Endotoxin induces differential regulation of mTOR-dependent signaling in skeletal muscle and liver of neonatal pigs

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Kimball, Scot R., Renán A. Orellana, Pamela M. J. O’Connor, Agus Suryawan, Jill A. Bush, Hanh V. Nguyen, M. Carole Thivierge, Leonard S. Jefferson, and Teresa A. Davis. Endotoxin induces differential regulation of mTOR-dependent signaling in skeletal muscle and liver of neonatal pigs. Am J Physiol Endocrinol Metab 285: E637–E644, 2003. —In the present study, differential responses of regulatory proteins involved in translation initiation in skeletal muscle and liver during sepsis were studied in neonatal pigs treated with lipopolysaccharide (LPS). LPS did not alter eukaryotic initiation factor (eIF)2B activity in either tissue. In contrast, binding of eIF4G to eIF4E to form the active mRNA-binding complex was repressed in muscle and enhanced in liver. Phosphorylation of eIF4E-binding protein, 4E-BP1, and ribosomal protein S6 kinase, S6K1, was reduced in muscle during sepsis but increased in liver. Finally, changes in 4E-BP1 and S6K1 phosphorylation were associated with altered phosphorylation of the protein kinase mammalian target of rapamycin (mTOR). Overall, the results suggest that translation initiation in both skeletal muscle and liver is altered during neonatal sepsis by modulation of the mRNA-binding step through changes in mTOR activation. Moreover, the LPS-induced changes in factors that regulate translation initiation are more profound than previously reported changes in global rates of protein synthesis in the neonate. This finding suggests that the initiator methionyl-tRNA (met-tRNAi) to the 40S ribosomal subunit (reviewed in Ref. 16). Thus eIF2B modulates the first step in translation initiation, and changes in its activity result in corresponding alterations in global rates of protein synthesis. Binding of mRNA to the 40S ribosomal subunit is mediated by a heterotrimeric complex referred to as eIF4F. The heterotrimeric complex referred to as eIF4F mediates the binding of mRNA to the 40S ribosomal subunit. The three proteins that comprise the eIF4F complex are eIF4A, an RNA helicase, eIF4E, the protein that binds to the m’GTP cap structure at the 5’-end of the mRNA, and eIF4G, a scaffolding protein that, in addition to eIF4A and eIF4E, also binds to the eIF3-40S ribosomal subunit complex. Thus mRNA binds to the 40S ribosomal subunit through the association of eIF4E with eIF4G. The binding of eIF4E to eIF4G is reversible and regulated by the association of eIF4E with the eIF4E-binding proteins (e.g., 4E-BP1). eIF4E complexed with 4E-BP1 can bind mRNA but cannot bind to eIF4G such that, when the eIF4E-mRNA complex is associated with 4E-BP1, the complex cannot bind to the 40S ribosomal subunit. The association of eIF4E with 4E-BP1 is regulated by phosphorylation of 4E-BP1, whereby eIF4E will bind to hypophosphorylated, but not hyperphosphorylated, 4E-BP1. Hyperphosphorylation of 4E-BP1 that occurs in response to treatment with growth factors or amino acids is mediated through a signal transduction pathway involving a protein kinase referred to as the mammalian target of rapamycin (mTOR; also known as FRAP or RAPT; see Ref. 13). mTOR also phosphorylates, and thereby activates, the 70-kDa ribosomal protein S6 kinase S6K1. Both phosphorylation events are, under some circumstances, associated with changes in global rates of protein synthesis, but more importantly they lead to a preferential increase in translation of mRNAs encoding particular proteins, particularly those mRNAs containing extensive se-
ondary structure in their 5′-untranslated region (UTR; reviewed in Ref. 13) or those mRNAs containing a terminal oligopyrimidine (TOP) tract adjacent to the 5′-cap structure (29).

A recent study (25) demonstrated that, in adult rats, the lipopolysaccharide (LPS)-induced repression of global rates of protein synthesis observed in gastrocnemius muscle is associated with both a decrease in eIF2B activity and decreased assembly of the active eIF4F complex. Such changes in translation initiation factors are quantitatively similar to those observed in a longer-term model of chronic abdominal sepsis (48, 49) and in rats treated with tumor necrosis factor (TNF)-α (26). Which of these steps, the met-tRNA binding or the mRNA-binding step, is rate controlling for global rates of protein synthesis in muscle during sepsis is unknown. In contrast to muscle, considerably less is known about the mechanism(s) involved in the increase in hepatic protein synthesis that occurs during sepsis. However, it has been reported that, during chronic abdominal sepsis, phosphorylation of S6K1 is enhanced in liver (4). The signal transduction pathway(s) that mediates the effects of LPS or TNF-α on eIF4F assembly and S6K1 phosphorylation have not been identified in either muscle or liver.

Little is known about the effects of sepsis in the neonate, a population whose protein synthesis rates are relatively high and uniquely sensitive to anabolic agents (5, 7–9, 11). In a recent study (35), LPS administration to neonatal pigs to promote cytokine production repressed protein synthesis in skeletal muscle and stimulated protein synthesis in liver. However, the magnitude of the changes in both tissues was smaller than previously reported for adult animals (25). In fact, in longissimus dorsi skeletal muscle, the decrease in protein synthesis was proportional to the decline in muscle RNA content, suggesting that a fall in ribosome number is causative in the effect of LPS on protein synthesis in that tissue (35). The relative insensitivity of protein synthesis to LPS treatment in neonates thus may provide a unique opportunity to examine the mechanisms by which LPS differentially regulates protein synthesis in skeletal muscle and liver. Overall, the results of the present study indicate that LPS administration results in enhanced signaling through mTOR in liver but repressed signaling in skeletal muscle. Moreover, the results suggest that eIF2B activity, rather than eIF4F assembly or S6K1 phosphorylation, may be rate controlling for global rates of protein synthesis in response to LPS administration.

**MATERIALS AND METHODS**

**Materials.** The anti-phospho-Thr2448 mTOR antibody was purchased from Cell Signal- ing Technology. The anti-mTOR antibody was purchased from Calbiochem.

**Animals.** Pigs (Landrace × Yorkshire × Hampshire × Duroc) were studied at 5–6 days of age. Before the study was performed (3 days), piglets were anesthetized, and catheters were surgically inserted in a jugular vein and a carotid artery, as described previously (5). 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. To minimize variability in the nutritional status of pigs at the time of study, pigs were fasted for 18 h, and 1 h before the start of the study, a constant infusion of dextrose (800 mg·kg⁻¹·h⁻¹) and an amino acid mixture (1.8 mmol total amino acids·kg⁻¹·h⁻¹; see Ref. 7) was begun to simulate a fed state. At time 0, animals were administered LPS (10 μg·kg⁻¹·h⁻¹) or an equal volume of saline (control), and 7.5 h later, a bolus dose of [³H]phenylalanine was administered intravenously. After the LPS infusion was initiated (8 h), pigs were killed with an intravenous dose of pentobarbital sodium (50 mg/kg body wt), and all tissue samples were rapidly removed, frozen in liquid nitrogen, and stored at −70°C until analysis. The protocol, previously described by Orellana et al. (35), 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.

**Measurement of eIF2B activity.** eIF2B activity in muscle and liver homogenates was measured by the exchange of [³H]GDP bound to eIF2 for nonradioactively labeled GDP, as described previously (22). Activity is expressed as the rate of GDP exchange.

**Quantitation of 4E-BP1-eIF4E and eIF4G-eIF4E complexes.** The association of eIF4E with 4E-BP1 or eIF4G was quantitated as described previously (21). Briefly, eIF4E was immunoprecipitated from muscle or liver homogenates using a monoclonal anti-eIF4E antibody. Proteins in the immunoprecipitate were resolved by SDS-PAGE and then transferred to polyvinylidene difluoride membranes. The membranes were then probed with either anti-4E-BP1 or anti-eIF4G antibodies and then developed using an enhanced chemiluminescence Western blotting kit (Amersham Pharmacia Biotech). The horseradish peroxidase coupled to the anti-rabbit secondary antibody was then inactivated by incubating the blot in 15% hydrogen peroxide for 30 min at room temperature, and the membranes were reprobed with the monoclonal anti-eIF4E antibody. Values obtained using the anti-4E-BP1 and anti-eIF4G antibodies were normalized for the amount of eIF4E present in the sample.

**Measurement of site-specific phosphorylation of S6K1 and mTOR.** Phosphorylation of S6K1 was assessed by Western blot analysis using an antibody specific for S6K1 when it is phosphorylated at the activating residue, Thr389. Phosphorylation of mTOR on Ser2448, a residue located in a repressor domain, was assessed by Western blot analysis using an antibody specific for mTOR phosphorylated on Ser2448. The membranes were then treated with hydrogen peroxide as described above and reprobed with an anti-mTOR antibody that recognizes both the phosphorylated and unphosphorylated forms of the protein. Values obtained using the anti-phospho-mTOR antibody were normalized for the total amount of mTOR present in the sample.

**Phosphorylation of 4E-BP1.** During SDS-PAGE, 4E-BP1 resolves into multiple isoelectric forms based on its phosphorylation state. The hyperphosphorylated γ-form is the slowest migrating form and does not bind to eIF4E. Phosphorylation of 4E-BP1 was assessed by Western blot analysis using an antibody that recognizes all three forms of 4E-BP1 resolved during electrophoresis. Results are expressed as the amount of protein present in the γ-form as a percentage of total 4E-BP1.

**Statistics.** Values shown are means ± SE. Statistical evaluation of the data was performed using an unpaired, two-
tailed t-test. Differences between means were considered significant at $P < 0.05$.

RESULTS

Because a change in eIF2B activity is the primary mechanism through which the met-tRNA-binding step is regulated (reviewed in Ref. 17), the guanine nucleotide exchange activity of eIF2B was measured in extracts of longissimus dorsi and liver from control and LPS-treated pigs. The difference in eIF2B activity between muscle and liver (Fig. 1) was directly proportional to the previously reported difference in protein synthesis (35). Thus eIF2B activity was greater in liver compared with muscle. However, no change in eIF2B activity was observed in either skeletal muscle or liver of LPS-treated compared with control pigs.

The best characterized mechanism through which the binding of mRNA to the 40S ribosomal subunit is regulated involves the reversible binding of eIF4G to eIF4E (reviewed in Ref. 38). In the present study, the amount of eIF4G bound to eIF4E was measured by immunoprecipitation of eIF4E followed by protein immunoblot analysis for eIF4G in the immunoprecipitate. As shown in Fig. 2, the amount of eIF4G present in the eIF4G-eIF4E complex in muscle was significantly reduced (−79%) by LPS treatment. In contrast, the binding of eIF4G to eIF4E in liver was enhanced (+77%) in response to LPS treatment.

To determine whether the changes in eIF4G binding to eIF4E were the result of altered association of eIF4E with the eIF4E-binding proteins, the eIF4E immunoprecipitates used for the analyses shown in Fig. 2 were also analyzed for 4E-BP1 content. In muscle, the amount of 4E-BP1 associated with eIF4E was increased (+53%) in LPS-treated compared with control pigs (Fig. 3A). In contrast, in liver, the amount of 4E-BP1 bound to eIF4E was reduced (−44%) in response to LPS treatment.

Binding of 4E-BP1 to eIF4E is regulated by phosphorylation of 4E-BP1. When 4E-BP1 is resolved by SDS-PAGE, it separates into three isoforms where the fastest migrating form (referred to as 4E-BP1α) is the least phosphorylated and the slowest migrating form (referred to as 4E-BP1γ) is the most highly phosphorylated form of the protein (15, 36). Because the γ-form is the only one that does not bind to eIF4E, the phosphorylation state of 4E-BP1 is presented as the proportion of the protein present in the γ-form. As shown in Fig. 3B, the amount of 4E-BP1 present in the hypophosphorylated γ-form was reduced (−60%) in muscle, but increased (+70%) in liver, of LPS-treated compared with control pigs.

Like 4E-BP1, S6K1 is downstream of mTOR (reviewed in Ref. 38). In in vitro studies, mTOR phosphorylates S6K1 on Thr389 (3). To determine whether phosphorylation of S6K1 (Thr389) is altered by LPS, muscle and liver extracts were analyzed by protein immunoblot analysis using an antibody that binds to S6K1 only when it is phosphorylated on Thr389. In the present study, phosphorylation of S6K1 on Thr389 was reduced (−74%) in muscle and increased (+387%) in liver of LPS-treated pigs compared with control animals (Fig. 4).

Phosphorylation of both 4E-BP1 and S6K1 is dependent on the activity of mTOR (reviewed in Ref. 38). One mechanism that has been proposed for regulation of mTOR activity involves phosphorylation of the protein on Ser2448, a residue that is present in a putative repressor domain (43). In the present study, the phosphorylation state of mTOR on Ser2448 was assessed by protein immunoblot analysis using an antibody that

![Fig. 1. Effect of lipopolysaccharide (LPS) treatment on eukaryotic initiation factor (eIF)2B activity in skeletal muscle and liver of neonatal pigs. Neonatal pigs were treated with LPS, and the rate of exchange of [3H]GDP bound to eIF2 for nonradiolabeled GDP was measured as described under MATERIALS AND METHODS. Results represent means ± SE for 9–10 animals/condition.](image)

![Fig. 2. Effect of LPS treatment on eIF4G association with eIF4E in skeletal muscle and liver of neonatal pigs. Neonatal pigs were treated with LPS as described under MATERIALS AND METHODS. The amount of eIF4G bound to eIF4E was measured as described under MATERIALS AND METHODS. Values for eIF4G were normalized for the amount of eIF4G present in the immuno- precipitate. C, control pigs; L, LPS-treated pigs. *$P < 0.02$ vs. control muscle. †$P < 0.025$ vs. control liver.](image)
specifies that the phosphorylated form of the
protein. As shown in Fig. 5, the relative phosphoryla-
tion of mTOR on Ser\textsuperscript{2448} was reduced (−51\%) in muscle
and enhanced (+83\%) in liver of LPS-treated compared
with control pigs. Interestingly, the changes in mTOR
phosphorylation were similar in magnitude to those
observed for 4E-BP1 and S6K1 phosphorylation (Figs.
3B and 5, respectively).

DISCUSSION

In the present study, no change in eIF2B activity
was observed in either longissimus dorsi or liver of
neonatal pigs after 8 h of LPS infusion, suggesting that
the met-tRNA\textsubscript{i}\textsuperscript{a}-binding step in translation initiation is
unaffected under such conditions. This finding is in
contrast to previous results showing that, in adult rats,
either TNF\textsubscript{α} (26) or LPS (25) administration or
chronic abdominal sepsis (48) reduces eIF2B activity in
gastrocnemius muscle. The basis for the differential
response observed in neonatal pigs compared with
adult rats may be because of species differences, dura-
tion of sepsis, or in the methods used to induce a
septic-like response. Alternatively, the difference may
be the result of the age of the animals being studied.
For example, skeletal muscle protein synthesis is stim-
ulated by insulin in 7-day-old pigs, an effect that is
severely attenuated by 28 days of age (6). Moreover, in
cells in culture, insulin stimulates eIF2B by modulat-
ing phosphorylation of the e-subunit of eIF2B (50).
Because plasma insulin concentrations are elevated in
LPS-treated pigs (35) and protein synthesis exhibits
heightened sensitivity to insulin in neonatal pigs (6), it
may be that insulin is better able to maintain eIF2B
activity in muscle of neonatal compared with adult
animals.

In contrast to the lack of effect on eIF2B activity in
neonatal pigs, LPS treatment decreased the amount of
eIF4G associated with eIF4E and increased the bind-
ing of 4E-BP1 to eIF4E in skeletal muscle. It is inter-
esting to note that the magnitude of the change in
eIF4G binding to eIF4E was greater than the change in

Fig. 3. Effect of LPS treatment on eIF4E association with 4E-BP1 and 4E-BP1 phosphorylation in skeletal muscle and liver of neonatal pigs. Neonatal pigs were treated with LPS as described under MATERIALS AND METHODS. A: amount of 4E-BP1 bound to eIF4E was measured in the same immunoprecipitates used for the analysis shown in Fig. 2. Values for 4E-BP1 were normalized for eIF4E recovery. Results represent means ± SE for 9–10 animals/condition. Results of typical blots are shown in inset. Muscle and liver samples were analyzed on separate immunoblots; therefore, the results from the 2 tissues cannot be compared directly. *P < 0.05 vs. control muscle. B: phosphorylation of 4E-BP1 was measured as described under MATERIALS AND METHODS and is expressed as the percentage of the protein present in the hyperphosphorylated γ-form. Results represent means ± SE for 9–10 animals/condition. Results of typical blots are shown in inset. The location of the α-, β-, and γ-forms of 4E-BP1 are noted on left of the inset. *P < 0.001 vs. control muscle. †P < 0.002 vs. control liver.

Fig. 4. Effect of LPS treatment on ribosomal protein S6 kinase (S6K1) (Thr\textsuperscript{389}) phosphorylation in skeletal muscle and liver of neonatal pigs. Neonatal pigs were treated with LPS as described under MATERIALS AND METHODS. Phosphorylation of S6K1 on Thr\textsuperscript{389} was measured by Western blot analysis using an anti-phospho-
Thr\textsuperscript{389} antibody as described under MATERIALS AND METHODS. Results represent means ± SE for 9 animals/condition. Results of typical blots are shown in inset. Muscle and liver samples were analyzed on separate immunoblots; therefore, the results from the 2 tissues cannot be compared directly. *P < 0.05 vs. control muscle. †P < 0.05 vs. control liver.
4E-BP1 binding. In part, this result may be because of alterations in eIF4E binding to other eIF4E-binding proteins, such as 4E-BP2 and/or 4E-BP3. It is also noteworthy that the magnitude of the change in the association of eIF4G with eIF4E in skeletal muscle of the neonate is similar to that reported in models of adult sepsis (26, 49), despite the small changes in muscle protein synthesis in the neonatal model (35). This suggests that other factors may be involved in maintaining the relatively high protein synthetic rates in skeletal muscle of the neonate during catabolic conditions such as sepsis and endotoxemia. In fact, it was previously reported that RNA content in both skeletal muscle and liver of neonatal pigs tended to change during LPS infusion such that, when normalized for RNA content, protein synthesis in longissimus dorsi of muscle and liver samples was analyzed on separate immunoblots; therefore, results from the 2 tissues cannot be compared directly. *P < 0.01 vs. control muscle. †P < 0.01 vs. control liver.

Fig. 5. Effect of LPS treatment on mammalian target of rapamycin (mTOR) (Ser\(^{2448}\)) phosphorylation in skeletal muscle and liver of neonatal pigs. Neonatal pigs were treated with LPS as described under MATERIALS AND METHODS. Phosphorylation of mTOR on Ser\(^{2448}\) was measured by Western blot analysis using an anti-phospho-Ser\(^{2448}\) antibody as described under MATERIALS AND METHODS. Values for phosphorylated mTOR [mTOR(P)] were normalized for total mTOR content. Results represent means ± SE for 10 animals/condition. Results of typical blots are shown in inset. Muscle and liver samples were analyzed on separate immunoblots; therefore, results from the 2 tissues cannot be compared directly. *P < 0.01 vs. control muscle. †P < 0.01 vs. control liver.

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growth (reviewed in Ref. 46). For example, during liver regrowth after partial hepatectomy, TOP mRNA translation correlates with increased activity of S6K1 and phosphorylation of rpS6 (1, 14, 18, 32). In contrast, cell cycle arrest is associated with selective translational arrest of TOP mRNA translation (12, 19, 28). Finally, in both flies (30) and mice (44), disruption of the gene encoding S6K1 results in organisms that have the same number of cells as do wild-type animals, but with smaller cells. In part, the small-cell phenotype observed in response to S6K1 deletion is a result of impaired regulation of TOP mRNA translation. Thus, in contrast to wild-type cells, in S6K1(−/−) cells, serum does not promote recruitment of TOP mRNAs into polysomes (20). One model that could account for the preferential recruitment of TOP mRNAs into polysomes is that phosphorylated, but not unphosphorylated, rpS6 might exhibit increased affinity for the TOP structure. The observation that rpS6 is located near the mRNA-binding site on the 40S ribosomal subunit (33, 34) makes such a model feasible. Thus it is not surprising that, in the present study, large changes in S6K1 phosphorylation are not associated with similar changes in global protein synthesis, because TOP mRNAs represent only a fraction of total mRNA.

Similarly, increased availability of eIF4E results in preferential translation of a subset of mRNAs. For example, addition of either eIF4E or eIF4F to cell-free translation systems results in a differential enhancement of mRNA translation; i.e., certain mRNAs are preferentially translated when eIF4F content is increased (39, 41, 45). This finding has been extended in recent years to show that overexpression of eIF4E in cells in culture leads to cell transformation as a result of increased translation of mRNAs encoding proteins important in the transformation process (reviewed in Ref. 10). One model that has been proposed to account for the observed changes in mRNA translation is that mRNAs with short, relatively unstructured 5′-UTR are translated preferentially compared with mRNAs with long, highly structured 5′-UTRs (reviewed in Ref. 13). In this model, increasing eIF4F availability upregulates the translation of mRNAs with highly structured 5′-UTRs. Thus, in the present study, the observed changes in eIF4G-eIF4E association may result in a preferential change in the translation of specific groups of mRNAs, an effect that might not be detected by measuring the incorporation of radioactive amino acid into total protein over a 30-min period. It is thus interesting to note that, during sepsis, the pattern of proteins secreted by the liver changes such that secretion of normally expressed proteins like albumin and transferrin falls dramatically (reviewed in Ref. 47). In contrast, synthesis and secretion of the acute-phase proteins from liver are enhanced. The importance of the translational control mechanisms described above in mediating the reported changes in liver protein synthesis is unknown. However, it is tempting to speculate that the changes in eIF4G-eIF4E complex formation and/or S6K1 phosphorylation that occur during sepsis might play an important role in modulating...
gene expression. In this regard, in unpublished studies, we have found that the distribution of the mRNA encoding rpS8 is predominantly polysomal in livers from neonatal pigs treated with LPS compared with a primarily nonpolysomal distribution in livers from control animals.

Direct support for a model wherein eIF2B is rate controlling for changes in global rates of protein synthesis, whereas eIF4F assembly and S6K1 activation are not, is provided by studies where L6 myoblasts were deprived of either leucine or histidine (21, 24). Deprivation of either amino acid was shown to cause decreased global rates of protein synthesis and eIF2B activity, but only deprivation of leucine repressed eIF4F assembly and S6K1 phosphorylation. Moreover, addition of insulin to leucine-deprived cells restored eIF4F assembly and S6K1 phosphorylation to values observed in control cells or histidine-deprived cells but had no effect on either global rates of protein synthesis or eIF2B activity. Thus, in that study, changes in eIF4F assembly and S6K1 phosphorylation had no effect on global rates of protein synthesis. Instead, changes in eIF4F assembly and S6K1 phosphorylation were shown to promote preferentially the translation of mRNAs encoding ornithine decarboxylase and elongation factor 1A (24).

The changes in 4E-BP1 binding to eIF4E observed in the present study were associated with alterations in 4E-BP1 phosphorylation, whereby the proportion of the protein in the hyperphosphorylated γ-form was reduced in skeletal muscle but enhanced in liver of LPS-treated pigs. Previous studies have shown that the feeding-induced increase in 4E-BP1 hyperphosphorylation that occurs in skeletal muscle is dependent on the activity of mTOR (2, 23). Thus treatment with the mTOR inhibitor rapamycin before feeding prevents the subsequent phosphorylation of 4E-BP1 and S6K1 in skeletal muscle. Moreover, in cells in culture, the phosphorylation of 4E-BP1 that occurs in response to a variety of stimuli, including insulin, IGF-I, and amino acids, is blocked by pretreatment with rapamycin (reviewed in Refs. 27 and 37). One mechanism that has been proposed for regulation of the protein kinase activity of mTOR involves phosphorylation of Ser1179, a residue in a domain that has been characterized as a repressor of mTOR function (43). In this regard, PKB phosphorylates Ser1179 on mTOR in vitro (31, 40), and activation of PKB in cells in culture is associated with enhanced phosphorylation of Ser1179 on mTOR as well as phosphorylation of proteins downstream of mTOR, such as 4E-BP1 and S6K1 (31, 42, 43). In the present study, we demonstrate for the first time that changes in 4E-BP1 and S6K1 phosphorylation during sepsis were mirrored by alterations in phosphorylation of Ser2448 on mTOR. Thus, in skeletal muscle of the neonate, phosphorylation of all three proteins is reduced, whereas in liver, phosphorylation is enhanced by LPS treatment.

**Perspectives.** Overall, the results of the present study demonstrate for the first time that LPS treatment of neonatal pigs represses signaling through the mTOR pathway in muscle while stimulating signaling through this pathway in liver. The observed changes in mTOR phosphorylation are associated with modulation of both S6K1 and 4E-BP1 phosphorylation, as well as alterations in eIF4G binding to eIF4E. In contrast, no change in eIF2B activity is observed in either muscle or liver of neonatal pigs in response to LPS treatment. The results therefore suggest that, in neonatal pigs, the lack of effect of LPS treatment on eIF2B activity likely accounts for the observed maintenance of global rates of protein synthesis.

The magnitude of the changes in the phosphorylation of 4E-BP1 and the association of eIF4E with eIF4G in skeletal muscle were similar to those reported in models of adult sepsis. In contrast, the magnitude of the reduction in muscle protein synthesis rates in neonatal sepsis in our recent paper (35) was less profound than that in adult sepsis models. This suggests that the relatively high synthesis rates of skeletal muscle proteins in the neonate may in part be maintained during neonatal sepsis through a mechanism that is distinct from eIF4F complex formation.

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**DISCLOSURES**

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