NOS3 is involved in the increased protein and arginine metabolic response in muscle during early endotoxemia in mice

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Sepsis is a severe catabolic condition that is characterized by loss of lean body mass, especially skeletal muscle proteins, which leads to muscle dysfunction (10, 51). Sepsis is, therefore, a major cause of death in intensive care units (1). The loss of muscle weight results from net proteolysis due to an imbalance between protein breakdown and synthesis (4). Amino acids from muscle protein enter the bloodstream and become available for the splanchnic area, kidneys, and immune cells (7, 42) for oxidation and energy production, for gluconeogenesis, and for protein synthesis (50). Bacterial lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria is known to play a major role in the pathogenesis of septic shock by activating Toll-like receptor-4 (3) and, subsequently, the activation of NF-κB and release of cytokines and induction of enzymes [e.g., inducible nitric oxide synthase (NOS2); see Ref. 34]. These cytokines and the ensuing production of nitric oxide (NO) are probably the initiating factors for the catabolic state in sepsis (10, 32, 51).

Under baseline conditions, nitric oxide synthase (NOS) enzymes produce low amounts of NO from the amino acid L-arginine. In normal skeletal muscle, all three isoforms of NOS are expressed (15, 23, 24, 33, 36, 39). NOS3 is mainly expressed in vascular endothelium within muscle tissue (14, 15) or linked to mitochondria-rich fibers (14, 25, 29). In accordance with this expression pattern, NOS3 mainly controls skeletal muscle blood flow (19) and mitochondrial respiration (25) but is not involved in contractile muscle function (20). NOS1 is expressed in the muscle in the neuromuscular end-plate, and NOS2 is located in intracellular structures in type II muscle fibers, where they regulate muscle contractility (14, 15; for review, see Ref. 43).

Sepsis and experimental endotoxemia upregulate NOS2 in many cells (32), including muscle fibers and cultured myocytes (13, 15, 16, 28, 46). NOS3 is also upregulated in muscle homogenate (13, 29), but downregulation of NOS3 has been reported in thoracic aorta homogenate (41), which may also indicate downregulation of NOS3 in vascular endothelium within the muscle. For NOS1, both upregulation (13) and downregulation (15, 16, 29) during endotoxemia/sepsis have been reported.

In septic shock, increased NO production and impaired mitochondrial and cellular oxygen uptake accompany muscle contractile dysfunction and fatigue, whereas inhibitors of NOS activity restore muscle contractility (5, 13, 15). Although the hypermetabolic response of muscle in endotoxemic rats did not seem related to increased NOS expression, the NOS-inhibitor L-NAME (35) reduced protein synthesis in skeletal muscle in normal rats (35). It was, therefore, suggested that NO plays a positive role in facilitating protein synthesis in muscle (37). The involvement of NO in the regulation of muscle protein metabolism in sepsis needs further study, if only because muscle is the major source for glutamine in the body. Besides being an important energy source for tissues, glutamine is converted to citrulline in the gut, which is then converted to arginine in the kidney (53). Changes in muscle glutamine release may therefore affect arginine production and consequently NO production in many organs.
This study aimed to investigate the role of NOS2 and NOS3 in muscle protein and arginine metabolism during early endotoxemia, using NOS2- and NOS3-deficient mice. Stable isotopes were used to study protein and arginine metabolism on the whole body and muscle level.

**MATERIALS AND METHODS**

**Animals.** Female C57BL6/J (wild type: WT), C57BL6/J:NOS2-/-, and C57BL6/J:NOS3-/- mice [16–26 g (mean 20 g/group), 2–3 mo old] were originally obtained from Jackson Laboratories and bred at the Department of Anatomy and Embryology (AMC, Amsterdam, The Netherlands). The mice were fed standard lab chow (Hope Farms, Woerden, The Netherlands) and subjected to standard 12:12-h light-dark cycle periods (7:30 AM to 7:30 PM). Room temperature was maintained at 25°C. Water was provided ad libitum throughout the experiment. Experiments were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (52) and approved by the Ethical Committee of Animal Research of Maastricht University.

**Experimental protocol.** The following six different experimental groups were studied in the protocol: WT (n = 8), NOS2-/- (n = 8), NOS3-/- (n = 9), WT/LPS-treated (n = 8), NOS2-/-/LPS-treated (n = 9), and NOS3-/-/LPS-treated animals (n = 9). All experiments started between 8 AM and 10 AM. As a model of sepsis, LPS (Escherichia coli O55:B5; 100 μg·200 μl saline·g⁻¹·h⁻¹ Sigma, St. Louis, MO) was given by intraperitoneal injection to mice (45). Control animals received a corresponding volume of saline. After injection with LPS or saline, food was withheld, but drinking water was provided ad libitum.

After LPS treatment (5 h), anesthesia and fluid maintenance was started as described previously (18). During the surgical procedures, the mice were kept at 37°C using a temperature controller (Technical Service, Maastricht University) and heat pads. Catheterization of the jugular vein, carotid artery, right renal vein, and inferior caval vein below the level of the renal vein was performed as described previously (18). A primed-constant infusion of stable isotopes (Mass Trace, Woburn, MA) was given via the jugular vein (Table 1). Plasma flow across the hindquarter was measured using an indicator-dilution technique with p-[glycyl-1-¹³C]aminohippuric acid (NEN Life Science Products, Boston, MA; see Ref. 18). Blood was collected from the inferior caval vein (venous blood), carotid artery (arterial blood), and renal vein (venous blood for renal metabolism; see Ref. 30), as described previously (18). Amino acid concentrations and tracer-to-tracee ratios (TTR) were determined in plasma using a fully automated LC-MS system after precolumn derivatization with o-phthalaldehyde (48, 49).

**Calculations.** Hindquarter metabolism is indicated as “muscle” metabolism throughout. Hindquarter substrate fluxes (net balances) were calculated by multiplying the inferior caval venous-arterial concentration difference with the mean hindquarter plasma flow and are expressed as nanomoles per 10 g body wt per minute (11, 18). A positive flux indicates net release and a negative flux net uptake.

The amino acid stable-isotope tracers were used to calculate organ disposal and production rates (8). The tracer net balance (nb), the disposal and production rate (nmol·10 g body wt⁻¹·min⁻¹) across the hindquarter, was calculated as:

\[ nb = \text{plasma flow} \times \left[ (A_1 \times \text{TTR}_{A_1}) - (V_1 \times \text{TTR}_{V_1}) \right] \]

where \([V_1] \) and \([A_1] \) are the venous and arterial plasma concentrations of substrates, and \(\text{TTR}_{A_1} \) and \(\text{TTR}_{V_1} \) are the TTR of the measured amino acid in the arterial plasma and venous plasma, respectively.

\[ \text{disposal} = nb/\text{TTR}_{V} \]

\( \text{TTR}_{V} \) was used as a surrogate precursor pool enrichment, because the venous TTR, compared with the arterial TTR, more closely resembles the precursor pool TTR (54).

\[ \text{production} = \text{disposal} + \text{net balance (tracee)} \]

Hindquarter protein metabolism was estimated from the \([^{2}H_3] \text{Phe} \) and \([^{2}H_2] \text{Tyr} \) tracers (8):

\[ \text{hindquarter PB} = \text{Phe production,} \]

where PB is protein breakdown and:

\[ \text{hindquarter PS} = \text{disposal Phe} - (\text{Phe} \rightarrow \text{Tyr}) \]

where PS is protein synthesis and (Phe → Tyr) represents the hydroxylation of phenylalanine to tyrosine in the hindquarter. Phe → Tyr is calculated as:

\[ (\text{Phe} \rightarrow \text{Tyr}) = PF \times \left[ (A_1 \times \text{TTR}_{A_1} \times \text{TTR}_{V_1} \times (A_1 \times \text{TTR}_{V_1} - [V_1 \times \text{TTR}_{V_1}]) \right] \]

where PF is hindquarter plasma flow. The term \((1 - FE)\) represents the fraction of total amino acids that bypasses metabolism in the hindquarter and appears in the output:

\[ \text{FE}_{\text{Tyr}} = \frac{[(A_1 \times \text{TTR}_{A_1} \times \text{TTR}_{V_1}) - [V_1 \times \text{TTR}_{V_1}])]}{(A_1 \times \text{TTR}_{A_1} \times \text{TTR}_{V_1})} \]

and

\[ \text{hindquarter net PB} = \text{PB} - \text{PS} \]

Since muscle hydroxylation is only minimal, phenylalanine flux can be considered as an indication of net protein breakdown (positive Phe flux) or net protein synthesis (negative Phe flux).

NO production and de novo arginine production in the hindquarter were calculated using \([^{15}N_2] \text{Arg} \) and \([^{13}C,^{2}H_2] \text{Cit} \) tracers (8). The rates of conversion of \([^{15}N_2] \text{Arg} \) to \([^{15}N] \text{Cit} \) (NO production), and of \([^{13}C,^{2}H_2] \text{Cit} \) to \([^{13}C,^{2}H_3] \text{Arg} \) (de novo arginine production) were calculated essentially as written for the conversion of Phe to Tyr. Because hindquarter NO production was at the limit of our detection level, these data are not shown.

**Statistical analysis.** Results are presented as means ± SE. One-way ANOVA was used to compare differences between groups under baseline conditions. Two-way ANOVA was used to compare differences between treatment groups, using “group” with three levels (WT, NOS2-/-, and NOS3-/-) and “LPS” with two levels (saline, LPS) as the factors. When significant differences were observed, post hoc Bonferroni analysis was used to discriminate between the groups. Significance was defined as \(P < 0.05\).

**RESULTS**

**Role of NOS2 and NOS3 in baseline muscle metabolism.** Muscle plasma flow was not significantly affected by the absence of NOS3 but was reduced by a factor 2 in NOS2-/- mice (\(P < 0.1\); Table 2).

In control mice, muscle glutamine, glycine, alanine, and arginine release were reduced in NOS2-/- mice, whereas only glycine release was reduced in NOS3-/- mice (Table 2, Fig. 1). In addition, the uptake of glutamic acid, citrulline, valine,


**Table 1. Tracer prime and infusion rates**

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Abbreviation</th>
<th>Prime, nmol/mouse</th>
<th>Continuous Infusion, nmol/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-[guanidino-¹⁵N₂]arginine</td>
<td>[¹⁵N₂]Arg</td>
<td>850</td>
<td>1,700</td>
</tr>
<tr>
<td>t-[laurodo-¹³C,H₂]citrulline</td>
<td>[¹³C,H₂]Cit</td>
<td>215</td>
<td>430</td>
</tr>
<tr>
<td>t-[ring-¹⁵N₂]phenylalanine</td>
<td>[¹⁵N₂]Phe</td>
<td>340</td>
<td>680</td>
</tr>
<tr>
<td>t-[ring-¹⁵N₂]tyrosine</td>
<td>[¹⁵N₂]Tyr</td>
<td>215</td>
<td>430</td>
</tr>
</tbody>
</table>
and leucine was reduced in NOS2−/− mice and that of citrulline also in NOS3−/− animals (Table 2).

Muscle protein synthesis and degradation were not affected by NOS2 or NOS3 deficiency in control mice (Fig. 2). In control WT mice, muscle arginine production consists largely of arginine released from protein breakdown and only 6% stemming from de novo arginine production from citrulline (Table 3). De novo arginine production was reduced in muscle of control NOS2−/− mice (Table 3).

Effect of LPS treatment on muscle metabolism in WT mice. Muscle plasma flow was not affected by LPS treatment (Table 2). After LPS administration, the net release of glutamine, glycine, histidine, phenylalanine, and total amino acids was significantly reduced (Table 2), whereas the release of serine and the uptake of citrulline were increased. Meanwhile, metabolism changed from net protein breakdown (positive phenylalanine flux) to net protein synthesis (negative phenylalanine flux; Table 2) because of a bigger increase in protein synthesis (Fig. 2, top) than in protein breakdown (Fig. 2, bottom). Although of relatively minor importance in muscle, phenylalanine hydroxylation to tyrosine also increased after LPS (Fig. 2, top).

After endotoxin challenge, muscle arginine turnover increased (Table 3). This mainly resulted from an increase in protein synthesis and breakdown, since de novo arginine production did not increase (Table 3). Both citrulline disposal and production more than doubled after LPS (Table 3).

Role of NOS2 and NOS3 in muscle metabolism during endotoxemia. In contrast to WT mice, NOS2−/− mice experienced only a small, nonsignificant reduction in net muscle glutamine release or release of total amino acids after LPS (Fig. 1). NOS3−/− mice responded to LPS almost in a similar way as WT mice for amino acid metabolism, but net protein synthesis (negative phenylalanine flux) was not observed in this group (Table 2). This latter observation is in line with the absence of an increase in muscle protein synthesis in NOS3−/−

Table 2. Fluxes across the hindquarter

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Flow</td>
<td>0.60±0.15</td>
<td>0.30±0.04</td>
<td>0.47±0.08</td>
<td>0.46±0.18</td>
<td>0.29±0.07</td>
<td>0.38±0.09</td>
</tr>
<tr>
<td>Glu</td>
<td>−4.1±1.8</td>
<td>1.8±1.1</td>
<td>−1.8±1.4</td>
<td>0.3±2.2</td>
<td>−0.8±1.1</td>
<td>0.1±0.9</td>
</tr>
<tr>
<td>Ser</td>
<td>5.0±2.1</td>
<td>3.4±1.3</td>
<td>5.2±1.2</td>
<td>8.6±1.2</td>
<td>5.1±1.3</td>
<td>8.0±1.5</td>
</tr>
<tr>
<td>Gly</td>
<td>30.2±9.2</td>
<td>7.2±1.8</td>
<td>11.4±2.6</td>
<td>10.4±3.9</td>
<td>6.4±3.4</td>
<td>9.3±2.9</td>
</tr>
<tr>
<td>His</td>
<td>0.7±0.8</td>
<td>3.2±2.2</td>
<td>3.4±2.4</td>
<td>−0.8±0.8</td>
<td>−0.1±1.1</td>
<td>−2.3±1.1</td>
</tr>
<tr>
<td>Cit</td>
<td>−3.0±1.1</td>
<td>−0.7±0.9</td>
<td>−0.5±0.9</td>
<td>−4.7±1.2</td>
<td>−2.8±0.7</td>
<td>−3.6±1.6</td>
</tr>
<tr>
<td>Ala</td>
<td>34.8±5.0</td>
<td>13.8±3.7</td>
<td>28.1±6.6</td>
<td>23.7±7.4</td>
<td>17.0±11.6</td>
<td>29.6±7.4</td>
</tr>
<tr>
<td>Arg</td>
<td>3.6±2.0</td>
<td>−1.0±1.3</td>
<td>3.0±1.5</td>
<td>1.2±4.1</td>
<td>0.2±2.4</td>
<td>0.7±1.8</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.2±1.3</td>
<td>−0.3±0.8</td>
<td>−1.9±1.4</td>
<td>0.2±1.3</td>
<td>−0.3±0.9</td>
<td>−1.2±1.4</td>
</tr>
<tr>
<td>Val</td>
<td>−3.4±1.3</td>
<td>−0.8±0.9</td>
<td>−3.0±1.0</td>
<td>−2.8±1.2</td>
<td>−3.4±1.4</td>
<td>−4.9±0.8</td>
</tr>
<tr>
<td>Met</td>
<td>2.4±0.6</td>
<td>1.7±0.5</td>
<td>2.1±0.3</td>
<td>2.1±1.6</td>
<td>1.3±0.8</td>
<td>1.9±0.7</td>
</tr>
<tr>
<td>Ile</td>
<td>−3.4±1.6</td>
<td>−1.5±0.7</td>
<td>−3.6±0.8</td>
<td>−2.5±2.1</td>
<td>−2.8±1.1</td>
<td>−4.0±1.7</td>
</tr>
<tr>
<td>Phe</td>
<td>1.3±0.6</td>
<td>0.5±0.6</td>
<td>0.1±0.6</td>
<td>−2.0±0.6</td>
<td>−1.0±1.2</td>
<td>0.2±0.3</td>
</tr>
<tr>
<td>Trp</td>
<td>−2.0±0.9</td>
<td>−2.0±0.2</td>
<td>−1.4±0.6</td>
<td>−1.9±1.0</td>
<td>−1.4±0.6</td>
<td>−3.8±1.4</td>
</tr>
<tr>
<td>Leu</td>
<td>−4.5±1.4</td>
<td>−0.6±1.0</td>
<td>−4.9±1.2</td>
<td>−2.9±2.2</td>
<td>−2.8±1.4</td>
<td>−6.3±1.6</td>
</tr>
<tr>
<td>Orn</td>
<td>−3.0±0.6</td>
<td>−1.6±1.0</td>
<td>−2.3±0.5</td>
<td>−0.2±0.7</td>
<td>−3.5±1.3</td>
<td>−6.7±4.9</td>
</tr>
<tr>
<td>BCAA</td>
<td>−11.4±4.1</td>
<td>−2.9±2.4</td>
<td>−2.3±10.3</td>
<td>−8.2±5.2</td>
<td>−9.0±3.7</td>
<td>15.2±3.7</td>
</tr>
<tr>
<td>Sum AA</td>
<td>122.6±24.4</td>
<td>57.7±18.3</td>
<td>94.7±18.5</td>
<td>65.1±20.1</td>
<td>50.3±39.4</td>
<td>52.4±22.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are nmol·10 g body wt−1·min−1, except for flow, where units are ml·10 g body wt−1·min−1. BCAA, branched-chain amino acid; sum AA, sum of amino acids; WT, wild type; NOS2−/− and NOS3−/−, nitric oxide synthase isoforms-2 and -3 deficient, respectively. Positive values indicate net release; negative values indicate net uptake. Statistics with 1-way ANOVA and post hoc Bonferroni test were used for baseline differences, and 2-way ANOVA using group and lipopolysaccharide (LPS) as factors were used for LPS effects: †group effect vs. WT (P < 0.05), ‡group effect vs. WT (P > 0.1), §group effect vs. NOS2−/−, ‡LPS effect, ґinteractive effect (group × LPS) vs. WT, and ✱interactive effect (group × LPS) vs. NOS2−/− (P < 0.05).
mice, in contrast to WT and NOS2\(^{-/-}\) mice (Fig. 2). In agreement, there was also no increase in muscle arginine turnover after LPS in these mice (Table 3). The doubling of citrulline disposal and production after LPS was not observed in NOS3\(^{-/-}\) mice.

**DISCUSSION**

In the present study, the role of NOS2 and NOS3 in muscle protein and amino acid metabolism under baseline conditions and during endotoxemia was investigated in mice. The major findings were that disruption of the NOS2 gene compromises muscle glutamine release in control mice and that muscle arginine and protein turnover increased in WT and NOS2\(^{-/-}\) but not in NOS3\(^{-/-}\) mice during endotoxemia, implying a crucial role for NOS3 in this response.

**Baseline**

NOS2 deficiency entails a reduction of glutamine release under baseline conditions. Because glutamine is synthesized in muscle from its precursors glutamic acid and branched-chain

**Table 3. Muscle arginine and citrulline metabolism**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LPS Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>NOS2(^{-/-})</td>
</tr>
<tr>
<td>Arg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disposal</td>
<td>25.9±4.7</td>
<td>26.3±10.5</td>
</tr>
<tr>
<td>production</td>
<td>29.5±3.2</td>
<td>25.3±9.4</td>
</tr>
<tr>
<td>Cit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disposal</td>
<td>5.6±1.6</td>
<td>2.7±2.1</td>
</tr>
<tr>
<td>production</td>
<td>2.6±0.9</td>
<td>2.0±1.4</td>
</tr>
<tr>
<td>Q(_{Cit\rightarrow Arg})</td>
<td>1.9±1.3</td>
<td>0.2±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are nmol-10 g body wt\(^{-1}\)min\(^{-1}\). Q\(_{Cit\rightarrow Arg}\), de novo arginine production from citrulline. Statistics with 1-way ANOVA and post hoc Bonferroni test were used for baseline differences, and 2-way ANOVA using group and LPS as factors were used for LPS effects: *group effect vs. WT, †LPS effect (P < 0.05); ‡LPS effects (P < 0.1), and §interactive effect (group × LPS) vs. WT.
NOS2 in muscle is related to NOS2 activity. Because the reduced glutamic acid in muscle, confirming that glutamine metabolism is a gluconeogenic amino acid that, like glutamine, is derived from alanine and glycine to a lesser extent. Alanine is an important amino acid substrate for protein synthesis. Although we observed no change in hindquarter flow, microvascular rarefaction (26) or shunting of hindquarter blood flow from artery to vein through nonnutritive routes may occur (9). The role of NOS3 in muscle protein metabolism during endotoxemia is not well explored, but NOS inhibition with L-NAME in LPS-treated rats did not modify the metabolic response in muscle, measured as myofibrillar protein breakdown (35), which seems in line with our data even though L-NAME is not a specific NOS3 inhibitor. As mentioned in the previous section, the net muscle release of glutamine and total amino acids was reduced in baseline NOS2−/− mice. LPS did not decrease this muscle release further in these mice, but a decrease was observed in WT and NOS3−/− mice, indicating the role of NOS2 in this response. The accelerated muscle efflux of glutamine that is observed in sepsis (2, 4, 42) was not found in our female C57BL6J mice or in female Swiss and FVB mice (Hallemees, unpublished observation). Whether this is related to the early endotoxemia phase in our study or is gender-related needs further study.

The increase in citrulline uptake and citrulline disposal in muscle during endotoxemia may be related to the role of muscle as an extraintestinal site of citrulline production or storage, from which it is released when the blood level declines (53). In these mice, arterial plasma citrulline increased from 70 to 90 mM after LPS (30). Unlike WT mice, NOS3−/− mice did not increase plasma citrulline production (production even decreased further in these mice, but a decrease was observed in WT and NOS3−/− mice, indicating the role of NOS2 in this response. This study was supported by Grants 902-23-098 and 902-23-239 from the Dutch Association of Scientific Research.

**Endotoxemia**

In WT mice, LPS stimulated muscle protein metabolism, as indicated by the almost twofold increase in protein synthesis, the tendency toward a higher protein breakdown, and the increased phenylalanine hydroxylation. The final result was a small but net increase in protein accumulation. The increase in muscle protein turnover in our model is different from the general feature of muscle wasting in sepsis as a result of increased net protein breakdown (4, 10) and diminished protein synthesis (21, 27, 38), of which the latter is observed in rats. However, no data on muscle protein metabolism are available from endotoxemic mice. The discrepancy could be related to the early stage of endotoxemia during which our measurements were done (after 6 h; see Ref. 6), gender-related differences in protein metabolism (22, 44, 47), the inflammatory response during sepsis (40, 55), or the LPS-induced stimulation of NO synthesis (31). Moreover, it is possible that mice and rats respond differently to LPS, since even mice strains differ in their metabolic response to sepsis (31). Finally, the isotope technique may be important because a flooding-dose isotope technique was used in the rat studies to measure incorporation in muscle protein, whereas our technique measured arterio-venous differences across the hindquarter. On the whole body level, a change toward a catabolic state was observed in response to endotoxemia in these mice (decreased protein synthesis with net protein breakdown; see Ref. 30), probably representing mainly reduced splanchnic protein metabolism (12).

**Role of NOS2 and NOS3 in Endotoxemia**

Unlike WT mice, NOS3−/− mice did not increase protein metabolism (protein breakdown and protein synthesis) in response to LPS. This indicates that NOS3 plays a role in the adaptation of muscle protein metabolism to endotoxemia through increased protein breakdown and protein synthesis. Similarly, NOS3−/− mice did not show an adaptive response of muscle arginine metabolism to LPS. Therefore, muscle arginine metabolism seems to be related to protein metabolism. Whether the NOS3 involved is present in muscle or endothelial cells is speculative, but most likely, diminished NO-mediated vasodilation in NOS3−/− mice limits the delivery of amino acid substrates for protein synthesis. Although we observed no change in hindquarter flow, microvascular rarefaction (26) or shunting of hindquarter blood flow from artery to vein through nonnutritive routes may occur (9). The role of NOS3 in muscle protein metabolism during endotoxemia is not well explored, but NOS inhibition with L-NAME in LPS-treated rats did not modify the metabolic response in muscle, measured as myofibrillar protein breakdown (35), which seems in line with our data even though L-NAME is not a specific NOS3 inhibitor. As mentioned in the previous section, the net muscle release of glutamine and total amino acids was reduced in baseline NOS2−/− mice. LPS did not decrease this muscle release further in these mice, but a decrease was observed in WT and NOS3−/− mice, indicating the role of NOS2 in this response. The accelerated muscle efflux of glutamine that is observed in sepsis (2, 4, 42) was not found in our female C57BL6J mice or in female Swiss and FVB mice (Hallemees, unpublished observation). Whether this is related to the early endotoxemia phase in our study or is gender-related needs further study.

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