using $NB_{[D_2]Tyr}$ as calculated in Equation *7*. $NB_{[D_4]Tyr}$, as calculated in Equation *8*, was used to calculate Phe → Tyr (Equation *9*).

$$FE_{[D_4]Tyr} = NB_{[D_2]Tyr}/(\text{flow} \times [AA_A] \times TTR_A)_{[D_2]TYR} \quad (7)$$

$$NB_{[D_4]Tyr} = \text{flow} \times \{([AA_V] \times TTR_V) - ([AA_A] \times TTR_A)\} \times (1 - FE)_{[D_4]Tyr} \quad (8)$$

$$\text{Phe} \rightarrow \text{Tyr} = NB_{[D_4]Tyr}/TTR_{A[D_5]Phe} \quad (9)$$

Across the portal-drained viscera, the total production rate of a substrate is the combination of the substrate derived from endogenous production plus the output of the substrate absorbed by the gut from the enterally infused meal that is not retained for disposal and escapes metabolism. The total production rate of a substrate can be corrected for the contribution of the substrate derived from nondisposed absorption. This gives a good approximate of the part of the substrate produced that represents endogenous production in the portal-drained viscera.

Whole-body phenylalanine and valine kinetics

The whole-body rate of appearance (RA; in nmol·kg $bw^{-1}\cdot min^{-1}$) of phenylalanine, valine, or tyrosine was derived as follows:

$$\text{Whole-body RA} = I/TTR_A \quad (10)$$

where I is the infusion rate (nmol·kg body $wt^{-1}\cdot min^{-1}$) of the tracers $[D_5]$phenylalanine, $[^{13}C]$valine, or $[D_2]$tyrosine.

The whole-body RA of phenylalanine was used as an indication of whole-body protein breakdown because this amino acid cannot be newly synthesized. The rate of whole-body phenylalanine hydroxylation was calculated accounting for the conversion of $[D_5]$phenylalanine to $[D_4]$tyrosine:

$$\text{Whole-body Phe} \rightarrow \text{Tyr} = \text{whole-body } RA_{Tyr} \times TTR_{A[D_4]Tyr}/TTR_{A[D_5]Phe} \quad (11)$$

where whole-body RA_{Tyr} is calculated with the use of $[D_2]$tyrosine. The whole-body rate of disappearance of $[D_5]$phenylalanine is a combination of the whole-body rate of protein synthesis and whole-body Phe → Tyr:

$$\text{Whole-body protein synthesis} = \text{whole-body protein breakdown} - \text{whole-body Phe} \rightarrow \text{Tyr}$$

Statistics

The results are presented as means ± SEMs. If the normality or equal variance test failed, data were transformed or log transformed where appropriate. The data were analyzed with a two-factor repeated-measures analysis of variance, with time (days –1, 0, 1, and 2) as a within-subject factor and treatment (arginine or alanine) as a between-subject factor. The level of significance was set at $P < 0.05$, and P values are given for the time effect, the group effect, and the time × group interaction. When an overall significance for time was observed, univariate F tests were used to evaluate contrasts among the different days to assess the immediate effect of endotoxin infusion plus fasting compared with fasting state baseline values (day 1 compared with day 0) and the long-term effect of the endotoxin infusion plus fasting period under fed conditions as compared with fed state baseline values (day 2 compared with day −1). Contrasts between day 0 and day 1 were assessed for the arginine variables in the control group and for the alanine variables within the arginine group after significant time × group interactions were observed. When overall significant time × group interactions were observed, pairwise group comparisons were performed on days 1 and 2 with the use of Bonferroni post hoc tests to adjust the nominal level of significance for the multiple comparisons made ($P < 0.05$ with correction).

RESULTS

Organ plasma flow, temperature, and blood gas values

The plasma flow across the hindquarter, liver, and kidneys did not change significantly over time (**Table 1**). Although an overall time effect for the portal-drained viscera plasma flow was observed, no significant effect of the fasting endotoxin infusion period was detected (day 1 compared with day 0 and day 2 compared with day −1). Organ plasma flow did not change significantly after L-arginine treatment. The body temperature of the pigs changed significantly over time (**Table 2**); the temperature increased after the fasting endotoxin infusion period (from day 0

TABLE 1
Plasma flow in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2)[1]

	Time				P[2]		
	Day –1	Day 0	Day 1	Day 2	Time	Time × group	Group
	mL·kg body wt^{-1}*·min*$^{-1}$						
Portal-drained viscera							
Control group	40 ± 3	37 ± 4	39 ± 4	48 ± 7	0.03	0.95	0.50
Arginine group	35 ± 2	35 ± 3	42 ± 6	46 ± 5			
Liver							
Control group	50 ± 9	59 ± 7	66 ± 11	55 ± 20	0.88	0.32	0.86
Arginine group	53 ± 3	69 ± 4	59 ± 8	54 ± 7			
Hindquarter							
Control group	31 ± 3	31 ± 5	33 ± 2	33 ± 7	0.28	0.06	0.19
Arginine group	22 ± 1	32 ± 4	40 ± 8	41 ± 8			
Kidneys							
Control group	38 ± 6	54 ± 12	68 ± 16	48 ± 9	0.42	0.78	0.12
Arginine group	30 ± 2	41 ± 3	45 ± 11	31 ± 4			

[1] $\bar{x}$ ± SEM; n = 6 in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis.
[2] Repeated-measures ANOVA.

to day 1) and remained significantly higher 1 d after cessation of the endotoxin infusion during intragastric feeding (day 2 compared with day −1). The arterial pH also increased significantly with endotoxin infusion during fasting (day 1 compared with day 0). Arterial hematocrit values, HCO_3^- concentrations, and the arterial pressure of carbon dioxide decreased significantly during the fasting endotoxin infusion period. Except for its lowering effect on the arterial pressure of carbon dioxide, L-arginine intervention had no significant effects on temperature or blood gas values.

Acute phase proteins

The concentrations of α_1-antitrypsin and of total protein decreased significantly as a result of the endotoxin infusion

TABLE 2
Temperature, hematocrit, and arterial blood gas values in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2)[1]

	Time				P[2]		
	Day –1	Day 0	Day 1	Day 2	Time	Time × group	Group
Temperature (°C)							
Control group	38.4 ± 0.4	38.1 ± 0.4	39.9 ± 0.3[3]	38.6 ± 0.1[4]	0.001	0.82	0.51
Arginine group	38.2 ± 0.4	38.4 ± 0.1	39.6 ± 0.4[3]	38.6 ± 0.2[4]			
Hematocrit							
Control group	27.5 ± 0.8	25.1 ± 1.3	23.1 ± 1.5[3]	21.5 ± 1.2	0.00	0.47	0.38
Arginine group	29.8 ± 0.4	26.6 ± 0.6	22.9 ± 0.8[3]	21.8 ± 1.1			
Arterial pH							
Control group	7.40 ± 0.01	7.43 ± 0.01	7.47 ± 0.01[3]	7.45 ± 0.01	0.04	0.74	0.73
Arginine group	7.40 ± 0.01	7.43 ± 0.01	7.45 ± 0.02[3]	7.46 ± 0.01			
P_aCO_2 (mmol/L)							
Control group	6.6 ± 0.3	6.0 ± 0.2	5.4 ± 0.2[3]	6.6 ± 0.1	0.00	0.17	0.03
Arginine group	6.1 ± 0.1	5.9 ± 0.2	4.6 ± 0.2[3]	5.9 ± 0.1			
P_aO_2 (mmol/L)							
Control group	12.7 ± 0.7	11.9 ± 0.4	12.3 ± 0.2	13.4 ± 0.6	0.08	0.86	0.70
Arginine group	12.9 ± 0.2	11.8 ± 0.4	13.1 ± 0.5	13.3 ± 0.3			
HCO_3^- (mmol/L)							
Control group	31.1 ± 1.0	29.8 ± 0.9	27.1 ± 1.2[3]	32.9 ± 1.4	0.00	0.48	0.06
Arginine group	29.8 ± 0.4	27.0 ± 0.8	21.8 ± 1.5[3]	29.8 ± 1.0			
SO_2 (%)							
Control group	96.8 ± 0.8	96.9 ± 0.4	96.9 ± 0.3	97.7 ± 0.3	0.03	0.33	0.58
Arginine group	97.4 ± 0.1	96.1 ± 0.2	97.1 ± 0.6	97.7 ± 0.3			

[1] $\bar{x}$ ± SEM; n = 6 in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis. P_aCO_2, arterial pressure of carbon dioxide; P_aO_2, arterial pressure of oxygen; SO_2, saturation of oxygen.
[2] Repeated-measures ANOVA.
[3] Significantly different from day 0, $P < 0.05$ (univariate F test).
[4] Significantly different from day −1, $P < 0.05$ (univariate F test).

TABLE 3
The acute phase proteins α_1-antitrypsin, haptoglobin, and fibrinogen, and total protein in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2)[1]

	Time				P^2		
	Day –1	Day 0	Day 1	Day 2	Time	Time × group	Group
	g/L						
α_1-Antitrypsin							
Control group	0.08 ± 0	0.08 ± 0	0.07 ± 0.01[3]	0.06 ± 0.01[4]	0.002	0.77	0.40
Arginine group	0.08 ± 0	0.09 ± 0	0.08 ± 0[3]	0.08 ± 0.01[4]			
Haptoglobin							
Control group	0.60 ± 0.03	0.59 ± 0.03	0.17 ± 0.01	0.17 ± 0.01	0.21	0.64	0.53
Arginine group	0.64 ± 0.03	0.61 ± 0.03	0.17 ± 0.01	0.18 ± 0.01			
Fibrinogen							
Control group	0.40 ± 1.14	1.41 ± 0.15	1.67 ± 0.23	1.55 ± 0.25	0.37	0.26	0.20
Arginine group	1.26 ± 0.90	1.49 ± 0.64	1.09 ± 0.15	1.60 ± 0.19			
Total protein							
Control group	30.6 ± 1.4	31.5 ± 1.8	26.0 ± 1.4[3]	23.4 ± 1.2[4]	0.00	0.57	0.74
Arginine group	30.7 ± 1.4	32.6 ± 1.4	26.9 ± 1.9[3]	25.5 ± 1.8[4]			

[1] $\bar{x}$ ± SEM; $n = 6$ in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis.
[2] Repeated-measures ANOVA.
[3] Significantly different from day 0, $P < 0.05$ (univariate F test).
[4] Significantly different from day −1, $P < 0.05$ (univariate F test).

during fasting (day 1 compared with day 0 and day 2 compared with day −1) (**Table 3**). The L-arginine infusion had no significant effect on concentrations of acute phase proteins or total protein.

Arterial concentrations

As shown in **Table 4**, the concentrations of glycine, glutamine, tyrosine, and the sum of the BCAAs decreased significantly and the phenylalanine concentration increased significantly after the endotoxin infusion during prolonged fasting (day 1 compared with day 0). The endotoxin infusion during fasting also resulted in a significant decrease in arginine concentrations in the control group and in alanine concentrations in the arginine group from days 0 to 1 ($P < 0.01$). The L-arginine and L-alanine interventions resulted in a significant increase in arginine and alanine concentrations in the arginine and control groups, respectively ($P < 0.01$). The L-arginine treatment resulted in a significant reduction in glutamine and tyrosine concentrations from day 0 to day 2.

Whole-body rate of appearance

After the pigs were exposed to endotoxin infusion during fasting on day 1, the whole-body RA of phenylalanine and Phe → Tyr were significantly higher than that on day 0, indicating an acceleration of protein breakdown and phenylalanine hydroxylation (**Table 5**). When feeding was resumed after cessation of the endotoxin infusion, the increase in protein breakdown and phenylalanine hydroxylation was attenuated (day 2 compared with day −1). L-Arginine intervention also resulted in a significant decrease in whole-body protein degradation on days 1 and 2, on the basis of the whole-body Ra of phenylalanine. L-Arginine intervention also resulted in a significant decrease in whole-body phenylalanine hydroxylation and protein synthesis, although not time dependently. This finding implies that L-arginine exerts an inhibitory effect on the whole-body protein turnover rate under endotoxemic conditions. The proportion of phenylalanine to tyrosine hydroxylation of total protein breakdown was 15–17% during the endotoxin challenge in the fasted state and 10% after the endotoxin challenge in the fed state.

Hindquarter

Fasting accompanied by endotoxin infusion resulted in a significant increase in the efflux of total AAs and BCAAs in the hindquarter from day 0 to day 1 and in a significant increase in alanine efflux in the arginine group and in a change from arginine influx to efflux in the control group (**Table 6**). The L-arginine infusion induced a significant influx of arginine. Endotoxin infusion during fasting (day 1 compared with day 0) resulted in phenylalanine efflux from the hindquarter, indicating net protein degradation (**Figure 2**). The L-arginine treatment resulted in a significant increase in phenylalanine disposal and production in the hindquarter (Figure 2), indicating that L-arginine stimulated both protein synthesis and degradation in this area. Nevertheless, the net balance of phenylalanine (net protein balance) across the hindquarter remained significantly unchanged after L-arginine treatment. The stimulating effect of L-arginine on protein turnover was not time dependent, suggesting that it was not dependent on the endotoxin infusion. The hydroxylation rate of phenylalanine in the hindquarter area (data not shown) was not significantly different from zero nor between the 2 treatment groups, either during or after the endotoxin challenge.

Portal-drained viscera

The influx of arginine across the portal-drained viscera was significantly higher in the arginine group than in the control group, whereas the efflux of alanine was significantly lower in the control group (**Table 7**). Endotoxin infusion during fasting resulted in a significant decrease in net protein catabolism from day 0 to day 1 (**Figure 3**). The L-arginine infusion had no significant effect on net protein balance, protein synthesis, or protein breakdown in the portal-drained viscera. The hydroxylation rate of phenylalanine across the portal-drained viscera during and after the endotoxin challenge was not significantly different from

TABLE 4
Arterial concentrations in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine[1]

	Time				P[2]		
	Day −1	Day 0	Day 1	Day 2	Time	Time × group	Group
			μmol/L				
Sum of AAs							
Control group	3386 ± 147	2375 ± 75	1937 ± 48[3]	3728 ± 209[4]	0.00	0.009	0.30
Arginine group	3330 ± 13	2648 ± 7	2597 ± 130	3218 ± 167			
Sum of BCAAs							
Control group	657 ± 24	580 ± 29	461 ± 20[3]	825 ± 49	0.00	0.86	0.90
Arginine group	707 ± 6	653 ± 7	548 ± 24[3]	787 ± 50			
Glutamine							
Control group	558 ± 43	345 ± 15	233 ± 15[3]	689 ± 65	0.00	0.01	0.01
Arginine group	538 ± 5	377 ± 3	187 ± 13[3,5]	325 ± 19[5]			
Glycine							
Control group	721 ± 59	633 ± 46	300 ± 2[3]	467 ± 29[4]	0.00	0.13	0.07
Arginine group	651 ± 7	626 ± 5	241 ± 17[3]	344 ± 32[4]			
Alanine							
Control group	353 ± 15	175 ± 14	369 ± 19	632 ± 45	0.00	0.00	0.00
Arginine group	383 ± 5	227 ± 3	126 ± 7[5]	363 ± 17[5]			
Arginine							
Control group	89 ± 7	70 ± 5	64 ± 3[3]	72 ± 10	0.00	0.00	0.00
Arginine group	90 ± 3	77 ± 1	465 ± 20[3,5]	359 ± 47[5]			
Tyrosine							
Control group	140 ± 9	45 ± 6	34 ± 2[3]	143 ± 16	0.00	0.05	0.38
Arginine group	134 ± 5	66 ± 3	31 ± 2[3]	95 ± 10[5]			
Valine							
Control group	364 ± 18	314 ± 15	246 ± 11[3]	418 ± 28	0.00	0.51	0.54
Arginine group	389 ± 4	363 ± 4	278 ± 15[3]	406 ± 24			
Isoleucine							
Control group	124 ± 4	120 ± 8	83 ± 3[3]	181 ± 10[4]	0.00	0.44	0.60
Arginine group	135 ± 3	127 ± 3	101 ± 9[3]	170 ± 12[4]			
Phenylalanine							
Control group	66 ± 3	52 ± 3	80 ± 4[3]	98 ± 4[4]	0.00	0.65	0.70
Arginine group	69 ± 2	61 ± 2	80 ± 2[3]	101 ± 7[4]			
Leucine							
Control group	169 ± 8	146 ± 8	132 ± 7	226 ± 14[4]	0.001	0.11	0.50
Arginine group	183 ± 4	169 ± 4	160 ± 7	211 ± 17[4]			

[1] $\bar{x}$ ± SEM; n = 6 in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis. AAs, amino acids; BCAAs, branched-chain amino acids.
[2] Repeated-measures ANOVA.
[3] Significantly different from day 0, $P < 0.05$ (univariate F test).
[4] Significantly different from day −1, $P < 0.05$ (univariate F test).
[5] Significantly different from control group, $P < 0.05$ (t test with Bonferroni correction).

zero and was not significantly different between the arginine and control groups (data not shown).

Liver

Compared with baseline, endotoxin infusion during fasting resulted in a significantly greater net uptake of valine across the liver during and after the endotoxin infusion (day 1 compared with day 0 and day 2 compared with day −1), indicating endotoxin-induced net protein synthesis in the liver (**Figure 4**). The L-arginine treatment induced a significant arginine influx across the liver, whereas the L-alanine infusion in the control group induced alanine influx across the liver (**Table 8**). The L-arginine infusion significantly decreased the disposal and production of valine to the same extent during and after the endotoxin infusion (Figure 4), implying a reduction in liver protein turnover in the absence of changes in the net balance of liver protein. Phe → Tyr in the liver approximated 10% of the disposal rate of phenylalanine during fasting, both before and after the endotoxin infusion (data not shown). During feeding, phenylalanine hydroxylation was not significantly different from zero because of the relatively low tyrosine enrichment in plasma.

Kidneys

In the control group, arginine efflux from the kidneys decreased significantly from day 0 to day 1 as a consequence of fasting during the endotoxin challenge. Endotoxin infusion during fasting had no significant effect on the net balance, synthesis, or breakdown of protein because no significant effects on the disposal, production, or net balance of phenylalanine across the kidneys were observed (**Figure 5**). Treatment with L-arginine induced significant arginine influx into the kidneys (**Table 9**), whereas L-alanine treatment in the control group increased

TABLE 5
Whole-body rate of appearance (Q Ra) of valine, tyrosine, and phenylalanine (protein degradation); whole-body conversion rate of phenylalanine to tyrosine ($Q_{Phe \rightarrow Tyr}$; phenylalanine hydroxylation); and the difference between the Q Ra of phenylalanine and $Q_{Phe \rightarrow Tyr}$ (protein synthesis) in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2)[1]

	Time				P[2]		
	Day –1	Day 0	Day 1	Day 2	Time	Time × group	Group
			μmol·kg body wt^{-1}*·min*$^{-1}$				
Q Ra of valine							
Control group	7.3 ± 0.9	5.7 ± 0.6	4.8 ± 0.4	7.3 ± 0.7	0.00	0.002	0.67
Arginine group	6.5 ± 0.9	4.7 ± 0.7	3.5 ± 0.3[3]	5.4 ± 0.7			
Q Ra of tyrosine							
Control group	3.6 ± 0.5	1.8 ± 0	1.5 ± 0.1[4]	3.0 ± 0.4	0.00	0.52	0.24
Arginine group	3.0 ± 0.1	1.9 ± 0	1.2 ± 0.1[4]	2.6 ± 0.2			
Q Ra of phenylalanine							
Control group	3.9 ± 0.5	2.4 ± 0.1	2.9 ± 0.2[4]	5.1 ± 0.3[5]	0.00	0.02	0.005
Arginine group	3.5 ± 0.1	2.4 ± 0.1	2.6 ± 0.1[4]	4.0 ± 0.2[3,5]			
$Q_{Phe \rightarrow Tyr}$							
Control group	3.6 ± 0.2	2.2 ± 0.1	2.4 ± 0.2[4]	4.9 ± 0.3[5]	0.002	0.22	0.02
Arginine group	3.2 ± 0	2.1 ± 0	2.2 ± 0.1[4]	3.6 ± 0.2[5]			
(Q Ra) − ($Q_{Phe \rightarrow Tyr}$)							
Control group	30.6 ± 1.4	31.5 ± 1.8	26.0 ± 1.4[4]	23.4 ± 1.2[5]	0.005	0.21	0.05
Arginine group	30.7 ± 1.4	32.6 ± 1.4	26.9 ± 1.9[4]	25.5 ± 1.8[5]			

[1] $\bar{x}$ ± SEM; n = 6 in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis.
[2] Repeated-measures ANOVA.
[3] Significantly different from control group, $P < 0.05$ (t test with Bonferroni correction).
[4] Significantly different from day 0, $P < 0.05$ (univariate F test).
[5] Significantly different from day −1, $P < 0.05$ (univariate F test).

alanine efflux significantly. The administration of L-arginine significantly increased the renal influx of glutamine, although not time dependently. L-Arginine also had no significant effect on the net balance, synthesis, or breakdown of protein in the kidneys (Figure 5). Moreover, Phe → Tyr across the kidneys was not affected significantly by L-arginine treatment (data not shown). Both during and after endotoxemia, irrespective of L-arginine infusion, Phe → Tyr was ≈20% of the rate of phenylalanine disposed.

DISCUSSION

Clinical trials using multiple-component test diets suggested anabolic- and nitrogen-retaining properties of L-arginine formulas

TABLE 6
Amino acid (AA) fluxes across the hindquarter in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2)[1]

	Time				P[2]		
	Day –1	Day 0	Day 1	Day 2	Time	Time × group	Group
Sum of AAs (μmol·kg body wt^{-1}·min^{-1})							
Control group	−4.0 ± 2.6	1.9 ± 1.4	6.0 ± 1.4[3]	−6.1 ± 3.6	0.00	0.46	0.49
Arginine group	−3.5 ± 3.0	1.4 ± 1.7	7.8 ± 4.1[3]	−6.6 ± 3.0			
Sum of BCAAs (μmol·kg body wt^{-1}·min^{-1})							
Control group	−1.6 ± 0.9	0.1 ± 0.1	0.9 ± 0.3[3]	−1.7 ± 0.7	0.009	0.81	0.89
Arginine group	−1.2 ± 0.4	0.2 ± 0.1	1.4 ± 0.6[3]	−1.6 ± 0.9			
Alanine (μmol·kg body wt^{-1}·min^{-1})							
Control group	−0.6 ± 0.3	0.6 ± 0.1	1.3 ± 0.4[3]	−1.6 ± 0.6	0.00	0.03	0.07
Arginine group	−0.1 ± 0.5	0.4 ± 0.2	1.8 ± 0.2[3]	−0.5 ± 0.5			
Glutamine (μmol·kg body wt^{-1}·min^{-1})							
Control group	0.8 ± 0.4	1.6 ± 0.2	1.4 ± 0.2	1.7 ± 0.6	0.51	0.14	0.68
Arginine group	1.2 ± 0.4	1.1 ± 0.3	1.4 ± 0.1	1.7 ± 0.4			
Arginine (nmol·kg body wt^{-1}·min^{-1})							
Control group	−445 ± 68	−149 ± 53	115 ± 76	−641 ± 71	0.00	0.26	0.05
Arginine group	−476 ± 115	−230 ± 57	−50 ± 112	−1438 ± 352			

[1] $\bar{x}$ ± SEM; n = 6 in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis. BCAAs, branched-chain amino acids.
[2] Repeated-measures ANOVA.
[3] Significantly different from day 0, $P < 0.05$ (univariate F test).

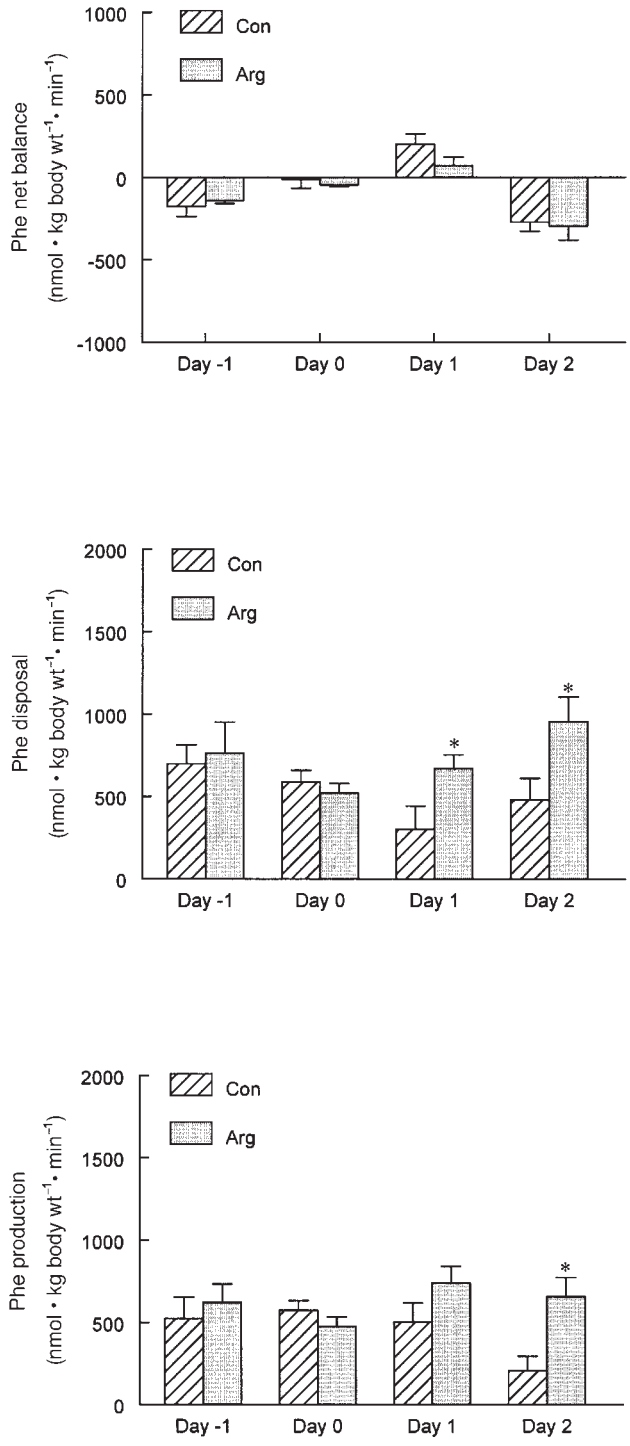

FIGURE 2. Mean (±SEM) rate of phenylalanine net balance, disposal (protein synthesis), and production (protein degradation) across the hindquarter in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group; Con) or L-arginine (Arg) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2). Repeated-measures ANOVA: there was a significant time effect on the net balance of phenylalanine ($P = 0.00$) and a significant group effect on phenylalanine disposal ($P = 0.01$) and production ($P = 0.05$). Univariate F test: phenylalanine net balance on day 1 was significantly different from day 0 ($P < 0.05$). *Significantly different from the control group, $P < 0.05$ (t test with Bonferroni correction).

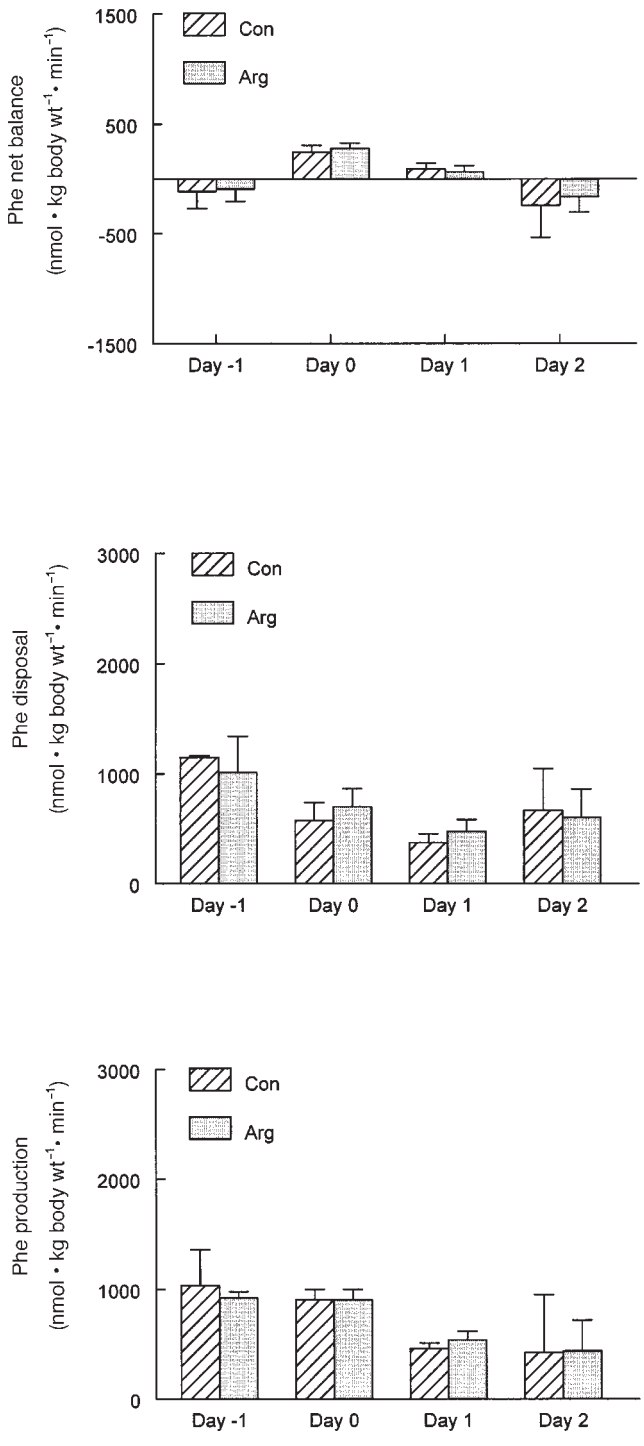

FIGURE 3. Mean (±SEM) rate of phenylalanine net balance, disposal (protein synthesis), and production (protein degradation) corrected for phenylalanine production derived from enteral infusion across the portal-drained viscera in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group; Con) or L-arginine (Arg) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2). Repeated-measures ANOVA: there was a significant time effect on the phenylalanine net balance ($P = 0.00$) and production ($P = 0.03$). Univariate F test: phenylalanine net balance and production on day 1 were significantly different from those on day 0 ($P < 0.05$).

TABLE 7
Amino acid (AA) fluxes corrected for the enteral infusion rate across the portal-drained viscera in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2)[1]

	Time				P[2]		
	Day –1	Day 0	Day 1	Day 2	Time	Time × group	Group
Sum of AAs (μmol·kg body wt^{-1}·min^{-1})							
Control group	21.2 ± 5.0	5.1 ± 1.4	1.5 ± 1.1	25.3 ± 6.3	0.01	0.84	0.94
Arginine group	17.6 ± 1.0	6.7 ± 3.0	3.0 ± 4.1	23.1 ± 4.2			
Sum of BCAAs (μmol·kg body wt^{-1}·min^{-1})							
Control group	5.5 ± 1.4	1.5 ± 0.6	0.0 ± 0.2	5.6 ± 1.9	0.04	0.36	0.26
Arginine group	4.6 ± 0.7	2.0 ± 0.9	0.5 ± 1.1	5.6 ± 1.0			
Alanine (μmol·kg body wt^{-1}·min^{-1})							
Control group	4.9 ± 1.2	1.0 ± 0.2	0.2 ± 0.2	11.1 ± 2.0[3]	0.00	0.005	0.03
Arginine group	4.5 ± 0.5	1.3 ± 0.3	1.1 ± 0.2[4]	5.9 ± 0.6[3,4]			
Glutamine (μmol·kg body wt^{-1}·min^{-1})							
Control group	−1.7 ± 0.6	−1.1 ± 0.3	−0.8 ± 0.2[5]	−0.9 ± 0.7[3]	0.00	0.72	0.22
Arginine group	−1.7 ± 0.4	−1.3 ± 0.4	0.0 ± 0.3[5]	0.1 ± 0.2[3]			
Arginine (nmol·kg body wt^{-1}·min^{-1})							
Control group	974 ± 196	292 ± 76	134 ± 35[5]	161 ± 170[3]	0.00	0.00	0.05
Arginine group	555 ± 141	295 ± 74	−613 ± 276[4,5]	−590 ± 545[3]			

[1] $\bar{x}$ ± SEM; n = 6 in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis. BCAAs, branched-chain amino acids.
[2] Repeated-measures ANOVA.
[3] Significantly different from day −1, $P < 0.05$ (univariate F test).
[4] Significantly different from control group, $P < 0.05$ (t test with Bonferroni correction).
[5] Significantly different from day 0, $P < 0.05$ (univariate F test).

(reviewed in 30). Intervention with L-arginine has been shown to exert beneficial effects on many experimental diseases. However, only few studies have examined the sole effect of L-arginine on protein synthesis and degradation in individual organs during stress or infections. The current in vivo study was undertaken to investigate the influence of L-arginine on both protein synthesis and degradation under endotoxemic conditions. For this purpose, L-arginine was administered intravenously during endotoxin infusion and administered intragastrically as a supplement in the diet after endotoxin infusion. We showed that under these endotoxemic conditions, L-arginine intervention increased protein turnover in the hindquarter and decreased protein turnover in the liver.

The model of endotoxemia used was characterized by increased whole-body and hindquarter protein breakdown, as measured with a stable phenylalanine isotope. In addition, a tyrosine tracer indicated that whole-body phenylalanine hydroxylation simultaneously increased. Supplementation with L-arginine under endotoxemic conditions reduced whole-body protein degradation and protein synthesis, ie, turnover, and decreased the rate of whole-body phenylalanine hydroxylation.

Hindquarter

The net protein breakdown that was observed across the hindquarter within the control group after endotoxin infusion reflects muscle catabolism, one of the main prominent features of the metabolic response to sepsis. Enteral nutrition 1 d after endotoxemia abolished this net protein degradation. The intravenous L-arginine infusion during endotoxemia and intragastric L-arginine infusion 1 d after endotoxemia not only promoted protein synthesis in the hindquarter area but also increased the protein breakdown to a similar extent, therefore not resulting in improved net protein balance. In addition, the efflux of total amino acids by the hindquarter was not affected significantly by the L-arginine infusion, neither during nor after endotoxemia, implying unchanged net protein balance across hindquarter muscle. Enhanced muscle protein synthesis was shown previously in traumatized rats in response to dietary L-arginine; unfortunately, concomitant muscle protein breakdown was not measured in this study (6). High protein turnover is supposed to facilitate a rapid response by means of rapid alteration of enzyme concentrations and elimination of damaged proteins (31). Therefore, although no improved net protein balance was achieved across the muscle by L-arginine infusion, increased muscle protein turnover can be of benefit under altered physiologic conditions such as septicemia.

Portal-drained viscera

Only a few studies of flux in the portal-drained viscera have been conducted because the portal vein is not easily sampled. This study showed that endotoxin infusion during fasting reduced the net catabolism of protein in the portal-drained viscera as a result of protein degradation. Although we detected no changes in phenylalanine disposal, a study of bacteremic rats showed stimulation of intestinal protein synthesis (32), and intravenous L-arginine supplementation decreased protein synthesis in the small intestine of bowel-resected rats (33). In our model of endotoxemia, L-arginine supplementation had no significant effect on the rate of protein synthesis or breakdown in the portal-drained viscera, neither during nor after endotoxemia. Because of the relatively lower protein turnover rate of gut enterocytes in pigs, the eventual effects of L-arginine on protein metabolism in the portal-drained viscera may have become manifest at a later stage of endotoxemia.

Liver

During endotoxemia, cytokine-driven synthesis of acute phase proteins by the liver is activated (34). The observed increase in net

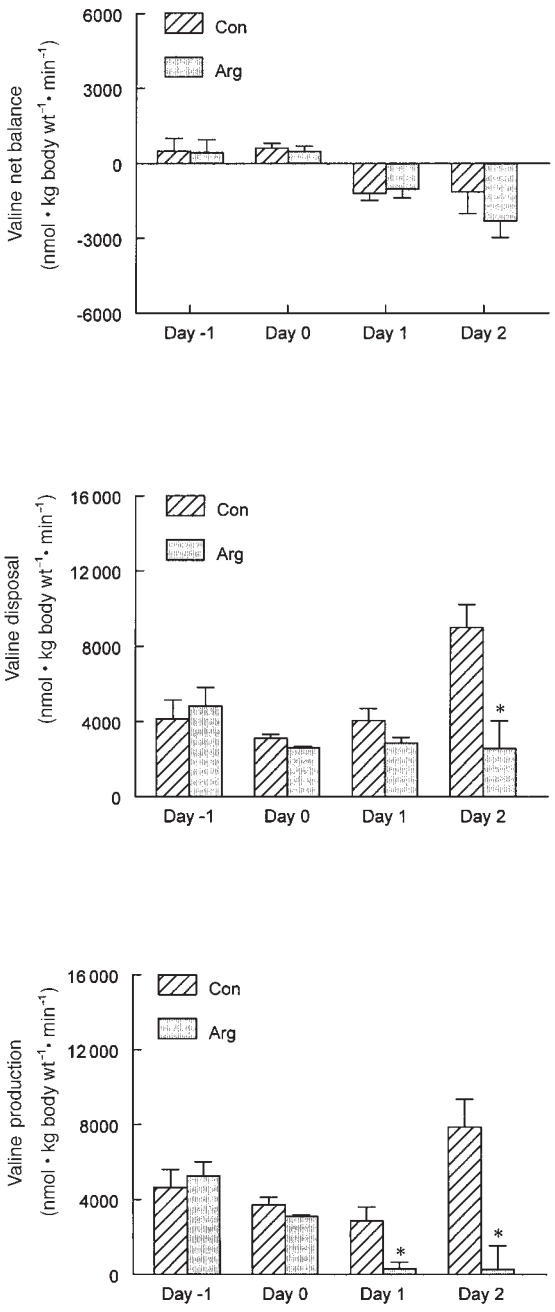

FIGURE 4. Mean (±SEM) rate of valine net balance, disposal (protein synthesis), and production (protein degradation) across the liver in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group; Con) or L-arginine (Arg) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2). Repeated-measures ANOVA: there was a significant time effect on the valine net balance ($P = 0.00$), disposal ($P = 0.01$) and production ($P = 0.04$) and a significant time × group effect on valine disposal ($P = 0.05$) and production ($P = 0.05$). Univariate F test: valine net balance on days 1 and 2 was significantly different from that on days 0 and −1, respectively, and valine disposal on day 1 was significantly different from day 0 ($P < 0.05$). *Significantly different from the control group, $P < 0.05$ (t test with Bonferroni correction).

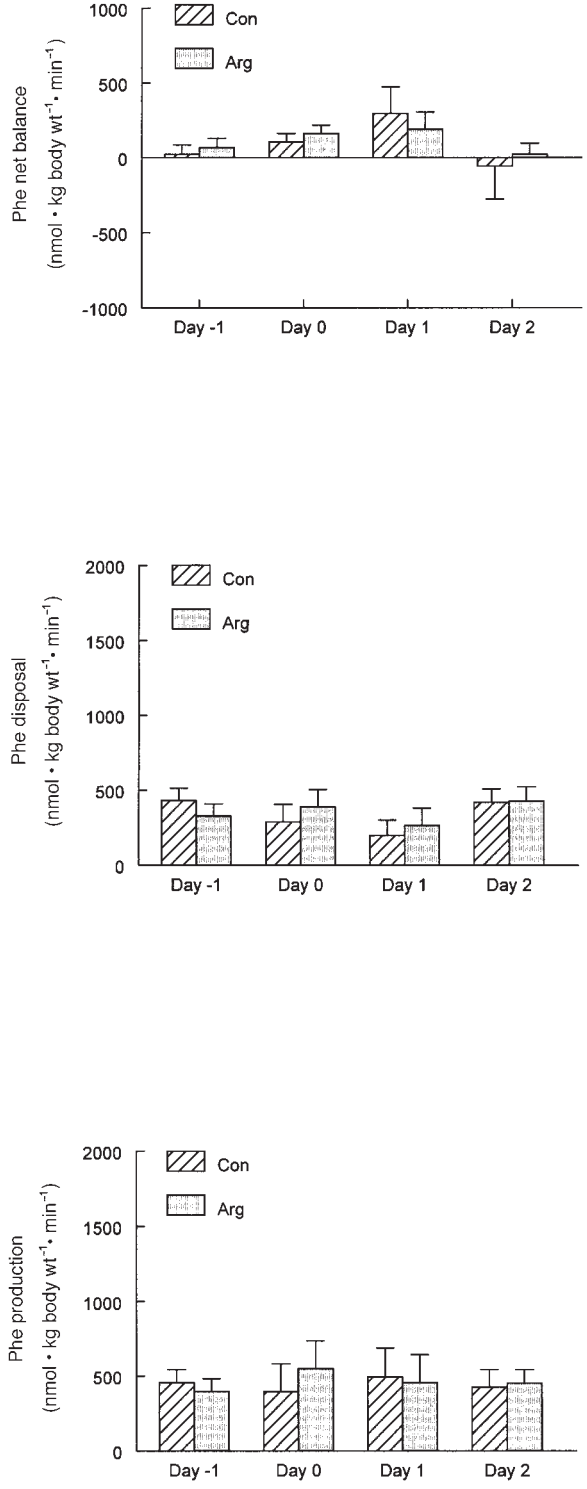

FIGURE 5. Mean (±SEM) rate of phenylalanine net balance, disposal (protein synthesis), and production (protein degradation) across the kidneys in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group; Con) or L-arginine (Arg) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2). Repeated-measures ANOVA: no significant effects were observed.

TABLE 8
Amino acid (AA) fluxes across the liver in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2)[1]

	Time				P[2]		
	Day –1	Day 0	Day 1	Day 2	Time	Time × group	Group
Sum of AAs (μmol·kg body wt^{-1}·min^{-1})							
Control group	−9.1 ± 5.2	−4.1 ± 1.8	−24.2 ± 2.2[3]	−21.8 ± 4.5	0.00	0.004	0.05
Arginine group	−11.0 ± 6.0	−6.2 ± 2.1	−12.1 ± 4.3[3]	−9.4 ± 5.4			
Sum of BCAAs (μmol·kg body wt^{-1}·min^{-1})							
Control group	−0.9 ± 2.2	−0.0 ± 0.3	1.3 ± 1.1[3]	−3.0 ± 1.7[4]	0.00	0.30	0.52
Arginine group	0.5 ± 2.2	0.4 ± 0.2	−1.6 ± 0.4[3]	−3.6 ± 0.8[4]			
Alanine (μmol·kg body wt^{-1}·min^{-1})							
Control group	−3.4 ± 2.6	−2.4 ± 0.5	−14.7 ± 1.2[3]	−7.2 ± 1.0	0.00	0.00	0.002
Arginine group	−2.5 ± 1.0	−3.4 ± 0.5	−5.0 ± 0.5[3,5]	−3.7 ± 0.3[5]			
Glutamine (μmol·kg body wt^{-1}·min^{-1})							
Control group	1.9 ± 0.3	1.3 ± 0.4	0.8 ± 0.2	1.0 ± 0.7	0.11	0.15	0.02
Arginine group	2.3 ± 0.3	1.3 ± 0.4	−0.2 ± 0.3	0.3 ± 0.2			
Arginine (nmol·kg body wt^{-1}·min^{-1})							
Control group	149 ± 432	127 ± 52	−613 ± 50[3]	−164 ± 116[4]	0.00	0.009	0.001
Arginine group	−34 ± 285	−21 ± 81	−4205 ± 484[3,5]	−1937 ± 375[4,5]			

[1] $\bar{x}$ ± SEM; n = 6 in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis. BCAAs, branched-chain amino acids.
[2] Repeated-measures ANOVA.
[3] Significantly different from day 0, $P < 0.05$ (univariate F test).
[4] Significantly different from day −1, $P < 0.05$ (univariate F test).
[5] Significantly different from control group, $P < 0.05$ (t test with Bonferroni correction).

liver protein synthesis during and after endotoxin infusion, therefore, appears to represent enhanced synthesis of acute phase proteins. However, the endotoxin-induced protein synthesis was not reflected by an increase in plasma acute phase protein concentrations. Plasma dilution resulting from fluid resuscitation during endotoxin infusion could have concealed possible increases in acute phase protein concentrations. As far as we know, no studies of the effect of L-arginine on hepatic protein metabolism during sepsis or endotoxemia are available. In our in vivo model, L-arginine supplementation during and after endotoxin-infusion exerted a pronounced reducing effect on hepatic protein synthesis and breakdown, ie, protein turnover, without affecting the net balance of protein. Nevertheless, the measured plasma acute phase protein concentrations did not decrease after the L-arginine infusion. Hence, whether the observed decrease in hepatic protein synthesis concerns delayed secretion of secretory proteins or rather con-

TABLE 9
Amino acid (AA) fluxes across the kidneys in pigs before endotoxin infusion in the fed (day −1) or fasting (day 0) state, during endotoxin infusion with intravenous L-alanine (control group) or L-arginine (arginine group) (day 1), and after endotoxin infusion with intragastric L-alanine or L-arginine (day 2)[1]

	Time				P[2]		
	Day –1	Day 0	Day 1	Day 2	Time	Time × group	Group
Sum of AAs (μmol·kg body wt^{-1}·min^{-1})							
Control group	−1.8 ± 4.0	4.8 ± 1.4	2.6 ± 4.8	−8.3 ± 7.6	0.03	0.71	0.33
Arginine group	−0.4 ± 2.7	6.4 ± 3.2	0.4 ± 4.5	−1.9 ± 1.8			
Sum of BCAAs (μmol·kg body wt^{-1}·min^{-1})							
Control group	0.6 ± 0.8	1.3 ± 0.7	1.1 ± 1.0	−0.8 ± 2.1	0.25	0.61	0.57
Arginine group	0.0 ± 0.8	2.1 ± 1.6	1.4 ± 1.2	0.0 ± 0.5			
Alanine (μmol·kg body wt^{-1}·min^{-1})							
Control group	−0.6 ± 0.4	0.2 ± 0.3	3.1 ± 1.0	3.6 ± 0.8[3]	0.00	0.009	0.02
Arginine group	−0.1 ± 0.7	0.0 ± 0.3	0.5 ± 0.6[4]	−1.4 ± 0.3[3,4]			
Glutamine (μmol·kg body wt^{-1}·min^{-1})							
Control group	0.6 ± 0.2	−0.1 ± 0.3	0.0 ± 0.2	0.0 ± 0.7	0.16	0.38	0.04
Arginine group	1.0 ± 0.2	0.2 ± 0.6	−1.5 ± 0.3	−1.1 ± 0.3			
Arginine (nmol·kg body wt^{-1}·min^{-1})							
Control group	356 ± 170	343 ± 86	179 ± 34[5]	10 ± 176	0.002	0.001	0.07
Arginine group	414 ± 149	472 ± 99	−532 ± 113[4,5]	−507 ± 142[4]			

[1] $\bar{x}$ ± SEM; n = 6 in the control group and 8 in the arginine group. Data were transformed or log transformed as appropriate before analysis. BCAAs, branched-chain amino acids.
[2] Repeated-measures ANOVA.
[3] Significantly different from day −1, $P < 0.05$ (univariate F test).
[4] Significantly different from control group, $P < 0.05$ (t test with Bonferroni correction).
[5] Significantly different from day 0, $P < 0.05$ (univariate F test).

cerns structural proteins of endogenous origin remains to be established. The nitric oxide that is generated by the hepatic inducible NOS II enzyme may be involved in the decreased hepatic protein turnover by means of inhibition of cell replication in the liver (35).

Phenylalanine 4-monooxygenase is considered to be predominantly located in the liver (36, 37). Our data indicated a large variability in the hydroxylation of phenylalanine to tyrosine across the liver because of the low enrichment of plasma tyrosine. Phenylalanine hydroxylation in the liver during fasting, irrespective of endotoxin or L-arginine infusion, accounted for ≈10% of phenylalanine disposal, a finding similar to that of a study performed on human liver in vivo (38).

Kidneys

Dietary intervention with L-arginine has been associated with amelioration of many experimental kidney diseases (39). The effect of L-arginine administration on renal protein synthesis and degradation under septic conditions, however, remains to be explored. In our model of hyperdynamic endotoxemia with well-preserved renal function, the renal synthesis and degradation of protein did not change significantly after treatment with L-arginine, as measured with a phenylalanine isotope. Phenylalanine is not only incorporated into protein because the kidneys of pigs appear to hydroxylate phenylalanine to tyrosine at a rate of 20% of the total phenylalanine disposal rate. This finding is consistent with that of a study in which phenylalanine hydroxylation was found to occur at a rate of 40% in human kidney (38). Neither endotoxemia nor L-arginine exerted any effect on the rate of renal phenylalanine hydroxylation.

During L-arginine supplementation the decline in hepatic protein turnover exceeded the increase in muscle protein turnover, accounting for the decrease in whole-body protein turnover that was observed after endotoxemia. Differences in the severity of the disease and related L-arginine requirements may underlie the way by which L-arginine influences protein metabolism. Because in this sepsis model it was shown that L-arginine infusion increased the whole-body production of nitric oxide (40), the indirect effects of nitric oxide may also be involved in the observed changes in protein metabolism.

On the basis of our results, intravenous or intragastric supplementation with L-arginine appears to be a promising means for increasing protein turnover in the hindquarter muscle after endotoxemia. Although we observed no significant decrease in acute phase protein concentrations, the decrease in protein turnover in the liver after L-arginine infusion under postendotoxemic conditions implies that L-arginine may reduce the severity of the hepatic response to tissue injury and inflammation.

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