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Endotoxemia reduces skeletal muscle protein synthesis in neonates

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Orellana, Renan A., Pamela M. J. O'Connor, Hanh V. Nguyen, Jill A. Bush, Agus Suryawan, M. Carole Thivierge, Marta L. Fiorotto, and Teresa A. Davis. Endotoxemia reduces skeletal muscle protein synthesis in neonates. *Am J Physiol Endocrinol Metab* 283: E909–E916, 2002. First published July 17, 2002; 10.1152/ajpendo.00220.2002.—Protein synthesis in skeletal muscle is reduced by as much as 50% as early as 4 h after a septic challenge in adults. However, the effect of sepsis on muscle protein synthesis has not been determined in neonates, a highly anabolic population whose muscle protein synthesis rates are elevated and uniquely sensitive to insulin and amino acid stimulation. Neonatal piglets (n = 10/group) were infused for 8 h with endotoxin [lipopolysaccharide (LPS), 0 and 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$]. Plasma amino acid and glucose concentrations were kept at the fed level by infusion of dextrose and a balanced amino acid mixture. Fractional protein synthesis rates were determined by use of a flooding dose of [^{3}H]phenylalanine. LPS infusion produced a septic-like state, as indicated by an early and sustained elevation in body temperature, heart rate, and plasma tumor necrosis factor-α, interleukin-1, cortisol, and lactate concentrations. Plasma levels of insulin increased, whereas glucose and amino acids decreased, suggesting the absence of insulin resistance. LPS significantly reduced protein synthesis in longissimus dorsi muscle by only 11% and in gastrocnemius by only 15%, but it had no significant effect in masseter and cardiac muscles. LPS increased protein synthesis in the liver (22%), spleen (28%), kidney (53%), jejunum (19%), diaphragm (21%), lung (50%), and skin (13%), but not in the stomach, pancreas, or brain. These findings suggest that, when substrate supply is maintained, skeletal muscle protein synthesis in neonates compared with adults is relatively resistant to the catabolic effects of sepsis.

sepsis; nutrition; infection; insulin; amino acids

SEPSIS, defined as a systemic inflammatory syndrome that occurs in response to the presence of pathogenic microorganisms or their toxins (6), is a major cause of mortality and morbidity in the pediatric population. Its incidence is >25 times higher in infants under 1 yr of age than in children from 1 to 14 yr of age (2). Despite the major physiological and developmental differences between infants and adults, most studies focused on metabolism during septic and critically ill states have been performed in adults. Clinical criteria established for the diagnosis of sepsis in adults include microbiological evidence of infection, an elevation >2 standard deviations in temperature, heart rate, and respiratory rate, and changes in the leukocyte count (6). Sepsis is known to cause metabolic acidosis, changes in fuel utilization for energy production (42), and anorexia (32, 41). Key factors in the metabolic response to sepsis likely include changes in the circulating levels of or responses to the inflammatory cytokines, i.e., tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1), and IL-6; the stress hormone cortisol; and the anabolic hormones insulin and insulin-like growth factor I (1, 23–25, 32, 40, 41, 42, 44).

Studies in both adult humans and animals suggest that sepsis induces profound changes in protein metabolism, as indicated by lean tissue wasting, decreased skeletal muscle mass, and increased nitrogen excretion (7). Rates of whole body protein turnover, protein degradation, and protein synthesis are elevated in sepsis (32, 41), but the changes in protein synthesis vary among tissues (38, 39). Liver protein synthesis increases in sepsis, probably due to the increased synthesis of acute-phase reactants (20, 34); however, skeletal muscle protein synthesis in adult rats decreases by as much as 50% as early as 4 h after induction of a septic state (25, 39). Reduced muscle protein synthesis and increased muscle proteolysis both contribute to the catabolism of muscle protein during sepsis in adults (7).

The mechanisms that regulate muscle protein mass in the healthy neonate are fundamentally different from those in the healthy adult. In the neonate, muscle protein synthesis rates are relatively high and are markedly stimulated by feeding (8, 13, 14). The response to feeding decreases rapidly with development and is modest or absent in the adult (8, 14, 29, 37). The stimulation of muscle protein synthesis in the neonate by feeding is mediated by the postprandial rise in both

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insulin and amino acids (8–11, 14, 33). At maturity, the stimulation of muscle protein synthesis by amino acids is blunted, and the response of muscle protein synthesis to insulin is lost (3, 19, 21, 27–29).

The muscle protein synthesis characteristics that are unique to neonates suggest that the protein-catabolic response to stress may be lower in the neonate than in the adult. However, no previous studies have assessed the effect of systemic inflammation on muscle protein synthesis in neonates. We hypothesized that the high anabolic drive of the newborn organism may protect the muscle against the catabolic state induced by sepsis. To address this hypothesis, we examined skeletal muscle protein synthesis in neonatal pigs infused with an *Escherichia coli* endotoxin, lipopolysaccharide (LPS), which in adult animals has been shown to replicate many of the metabolic effects of sepsis (17, 40). For purposes of comparison, protein synthesis also was measured in tissues that are less responsive to anabolic stimuli, such as feeding, insulin, and amino acids, and that exhibit little or no developmental change in protein synthesis (11).

METHODS

Animals. Two crossbred (Landrace × Yorkshire × Hampshire × Duroc) pregnant sows (Agriculture Headquarters, Texas Department of Criminal Justice, Huntsville, Texas) were housed in lactation crates in individual environmentally controlled rooms for 1–2 wk before farrowing. They were fed a commercial diet (5084, PMI Feeds, Richmond, IN) and provided with water ad libitum. After farrowing, piglets were allowed to remain with the sow and were not given supplemental creep feed. Three days before the study, piglets were anesthetized with isoflurane anesthesia (Aerrane; Anaquest, Madison, Wisconsin), and catheters were inserted into a jugular vein and carotid artery by use of sterile techniques (43). Catheters were filled with heparinized saline, tied, and secured to the back of the animal with sterile dressings to avoid contamination, and the piglets were returned to the sow and allowed to suckle freely until studied. The study was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals.*

Experimental design. Twenty piglets (5–6 days of age; 2.2 ± 0.37 kg) from two litters were assigned randomly to control (n = 10) and LPS (n = 10) treatment groups. Before the study began, the animals were removed from the sow and placed in individual cages in a heated room (84°F), with free access to water but no feed. After fasting 16–18 h, the animals were placed in a sling restraint system. The arterial and venous catheters were accessed for infusion of dextrose and an amino acid mixture and for blood sampling, respectively. Between sampling times, the catheter was filled with normal saline solution containing 30 IU/ml of heparin sodium. One hour before the LPS infusion was initiated (−1 h), animals were infused with dextrose at a rate of 800 $mg \cdot kg^{-1} \cdot h^{-1}$ and a balanced amino acid mixture at a rate of 1.8 mmol total amino $acids \cdot kg^{-1} \cdot h^{-1}$ to simulate a normal fed state. The infusion was continued for 9 h. This amino acid mixture (11) contained (in mm) 20.1 arginine, 12.9 histidine, 28.6 isoleucine, 34.3 leucine, 27.4 lysine, 10.1 methionine, 12.1 phenylalanine, 21.0 threonine, 4.4 tryptophan, 34.1 valine, 27.3 alanine (38% provided as alanyl-glutamine), 12 aspartate, 6.2 cysteine, 23.8 glutamate, 17.1 glutamine (100% provided as alanyl-glutamine), 54.3 glycine (4% provided as glycyl-tyrosine), 34.8 proline, 23.8 serine, 2.0 taurine, and 7.2 tyrosine (83% provided as glycyl-tyrosine).

One hour after the initiation of the dextrose-amino acid infusion (0 h), the LPS group received a continuous infusion ($10\ \mu g \cdot kg^{-1} \cdot h^{-1}$) of *E. coli* endotoxin (lyophilized *E. Coli* Serotype 0111-B4, Sigma Chemical, St. Louis, MO) that was continued for 8 h while the control group received an equal volume of sterile normal saline solution (0.9% sodium chloride) at the same rate as the LPS infusion. The dose of LPS chosen was based on prior reports on LPS porcine models (17, 26) and pilot studies showing that higher doses ($15\ \mu g \cdot kg^{-1} \cdot h^{-1}$) did not allow animal survival, and a lower dose ($7.5\ \mu g \cdot kg^{-1} \cdot h^{-1}$) did not increase body temperature. Baseline and hourly measurements of rectal temperature, heart rate, and circulating concentrations of TNF-α, IL-1, cortisol, insulin, glucose, branched-chain amino acids (BCAA), blood urea nitrogen (BUN), and lactate were obtained.

Protein synthesis in vivo. Tissue protein synthesis was measured in vivo using a modification of the flooding dose technique described by Davis et al. (12) and Garlick et al. (18). Seven hours and 30 min after the LPS infusion began, pigs were injected via the jugular vein catheter with 1.5 mmol/kg body wt (1 mCi/kg body wt) of a flooding dose of L-[4-^{3}H]phenylalanine (Amersham, Arlington Heights, IL). Blood samples were taken at 5, 15, and 30 min after the injection for measurement of the specific radioactivity of the extracellular free pool of phenylalanine. Immediately after the 30-min blood sample was obtained, and 8 h after the LPS infusion was initiated, pigs were euthanized with an intravenous dose of pentobarbital sodium (50 mg/kg body wt). Longissimus dorsi, gastrocnemius, masseter, diaphragm, and cardiac muscles, liver, stomach, jejunum, lung, pancreas, kidney, and brain were rapidly removed, frozen in liquid nitrogen, and stored at −70°C until analysis (11).

Frozen tissues were processed as previously described (8, 12, 18). Briefly, samples were homogenized in 0.2 M perchloric acid (PCA), and the homogenate supernatants containing the tissue free amino acid pools were separated from the PCA-insoluble precipitates and neutralized. The PCA-insoluble precipitates were then washed and solubilized, and the protein content of the solubilized pellet was determined by the method of Smith et al. (36). The remaining solution was reacidified, and the acid-soluble fraction was assayed for RNA as described by Munro and Fleck (31). The protein pellet was washed and hydrolyzed for 24 h with 6 N HCl. The protein hydrolysate, homogenate supernatant, and blood supernatant were dried (Speedvac; Savant Instruments, Farmingdale, NY), washed three times with distilled water, and resuspended in distilled water for determination of phenylalanine-specific radioactivity. Phenylalanine in the protein hydrolysate, homogenate supernatant, and blood supernatant was separated from other amino acids by anion exchange chromatography (AS8 column; Dionex, Sunnyvale, CA). Amino acids were postcolumn derivatized with orthophethalaldehyde reagent and detected with an on-line fluorometer. Fractions were collected, and the radioactivity associated with the phenylalanine peak was measured in a liquid scintillation counter (TM Analytic, Elk Grove Village, IL).

Substrate, TNF-α, IL-1, cortisol, and insulin assays. Heparinized blood samples, obtained every hour from 1 to 8 h of the LPS infusion, were centrifuged, and the plasma was aliquoted and stored at −70°C until analyzed. Plasma glucose and lactate concentrations were determined by a glucose oxidase reaction (YSI 2300 STAT Plus; Yellow Springs In-

struments, Yellow Springs, OH). Plasma concentrations of total BCAA were measured by analysis of leucine, isoleucine, and valine deamination by leucine dehydrogenase with stoichiometric reduction of NAD measured by spectrophotometry, as previously described by Beckett et al. (4). BUN concentrations were measured by a multilayered urease reaction (Vitros Chemistry Products, Rochester, NY). Immunoreactive TNF-α and IL-1 concentrations were measured using swine solid-phase sandwich ELISAs with swine antibodies to the respective cytokines and swine standards (Biosource International, Camarillo, CA). Plasma cortisol concentration was determined using a human radioimmunoassay (RIA) kit with the appropriate standardization (Diagnostic Systems Laboratory, Webster, TX). Plasma insulin concentrations were measured using a porcine insulin RIA kit (Linco, St. Charles, MO).

Calculations. The fractional rate of protein synthesis (K_s), the percentage of protein mass synthesized in a day, was calculated as

$$K_s\ (\%/\text{day}) = [(S_B/S_A) \times (1{,}440/t)] \times 100$$

where S_B is the specific radioactivity of the protein-bound phenylalanine; S_A is the mean specific radioactivity of the tissue free phenylalanine during the labeling period determined from the amount at the time of tissue collection and corrected by linear regression of the change in blood specific radioactivity against time; and t is the time of labeling in minutes. We have demonstrated that the specific radioactivity of the tissue free phenylalanine after a flooding dose of phenylalanine is in equilibrium with the aminoacyl-tRNA specific radioactivity; hence, the tissue free phenylalanine reflects the specific radioactivity of the tissue precursor pool (12). Because most of the RNA in tissues is ribosomal RNA, the RNA-to-protein ratio (mg RNA/g protein) was used as an estimate of the protein synthetic capacity (C_s). Protein synthetic efficiency (K_{RNA}) was estimated as the total protein synthesized per total RNA (g protein·day^{-1}·g RNA^{-1}) (8).

Data analysis. Repeated-measures analysis of variance was used to detect differences between control and LPS groups over the time period. Treatment was the grouping factor for different parameters. Difference between groups in the rate of tissue protein synthesis was determined by a *t*-test. Results are presented as means ± SE. Probability values of <0.05 were considered statistically significant and are presented in Figs. 1–5 but not in the text. SPSS statistical software was used to analyze the data.

RESULTS

Indicators of sepsis. Body temperature of the animals infused with LPS increased within 2 h of the start of the LPS infusion and remained >2 SD above control values throughout the experiment (Fig. 1). Heart rate of the LPS group also was elevated by 2 h after initiation of the LPS infusion and remained higher throughout the experiment (Fig. 1). In contrast to control animals, LPS-treated pigs were tachypneic, had rigors and frequent, loose, watery stools, and responded poorly to tactile stimulation.

Cytokines, hormones, and substrates. TNF-α concentrations of the LPS group peaked 1 h after initiation of the LPS infusion, remained higher for 4 h, and then declined (Fig. 1). Peak IL-1 plasma concentration was reached 1 h after the TNF-α peak (Fig. 1). Similarly, cortisol increased significantly from the 2nd h in the LPS group and remained elevated throughout the experiment (Fig. 2). The elevated plasma cortisol concentration in both groups before the LPS infusion likely reflected the initial stresses of manipulation and fasting. Lactate in plasma was elevated in both groups before the LPS infusion, probably reflecting the relatively long period of fasting before the experiment, and it decreased rapidly after initiation of glucose and amino acid infusion. In the LPS group, serum lactate increased again after 3 h of initiation of LPS and remained elevated thereafter (Fig. 2).

Plasma insulin concentration in both groups increased in response to the glucose and amino acid infusion (Fig. 2). Plasma insulin concentration of the

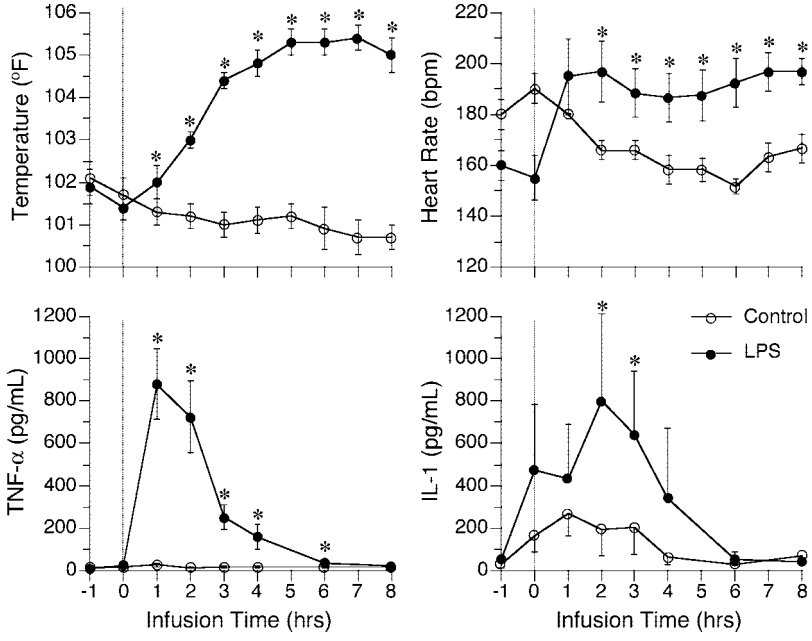

Fig. 1. Temperature, heart rate, tumor necrosis factor (TNF)-α, and interleukin (IL)-1 in lipopolysaccharide (LPS)-treated and control pigs during LPS infusion. Values are means ± SE; n = 10/group. bpm, Beats/min. Temperature and heart rate increased significantly ($P < 0.05$) in the LPS group after endotoxin infusion and remained higher throughout. TNF-α increased abruptly 1 h after initiation of LPS ($P < 0.05$) and returned to control levels by 8 h. IL-1 increased 2 h later in the LPS pigs ($P < 0.05$). *Significantly different from control value ($P < 0.05$).

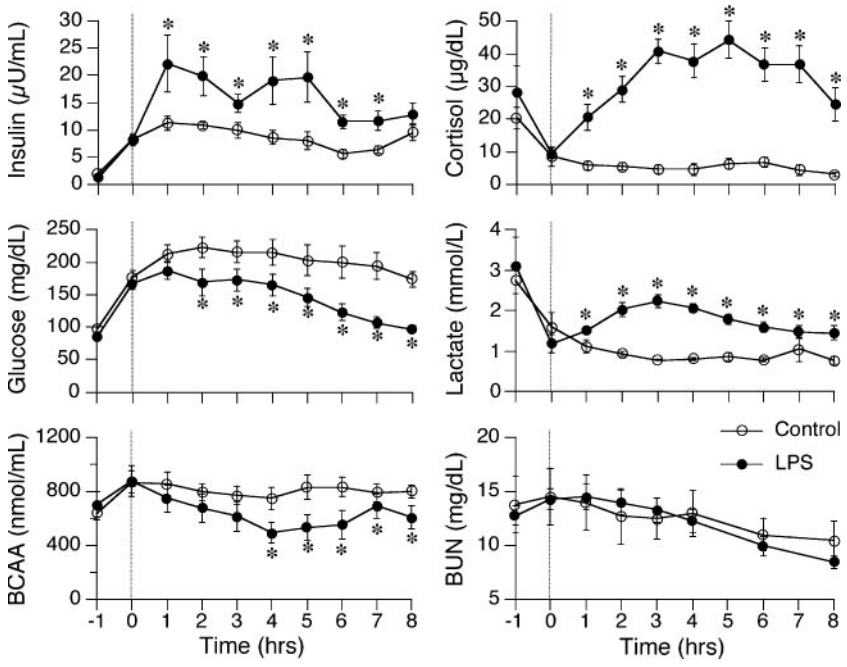

Fig. 2. Plasma insulin, cortisol, glucose, lactate, branched-chain amino acids (BCAA), and blood urea nitrogen (BUN) in LPS and control pigs during LPS infusion. Values are means ± SE; n = 10/group. Cortisol increased significantly ($P < 0.05$) by 1 h of endotoxin infusion and remained higher throughout. Dextrose and amino acid mixture infusions to simulate fed state raised insulin, glucose, and BCAA by *time 0* ($P < 0.05$) in both groups. Endotoxin infusion raised plasma insulin further by 1 h and decreased blood glucose levels 2 h after LPS was initiated ($P < 0.05$). BCAA levels were lower in the LPS group throughout the experiment ($P < 0.05$). There was no difference in BUN between control and LPS groups over time ($P < 0.05$). Lactate was significantly higher in LPS-infused pigs ($P < 0.05$). These changes were maintained throughout the LPS infusion. *Significantly different from control value ($P < 0.05$).

LPS group increased further with LPS infusion and remained significantly higher than that of the control group throughout the experiment. Whole blood glucose concentrations of both groups rose after initiation of the dextrose infusion. One hour after the start of the LPS infusion, the blood glucose concentration of the LPS group decreased and remained lower than that of the control group. Plasma BCAA concentration of both groups increased in response to the amino acid infusion. After 1 h of LPS infusion, levels were at plateau in controls but began to decrease in the LPS group; after 5 h there was no further change. BUN decreased progressively in both groups; the change over time was not different between the two groups (Fig. 2).

Protein synthesis. Fractional rates of protein synthesis in longissimus dorsi and gastrocnemius muscles were 11 and 15% lower, respectively, in the LPS vs. the control group (Fig. 3). There was no statistically significant effect of LPS infusion on masseter or cardiac muscle fractional protein synthesis rates (Fig. 3). Protein synthesis in the diaphragm, however, was 21% higher in the LPS group than in controls (Fig. 3).

LPS infusion resulted in higher fractional rates of protein synthesis in liver (22%), spleen (28%), jejunum (19%), lung (50%), kidney (53%), and skin (13%) (Fig. 4). Protein synthesis rates in stomach, pancreas, and brain were unaffected by LPS infusion (Fig. 5).

The RNA-to-protein ratio, an indicator of ribosome number, and thus of the protein synthetic capacity of the tissue, was not significantly affected by LPS in most tissues (Table 1). Protein synthetic efficiency, the total protein synthesized per total RNA, was significantly ($P < 0.05$) increased in spleen (44%), lung (25%), and kidney (50%). Interestingly, ribosome number decreased and protein synthetic efficiency increased in brain ($P < 0.05$).

DISCUSSION

Neonates are highly anabolic and are characterized by a high rate of skeletal muscle protein synthesis that is uniquely sensitive to stimulation by insulin and amino acids (8, 10, 11, 13–15, 33). However, the effect of sepsis on skeletal muscle protein synthesis in neonates has not been examined previously. In the current study, we infused endotoxin in neonatal pigs to assess the short-term effect of a septic-like state on skeletal muscle protein synthesis. Glucose and amino acids were infused to simulate a fed state (8, 10, 11). Protein synthesis in fast-twitch, glycolytic skeletal muscle of neonatal pigs infused with endotoxin was reduced by only 11–15% relative to the control group, which is a four- to fivefold smaller response than changes re-

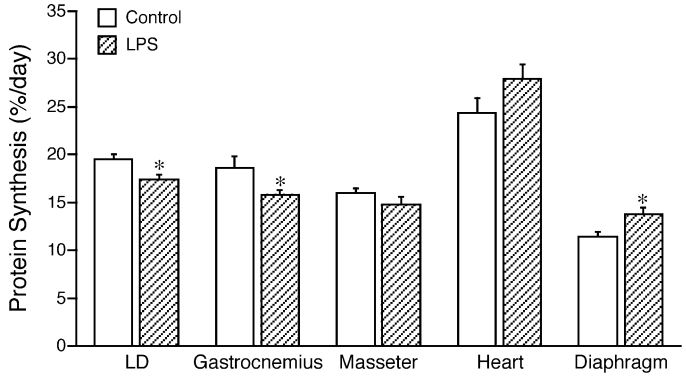

Fig. 3. Fractional protein synthesis rates in longissimus dorsi (LD), gastrocnemius, masseter, heart, and diaphragm muscles in LPS and control pigs after an 8-h LPS infusion. Values are means ± SE; n = 10/group. LPS infusion modestly reduced protein synthesis in LD and gastrocnemius muscles ($P < 0.05$) and increased protein synthesis in diaphragm ($P < 0.05$). *Significantly different from control value ($P < 0.05$).

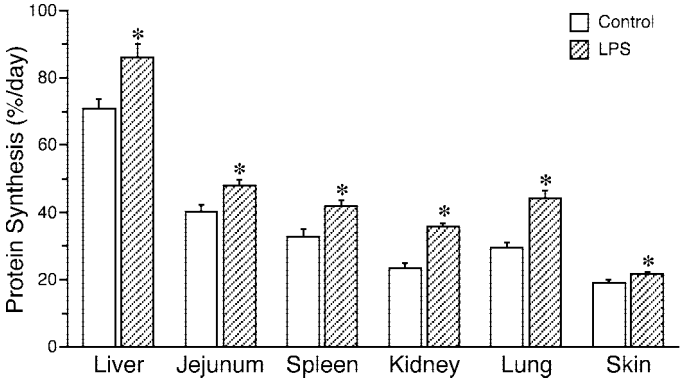

Fig. 4. Fractional rates of protein synthesis in liver, jejunum, spleen, kidney, lung, and skin in LPS and control pigs after an 8-h LPS infusion. Values are means ± SE; $n = 10$/group; ($P < 0.05$). Protein synthesis in liver, jejunum, spleen, kidney, lung, and skin was increased in the LPS group ($P < 0.5$). *Significantly different from control value ($P < 0.05$).

ported in adult models of sepsis, including LPS infusion models (25, 39). This finding suggests that, when substrate supply is maintained, skeletal muscle protein synthesis in neonatal pigs is relatively resistant to the catabolic effects of acute endotoxin exposure compared with that in adults (25, 39).

Neonatal model of endotoxemia. Ethical considerations preclude measuring in vivo protein synthesis in a vulnerable population such as the human infant. The neonatal pig has proven useful for metabolic-hormonal studies because of the similarity between the neonatal pig and the human infant in several aspects of anatomy, developmental physiology, and metabolism (30). Although neonates have limited muscle protein stores and could be particularly vulnerable to the protein catabolic effects of sepsis, we hypothesized that their high anabolic drive and unique sensitivity to insulin and amino acids (9–11) render them resistant to the sepsis-induced reduction in muscle protein synthesis. In the current study, we used endotoxemia to develop a septic-like state in the neonatal pig, because previous studies in adult animals, including pigs, have shown that LPS administration reproduces many of the metabolic derangements of sepsis shortly after LPS is infused (17, 40). Different models have been used to induce experimental sepsis (17, 25, 26, 39, 40); however, the end point used in this study was a clinical state of inflammation already defined by objective criteria (5). The dose of LPS we chose was based on pilot studies to guarantee the survival of the animal without cardiorespiratory support. Although differences in response may exist between species, the response to endotoxin has been validated in the adult swine (17, 26, 40).

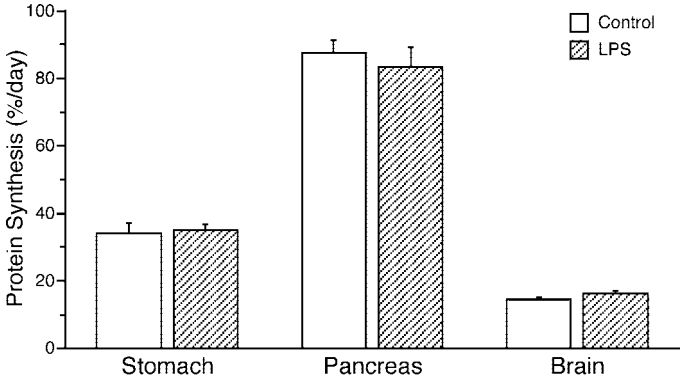

Fig. 5. Fractional rates of protein synthesis in stomach, pancreas, and brain in LPS and control pigs after an 8-h LPS infusion. Values are means ± SE; $n = 10$/group. There were no significant differences in protein synthesis in the 2 groups ($P > 0.05$).

Table 1. *Percent changes in protein synthetic capacities and efficiencies in different tissues in endotoxin-infused neonatal pigs compared with controls*

	C_s (% change)	K_{RNA} (% change)
Longissimus dorsi	−9.9	+0.4
Gastroenemius	−11.2	+4.3
Diaphragm	+18.3	+6.0
Heart	−1.9	+15.7
Masseter	+8.1	−7.8
Liver	+7.9	+13.9
Spleen	−11.8	+44.5*
Kidney	+3.6	+46.9*
Lung	+17.4	+26.5*
Jejunum	+3.8	+11.4
Pancreas	−1.9	−9.5
Brain	−8.8*	+28.3*
Stomach	+7.1	−2.8
Skin	+10.5	+5.2

Values are percent changes in protein synthetic capacity (C_s, RNA-to-protein ratio) and efficiency (K_{RNA}, total protein synthesized per total RNA) in LPS-infused vs. control neonatal pigs. *Significant change in LPS-infused vs. controls ($P < 0.05$).

In the current study, an 8-h infusion of LPS stimulated the clinical signs of sepsis, which included an elevation of temperature and heart rate along with elevations in blood cytokine and hormone levels. An early TNF-α elevation followed by a rise in cortisol and IL-1 was demonstrated during the continuous endotoxin infusion, similar to the pattern observed in previous studies on endotoxemic models in mature animals (16, 40). Lactate elevation was also seen in the LPS-infused animals, which may reflect an attempt to maintain a gluconeogenic substrate supply for the high energy needs incurred by stress and/or the impaired perfusion state that is characteristic of sepsis and that obliges the cell to anaerobic metabolism (42).

The fact that there was no difference over time in BUN between LPS and control groups suggests that there was no effect on ureagenesis in our model of acute endotoxemia. This finding contrasts with those of endotoxin studies in adult pigs, in which elevation of BUN ensued as early as 4 h as a reflection of a catabolic state (20, 40).

Insulin sensitivity. In adult humans, LPS infusion triggers hyperglycemia and an increase in circulating insulin levels similar to symptoms observed in sepsis (1, 35, 43). Studies have shown that LPS increases pancreatic insulin secretion (23) and that hyperglycemia during endotoxin infusion may occur from other

counterregulatory hormones (e.g., glucagon, cortisol, and epinephrine) or insulin resistance (17, 23, 24, 43). In the current study, we found that circulating concentrations of insulin increased in the neonate in response to LPS infusion; however, in contrast to the adult, glucose decreased. Although changes in plasma insulin and glucose are difficult to interpret unequivocally, as they relate to insulin sensitivity, the results of the current study are consistent with an absence of insulin resistance for glucose metabolism in acute endotoxemia in the neonate. Hypoglycemia is commonly observed as the initial manifestation of sepsis in the neonate, and previous studies have shown that LPS may induce hypoglycemia in the suckling rat, with factors other than diminished glycogen reserve playing a role in glucose dyshomeostasis (44). Whether the hypoglycemia in the presence of hyperinsulinemia in neonatal sepsis is due to a lack of insulin resistance, decreased gluconeogenic capacity, or both, remains to be demonstrated.

The decrease in serum BCAA levels after LPS administration also suggests a lack of insulin resistance for amino acid metabolism. Previous studies in adult septic animals have allowed the subject to feed ad libitum, but they become anorectic (6), and the lack of substrate availability likely contributes to the enhanced protein catabolism. In our study, we infused a balanced amino acid solution that resulted in serum amino acid levels similar to those in the fed state. The high sensitivity to insulin in the neonatal muscle (9, 11, 14, 33), and an apparent lack of development of insulin resistance in the early septic-like state in the neonate, likely account for the lower serum BCAA levels in the LPS group. However, more detailed study is required to ascertain whether the enhanced insulin sensitivity of protein metabolism in neonatal muscle is maintained during an acute septic challenge in the neonate.

Protein synthesis. Studies in adult animals have shown that sepsis reduces skeletal muscle protein synthesis, a reduction that occurs as early as 4 h and is maintained as long as 24 h after an endotoxin challenge (25, 39). In the current study, LPS infusion also reduced muscle protein synthesis in neonatal pigs and, similar to findings in adult studies, the sepsis-induced reduction in skeletal muscle protein synthesis was more prominent in fast-twitch glycolytic than in slow-twitch oxidative muscles of the neonatal pig (22, 39). Muscle protein synthesis rates in skeletal muscles primarily composed of fast-twitch, glycolytic fibers, i.e., gastrocnemius and longissimus dorsi, which predominate in the neonatal pig, were reduced by 11 to 15% in response to LPS infusion. In contrast, the masseter muscle, which has primarily oxidative properties, and the cardiac muscle showed no change in protein synthesis in response to LPS. Interestingly, protein synthesis in the diaphragm, a muscle of mixed composition, was significantly increased, perhaps due to the increased respiratory work related to tachypnea and hyperpnea observed in this study.

Although acute LPS infusion in the neonatal model used in the current study reduced protein synthesis in fast-twitch, glycolytic muscles, the reduction was about fourfold less than that reported previously in adult models of sepsis, including LPS infusion models (25, 39). Because insulin resistance has been implicated in the reduction in protein synthesis in different adult models of sepsis (22), we speculate that the modest reduction in muscle protein synthesis in this neonatal model of endotoxemia may be due to the unique sensitivity of neonatal muscle protein synthesis to insulin and amino acids. In the current study, pigs were infused with glucose and amino acids to simulate the fed level and to induce endogenous production of insulin similar to that observed in the fed state. Because the sensitivity of muscle protein synthesis to these anabolic agents is markedly elevated in the neonate (8–11, 33), and there was no readily apparent insulin resistance in this model, the elevation in insulin and/or amino acids to fed levels (9, 33, 38) may have circumvented some of the catabolic effects induced by the septic-like state (7). Whether the further elevation in insulin, induced by LPS infusion, blunted the reduction in muscle protein synthesis by endotoxin is open to speculation, but this potential effect also could have been counteracted by an LPS-induced reduction in amino acid concentration. Further study is required to determine the role of hormones and substrate availability in the regulation of muscle protein synthesis in the neonate during sepsis.

Studies in different adult models of sepsis have shown that, although protein synthesis is reduced in skeletal muscle during the inflammatory response, whole body protein synthesis is enhanced as a result of increased visceral tissue protein synthesis (6). The increase in hepatic protein synthesis reflects the stress-induced synthesis of acute-phase reactants (6, 20, 41, 40). In the current study, we found an increase in hepatic protein synthesis in neonatal pigs infused with LPS. Other organs that presented a similar increased protein synthesis response were the small intestine, kidney, spleen, and lung. In healthy neonatal animals, visceral protein synthesis is not dependent on insulin, and different mechanisms regulate the growth of peripheral and visceral tissues in the neonate (10, 11). The results of the current study demonstrate that the regulation of protein synthesis in skeletal muscle differs from that in other tissues of the neonate during endotoxemia. In the acute phase of sepsis, accumulation and migration of inflammatory cells, lymphocyte activation, and extrahepatic acute-phase reactant synthesis in some of the visceral tissues likely play a role in increasing protein synthesis (6). Prior studies in adult rats showed that skin protein synthesis is not affected by sepsis (6). In our study, skin protein synthesis was increased in LPS-treated neonatal pigs.

The LPS-induced changes in tissue protein synthesis were likely due to changes in the efficiency of the translational process rather than changes in ribosome number, similar to reports in adult septic rats (39). Although we were unable to detect significant reduc-

tions in translational efficiency in muscles containing primarily fast-twitch, glycolytic fibers in LPS-infused pigs, despite reductions in fractional protein synthesis rates, this was not surprising given the small (−11 to −15%) decline in muscle protein synthesis rates. Moreover, significant elevations in translational efficiency were observed in visceral organs (spleen, lung, and kidney) in which protein synthesis rates were markedly (+28 to +53%) increased. Further study is required to determine whether the LPS-induced changes in protein synthesis in neonates are associated with changes in activity of the factors that regulate translation initiation.

Perspectives. In summary, in the present study we demonstrate for the first time that neonatal animals, unlike adults, are uniquely resistant to a sepsis-induced reduction in muscle protein synthesis after a short-term infusion of LPS. The modest reduction in protein synthesis in fast-twitch skeletal muscle of LPS-infused neonatal pigs contrasts with a more profound reduction in skeletal muscle protein synthesis in different models of adult sepsis (25, 39). The reduction of circulating glucose and amino acid concentrations in the presence of an LPS-induced elevation in insulin levels suggests a lack of insulin resistance in this model, although further study is required. The results of the current study support the hypothesis that, when substrate supply is maintained, the high rate of neonatal muscle protein synthesis is largely maintained during acute endotoxemia because of the high anabolic drive and unique sensitivity of neonatal muscle protein synthesis to stimulation by insulin and/or amino acids.

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