Modulation of muscle protein synthesis by insulin is maintained during neonatal endotoxemia

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BACTERIAL ENDOTOXIN [lipopolysaccharide (LPS)] elicits a systemic inflammatory response that has profound consequences on human metabolic homeostasis (1, 45). The physiological response to LPS mimics the metabolic processes triggered by the generalized immune response observed during sepsis, such as insulin resistance for glucose and amino acid metabolism (1, 39, 46) and the profound reduction of muscle protein synthesis in adult animals (26, 29). Hormones and acute-phase reactants that exert and regulate the stress response during sepsis, such as tumor necrosis factor-α (TNF-α), cortisol, insulin, and insulin-like growth factor I (17, 44, 45), affect the metabolic homeostasis in the normal host, and LPS has been recognized as a potent stimulus that elicits this innate immune response (17, 18, 44, 49).

In healthy adult humans, insulin promotes an anabolic state, augmenting cellular glucose uptake and enhancing muscle protein deposition (23, 30, 41). During experimental sepsis, LPS has been shown to induce pancreatic insulin secretion (28) and to increase circulating insulin levels similar to that observed in naturally occurring sepsis (32, 46). During sepsis, there is a hyperglycemic response in the face of hyperinsulinemia, which may occur as a result of elevations in stress-related counterregulatory hormones (e.g., glucagon, cortisol, and epinephrine), insulin resistance (39, 46), or alterations in cellular glucose transporters (21, 24). However, in mature organisms, high levels of insulin have failed to promote an increase in muscle protein synthesis during experimental sepsis (26, 42), suggesting that insulin resistance may contribute to the profound and sustained reduction in muscle protein synthesis during sepsis in mature organisms (29, 43). In adult rats, the LPS-induced reduction in muscle protein synthesis has been associated with depression of the insulin-dependent translation initiation signaling (29, 43), an effect that has been reproduced in different experimental models of sepsis (27, 37, 38).

In contrast to adults who present a profound and sustained reduction in muscle protein synthesis, neonatal animals present only a moderate decrease in muscle protein synthesis when challenged acutely with endotoxin (38), and this metabolic effect is maintained for at least 24 h (37). This difference has been attributed to the high anabolic drive that is required to sustain growth in young organisms (15, 35). In the healthy neonate, contrary to adults, muscle protein synthesis rates are relatively high and are markedly stimulated by the postprandial rise in both insulin and amino acids (12, 13, 35). This response of muscle protein synthesis to stimulation by insulin and amino acids decreases rapidly with development and is modest or absent in the adult (12, 16, 48). The high anabolic drive and unique sensitivity of neonatal muscle protein synthesis to stimulation by insulin and/or amino acids suggests that the protein catabolic response to stress may differ in the neonate compared with the adult. During experimental sepsis, neonatal animals present with an inability to maintain normoglycemia (20, 22) due to immaturity of hormonal actions and responses (22, 50). By contrast, neonates only exhibit a modest reduction in muscle protein synthesis (38), although the change in cel-
lular signaling to translation initiation is more profound than the degree of reduction in muscle protein synthesis (27, 37).

To determine whether the enhanced responsiveness of muscle protein synthesis to insulin is maintained during endotoxemia in the neonate, we examined skeletal muscle protein synthesis in neonatal pigs infected with *Escherichia coli* endotoxin (LPS), and insulin was maintained at fasting (2 μU/ml) and fed (10 μU/ml) levels by providing somatostatin and exogenous insulin. Dextrose and a balanced amino acid mixture were infused to maintain amino acids and glucose levels similar to those observed during fasting. We measured glucose and amino acid disposal rates, protein synthesis in muscles of different fiber types, and, for comparison, protein synthesis in other tissues that have shown to be less responsive to insulin and exhibit little or no developmental change in protein synthesis (14). Our results suggest that the response of neonatal muscle protein synthesis to insulin is largely maintained during acute endotoxemia, likely acting as a protective mechanism against catabolism during acute systemic inflammation in newborn animals.

**METHODS**

**Animals.** Crossbred (Landrace × Yorkshire × Hampshire × Duroc) pregnant sows (Agriculture Headquarters, Texas Department of Criminal Justice, Huntsville, TX) were housed in lactation crates in individual, environmentally controlled rooms for 1 to 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, WI), and catheters were inserted into a jugular vein and carotid artery by use of sterile techniques (47). 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.** Thirty piglets (5–6 days of age; 1.9 ± 0.31 kg) were assigned randomly to control (n = 15) and LPS (n = 15) treatment groups. Three days were allowed for recovery between catheter insertion and infusion. 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 14–16 h, the animals were placed in a sling restraint system. The arterial and venous catheters were accessed for infusion of hormones, 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 sodium heparin.

**Pancreatic glucose-amino acid clamps.** Pancreatic glucose-amino acid clamps were performed in each animal, following techniques previously described (Fig. 1) (34, 47). Each animal was placed, unanesthetized, in a sling restraint system, and the average basal concentration of whole blood glucose and plasma branched-chain amino acid (BCAA) concentrations was measured in each individual animal over a 30-min period. The clamp was then initiated with a primed (20 μg/kg), continuous (100 μg·kg⁻¹·h⁻¹) somatostatin (Bachem, Torrance, CA) infusion to block endogenous insulin secretion (−60 t). Because the secretion of glucagon by the pancreas is also inhibited by somatostatin, a continuous infusion of replacement glucagon (150 ng·kg⁻¹·h⁻¹; Eli Lilly, Indianapolis, IN) was provided 10 min after the somatostatin infusion began and was continued to the end of the clamp period. Simultaneously with the glucagon infusion, insulin was infused at 7 ng·kg⁻¹·min⁻¹ to achieve plasma insulin concentrations of 2–5 μU/ml to simulate a fasting insulinemic state (Fig. 1). Glucose and amino acids were clamped to the individual basal fasting levels achieved during the first hour (−60 to 0 t) and maintained during the 8-h infusion by monitoring the blood glucose and serum BCAA every 5 min and adjusting the infusion rates of dextrose and a balanced amino acid mixture to maintain plasma BCAA and blood glucose within 10% of the desired level (16, 47). One hour after the initiation of the somatostatin infusion, the LPS group received a continuous infusion (10 μg·kg⁻¹·h⁻¹) of *E. coli* endotoxin (lyophilized *E. coli* serotype 0111:B4; Sigma Chemical, St. Louis, MO) that was continued for 8 h, and the control group received an equal volume of sterile normal saline solution (0.9% sodium chloride) at the same rate as the LPS infusion (Fig. 1). On the basis of pilot work, prior studies in neonatal piglets (38), and prior reports on LPS porcine models (6, 18), this dose of LPS produces a septic-like response during a short, 8-h infusion and allows animal survival without cardiorespiratory support. Baseline and hourly measurements of rectal temperature, heart rate, and circulating concentrations of cortisol, insulin, glucose, BCAA, and C-peptide were obtained.

After 6 h of LPS-saline infusion (6 t), insulin infusion was either increased to 40 ng·kg⁻¹·min⁻¹ (LPS + insulin, n = 7; control + insulin, n = 8) to replicate fed levels of insulin (10 μU/ml), or it remained at replacement levels (LPS, n = 8; control, n = 7). Blood glucose and serum BCAA continued in the targeted fasting range in both groups. All animals were killed 8 h after the LPS infusion began (8 t), i.e., 2 h after increasing the insulin infusion rate in the fed-insulin-level groups (6 t), thereby providing sufficient time to achieve a steady state for the targeted circulating glucose, amino acid.

**Fig. 1.** Schematic representation of endotoxemic pancreatic-substrate clamps and tracer infusion indicating times of hormonal infusion, tracer administration, and insulin clamp. AA, amino acid.
acids, and insulin levels before the animals were subjected to a flooding dose of the tracer.

**Measuring protein synthesis in vivo.** Tissue protein synthesis was measured in vivo using a modification of the flooding dose technique (19). Seven hours and 30 min after the LPS infusion was initiated, 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 [4-3H]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). 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 (38, 48).

Frozen tissues were processed as previously described (12, 19). Briefly, samples were homogenized in 0.2 M perchloric acid, and the homogenate supernatants containing the tissue free amino acid pools were determined by the method of Smith et al. (40), and total RNA concentration was estimated (33). Phenylalanine-specific radioactivity was measured by anion exchange chromatography (AS8 column; Dionex, Sunnyvale, CA). Amino acid 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, cortisol, and insulin assays.** Whole blood glucose concentrations were determined by a glucose oxidase reaction (YSI 2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH). Heparinated 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 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 (3). 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). Plasma C-peptide levels were measured using an immunochromiluminometric assay (Esoterix, Calabasas Hills, CA).

**Calculations.** Whole body net glucose disposal rates (mg glucose·kg⁻¹·min⁻¹) and whole body net amino acid disposal rates (mmol leucine·kg⁻¹·h⁻¹) were calculated from the infusion rates of dextrose and amino acid solution needed to maintain baseline fasting levels of blood glucose and serum amino acids, respectively.

The fractional rate of protein synthesis (Ks), the percentage of protein mass synthesized in a day, was calculated as $K_s \,(\% / \text{day}) = (\frac{[S_0/S_A] 	imes (1,440/t)}{100},$ where $S_0$ 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, 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 following 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. 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, i.e., ribosome number. Protein synthetic efficiency was estimated as the total protein synthesized per total RNA (g protein·day⁻¹·g RNA⁻¹) (12).

**Data analysis.** Repeated-measures analysis of variance was performed to detect differences between control and LPS groups over the time period by using statistical software (Minitab for Windows). Treatment was the grouping factor for different parameters. Analysis of variance was used to determine the effect of LPS, insulin, and their interaction on protein synthesis. $t$-Tests were also performed to examine the specific effects of each treatment group. Results are presented as means ± SE. Probability values of <0.05 were considered statistically significant and are presented in the figures but not in the text.

**RESULTS**

**Indicators of a systemic inflammatory response.** LPS-infused animals presented with higher temperature (controls, 101.5 ± 0.3°C; LPS, 104.1 ± 0.3°C; $P < 0.05$), tachycardia (controls, 180 ± 3 beats/min; LPS, 232 ± 3 beats/min; $P < 0.05$), and a tendency for elevated cortisol levels (controls, 2.2 ± 1 mg/ml; LPS, 15.1 ± 5.3 mg/ml; $P = 0.07$) compared with controls. An elevation in these parameters indicates that a stress response was activated by endotoxin infusion.

**Hormones and substrates during pancreatic-substrate clamps.** After the infusion of somatostatin began, C-peptide was not detectable in plasma of either LPS or control animals, indicating endogenous insulin blockade. After 14–16 h of fasting, baseline insulin levels were obtained for each individual animal, and those fasting levels were maintained during the entire period of LPS infusion in both control and experimental animals ($P > 0.05$, baseline vs. fasting insulin levels, Table 1). In the insulin-stimulated group, insulin levels similar to those observed in the fed state were obtained by providing exogenous insulin in both control and LPS-infused animals during the last 2 h of LPS infusion, and they were different from those in the fasting condition in both control and LPS-infused animals ($P < 0.05$; Table 1). In controls, whole blood glucose and plasma BCAAs were maintained at fasting levels during the entire experimentation ($P > 0.05$; Table 1), even when exogenous insulin was supplemented ($P < 0.05$; Table 1). Even though blood glucose was reduced from baseline when insulin was maintained at fasting levels in LPS-infused animals ($P = 0.04$), its value remained within the physiological fasting range (Table 1). In fasting LPS-infused animals, plasma BCAAs were maintained at baseline levels during the entire experimentation ($P > 0.05$; Table 1). When exogenous insulin was supplemented, LPS-infused animals maintained baseline levels.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Control</th>
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<tr>
<td>Insulin, μU/ml</td>
<td>3 ± 0.6</td>
<td>3 ± 0.4</td>
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<tr>
<td>- Insulin</td>
<td>3 ± 0.6</td>
<td>3 ± 0.8</td>
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<tr>
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<td>12 ± 3.0*</td>
<td>13 ± 8.0*</td>
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<td>+ Insulin</td>
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<td>75 ± 4</td>
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<tr>
<td>BCAA, mmol/ml</td>
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<td>509 ± 36</td>
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<td>613 ± 76</td>
<td>480 ± 21</td>
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<tr>
<td>+ Insulin</td>
<td>547 ± 43</td>
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Values are means ± SE; $n = 7–8$ / group. BCAA, branched-chain amino acid; LPS, lipopolysaccharide. −Insulin groups were provided replacement insulin to achieve fasting levels (2–4 μU/ml); +Insulin groups were provided replacement insulin to achieve fed levels (10–12 μU/ml). *Significantly different from baseline value ($P < 0.05$).
of blood glucose and plasma BCAA during the entire infusion ($P > 0.05$; Table 1).

**Glucose and amino acid disposal rates during pancreatic-substrate clamps.** Both glucose and amino acid infusion rates were significantly increased from baseline by both insulin and LPS infusion ($P < 0.05$; Fig. 2). In control and LPS-infused animals, insulin enhanced whole body glucose disposal rates ($P < 0.05$; Fig. 2). Compared with fasting controls, LPS augmented whole body glucose disposal rates ($P < 0.03$; Fig. 2). We did not find an interaction of LPS and insulin on glucose disposal rates, indicating that the effects of insulin and LPS were additive ($P > 0.05$; Fig. 2). In fasting animals, LPS increased whole body net amino acid disposal rates ($P < 0.01$; Fig. 2). Insulin also enhanced whole body net amino acid disposal rates in control and LPS-infused animals ($P < 0.05$; Fig. 2). However, there was no interaction of LPS and insulin on whole body amino acid disposal, indicating that the response to insulin was not affected by LPS ($P > 0.05$; Fig. 2).

**Protein synthesis in muscle of different fiber types during endotoxemic pancreatic-substrate clamps.** In the presence of fasting insulin levels, LPS reduced protein synthesis in gastrocnemius ($-26\%$, $P < 0.04$), stimulated protein synthesis in diaphragm ($+43\%$, $P < 0.01$), and had no effect on masseter and heart muscle ($P > 0.05$; Fig. 3A). Increasing insulin to fed levels accelerated muscle protein synthesis rates in gastrocnemius (controls by 58%, LPS by 60%), masseter (controls by 50%, LPS by 43%), heart (controls by 34%, LPS by 40%), and diaphragm (controls by 54%, LPS by 29%, $P < 0.05$; Fig. 3A). The response to insulin was proportionally similar in LPS and controls, and there was no interaction between LPS and insulin in regard to the effect on protein synthesis.

The protein synthetic capacity of the tissue, measured by the RNA-to-protein ratio, was not affected by LPS or insulin in all muscles ($P > 0.05$; data not shown). In animals maintained at fasting insulin levels, the protein synthetic efficiency, as indicated by the total protein synthesized per total RNA, was significantly decreased by LPS in gastrocnemius ($-25\%$, $P < 0.03$; Fig. 3B), increased in the diaphragm ($+52\%$, $P < 0.01$; Fig. 3B), and was not altered in masseter or heart muscles ($P > 0.05$; Fig. 3B). Raising insulin to fed levels accelerated protein synthetic efficiency in gastrocnemius (controls by 38%, LPS by 60%), masseter (LPS only, by 25%), masseter (controls only, by 37%), and diaphragm (controls by 73%, LPS by 26%, $P < 0.05$; Fig. 3B).

**Protein synthesis in viscera.** In the presence of fasting insulin levels, LPS stimulated protein synthesis in liver ($+28\%$, $P < 0.02$), lung ($+36\%$, $P < 0.04$), spleen ($+27\%$, $P < 0.05$), pancreas ($+44\%$, $P < 0.05$), and kidney ($+43\%$, $P < 0.01$) compared with controls (Fig. 4A). Raising insulin to fed levels increased protein synthesis in the spleen of fasting controls ($+35\%$, $P < 0.02$) only but did not alter protein synthesis in liver, lung, pancreas, and kidney in both groups ($P > 0.05$; Fig. 4A). Neither LPS nor insulin influenced protein synthesis in the spleen of fed animals.
synthesis in jejunum, stomach, skin, and brain (P > 0.05; data not shown).

LPS, but not insulin (P > 0.05; Fig. 4B), accelerated protein synthetic efficiency in liver (P < 0.01; Fig. 4B). Similar to previous publications (38), LPS did not affect the protein synthetic capacity of the viscera of neonatal animals (P > 0.05; data not shown). In the present study, insulin did not affect the protein synthetic capacity of the liver, lung, spleen, pancreas, kidney, jejunum, stomach, skin, or brain in controls or LPS-infused animals (P > 0.05; data not shown). In the presence of fasting insulin levels, LPS stimulated fractional protein synthesis efficiency in the lung (P = 0.00; Fig. 4B), spleen (P = 0.06; Fig. 4B), pancreas (P = 0.01; Fig. 4B), and kidney (P = 0.00; Fig. 4B). Raising insulin to fed levels stimulated the protein synthetic efficiency in the spleen of controls (P < 0.05; Fig. 4B).

DISCUSSION

Previously, we have examined the effects of sepsis on protein synthesis in skeletal muscle of neonatal pigs by infusing endotoxin (LPS), and glucose, amino acids, and insulin levels were maintained at levels similar to those seen in the fed state (37, 38). In those studies, LPS reduced muscle protein synthesis only in fast-twitch muscles of neonatal pigs, and the reduction was no more than 11–15% relative to the control group. By contrast, previous models of experimental sepsis in which adult septic animals were allowed to decrease their feed intake have shown a more profound reduction in muscle protein synthesis in fast-twitch muscles (29, 43). The lack of substrate availability may account for the decrease in muscle protein synthesis. In the present study, we infused endotoxin in neonatal pigs, and glucose and BCAA were maintained at fasting levels, but insulin levels were maintained either at fasting (2 μU/ml) or fed (10 μU/ml) levels by infusing somatostatin to block endogenous insulin secretion (34) and infusing insulin to achieve the desired levels. Our results show that the reduction in protein synthesis in skeletal muscle of LPS-infused neonatal pigs was more profound when insulin and amino acids were kept at the fasting level, but the ability of muscle protein synthesis to respond to insulin was maintained, contrary to reports in mature animals (29, 43).

Effects of endotoxemia in neonatal animals. Although neonatal animals have limited muscle protein stores, they maintain a high anabolic drive and a unique sensitivity to insulin and amino acids (15), even when faced by catabolic insults such as endotoxin infusion (38). The neonatal animal has unique metabolic responses to acute inflammation compared with the adult (50, 51). Considering the similarities between the neonatal pig and the human infant in physiology and metabolism, the reproducible response to LPS administration (37, 38), and the prominent response of muscle protein synthesis to insulin and amino acids in the neonatal pig (15), our neonatal model of endotoxemia may provide a close reproduction of the influence of development on the effect of acute inflammation on muscle protein synthesis. In the present study, 8-h infusion of LPS simulated a septic-like state characterized by a febrile response, tachycardia, and elevation of serum cortisol. The metabolic consequences of endotoxin infusion in swine have been validated in prior publications (5, 18, 44).

Glucose disposal and response to insulin during LPS infusion. During acute sepsis and inflammation, the balance between blood glucose and whole body glucose uptake depends on hormones that promote hyperglycemia (such as cortisol, glucagon, and epinephrine), the presence or absence of insulin resistance, altered insulin homeostasis, and the effect of specific mediators such as endotoxin (LPS) (11, 46). In the present experiment, both insulin and LPS augmented whole body glucose disposal in neonatal pigs. Moreover, fasted LPS-infused animals required higher infusion rates of dextrose to maintain baseline fasting blood glucose compared with fasting controls, even when faced by a similar plasma insulin concentration. Previously, it has been demonstrated (11, 31) that, during the euglycemic and hypoglycemic stages of sepsis, glucose uptake is elevated and is independent of changes in glucose and insulin. In humans, glucose utilization has been reported to be augmented with exogenous insulin administration in controls but not in septic subjects, suggesting impairment of the action of insulin by the inflammatory response (9, 11). In our study, acute LPS infusion had an additive effect to the insulin-related increase in whole body glucose disposal when insulin was raised to fed levels. Hyperglycemia is observed as a frequent manifestation of acute sepsis in the neonatal animal (22, 50), and hyperglycemia associated with insulin resistance complicates sepsis in later stages (45, 46). LPS has been shown to induce insulin resistance rapidly when infused into adult humans (1), but evidence suggests that
developmental factors may affect insulin sensitivity during LPS infusion (2, 20, 50, 51). It becomes important to determine whether insulin resistance develops more gradually in the neonate or whether the failure to develop insulin resistance for glucose metabolism is related to direct effects of endotoxin (11) and not to other inflammatory insults.

**Amino acid disposal and response to insulin during LPS infusion.** In the present study, both insulin and LPS augmented whole body amino acid disposal in neonatal pigs. Fasted LPS-infused animals required higher infusion rates of the amino acid mixture to maintain baseline fasting plasma BCAA compared with those in the control group, even when faced by a similar plasma insulin concentration. Raising insulin to fed levels increased whole body amino acid disposal in both control and LPS-infused animals, but the response to insulin in LPS-infused animals was less pronounced. The balance between plasma amino acids and whole body amino acid uptake during acute sepsis and inflammation depends on a catabolic response that mobilizes amino acids from muscle to immunogenic organs for synthesis of acute-phase reactants (10, 45, 46), but the specific protein synthetic and degradative response of each organ to alter protein synthesis or degradation differs (4, 6, 7, 10). LPS is known to cause amino acid mobilization from muscle to the liver to aid in the generation of defense proteins (4, 10), but insulin does not stimulate amino acid uptake in liver of neonatal animals in normal conditions (36). In our study, LPS increased liver protein synthesis at all insulin levels, suggesting that the innate metabolic response to LPS infusion that provides amino acids preferentially to the liver was maintained. The lack of interaction between insulin and LPS on whole body amino acid disposal rates might suggest that different mechanisms regulate whole body amino acid disposal during acute inflammation (5, 6), and those mechanisms can affect amino acid oxidation as well as protein synthesis or degradation. Protein degradation in muscle may also provide amino acids to supply the substrate required to sustain both the immunologic and metabolic responses triggered by endotoxin. It is known that insulin decreases proteolysis in normal subjects (4, 39, 46), but the effect of insulin on proteolysis in catabolic conditions is controversial and appears to be regulated by the degree or nature of the catabolic insult (39).

**Effects of insulin on protein synthesis in muscle during LPS infusion.** Previous studies suggest that systemic inflammation targets skeletal muscle and causes muscle catabolism and atrophy in adults and pediatric patients (4, 45). Our previous studies have shown that protein synthesis rates in skeletal muscles such as the gastrocnemius, which are primarily composed of fibers of oxidative metabolism, also responded to insulin stimulation by a similar proportion, suggesting that the protective effect of insulin against an LPS-induced decrease in protein synthesis occurs in muscles of different fiber types.

Raising insulin to fed levels augmented protein synthesis rates in skeletal muscle by 40 to 60% in both LPS-treated and control animals, and this response was independent of fiber type. This suggests that the enhanced ability of muscle protein synthesis to respond to insulin is maintained during an acute inflammatory insult induced by LPS in neonatal animals and that the LPS-induced reduction in muscle protein synthesis is blunted when insulin is maintained at fed levels. Whether a protective effect of insulin against catabolism is enhanced in neonatal animals, which are very sensitive to insulin, and whether insulin counteracts the effects of LPS on protein degradation in muscle, in addition to the blunting of the LPS-associated reduction in muscle protein synthesis, is open to speculation and requires further study.

Changes in the efficiency of the translational process, rather than changes in ribosome number, arise in response to LPS in adult septic rats (43). In a previous study, we did not detect significant reductions in translational efficiency or ribosome number in muscles containing primarily fast-twitch fibers in LPS-infused neonatal pigs when insulin and amino acids were maintained at fed levels (38). In the present study, neither LPS nor insulin infusion altered ribosomal number. However, LPS infusion reduced translational efficiency in fast-twitch glycolytic muscle in the presence of fasting insulin and substrate concentrations, and the raising of insulin to the fed level augmented translational efficiency in LPS-infused animals as well as in controls. This suggests that insulin can stimulate the translational process in neonatal animals even in the presence of a catabolic insult such as LPS infusion. The finding in neonatal animals contrasts with prior reports (26, 42) in septic adult animals in which insulin resistance to protein metabolism occurs and is manifested by a diminished response of protein synthesis to insulin and a decrease in the translational process that leads to protein synthesis in muscle.

**Effects of insulin on protein synthesis in viscera during LPS infusion.** 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 (5, 6). In the present study, LPS increased protein synthesis in the liver, spleen, lung, and kidney, and this metabolic response was not affected by insulin administration, suggesting that protein synthesis in those organs is not regulated by insulin during the systemic inflammatory response. Likely, LPS-mediated activation, accumulation and migration of inflammatory cells, and extrahepatic acute-phase reactant synthesis in some of the visceral tissues may play a function in increasing protein synthesis (7, 25). Similarly, in healthy neonatal animals, visceral protein synthesis is not dependent on insulin, and different processes regulate the growth of peripheral and visceral tissues (8, 14). The results of the present study demonstrate that the regulation of protein synthesis in skeletal muscle differs from that in other tissues of the neonate during endotoxemia. In visceral tissue, insulin stimulated protein synthetic efficiency only in the spleen and pancreas of controls, and LPS did not affect the insulin sensitivity for protein metabolism in those tissues. In previous studies where LPS was infused in neonatal pigs and insulin and amino acids were...
maintained in the fed state, the translational efficiency was not altered by 8 h of endotoxin infusion (38).

In summary, we have demonstrated for the first time that muscle protein synthesis in neonatal animals, unlike adults, retains its enhanced ability to respond to insulin even in the presence of an acute catabolic insult, such as endotoxin infusion, at least after a short-term infusion of LPS. The reduction in protein synthesis in fast-twitch skeletal muscle of LPS-infused neonatal pigs is more profound when the neonatal animal is exposed to insulin and amino acid levels similar to those seen during fasting, and this reduction in muscle protein synthesis can be minimized by insulin administration. Thus the results of the present study support the hypothesis that muscle protein synthesis in neonates retains its high sensitivity to insulin during acute endotoxia, even in the presence of fasting concentrations of plasma amino acids. Furthermore, the presence of fasting insulin concentrations potentiates the catabolic response by further decreasing muscle protein synthesis during neonatal sepsis.

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