Endotoxin transiently inhibits protein synthesis through Akt and MAPK mediating pathways in C2C12 myotubes

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Tarabees R, Hill D, Rauch C, Barrow PA, Loughna PT. Endotoxin transiently inhibits protein synthesis through Akt and MAPK mediating pathways in C2C12 myotubes. Am J Physiol Cell Physiol 301: C895–C902, 2011. First published July 20, 2011; doi:10.1152/ajpcell.00387.2010.—In this study, the effect of lipopolysaccharide (LPS) on protein synthesis (PS) and intracellular signaling factors that regulate it have been investigated in C2C12 murine-derived myotubes. In particular, the role of Akt/mammalian target of rapamycin (mTOR), P70S6K, and 4E-BP1/Akt/mTOR pathway. Akt (protein kinase B) is a member of the family of serine/threonine protein kinases (7) that are stimulated by a number of receptors and effectors including ribosomal S6 kinase (S6K1) and 4E binding protein 1 (4E-BP1) in the regulation of skeletal muscle mass has been reviewed by Glass (15). Translation is conventionally divided into three main stages: initiation, elongation, and termination. mTOR controls the initiation and elongation process stages of translation (55) through its downstream effectors including ribosomal S6 kinase (S6K1) and 4E-BP1 (55). The direct contribution of the mitogen-activated protein kinase (MAPK) family and their proposed downstream target MAPK-integrating kinase 1 (Mnk1) in the regulation of the translation initiation process through eukaryotic initiation factor (eIF-4E) has also been shown by Fukunaga and Hunter (13). A number of studies have measured the changes in the signaling pathways activation that may regulate PS without direct measurement of this process itself. Recent studies suggest that in some cases changes in the activation of the pathways that regulate translation may not always correlate with the expected changes in PS (2, 32). Thus a better understanding of the role of signaling pathways in particular that of catabolic pathways that lead to accelerated rate of protein loss (11). Clinical studies in humans and in a variety of animal models clearly demonstrate that PD contributes to the loss of skeletal muscle protein and impaired contractile function observed in response to sepsis or endotoxin administration (37). In a number of clinical conditions and experimental models, it has been shown, however, that a change in rate of PS is the primary initiator of altered muscle mass (28, 50). In vivo studies examining LPS and muscle PS (29, 52) suggest a suppression of PS in some but not all muscle types. To date, two studies have examined the direct effect of LPS on PS in cultured muscle cells in vitro (10, 43), and the results are contradictory. Thus Frost et al. (10) showed that LPS alone has no effect on protein synthesis, but the combination of LPS and interferon-γ induced an 80% decrease in the PS. In contrast, Russell et al. (43) demonstrated that LPS alone induced a 50–60% decrease in PS in the same C2C12 cell line.

PS in skeletal muscle is regulated at the transcriptional or translational level (30), with translational control being the common mechanism in most acute responses (20). One of the most extensively studied anabolic pathways is the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Akt (protein kinase B) is a member of the family of serine/threonine protein kinases (7) that are stimulated by a number of receptors and cytokines, including interferon-γ induced an 80% decrease in the PS. In contrast, Russell et al. (43) demonstrated that LPS alone induced a 50–60% decrease in PS in the same C2C12 cell line.

Muscle wasting is a common syndrome associated with severe catabolic diseases such as sepsis, cancer, and AIDS. Sepsis is an inflammatory condition that causes severe and rapid loss of body protein, much of which originates from skeletal muscle (17). Lipopolysaccharide (LPS) is an integral part of the cell wall of Gram-negative bacteria and a potent activator of the innate immune system through recognition by Toll-like receptor 4 (TLR4) (40). TLR4 is a part of a large family of receptors that recognize pathogen-associated molecular patterns and plays an indispensable role in the transduction of the LPS signal and subsequently the stimulation of inflammatory cytokines gene expression (22). The dynamic balance between rates of protein synthesis (PS) and protein degradation (PD) governs the skeletal muscle protein mass maintenance. To date most studies investigating the effects of sepsis upon skeletal muscle protein metabolism have examined the regulation of muscle; lipopolysaccharide; signaling

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LPS INHIBITS PROTEIN SYNTHESIS IN SKELETAL MYOCYTES

the Akt and MAPKs (p38 and ERK1/2) in sepsis is important for developing successful therapies to retard the loss in lean body mass and reduce morbidity and mortality (26, 48). For these reasons, our study aimed to examine the subcellular mechanisms responsible for the LPS-associated changes in PS in murine C2C12 myotubes and the possible role of Akt/ MAPK signaling pathways. In addition, we measured the direct effect of LPS alone and with specific pathway inhibitors on PS as a short-term (3 h) or long-term (18 h) response.

MATERIALS AND METHODS

Cell culture. C2C12 mouse myoblasts were maintained in Dulbecco’s modified Eagle’s medium (DMEM) (high glucose with l-glutamine and penicillin and streptomycin) (Invitrogen) plus 10% (vol/vol) fetal bovine serum (Invitrogen) at 37°C with 5% CO2. When the cells reached 80% confluence, they were then cultured in differentiation medium (DM) (DMEM, high glucose with l-glutamine and penicillin and streptomycin, plus 2% (vol/vol) horse serum). On day 6 of differentiation, the cells were characterized as myotubes by morphological criteria (4).

Western blotting. C2C12 myotubes were treated with LPS (Salmonella typhimurium, Sigma) (10 ng/ml, 100 ng/ml, and 1 μg/ml) for 5 and 30 min and 1, 3, and 18 h. Then cells were washed with sterile cold PBS containing Na2VO4 (400 μM) and scraped in lysis buffer [76.5 mM Tris·HCl (pH 6.8), glycerol 10% (vol/vol), SDS 2% (wt/vol)] supplemented with 1 mM sodium metavanadate (Na2VO4), 10 mM leupeptin, 1 mM AEBSF (Pefabloc), and the protease inhibitors. The protein concentration of the samples was measured using the BCA protein assay method kit (Fierce) with bovine serum albumin (BSA) as standard. An equal amount of protein (25 μg) was loaded into each lane. Phosphorylated protein concentrations were determined for [PKB/Akt, p38, extracellular regulated protein kinase (ERK)1/2, mammalian target of rapamycin (mTOR), P70S6K, eIF-4E, 4E-BP1] by SDS-PAGE and Western blot analysis as described previously (2, 41). Briefly, following electrophoresis, the protein was transferred onto a PVDF membrane (GE Healthcare) using a semidy transfer unit (Bio-Rad) and blocked with blocking buffer [PBS containing 0.1% (vol/vol) Tween 20 and 5% (wt/vol) Marvel nonfat milk] for 1 h. The membranes were probed with the appropriate primary antibodies diluted in PBS containing 0.1% (vol/vol) Tween 20 and 2% (wt/vol) BSA overnight. After being washed with PBST (PBS + 0.1% Tween 20), the membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. After washing was completed, the membranes were developed using ECL plus Western blotting Detection Agent (GE Healthcare). Bands on the X-ray films were quantified using densitometry software (Quantity One software, Bio-Rad). The primary antibodies p-ERK1/2 (Thr202/Tyr204), p-Akt1/2/3 (Ser473), p-p38 (Thr180/Tyr182), Akt1/2 (N-19), p38 (H-147), and the HRP-conjugated secondary antibody (donkey-Anti-rabbit HRP conjugated IgG) were obtained from Santa Cruz. Phosphoplus p70S6 kinase (Thr389, Thr421/Ser424) Antibody Kit, phospho-4E-BP1 (Ser65), phospho-eIF-4E (Ser209), phospho-Mnk1 (Thr197/202) antibody, and phospho-mTOR (Ser2448) were obtained from Cell Signaling. All the data were normalized to β-actin (Cell Signaling).

Protein synthesis measurement in C2C12 myotubes. C2C12 myotubes were treated with LPS (1 μg/ml) for 3 and 18 h. To each well 2 μCi [3H]phenylalanine (1 μCi/ml) (GE Healthcare) were added at the last hour of each treatment. Protein synthetic rate was expressed as incorporation of [3H]phenylalanine per milligram of total trichloroacetic acid (TCA) precipitated protein as previously described (49). Briefly, the cells were treated with LPS and incubated with the 2 μCi [3H]phenylalanine (GE Healthcare) for 1 h and were then washed three times with cold PBS (GIBCO) and 500 μl 5% (wt/vol) TCA (BDH, UK). The plates were scraped and washed twice with 400 μl 5% TCA, and the pooled protein in TCA was placed on ice for 1 h. The pelleted protein was washed three times with 5% TCA to remove free [3H]phenylalanine and then dissolved in 400 μl 0.1 M NaOH (Sigma). Samples were then heated in 0.1% (wt/vol) sodium dodecyl sulfate (SDS) (Sigma) for 2 h at 70°C to solubilize the precipitate. Protein concentration was determined using the Bradford Assay (Bio-Rad) with BSA as standard. [3H]phenylalanine incorporation was measured by the liquid scintillation analyzer (Packard Bioscience). The values were expressed as counts per minute per microgram-precipitated protein.

Pathway inhibitors. TLR4 inhibitor polymyxin B (PMB, 5 μg/ml, Sigma) was added 2 h before the addition of LPS, while P38 inhibitor (LY-0294002, 50 μM, Cell Signaling), MEK inhibitor (PD-098059, 40 μM, Cell Signaling), and MAPK inhibitor (SB-203580, 10 μM, Sigma) were added 1 h before the addition of LPS. Then C2C12 murine myotubes were treated with LPS (1 μg/ml) for 3 and 18 h. The optimal doses of inhibition were obtained from the previous literature for PMB (12), SB-203580 (19), PD-098059 (39, 46), and for LY-0294002 (39). The high doses of PD-098059 and LY-0294002 were according to the manufacture’s recommendations.

Statistics. Data were expressed as means ± SE. The statistical differences among the groups were assessed by one- and two-way analysis of variance with Tukey’s post hoc using GraphPad software (GraphPad) if a significant overall effect was detected or simply by Student’s t-test (Microsoft Excel) when two groups were compared. The statistical difference was set at P < 0.05.

RESULTS

LPS-induced changes in the activity-related phosphorylation of Akt and mTOR. To characterize the effect of LPS (bacterial endotoxin) on the signaling pathways mediating the protein synthesis, C2C12 myotubes were incubated at various concentrations of LPS (10 ng/ml, 100 ng/ml, and 1 μg/ml) at a number of time points (5 and 30 min and 1, 3, and 18 h). The effect of LPS on the activity-related phosphorylation state of Akt was presented in (Fig. 1A). LPS induced a rapid but transient decrease in the Akt phosphorylation lasting only for 30 min. LPS significantly decreased the Akt phosphorylation at 5 and 30 min in a dose-dependent manner (Fig. 1A). Later, the LPS had no effect on Akt phosphorylation compared with the control levels at other time points (Fig. 1A). In contrast, the mTOR exhibited a different time course of response with regard to activity-related phosphorylation as a concentration-dependent reduction in phosphorylation at all the time points starting early at 5 min and continued to the 18-h time point (Fig. 1B).

LPS induced changes in the activity-related phosphorylation of MAPK (p38, and ERK1/2) and their downstream target Mnk1. To investigate the possible role of the other signaling pathways known to regulate the anabolic pathways, the level of phosphorylated p38, ERK1/2, and their substrate Mnk1 were measured in response to LPS administration (Fig. 2, A–C). LPS-induced increases in p38 activity-related phosphorylation at all time points peaked at 30-min, 1-h, and 3-h time points (Fig. 2A). The effect of the LPS on the phospho-ERK1/2 is summarized in Fig. 2B. Incubation of the C2C12 myotubes with LPS (10 ng/ml, 100 ng/ml, and 1 μg/ml) significantly induced a dose-dependent increase in ERK1/2 phosphorylation at all the time points with the exception of the 3-h time point (Fig. 2B). In Fig. 2C, the activity-related phosphorylation of the Mnk1 was shown. Mnk1 has previously been shown as in vitro downstream target of the MAPKs (p38 and ERK1/2). Incubation of the C2C12 myotubes with...
LPS (10 ng/ml, 100 ng/ml, and 1 μg/ml) significantly increased the Mnk1 phosphorylation at 1- and 3-h time points only (Fig. 2C).

Effect of LPS on the regulation of translation in myotube PS. In vivo, it has previously been shown that LPS and sepsis significantly altered the PS via the alteration of the translation initiation step of the protein synthesis. The effect of the LPS (10 ng/ml, 100 ng/ml, and 1 μg/ml) administration on the translation initiation signaling stream particularly p70S6K, 4E-BP1, and eIF-4E is summarized in Fig. 3, A–C. Incubation of the C2C12 myotubes with LPS significantly decreased the p70S6K phosphorylation in a dose-dependent manner at the time points, and this effect was maximally seen at the 5-min and 1-h time points (Fig. 3A). In contrast, LPS only decreased the 4E-BP1 phosphorylation at the 1-h time point (Fig. 3B).

This decrease in 4-BP1 phosphorylation at the 1-h time points was then followed by a decrease on the eIF-4E phosphorylation at the 3-h time point (Fig. 3C).

Effect of LPS on the muscle cell PS rate. From the previous results, we can conclude that LPS significantly decreased the anabolic signaling pathway (Akt/mTOR and their downstream targets). We subsequently measured the direct effect of LPS on the PS rate (expressed as incorporation of [3H]phenylalanine per microgram of total TCA precipitable protein) after stimulation of C2C12 murine myotubes with LPS (1 μg/ml) for 3 h (short-term effect) and 18 h (long-term effect). Our result showed that at 3 h there was a significant decrease in PS rate by 49% (P < 0.05) compared with the control untreated cells, n = 6) (Fig. 4), but no significant change was observed at the 18-h time point (Fig. 5).
Effect of inhibitors on the muscle cell PS rate. The effect of LPS and signaling pathways was verified using specific pathway inhibitors. PMB (5 μg/ml), TLR4 inhibitor LY-0294002 (50 μM), PI3K inhibitor PD-098059 (PD, 40 μM), MEK inhibitor, and SB-203580 (SB, 10 μM), MAPK inhibitor were added to fully differentiated C2C12 alone or with LPS (1 μg/ml). Incubation of C2C12 myotubes with LPS in combination with PMB or with PMB alone showed no changes in the PS rate compared with the control level at both 3- and 18-h time points, but PMB and LPS together prevented the decreases in PS seen at 3 h that were observed with LPS alone (Fig. 4). Incubation of C2C12 with LPS plus SB-203580 significantly decreased the PS at the 18-h time point by 41% \( (P < 0.01) \) with no significant changes observed at the 3-h time point (Figs. 4 and 5), whereas incubation of cells with SB-203580 alone significantly decreased protein synthesis by 63% \( (P < 0.05) \) and 40% \( (P < 0.01) \) at the 3-h and 18-h time points, respectively (Figs. 4 and 5). Incubation of cells with LPS and PD98059 had no effect on PS at 3 h (Fig. 4), whereas it significantly decreased the PS rate by 59% \( (P < 0.001) \) at the 18-h time point (Fig. 5). In contrast, incubation of cells with PD98059 alone significantly decreased PS by 64% \( (P < 0.05) \) at 3 h (Fig. 4), whereas no such decrease was observed at the 18-h time point (Fig. 5). Finally, incubation of cells with LPS and LY-0294002 induced a significant reduction in PS by 81% \( (P < 0.05) \) and 80% \( (P < 0.001) \) at the 3- and 18-h time point, respectively, compared with the control cells (Figs. 4 and 5). The efficiency of pathways inhibition was verified by Western blotting. Our Western blotting results have shown that LY-0294002 and PD-098059 completely abolished the activity-related phosphorylation of Akt and ERK1/2, respectively, had no effect on the total Akt or total ERK1/2 (Fig. 6, A, C, and D), whereas SB-203580, which acts to inhibit p38 action downstream, showed no effect on the activity-related phosphorylation of p38 (Fig. 6, B and D). Similarly, incubation of cells with LPS in combination with PMB had no effect on the activity-related phosphorylation of Akt, p38, and ERK1/2 compared with the control untreated cells (Fig. 6, A–D). In contrast, inhibition of the p38 and ERK1/2 significantly decreased the Akt phosphorylation compared with the control cells \( (P < 0.05) \) (Figs. 6, A and D).

DISCUSSION

Sepsis is an acute inflammatory condition characterized by accelerated rate of protein turnover and decreased protein synthetic rate in skeletal muscle (17). In vivo it has been shown that endotoxin induced a significant decrease in the rate of PS in the gastrocnemius muscle of the rat (29). It has also been observed that sepsis itself significantly decreased the rate of PS in the gastrocnemius but not, however, in the slow-twitch soleus muscle (52). While in vitro the effect of LPS was varied, with Frost et al. (10) showing no changes in PS rate when LPS was used alone in C2C12 differentiated myotubes, but the subsequent study of Russell et al. (43) showing a significant decrease in the PS rate in response to LPS in the same cell line. Our data showed that LPS significantly decreased the rate of PS in C2C12 myotubes by 50% but that this effect was
transient (3 h) and the PS returned to control levels by 18 h. Studies in adult animal model of sepsis have shown that LPS challenge significantly decreased the skeletal muscle protein synthesis, a reduction occurring as early as 4 h and maintained for up to 24 h (29, 52). The absence of changes in PS observed by Frost et al. (10) compared with the present study may be due to the fact that it was measured over a much longer time period between 10 and 14 h following LPS administration, and thus earlier transient effects may not have been observed. Whether the mechanisms of action of endotoxin on protein metabolism when acting on muscle cells in vitro is the same as in vivo is unclear. LPS has been demonstrated to decrease muscle protein content, and it is likely that decreasing the rate of PS and translation efficiency contributes to this (1, 20, 31). The observation that the effect of LPS was diminished by the LPS-neutralizing agent PMB (12), and data from previous studies suggest that LPS signaling transduction is mediated mainly through TLR-4 (22). The process of PS is divided into three distinct stages: initiation, elongation, and termination. Sepsis-induced inhibition of PS is a primarily a result of the decrease in the translation efficiency and not a decrease in the number of ribosomes (5, 29, 52). The process of mRNA translation regulation is primarily the function of a group of eukaryotic initiation factors (eIFs). The active eIF-4F complex controls the peptide-chain initiation by regulating the recruitment of the 43S preinitiation complex to mRNA (38). The formation of the active eIF-4F complex depends on the availability of eIF-4E,
and the interaction between eIF-4G and eIF-4E is controlled partially by the eIF-4E binding protein (4E-BP1) (16). Increased phosphorylation of 4E-BP1 releases it from eIF-4E facilitating the formation of the active eIF-4F complex (27). We have shown that LPS significantly decreased the basal phosphorylation of 4E-BP1 and subsequently decreased the availability of eIF-4E. These changes in 4E-BP1 and eIF-4E activity-related phosphorylation are suggestive of a fall in PS and similar to changes observed in vivo (52).

The activation of p70^S6K kinase is essential to obtain normal muscle fiber size in vivo (34). We observed a significant decrease in activity-related phosphorylation of p70^S6K in response to LPS, which correlates well with in vivo studies (29, 52) but is in contrast to the finding of Frost et al. (10), who observed no such decrease in response to LPS alone. The decreased phosphorylation of 4E-BP1 and of p70^S6K in response to LPS that we observed in the present study would both contribute to reduced translation initiation efficiency and are consistent with previous findings in vivo (27, 29).

Upstream regulators of protein synthesis are known to include Akt (protein kinase B) and the MAPKs (p38 and ERK1/2). Although, in the present study, LPS significantly decreased the phosphorylation of Akt for only 30 min, its downstream target mTOR exhibited a decreased activity-related phosphorylation over a longer time period, suggesting that LPS mediated its effect on PS mainly through Akt/mTOR and downstream targets including p70^S6K, eIF-4E, and 4E-BP1 (3, 14, 27, 29, 35). To investigate the role of Akt inhibition on PS, we used the PI3K inhibitor LY-0294002 (39). Incubation of C2C12 cells with LPS plus LY-0294002 both significantly decreased the rate of PS (Figs. 4 and 5), indicating that Akt plays a key role in the regulation of PS in skeletal muscle. Decreased Akt total protein has been seen in a rodent model of sepsis (6) as well as in the C2C12 model of sepsis (44, 48). Akt is a well-known upstream regulator of translation initiation with increased Akt phosphorylation increasing both eIF-2B and eIF-4F assembly via the phosphorylation and subsequent inactivation of GSK-3/β and 4E-BP1 (33).

Fig. 5. The effect of LPS (1 μg/ml) and LPS in combination with specific inhibitor and the specific inhibitors alone of the on the protein synthesis rate. C2C12 myotubes were incubated with either LPS (1 μg/ml) alone or with PMB (5 μg/ml), or SB (10 μM), or PD (40 μM), or LY (50 μM) for 18 h (long-term response), or the C2C12 myotubes were incubated either with PMB (5 μg/ml) alone, or SB (10 μM) alone, or PD (40 μM) alone, or LY (50 μM) alone for 18 h (long-term response). The protein synthesis rate was determined by measuring the direct [3H]phenylalanine incorporation into the cell protein (CPM/μg protein). Data are represented as means ± SE (a.u). Asterisks are statistically different from control cells (**P < 0.01 and ***P < 0.001); n = 6.

Fig. 6. Changes in the phospho-Akt (A), phospho-p38 (B), and phospho-ERK1/2 (C). C2C12 myotubes were incubated either with LPS alone or with LPS and PMB (5 μg/ml); LPS and SB (10 μM); LPS and PD (40 μM); or LPS and LY (50 μM) (D). Values are represented as means ± SE. Asterisks are statistically different compared with control cells (**P < 0.05, ***P < 0.01, and ****P < 0.001); n = 4. The presented blots illustrated the effect of LPS alone and LPS in combination with specific inhibitors on activity-related phosphorylation of Akt, p38, and ERK1/2. The data were normalized to β-actin as an internal loading control.
The MAPK family is considered a fundamentally important ubiquitous intracellular signaling system involved in the regulation of cell growth, differentiation, and survival (45). In the present study, LPS significantly increased the p38 phosphorylation at all time points (with the exception of 1 h) and of ERK1/2 (with the exception of the final 18-h time point). Increased muscle p38 phosphorylation has been shown in many pro-catabolic conditions including acute quadriplegic myopathy (8), neurogenic atrophy (8), aging (57), and sepsis (21). Although similar increases in p38 phosphorylation have been observed in rats administered LPS (21), a diminished ERK1/2 and p38 phosphorylation was found by Vary et al. (51) in which chronic abdominal sepsis had been induced. Interestingly, however, in the later study, these falls in ERK1/2 and p38 phosphorylation were prevented when the activity of TNF-α was inhibited by the administration of TNF binding protein to the rats with sepsis. One of the downstream targets of both p38 and ERK1/2 MAPKs is Mnk1 (13, 56). Mnk1 has been shown to be involved in the process of PS in the main via its downstream target eIF-4E (14). Our data demonstrated that LPS significantly increased p38 and ERK1/2 phosphorylation, and this effect was associated with increased basal phosphorylation of Mnk1, suggesting that Mnk1 be considered as one of the MAPK (p38 and ERK1/2) substrates (13, 56). Although, LPS increased the phosphorylation of MAPKs (p38 and ERK1/2) and Mnk1, this was not sufficient to prevent the LPS-induced significant decrease in PS at the 3-h time point. A possible explanation for the inability of LPS increases in p38 and ERK1/2 phosphorylation to elevate PS (or prevent a decrease) through Mnk1 and eIF-4E may be a consequence of deceased 4E-BP1 that we observed under such conditions (see above). An increased association of eIF-4E with this binding protein would decrease the ability of Mnk1 to phosphorylate eIF-4E. It has been shown that in a number of species that sepsis induces reduced phosphorylation of eIF4G in vivo thus inhibiting eIF-4F complex formation and translation initiation (36). In C2C12 cells the phosphorylation of eIF4G has, however, been shown to be independent of ERK (58), though a role for p38 cannot be precluded. The reduced levels of Akt phosphorylation at early time points might also reduce the phosphorylation of eIF-4G.

Our data showed that inhibition of p38, ERK1/2 activity by SB-203580, and PD-98059, respectively, when administered to C2C12 cells alone, significantly decreased the rate of PS (Figs. 4 and 5), suggesting that these MAPKs are normally directly involved in the regulation of PS, and this is most probably via the Mnk1/eIF-4E cascade. The involvement of these MAPKs in the regulation of PS has been shown in several studies (23, 25) with regulation of PS in two possible ways. First, they can phosphorylate eIF-4E via Mnk1, and in turn, eIF-4E phosphorylation regulates translation initiation (56). Second, MAPK may be involved in the regulation of the formation of eIF-4F complex (24). Surprisingly, incubation of the cells with both SB-203580 and PD-98059 alone significantly decreased the PS rate, but in combination with LPS, no such decrease in the rate of PS has been observed at the 3-h time point. The effects of these MAPK inhibitors in inducing a decrease in PS in control cells might be expected, but the inhibition of this decrease when both inhibitor and LPS are present is more difficult to explain. One possible explanation is possible cross talk between Akt and MAPK pathways that has been reported in some studies in skeletal muscle (9, 32, 42) or the role of other factors (46, 54). Such a potential factor could be p53, which in other cell types (but not to date in skeletal muscle cells) has been shown to inhibit translation initiation through its action on 4E-BP1 and p70S6K (18). In turn, it has been shown in some cell types that p53 is upregulated by MAPK action. This role of p53 in the regulation of PS in skeletal muscle needs further examination.

Although our data showed that LPS alone significantly decreased the protein synthetic rate in C2C12 myotubes by 50%, this effect was mediated most likely via Akt/mTOR signaling cascade. This effect was mediated via the alteration of the translation initiation efficiency. Furthermore, the direct involvement of MAPK in the regulation of global muscle cell PS has been shown. However, further investigations are required to clarify how MAPK regulates the global PS in vivo and in vitro. It is interesting that the transient effects of LPS that we observe on protein synthesis in this study are similar to transient responses in physiological parameters that have been observed in vivo and which have been termed endotoxin resistance or tolerance (20). Whether the current observations indicate a similar response in vitro is unclear.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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