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Sepsis attenuates the anabolic response to skeletal muscle contraction

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Abstract

Electrically stimulated muscle contraction is a potential clinical therapy to treat sepsis-induced myopathy; however, whether sepsis alters contraction-induced anabolic signaling is unknown. Polymicrobial peritonitis was produced by cecal ligation and puncture (CLP) in male C57BL/6 mice and time-matched, pair-fed controls (CON). At ~24 h post-CLP, the right hindlimb was electrically stimulated via the sciatic nerve to evoke maximal muscle contractions and the gastrocnemius was collected 2 h later. Protein synthesis was increased by muscle contraction in CON mice. Sepsis suppressed the rate of synthesis in both the non-stimulated (31%) and stimulated (57%) muscle versus CON. Contraction of muscle in CON mice increased the phosphorylation of mTORC1 substrates S6K1 Thr³⁸⁹ (8-fold), S6K1 Thr⁴²¹/Ser⁴²⁴ (7-fold) and 4E-BP1 Ser⁶⁵ (11-fold). Sepsis blunted the contraction-induced phosphorylation of S6K1 Thr³⁸⁹ (67%), S6K1 Thr⁴²¹/Ser⁴²⁴ (46%) and 4E-BP1 Ser⁶⁵ (85%). Conversely, sepsis did not appear to modulate protein elongation as eEF2 Thr⁵⁶ phosphorylation was decreased similarly by muscle contraction in both groups. MAPK signaling was discordant following muscle contraction in septic muscle; phosphorylation of ERK Thr²⁰²/Tyr²⁰⁴ and p38 Thr¹⁸⁰/Tyr¹⁸² was increased similarly in both CON and CLP mice while sepsis prevented the contraction-induced phosphorylation of JNK Thr¹⁸³/Tyr¹⁸⁵ and c-JUN Ser⁶³. The expression of IL-6 and TNF- α mRNA in muscle was increased by sepsis, and contraction increased TNF- α to a greater extent in muscle from septic than CON mice. Injection of the mTOR inhibitor Torin2 in separate mice confirmed that contraction-induced increases in S6K1 and 4E-BP1 were mTOR-mediated. These findings demonstrate that resistance to contraction-induced anabolic signaling occurs during sepsis and is predominantly mTORC1-dependent.

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Keywords

anabolic resistance; skeletal muscle metabolism; resistance exercise; inflammation; cecal ligation and puncture

1. Introduction

Sepsis and critical illness is accompanied by a significant loss of skeletal muscle mass which is linked to prolonged hospital stay, ventilation time, recovery and return of functional strength (1). Muscle mass is tightly regulated by the balance between rates of protein synthesis and degradation; hence a sustained perturbation of either metabolic process can alter muscle mass. During sepsis, loss of muscle mass is due to both an increased rate of protein degradation as well as a decreased rate of synthesis within skeletal muscle (2). However, should the sepsis-induced increase in protein breakdown be overcome by enhancement of rates of protein synthesis muscle mass is likely to be maintained. Therefore, the evaluation of protein synthesis is of central importance to determining the effects of sepsis on muscle metabolism.

Protein synthesis is a highly regulated process predominately controlled by the mammalian target of rapamycin (mTOR) protein complex 1 (mTORC1). This Ser/Thr kinase serves as a central point of integration for a variety of signals including changes in energy/nutrient status, hypoxia, and anabolic signals. Activation of mTOR results in the phosphorylation of its immediate downstream substrates 70kDa ribosomal protein S6 kinase-1 (S6K1) and eukaryotic initiation factor (eIF) 4E binding protein (4E-BP1), which promotes translation initiation, mRNA elongation and protein synthesis via subsequent phosphorylation events. Furthermore, proteins outside of the canonical mTOR pathway, including those in the mitogen activated protein kinase (MAPK) family, may also contribute to induction of protein synthesis (3, 4). The decreased rate of muscle protein synthesis observed during sepsis is multifactorial and includes suppression of mTOR kinase activity, increased local and systemic production of inflammatory mediators [tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), nitric oxide synthase-2 (NOS-2)], and decreased insulin-like growth factor-I (IGF-I) concentrations which reduce translational efficiency (5). Additionally, sepsis-induced resistance to anabolic stimulation by either the amino acid leucine or the hormone insulin may contribute to the loss of muscle mass (6, 7).

Electrically stimulated muscle contraction is a potent anabolic stimulus but its efficacy to induce mTORC1-mediated protein synthesis in septic muscle remains to be determined. While the clinical use of electrical stimulation to evoke muscle contractions is growing, results of reported trials specifically within septic patients remain equivocal; one study showed maintenance of muscle strength and faster recovery of functional movements following stimulation while another reported no differences versus the control condition (8, 9). Further, outcome measures have predominately focused on changes in muscle strength or mass while signaling pathways important in protein synthesis and degradation remain largely unexplored.

Therefore, in the current work an animal model of sepsis (cecal ligation and puncture-CLP (10)) was used to investigate whether sepsis altered the anabolic response to a single bout of electrically stimulated muscle contraction. It was hypothesized that skeletal muscle contraction would overcome the sepsis-induced suppression of protein synthesis and mTORC1 signaling to potentially support the future use of this therapy in the attenuation of critical illness myopathy. Results however showed that a single bout of maximal muscle stimulation was not sufficient to enhance protein synthesis nor mTOR signaling and future work will therefore be required to determine whether repeated bouts of muscle stimulation could confer beneficial effects on time to recovery from sepsis-induced myopathy.

2. Methods

2.1 Animals

Viral antibody free male C57BL/6 mice aged 11–12 weeks old were purchased from Charles River Laboratories (Wilmington, MA) and acclimated to the animal facility at the College of Medicine at Penn State Hershey for at least 1 week prior to experimental use. Mice were housed in shoe-box cages with corn cob bedding under controlled environmental conditions (12:12 light:dark), and were provided Teklad Global 2019 (Harlan Teklad, Boston, MA) and water *ad libitum* until the start of the experiment. All experimental procedures were performed in accordance with the National Institutes of Health guidelines for the use of experimental animals and were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine.

2.2 Induction of polymicrobial peritonitis

Sepsis was produced by CLP (10). Mice were anesthetized via inhalation of isoflurane (2–3% in O₂ with 1.5% maintenance) and a laparotomy was performed after shaving and aseptic preparation of the abdomen. The cecum was ligated, punctured twice with a 23G needle (through and through), and a small amount of cecal material was extruded before returning the cecum and closing the incision through the muscle layer with 5-0 surgical suture. The incision through the skin was secured with 9 mm metal wound clips and all animals received 1 mL of warm 0.9% sterile saline subcutaneously before being returned to their individual cage for recovery. Control mice underwent an identical laparotomy except the cecum was not ligated or punctured. Following surgery all mice were individually housed and allowed free access to water although food was withheld to standardize the nutritional status across groups.

2.3 Muscle contraction protocol

Approximately 18–24 h after the induction of sepsis, mice were anesthetized via isoflurane (3–4% in O₂ with 2% maintenance) and prepared for the muscle contraction protocol that was performed exactly as described previously (11). Briefly, a small incision was made through the skin and muscle to expose the sciatic nerve. Needle electrodes were placed over the nerve and maximal muscular contractions were evoked using a current stimulator (Model A365, World Precision Instruments, Sarasota, FL) interfaced with Powerlab 4/35 and LabChart software (ADI Instruments, Colorado Springs, CO). The stimulation protocol included 10 sets of 6 contractions (each lasting 3 s) with 10 s rest between each contraction

and 60 s rest after each set of 6 contractions (11). To ensure maximal contractions were induced the current was set to 1 milliamperes (mA) with a pulse height of 6 volts (V). Each contraction produced a concentric shortening of the triceps surae complex (gastrocnemius, plantaris, soleus) along with a concomitant lengthening of the tibialis anterior and extensor digitorum longus muscle. The contralateral leg served as the non-contracted control. Immediately following the last contraction, mice were administered 1 mL of warm sterile saline subcutaneously and allowed to recover for 2 h. Mice remained in the fasted state throughout the recovery period but had free access to water. Two hours following the last muscle contraction, the gastrocnemius and plantaris muscle complex (referred to as muscle from here on) was excised from the stimulated and non-stimulated leg and immediately frozen between aluminum blocks precooled to the temperature of liquid nitrogen. Blood was collected from the vena cava in heparinized syringes and centrifuged ($10,000 \times g$ for 10 min) for isolation of plasma. Both frozen tissue and plasma were stored at -80°C until analysis. The experimental time point of 2 h post electrically stimulated muscle contraction was chosen based on a previous investigation showing induction of S6K1 Thr³⁸⁹ phosphorylation by 30 min (11) and to stay within a 24 h time period following CLP surgery.

2.4 Torin2 administration

To investigate the role of mTOR in contraction-induced protein synthetic signaling, a separate group of 8 week old male C57BL/6 mice ($n=7$) were fasted overnight and given the ATP-competitive inhibitor of mTOR, Torin2 (9–6-aminopyridin-3-yl)-1-(3-trifluoromethyl)phenyl)benzo[h][1,6]naphthyridin-2(1H)one). Torin2 powder (EMD Millipore, Darmstadt, Germany) was dissolved at 25 mg/mL in 100% N-methyl-2-pyrrolidone (NMP) before being diluted 1:4 with sterile 50% PEG400. Torin2 (20 mg/kg) was injected 15 min prior to the initiation of electrically stimulated muscle contraction and muscles were collected 4 h following cessation of muscle contraction. Control mice ($n=5$) were injected with the same volume of vehicle solution (i.e., NMP diluted 1:4 with 50% PEG400) based on individual body weight.

2.5 Western blotting

Half of the frozen gastrocnemius complex (50–90 mg) was homogenized using a mechanical homogenizer in 10 volumes of ice cold buffer consisting of (in mmol/L): 50 HEPES, 0.1% Triton-X, 4 EGTA, 10 EDTA, 15 sodium pyrophosphate, 100 β -Glycerophosphate, 25 sodium fluoride, 5 sodium orthovanadate and Protease inhibitor cocktail (P8340 SIGMA-ALDRICH) (St. Louis, MO). Protein concentration was quantified using the Bio-Rad Protein Assay Dye reagent concentrate (Hercules, CA) and SDS-PAGE was carried out using equal amounts of total protein per sample. The membranes were incubated overnight at 4°C with primary antibody (Cell Signaling, Beverly, MA, unless otherwise noted). Antibodies included Regulated in Response to DNA damage-1 (REDD1) (ProteinTech, Chicago, IL), S6K1, S6K1 (Thr³⁸⁹ and Thr⁴²¹/Ser⁴²⁴), ribosomal protein S6 (rpS6), rpS6 (Ser^{240/244} and Ser^{235/236}), 4E-BP1 (Bethyl Laboratories, Montgomery, TX), 4E-BP1 (Ser⁶⁵), programmed cell death protein 4 (PDCD4), PDCD4 (Ser⁶⁷) (Abcam, Cambridge, MA), eIF4B, eIF4B (Ser⁴²²), eukaryotic elongation factor-2 (eEF2), eEF2 (Thr⁵⁶), extracellular-signal-regulated kinases (ERK)1/2 (Thr²⁰²/Tyr²⁰⁴), p42/44 MAPK, p38

(Thr¹⁸⁰/Tyr¹⁸²), p38, c-JUN N-terminal kinase (JNK), JNK (Thr¹⁸³/Tyr¹⁸⁵), c-Jun (Ser⁶³), GAPDH, Akt (Ser⁴⁷³) and Akt. The FluorChem M Multifluor System (ProteinSimple, San Jose, CA) was used for visualization following exposure to ECL reagent (Thermo Scientific, Waltham, MA). Images were analyzed using AlphaView (ProteinSimple) and Image J software (NIH).

2.6 Protein synthesis

A separate group of age-matched male C57BL/6 mice were used to determine the *in vivo* rate of muscle protein synthesis in the gastrocnemius 24 h post induction of sepsis and 2 h after the cessation of the muscle contraction protocol. All experimental protocols were performed as described above except that at 2 h post-muscle contraction mice were injected with L-[2,3,4,5,6-³H]phenylalanine [Phe; 150 mM, 50 μ Ci/ml; 0.5 mL] for an additional 15 min prior to tissue collection. Mice were then anesthetized with isoflurane and blood was collected from the vena cava for measurement of plasma Phe concentration and radioactivity. Muscles were excised and immediately clamped between aluminum blocks precooled to the temperature of liquid nitrogen. High performance liquid chromatography (HPLC) was used for the measurement of specific radioactivity of plasma Phe levels in the supernatant from trichloroacetic acid (TCA) treated plasma extracts. The global rate of [³H]Phe incorporation into protein within the muscle was assessed as previously described by our laboratory (11).

2.7 Measurement of mRNA expression

Total RNA was extracted using Tri-reagent (Molecular Research Center, Cincinnati, OH) and RNeasy mini kit (Qiagen, Valencia, CA) following the manufacturers' protocols. Half of the gastrocnemius complex (50–90 mg) was homogenized in Tri-reagent followed by chloroform extraction, according to the manufacturer's instructions. An equal volume of 70% ethanol was added to the aqueous phase, and the mixture was loaded on a Qiagen mini spin column. The Qiagen mini kit protocol was followed from this step onward, including the on-column DNase I treatment to remove residual DNA contamination. RNA was eluted from the column with RNase-free water and an aliquot was used for quantitation (NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA). The quality of the RNA was analyzed on a 1% agarose gel. Total RNA (1.5 μ g) was reverse transcribed using superscript III RT (Invitrogen, Carlsbad, CA) in a total volume of 20 μ l following the manufacturer's instructions. Real-time quantitative PCR was performed with 0.5–1 μ l of the reverse transcription reaction in a QuantStudio™ 12K Flex Real-Time PCR System using TaqMan gene expression assays (Applied Biosystems, Foster City, CA) for IL-6 Mm00446190_m1; tumor necrosis factor alpha (TNF- α) Mm00443258_m1 and ribosomal protein L32, (Rpl32) Mm02528467_g1. The comparative quantitation 2^{-C_t} method was used in presenting gene expression of target genes in reference to the endogenous control.

2.8 Statistical Analysis

All data were analyzed using commercial statistic software (SigmaPlot, Systat, San Jose, CA) using a repeated measures two-way ANOVA (contraction $\times$ sepsis) with Student-

Neuman-Keuls post hoc test when appropriate. Data are presented as mean $\pm$ SE and considered significant when $P < 0.05$.

3. Results

3.1 Muscle protein synthesis

The rate of muscle protein synthesis measured *in vivo* was increased 22% by muscle contraction in control mice 2 h after cessation of electrical stimulation (Figure 1A). Sepsis decreased protein synthesis in the non-stimulated (32%) muscle and completely prevented any contraction-induced increase (Figure 1A). Calculation of the delta change between the non-stimulated and stimulated muscle in the same animal showed a greater increase in rate of synthesis following muscle contraction in control versus septic mice (Figure 1B).

3.2 Contraction-induced mTORC1 signaling is abrogated by sepsis

Subsequent analysis was performed to elucidate the mechanism by which sepsis antagonized contraction-induced increases in muscle protein synthesis. REDD1, an upstream negative regulator of mTORC1, was increased by sepsis in both the stimulated and non-stimulated muscle (Figure 2A). Contraction tended ($p=0.06$) to decrease REDD1 in control mice, but not in muscle from septic mice. Accordingly, stimulated muscle contraction increased mTORC1 signaling in skeletal muscle of control mice; phosphorylation of S6K1 Thr³⁸⁹ (8-fold), S6K1 Thr^{421/Ser424} (7-fold), ribosomal protein S6 (rpS6) Ser^{240/244} (8-fold), rpS6 Ser^{235/236} 10-fold) (data not shown), and 4E-BP1 Ser⁶⁵ 11-fold) were all significantly increased 2h after muscle contraction (Figure 2B–E). Sepsis prevented this contraction-induced increase in mTORC1 signaling as no increase was observed in the stimulated leg compared with the respective contralateral control (Figure 2B–E). Further, the contraction-induced phosphorylation of each of these proteins was significantly lowered in septic compared with control mice.

S6K1 phosphorylates several substrates (in addition to rpS6) which have the potential to control translation initiation and mRNA elongation, including PDCD4, eIF4B and eEF2 kinase (3, 12). We detected a sepsis-induced increase in the phosphorylation of eIF4B Ser⁴²² and a tendency for increased PDCD4 Ser⁶⁷ phosphorylation in septic muscle (Figure 3A and 3B). Further, eIF4B phosphorylation was only increased by muscle contraction in control animals while PDCD4 phosphorylation was not altered by either condition. Elongation may also be regulated by mTORC1 via S6K1-mediated phosphorylation of eEF2 kinase which increases phosphorylation and activation of eEF2 to suppress elongation (3). Our results indicate that muscle contraction decreased eEF2 Thr⁵⁶ in both control and septic muscle (Figure 3C). Moreover, sepsis did not alter eEF2 Thr⁵⁶ phosphorylation compared to control values in either the basal state or after stimulation (Figure 3C).

3.3 Contraction-induced increases in MAPK signaling is differentially altered by sepsis

Contrary to the response of the mTORC1 substrates described above, sepsis did not suppress the muscle contraction-induced increase in the phosphorylation of ERK Thr^{202/Tyr204} and p38 Thr^{180/Tyr182} compared with control values (Figure 4A and B). However, in contradistinction, the contraction-induced phosphorylation of JNK Thr^{183/Tyr185} and its

substrate c-JUN Ser⁶³ was abrogated by sepsis so that muscle from septic mice did not differ between the non-contracted and contracted condition (Figure 4C and D).

3.4 Sepsis increases mRNA expression of inflammatory cytokines IL-6 and TNF- α

Inflammation may also contribute to skeletal muscle loss observed during disease and the expression of IL-6 and TNF- α has been linked to muscle catabolism (13–15). Therefore, changes in these two pro-inflammatory cytokines was assessed as a possible mechanism of the sepsis-induced suppression of protein synthesis in the contracted muscle. Presently, mRNA expression of IL-6 was increased almost 80-fold in septic muscle compared with the respective control group (Figure 5A). Muscle contraction did not significantly alter the expression of this cytokine. Similarly, TNF- α expression was elevated in the non-stimulated and stimulated muscle of the septic mice versus controls (Figure 5B) with the sepsis-induced increase being exaggerated by muscle contraction.

3.5 Torin2 and muscle contraction

To determine whether muscle contraction-induced increases in S6K1 and 4E-BP1 were mediated by mTOR under our current experimental conditions, we treated an additional control (non-septic) group of mice with the mTOR inhibitor, Torin2, prior to muscle contraction. Torin2 inhibited basal and contraction-induced phosphorylation of rpS6 Ser^{240/244}, 4E-BP1 Ser⁶⁵, Akt Ser⁴⁷³ and mTOR Ser²⁴⁸¹ (Figure 6A–D). In contrast, basal levels of S6K1 Thr³⁸⁹ were not reduced compared to control levels, but Torin2 did prevent the contraction-induced increase in its phosphorylation (Figure 6E). Torin2 treatment did not influence the phosphorylation of ERK1/2 Thr²⁰²/Tyr²⁰⁴ as increases in response to muscle contraction were observed in muscle from both control and Torin2 treated mice (Figure 6F).

4. Discussion

The use of electrically stimulated muscle contraction has been promoted as a means to limit loss of muscle and speed recovery in the critically ill; however, little is known regarding the mechanism by which sepsis alters the cellular changes contributing to contraction-induced muscle adaptations under tightly controlled experimental conditions. Herein we show that CLP-induced sepsis prevents the anabolic response to electrically stimulated muscle contraction in the gastrocnemius and plantaris of mice. Importantly, the sepsis-induced decrease in muscle protein synthesis is not rescued by electrically stimulated muscle contraction. Similarly, sepsis prevents the contraction-induced increase in anabolic mTORC1 signaling observed in control mice. As the signaling pathway through which muscle contraction activates mTOR remains elusive, identification of a precise mechanistic target related to the sepsis-induced suppression of anabolic signaling is challenging, although as is shown here, elevations in the pro-inflammatory cytokines IL-6 and TNF- α in addition to suppressed mTORC1 signaling may contribute.

4.1 Sepsis-induced changes in skeletal muscle protein synthesis and signaling

The sepsis-induced decrease in skeletal muscle protein synthesis is well characterized and at least partially attributable to impaired translation initiation and translational efficiency which is predominately controlled by proteins signaling to and within the canonical

mTORC1 signaling pathway (16). For example, REDD1, which is a negative regulator of mTORC1 via TSC2 dependent inactivation of Rheb is increased in septic muscle. As REDD1 null mice have increased rates of skeletal muscle protein synthesis, REDD1 may represent a future therapeutic target for the treatment of sepsis-induced muscle disease (17). In contrast to the sepsis-mediated increase in REDD1 protein expression, we did not observe a significant decrease in the phosphorylation of S6K1 and 4E-BP1 (in the basal non-contracted muscle). In both rat and mouse models of CLP, phosphorylation of 4E-BP1 on Thr^{37/46} has been reported to be decreased (18–21); however, this is among the first report of phosphorylation of the Ser⁶⁵ site within septic muscle. Phosphorylation of this residue may be a better indicator of 4E-BP1 activation and maintenance of eIF4E dissociation as 4E-BP1 undergoes hierarchical phosphorylation (i.e. Thr^{37/46} and Thr⁷⁰ phosphorylation is required for Ser⁶⁵ phosphorylation) (22).

One of the few prior investigations to show reductions in skeletal muscle S6K1 phosphorylation following CLP was performed in 12 month old Balb/c mice in contrast to the 12 week old C57BL/6 mice used presently (19). Both the genotype and age difference may have contributed to the different basal response to sepsis; Balb/c mice experience higher levels of pro-inflammatory cytokines and reduced survival compared with C57BL/6 mice following CLP (23), and aged mice (60 weeks old) experience greater body weight loss, inflammation and tissue damage than younger mice in response to viral infection (24). Therefore, the older Balb/c mice may have been more susceptible to infection causing greater reductions in S6K1 and 4E-BP1 phosphorylation in muscle following CLP compared with the young C57BL/6 used herein (19).

4.2 Electrically stimulated muscle contraction during sepsis

Our data indicate an acute bout of electrically stimulated muscle contraction increased mTORC1 (S6K1, 4E-BP1 and downstream substrates rpS6, eIF4B and eEF2) as well as MAPK (ERK, p38, JNK, and cJUN) signaling in control non-septic muscle in association with an increase in muscle protein synthesis similar to previous reports (17, 25, 26). Sepsis prevented the contraction-induced changes in several of these proteins providing evidence that the anabolic response of septic muscle to a mechanical stimulus was suppressed. These findings contrast the protein synthetic/mTORC1 response to IGF-I which was not reduced during sepsis, but is in agreement with that after leucine gavage in which the sepsis-induced decrease in protein synthesis and phosphorylation of S6K1, rpS6 and 4E-BP1 was also not overcome (6). It is notable that the maintenance of contraction-induced increases in ERK, p38 signaling and mRNA elongation (assessed by eEF2 Thr⁵⁶ phosphorylation) was not sufficient to overcome the sepsis-induced decrease in protein synthesis. Lastly, use of the mTOR inhibitor Torin2 confirmed the important role of mTOR in the anabolic response to muscle contraction and accordingly mimicked the effects observed in septic muscle in which mTOR signaling is also suppressed.

4.3 IL-6 and TNF- α expression in response to sepsis and/or muscle contraction

Increased inflammatory cytokine levels as is currently observed, is a hallmark of sepsis and may have implications on the imbalance between rates of protein synthesis and degradation responsible for the loss of muscle mass (13–15). A role for TNF- α in septic animal models

is well established and preventing its synthesis, reducing circulating levels or pretreating with TNF binding protein offsets sepsis-related decreases in muscle mass, protein synthesis and translation initiation (18, 27, 28). Further, TNF- α can also act in an autocrine and/or paracrine manner stimulating the release of other pro-inflammatory, pro-catabolic cytokines including IL-6 and IL-1 (29). Accordingly, IL-6 overexpression can lead to muscle atrophy and decreased muscle protein synthesis (15); however, its significance during sepsis has been questioned as LPS-induced loss of body weight did not differ between control and IL-6 deficient mice (30). Lastly, interferon- γ (IFN- γ), another pro-inflammatory cytokine elevated during sepsis, increases the phosphorylation of eIF4B Ser⁴²² *in vitro* and therefore may explain the significant increase observed in septic mice.

While increased expression of these cytokines is associated with detrimental effects under septic conditions, counter intuitively, muscle expression of IL-6 and TNF- α can also be amplified by muscle contraction (31, 32). However, as the magnitude of increase induced by exercise in the present work is lower in the control animals than in the septic mice, we speculate there may be a critical threshold of expression above which levels become harmful.

4.4 Use of electrical stimulation in septic patients

Electrical muscle stimulation has been used sporadically in patients as a means to offset the loss of muscle mass and strength associated with critical illness. Although few trials using electrical stimulation have been performed specifically in septic patients, beneficial effects in patients with intensive care unit (ICU)- and mechanical ventilation-acquired weakness have been described (8, 9, 33). For example, twice daily neuromuscular electrical stimulation improved muscle strength and prevented muscle atrophy assessed by maintenance of cross sectional area in both type I and type II muscle fibers of sedated ICU patients (9, 33). However, the increase in cross sectional area reported was not associated with changes in the phosphorylation of S6K1 or Akt similar to our current findings. Regardless, this work provides evidence that repetitive bouts of muscle stimulation repeated over a longer period of time may prevent atrophy and strength loss although additional work is needed to determine the cellular mechanisms responsible for these changes.

4.5 Conclusion

Sepsis is characterized by a significant loss of muscle mass, strength and contractility stemming from the dysregulation of several physiological processes including increased inflammation and inhibition of protein synthesis. The efficacy of therapies to offset the loss of muscle mass are critical to speed recovery and enable complete return of functional capacity; however, studies on intracellular signaling important in regulating the functional changes produced by electrically stimulated muscle contraction have been lacking. Therefore, the results of this work are novel in that they show sepsis prevents the acute increase in protein synthesis induced by electrically stimulated muscle contraction and the mechanism for this inhibitory effect is likely mTOR-dependent. Further research is needed to determine the implications of these findings in a chronic setting or during recovery, as emerging evidence supports the use of chronic neuromuscular electrical stimulation in this patient population.

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Abbreviations

mTORC1	mammalian target of rapamycin (mTOR) complex 1
DEPTOR	DEP domain-containing mTOR-interacting protein
Raptor	regulatory-associated protein of mTOR
MLST8	mammalian lethal with SEC13 protein 8
PRAS40	Proline-rich Akt Substrate of 40 kD
S6K1	70kDa ribosomal protein S6 kinase-1
eIF	eukaryotic initiation factor
4E-BP1	eukaryotic initiation factor-binding protein-1
MAPK	mitogen activated protein kinase
TNF-α	tumor necrosis factor-alpha
IL-6	interleukin-6
NOS-2	nitric oxide synthase-2
IGF-1	insulin-like growth factor-I
CLP	cecal ligation and puncture
REDD1	Regulated in Response to DNA damage-1
rpS6	ribosomal protein S6
PDCD4	programmed cell death protein 4
eEF2	eukaryotic elongation factor-2
ERK	extracellular-signal-regulated kinases
JNK	c-JUN N-terminal kinase
Phe	phenylalanine
HPLC	high performance liquid chromatography
LPS	lipopolysaccharide
TSC2	tuberous sclerosis complex 2
IFN-γ	interferon- γ

ICU	intensive care unit
APACHE-II	Acute Physiology and Chronic Health Evaluation II

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Figure 1.

Muscle protein synthesis is suppressed by sepsis. The rate of protein synthesis was assessed *in vivo* in the gastrocnemius 2 h post cessation of electrically stimulated muscle contraction (A). The delta change between the non-contracted and contracted muscle is shown in (B). Open bars represent control (Con) mice (n=7–10) and shaded bars correspond to septic mice (n=7–10). ‘Non-Stim’ indicates control condition, while ‘Stim’ indicates muscle underwent electrically stimulated muscle contraction. Horizontal bars indicate statistical differences between groups ($P < 0.05$). Values are expressed as means $\pm$ SE.

Figure 2.

Sepsis modulates mTORC1 related signaling and prevents contraction-induced changes in muscle. REDD1 (A), S6K1 (B and C), 4E-BP1 (D) and rpS6 (E) phosphorylation was assessed 2 h after cessation of muscle contraction in the gastrocnemius. Bar graphs represent quantification of Western blot images relative to the total amount of the respective protein with the control non-contracted value set to 100%. GAPDH was used to verify loading for REDD1 as there is no corresponding total protein (A). Open bars represent control (Con) mice (n=9) and shaded bars correspond to septic mice (n=7). 'Non-Stim' indicates control condition, while 'Stim' indicates muscle underwent electrically stimulated muscle contraction. Horizontal bars with * indicates statistical differences between groups (P<0.05). Dashed horizontal bars indicate a trend for significance was detected and the p-value is indicated on the graph. Values are expressed as means $\pm$ SE.

Figure 3.

Sepsis and contraction induced changes in S6K1 substrates. Phosphorylation of eIF4B (A), PDCD4 (B), and eEF2 (C) was assessed 2 h after cessation of muscle contraction in the gastrocnemius. Bar graphs represent quantification of Western blot images relative to the total amount of the respective protein with the control non-contracted value set to 100%. Open bars represent control (Con) mice (n=9) and shaded bars correspond to septic mice (n=7). 'Non-Stim' indicates control condition, while 'Stim' indicates muscle underwent electrically stimulated muscle contraction. Horizontal bars with * indicates statistical differences between groups ($P < 0.05$). Dashed horizontal bars indicate a trend for significance was detected and the p-value is indicated on the graph. Values are expressed as means $\pm$ SE.

Figure 4.

Differential response of MAPK signaling following sepsis and electrically stimulated muscle contraction. Phosphorylation of ERK (A), p38 (B), JNK (C) and cJUN was measured in the gastrocnemius 2 h after cessation of muscle contraction. Bar graphs represent quantification of Western blot images relative to the total amount of the respective protein or GAPDH (cJUN) with the control non-contracted value set to 100%. Open bars represent control (Con) mice (n=9) and shaded bars correspond to septic mice (n=7). 'Non-Stim' indicates control condition, while 'Stim' indicates muscle underwent electrically stimulated muscle contraction. Horizontal bars with * indicates statistical differences between groups ($P < 0.05$). Dashed horizontal bars indicate a trend for significance was detected and the p-value is indicated on the graph. Values are expressed as means $\pm$ SE.

Figure 5.

Expression of inflammatory cytokines is increased by sepsis and/or muscle contraction. Bar graphs represent relative mRNA expression of IL-6 (A) or TNF- α (B) normalized to RPL32 in gastrocnemius with the control non-contracted value set to 100 arbitrary units (AU) and all other groups expressed relative to this value. Open bars represent control (Con) mice (n=9) and shaded bars correspond to septic mice (n=7). 'Non-Stim' indicates control condition, while 'Stim' indicates muscle underwent electrically stimulated muscle contraction. Horizontal bars with * indicates statistical differences between groups ($P < 0.05$).

Dashed horizontal bars indicate a trend for significance was detected and the p-value is indicated on the graph. Values are expressed as means $\pm$ SE.

Figure 6.

Effect of Torin2 on electrically stimulated muscle contraction induced changes in mTOR signaling. Phosphorylation of rpS6 (A), 4E-BP1 (B), Akt (C), mTOR (D), S6K1 (E) and ERK (F) was measured in gastrocnemius after treatment with Torin2 (20mg/kg) and electrically stimulated muscle contraction. Bar graphs represent quantification of Western blot images normalized to the total amount of respective protein with the control non-contracted value set to 100%. Striped bars correspond to Torin2 (Torin) treated mice (n=7) and open bars represent control (Con) mice (n=5). 'Non-Stim' indicates control condition,

while 'Stim' indicates muscle underwent electrically stimulated muscle contraction. Horizontal bars with * indicates statistical differences between groups ($P < 0.05$). Values are expressed as means $\pm$ SE.