Loss of muscle strength during sepsis is in part regulated by glucocorticoids and is associated with reduced muscle fiber stiffness

Nima Alamdari,1* Gianluca Toraldo,2* Zaira Aversa,1 Ira Smith,1 Estibaliz Castillero,1 Guillaume Renaud,3 Rizwan Qaisar,3 Lars Larsson,3 Ravi Jasuja,2 and Per-Olof Hasselgren1

1Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; 2Department of Medicine, Section of Endocrinology, Diabetes, and Nutrition, Boston Medical Center, Boston University, Boston, Massachusetts; and 3Department of Neuroscience, Clinical Neurophysiology, Uppsala University, Uppsala, Sweden

Submitted 21 November 2011; accepted in final form 21 September 2012

Alamdari N, Toraldo G, Aversa Z, Smith I, Castillero E, Renaud G, Qaisar R, Larsson L, Jasuja R, Hasselgren PO. Loss of muscle strength during sepsis is in part regulated by glucocorticoids and is associated with reduced muscle fiber stiffness. Am J Physiol Regul Integr Comp Physiol 303: R1090–R1099, 2012. First published September 26, 2012; doi:10.1152/ajpregu.00636.2011.—Sepsis is associated with impaired muscle function but the role of glucocorticoids in sepsis-induced muscle weakness is not known. We tested the role of glucocorticoids in sepsis-induced muscle weakness by treating septic rats with the glucocorticoid receptor antagonist RU38486. In addition, normal rats were treated with dexamethasone to further examine the role of glucocorticoids in the regulation of muscle strength. Sepsis was induced in rats by cecal ligation and puncture, and muscle force generation (peak twitch and tetanic tension) was determined in lower extremity muscles. In other experiments, absolute and specific force as well as stiffness (reflecting the function of actomyosin cross bridges) were determined in isolated skinned muscle fibers from control and septic rats. Sepsis and treatment with dexamethasone resulted in reduced maximal twitch and tetanic force in intact isolated extensor digitorum longus muscles. The absolute and specific maximal force in isolated muscle fibers was reduced during sepsis together with decreased fiber stiffness. These effects of sepsis were blunted (but not abolished) by RU38486. The results suggest that muscle weakness during sepsis is at least in part regulated by glucocorticoids and reflects loss of contractility at the cellular (individual muscle fiber) level. In addition, the results suggest that reduced function of the cross bridges between actin and myosin (documented as reduced muscle fiber stiffness) may be involved in sepsis-induced muscle weakness. An increased understanding of mechanisms involved in loss of muscle strength will be important for the development of new treatment strategies in patients with this debilitating consequence of sepsis.

* N. Alamdari and G. Toraldo contributed equally to the study.

Address for reprint requests and other correspondence: P.-O. Hasselgren, 330 Brookline Ave., ST 919, Boston, MA 02215 (e-mail: phasselg@bidmc.harvard.edu).

RU38486 (37) on force generation in isolated extensor digitorum longus (EDL) muscles and in isolated muscle fiber preparations from septic rats.

The experiments on individual muscle fibers were performed in muscle fibers that had been chemically “skinned.” The skinning procedure removes membranes of the sarcolemma, sarcoplasmic reticulum, and mitochondria, resulting in exposure of the contractile proteins and allowing for measurement of contractile strength at the level of myofilaments without the influence of membrane events (10). Skinned fiber preparations were used in previous reports in which conditions characterized by muscle weakness were studied, including muscle atrophy caused by hemiparesis (10), acute quadriplegic myopathy (33), aging (5, 24), endotoxemia (3, 49), and sepsis (52).

Muscle force generation is dependent on cross-bridge formation between the contractile proteins actin and myosin (14, 20, 38). Cross-bridge function can be assessed by determining muscle fiber stiffness (the relationship between change in force and change in fiber length). The stiffness of activated muscle fibers reflects the number of attached cross bridges or the compliance of individual cross bridges (12, 19, 43). Reduced muscle fiber stiffness was reported previously in different conditions characterized by muscle weakness, including hindlimb unloading (28), nebulin-related myopathy (35), and in a recently developed intensive care unit model in rats (34), but the influence of sepsis on muscle fiber stiffness has not been reported.

Results in the present study suggest that sepsis-induced muscle weakness is at least in part regulated by glucocorticoids and reflects reduced function of the cross bridges between actin and myosin (documented as reduced muscle fiber stiffness). The results are important because they expand our understanding of mechanisms behind muscle weakness during sepsis.

METHODS

Animals and experimental design. Four series of experiments were performed. In the first series of experiments, we tested the effect of sepsis on force generation in intact skeletal muscle. Sepsis was induced in male Sprague-Dawley rats (50–60 g body wt) by cecal ligation and puncture (CLP) as described in detail previously (1, 30, 45, 46, 55, 57, 59). In short, with rats under general anesthesia induced by pentobarbital (50 mg/kg administered ip), the abdomen was opened through a midline incision and the cecum was ligated below the ileocecal junction with a 3–0 silk ligature and punctured twice with an 18-gauge needle. Control rats underwent sham operation consisting of laparotomy and manipulation, but no ligation or puncture, of the cecum. Rats were resuscitated with 10 ml/100 g body wt of saline administered subcutaneously on the back at the time of sham operation or CLP to prevent hypovolemia and septic shock. Animals had free access to water, but food was withheld after the
surgical procedures to avoid the influence of differences in food intake on changes in muscle strength between sham-operated and septic rats. Sixteen hours after sham operation or CLP and with rats under pentobarbital anesthesia, the white, fast-twitch extensor digitorum longus (EDL) and the red, slow-twitch soleus muscles were harvested for determination of muscle strength and contractility. After removal of the muscles, the rats were euthanized by exsanguination. The septic model used here is clinically relevant because it resembles the situation in patients presenting with septic peritonitis caused by perforated viscus and intraabdominal abscesses. Of note, small, growing rats weighing 50–60 g were used in several previous reports to study the effects of sepsis on total and myofibrillar protein breakdown rates determined as release of tyrosine and 3-methylhistidine, respectively, from incubated EDL and soleus muscles (18, 54). Rats weighing 50–60 g have lower extremity muscles that are thin enough to allow for in vitro incubations with maintained tissue oxygenation and viability. Rats of the same size were used in the present experiments when intact muscles were studied in vitro to make comparisons with previous studies possible.

In a second series of experiments, septic rats were treated with 10 mg/kg of the glucocorticoid receptor antagonist RU38486 (37) or vehicle administered intraperitoneally 2 h before CLP as described in detail previously (1, 45). Sham-operated rats that were treated with vehicle were also included in this experiment. EDL muscles were removed 16 h after sham operation or CLP for determination of muscle strength and contractility. We found in previous studies that treatment of rats with RU38486 prevented sepsis-induced metabolic changes, including muscle proteolysis and activation of the ubiquitin-proteasome pathway (53) and the regulation of the transcription factor FOXO1 (45) and the nuclear cofactor p300 (1), but the effects of RU38486 on sepsis-induced changes in muscle strength and contractility are not known.

In a third series of experiments, rats were treated with dexamethasone (10 mg/kg) or corresponding volume of vehicle (0.25% ethanol) administered intraperitoneally as described previously (1, 30, 45, 46, 58). Rats had free access to water but food was withheld after the injections. Muscles were harvested 16 h after administration of dexamethasone or vehicle for determination of muscle strength and contractility.

In a final series of experiments, the effects of sepsis and RU38486 on single muscle fiber contractility were examined. To increase the amount of tissue for the preparation of single muscle fibers, bigger rats (male Sprague-Dawley rats weighing 300–325 g) were used in these experiments. Although it is possible that small, growing rats of the same size that were used for the study of isolated intact muscles could have been used also when individual muscle fibers were examined, we wanted to make certain that we had enough tissue available for the muscle fiber experiments and this is why we used bigger rats in these experiments. In addition, using bigger rats allowed us to make comparisons with previous experiments in which adult rats weighing ~300 g were used to study isolated muscle fibers (26). The experimental conditions were otherwise identical to those described above for induction of sepsis and treatment with RU38486 in small rats. Of note, we reported previously that the catabolic response in skeletal muscle to sepsis induced by CLP was similar in small, growing rats and in rats of the same size used in the fourth series of experiments in the present study (59). In addition, mortality rates were not different after CLP among small and big rats, 25% and 22%, respectively (59), similar to mortality rates observed 16 h after CLP in the present study (varying between 20% and 30% in the different experiments). To

Fig. 1. Effect of sepsis in rats on maximal twitch (A) and tetanic (B) force in extensor digitorum longus (EDL) muscles from sham-operated and septic (CLP) rats. The corresponding results for soleus muscles are shown in C and D. Results are means ± SE with n = 6–8 per group. *P < 0.05 vs. sham by Student’s t-test.
farther validate the use of bigger rats in the present study, we
determined mRNA levels for the ubiquitin ligase atrogin-1 and
muscle RING-finger protein-1 (MuRF1) (commonly used “molecular
markers” of muscle wasting). Atrogin-1 and MuRF1 mRNA levels
were determined by real-time PCR as described in detail previously
(1, 30, 45, 46, 58).

All animal surgeries were performed at the Beth Israel Deaconess
Medical Center (Boston, MA). Animals were treated and cared for in
accordance with the National Research Council’s Guide for the Care
and Use of Laboratory Animals. The experimental protocols were
approved by the Institutional Animal Care and Use Committee at the
Beth Israel Deaconess Medical Center (Boston, MA).

Force generation in intact muscles. Sixteen hours after sham
operation or CLP, muscles were dissected with intact tendons and
used for determination of peak twitch and tetanic tension using
methods described in detail previously (13) with minor modifications.
In short, muscles were mounted in an experimental chamber and
maintained in oxygenated Krebs-Ringer solution that was circulated
through the chamber at room temperature (20°C). The proximal
tendon was attached to a rigid post, and the distal tendon was attached
to a Kulite BG1000 transducer (Kulite Semiconductor Products,
Leonia, NJ) with 4-0 silk. The muscle was stimulated with a Grass
S48 stimulator (Quincy, MA) through two platinum field electrodes
attached to the chamber walls. The transducer output was recorded
using ASI dynamic muscle control and analysis software from Aurora
Scientific (Aurora, Canada). Twitch kinetics and amplitude (Pt) were
determined before measurement of the tetanus amplitude (Po). Force
responses were obtained by stimulating the muscle at supramaximal
voltage (2 ms pulse duration) while stretching the muscle between
responses were obtained by stimulating the muscle at supramaximal
force curves were shifted to the right at higher temperatures.

By Segal et al. (42), maximal force was generated at
/2H11011
20°C (25,
/2H11006
33–35). All bundles were cryo-protected within 1 wk after skinning by
transferring the bundles every 30 min to relaxing solution containing
increasing concentrations (0, 0.5, 1.0, 1.5, and 2.0 M) of sucrose and
subsequently frozen in liquid propane chilled with liquid nitrogen
(10). The frozen bundles were stored at –160°C until use. One day
before experimentation, a bundle was transferred to a 2.0 M sucrose
solution for 30 min, subsequently incubated in solutions with decreasing
sucrose concentration (1.5 to 0.5 M), and finally kept in a skinning
solution at –20°C.

Single muscle fiber experimental procedure. The single muscle
fiber experimental procedure was described in detail recently (23, 33,
34). In short, on the day of experiment, a 1- to 2-mm long fiber
segment was left exposed to the experimental solution between
connectors leading to a force transducer (model 400A, Aurora Scientific)
and a lever arm system (model 308B, Aurora Scientific) (32).

The apparatus was mounted on the stage of an inverted microscope
(model IX70; Olympus). While the fiber segments were in relaxing
solution, the sarcomere length was set to 2.65–2.75 µm by adjusting
the overall segment length (25). The diameter of the fiber segment
between the connectors was measured through the microscope at a
magnification of ×320 with an image analysis system before the
mechanical experiments. Fiber depth was measured by recording the
vertical displacement of the microscope nose piece while focusing
on the top and bottom surfaces of the fiber. The focusing control of
the microscope was used as a micrometer. Fiber CSA was calculated
from the diameter and depth, assuming an elliptical circumference,
and was corrected for the 20% swelling that is known to occur
during skinning (32).

Relaxing and activating solutions contained 4 mM Mg-ATP, 1 mM
free Mg²⁺, 20 mM imidazole, 7 mM EGTA, 14.5 mM creatine

\[
\text{Maximal twitch force (mN/m)²} \\
\text{Maximal tetanic force (mN/m)²}
\]

Fig. 2. Effect of sepsis on normalized maximal peak twitch (A) and
tetanic (B) force in EDL muscles from sham-operated and septic (CLP)
rats. Results are means ± SE with \( n = 6–8 \) per group. \(* P < 0.05 \) vs. sham by
Student’s t-test.
phosphate, and KCl to adjust the ionic strength to 180 mM. The pH was adjusted to 7.0. In preliminary experiments, the concentrations of free Ca\(^{2+}\) were 10^{-9} M (relaxing solution) and 10^{-6.2}, 10^{-6.0}, 10^{-5.8}, 10^{-5.5}, 10^{-5.2}, 10^{-4.9}, and 10^{-4.5} M (activating solutions), expressed as pCa (i.e., \(-\log [Ca^{2+}]\)). Maximum activation occurred at a free calcium concentration of 10^{-4.5} M, and in continued experiments, fibers were studied at 10^{-9} M and 10^{-4.5} M free Ca\(^{2+}\). Apparent stability constants for Ca\(^{2+}\)-EGTA were corrected for temperature (15°C) and ionic strength (180 mM). A computer program (9) was used to calculate the concentrations of each metal, ligand, and metal-ligand complex.

Immediately preceding each activation, the fiber was immersed at 15°C for 10–20 s in a solution with reduced Ca\(^{2+}\)-EGTA buffering capacity. This solution was identical to the relaxing solution except that the EGTA concentration was reduced to 0.5 mM, resulting in more rapid attainment of steady-state force during subsequent activation. Maximum active tension (P_o) was calculated as the difference between the total tension in the activating solution (pCa 4.5) and the resting tension measured in the same segment while in the relaxing solution. All contractile measurements were carried out at 15°C to preserve the structure and function of the muscle fibers as described in previous reports (25, 32). Contractile recordings were not accepted if sarcomere length during isometric tension development changed by more than 0.10 \(\mu\)m compared with sarcomere length while the fiber was relaxed or if force changed more than 10% during repeated activations (32). Specific tension was calculated as maximum tension (P_o) normalized to CSA.

**Stiffness.** Changes in the function of actomyosin cross bridges were monitored by determining muscle fiber stiffness as described recently (34, 35). Once steady-state isometric force was reached, small-amplitude sinusoidal changes in length (\(\Delta L: \pm 0.2\%\) of fiber length) were applied at 500 Hz at one end of the fiber (27). The resultant force response (\(\Delta F\)) was measured, and the mean of 20 consecutive readings of \(\Delta L\) and \(\Delta F\) was used to determine stiffness (\(\Delta F/\Delta L\)). The actual elastic modulus of each fiber (\(E\)) was calculated as the difference between \(E\) in activating solution and resting \(E\) measured in the same segment in the relaxing solution. \(E\) was determined by using the equation \(E = (\Delta F/\Delta L) \times (fiber\ length/CSA)\) (28).

**Statistics.** Results are reported as means ± SE. For statistical analysis, Student’s \(t\)-test was used for comparisons between two groups and analysis of variance (ANOVA) was used when results from more than two groups were compared. \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Muscle strength is reduced in septic rats.** Sixteen hours after induction of septic peritonitis by CLP in rats, absolute maximal twitch force was reduced by \(\sim 60\%\) and maximal tetanic force by \(\sim 70\%\) in EDL muscles (Fig. 1, A and B). The corresponding changes in soleus muscles were less pronounced with an \(\sim 35\%\) reduction of maximal twitch force and no changes in maximal tetanic force noted in septic rats (Fig. 1, C and D). Thus, similar to sepsis-induced metabolic changes in skeletal muscle (18, 55), the effects of sepsis on muscle strength and contractility were more pronounced in fast-twitch than in slow-twitch muscle.

---

**Fig. 3.** Effect of sepsis on the dynamics of muscle contractility. A: time to maximal twitch; B: half relaxation time after maximal twitch; C: time to maximal tetanic force; D: half relaxation time after maximal tetanic force in EDL muscles from sham-operated and septic (CLP) rats. Results are means ± SE with \(n = 6–8\) per group. *\(P < 0.05\) vs. sham by Student’s \(t\)-test.
Because the influence of sepsis on muscle function was more pronounced in EDL than in soleus muscles, EDL muscles were used for further studies in the present report. Reduced absolute muscle strength may reflect loss of muscle mass or may be the result of more specific changes affecting contractility. To address that question, we analyzed specific (normalized) twitch and tetanic forces (i.e., peak twitch and tetanic tensions normalized to muscle cross-sectional area). We found that the normalized peak twitch and tetanic forces were reduced by ~60% and 80%, respectively, in EDL muscles 16 h after CLP (Fig. 2).

Fig. 4. Effect of the glucocorticoid receptor antagonist RU38486 on maximal twitch (A) and tetanic (B) force in EDL muscles from septic (CLP) rats. Sham and one group of CLP rats were treated with vehicle and one group of septic rats (CLP + RU) was treated with RU38486 (10 mg/kg) as described in METHODS. Results are means ± SE with n = 8–10 per group. *P < 0.05 vs. sham; †P < 0.05 vs. CLP by ANOVA.

The almost identical changes in normalized and absolute maximal twitch and tetanic tension (see Fig. 2, A and B, and compare with Fig. 1, A and B) suggest that the decrease in muscle strength was not primarily caused by reduced muscle mass under the present experimental conditions.

In addition to measuring muscle twitch and tetanic force, the dynamics of muscle contractility were analyzed by measuring time to maximal twitch and half relaxation time after the establishment of maximal twitch and tetanic force. Time to maximal twitch force and the increased half relaxation time after maximal twitch suggest that muscle calcium handling may be affected by sepsis.

Glucocorticoids are involved in sepsis-induced muscle weakness.

We next tested the role of glucocorticoids in sepsis-induced muscle weakness by treating rats with the glucocorticoid receptor antagonist RU38486 (37). Treatment of septic rats with RU38486 resulted in improved maximal twitch and tetanic force (Fig. 4). Of note, although a statistically significant improvement of muscle strength was observed in septic rats treated with RU38486, muscle force was not normalized but remained significantly lower than in sham-operated rats.

Muscle strength is reduced in dexamethasone-treated rats.

To test whether glucocorticoids by themselves may influence muscle function, rats were treated with dexamethasone followed 16 h later by measurement of muscle strength (the same time interval that was used in the septic rats). Treatment with dexamethasone resulted in reduced maximal twitch and tetanic force in EDL muscles (Fig. 5). Of note, the reduction of maximal twitch and tetanic force in dexamethasone-treated rats was less pronounced than in septic rats (compare with Fig. 1, A and B).

Sepsis reduces single muscle fiber contractility. Because changes in muscle strength measured in intact muscles may reflect changes in membranes of the sarcolemma, sarcoplasmic reticulum, and mitochondria, as well as changes in the extracellular
matrix, rather than changes in the contractile proteins or the interaction between contractile proteins, we performed additional experiments using isolated, skinned muscle fibers. To increase the amount of tissue available for the preparation of single muscle fibers, larger (adult) rats were used in these experiments than in the experiments in which isolated intact muscles were studied. Although we reported previously that sepsis-induced changes in muscle protein turnover rates as well as mortality rates were similar in adult and small, growing rats (59), here we determined mRNA levels for atrogin-1 and MuRF1 in muscles from sham-operated and septic rats weighing 300–325 g to further validate the use of adult rats in the present study. Similar to several previous reports in which small, growing rats were studied (1, 30, 57), the expression of atrogin-1 and MuRF1 was substantially upregulated during sepsis in the bigger rats (Fig. 6) further supporting the notion that the catabolic response to sepsis is similar in small, growing and adult rats (although, of course, the observations do not necessarily mean that the effects of sepsis on muscle strength are the same in young and adult rats).

When isolated muscle fibers were examined, we found that the CSA measured at a fixed sarcomere length did not differ significantly between fibers from control and septic EDL muscles (1,260 ± 60 μm² in control vs. 1,430 ± 70 μm² in septic rats; NS). The absolute maximum force in isolated fibers was reduced during sepsis by 22% (Fig. 7A). Because the muscle fiber CSA was similar in fibers from septic and control rats, the reduced absolute maximum force in EDL muscle fibers reflected reduced specific force, i.e., force normalized to muscle fiber CSA (Fig. 7B).

Muscle fiber stiffness is decreased during sepsis. The unchanged muscle fiber diameters and reduced specific force in the septic EDL muscle fibers suggest that reduced muscle fiber stiffness is decreased during sepsis. The un}

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

**Fig. 6.** Effect of sepsis in adult rats on atrogin-1 (A) and muscle RING-finger protein-1 (MuRF1) (B) mRNA expression levels in EDL muscles from rats weighing 300–325 g. Muscles were studied 16 h after sham-operation or induction of sepsis by CLP. Results are means ± SE with n = 8 in each group. *P < 0.05 vs. sham by Student’s t-test.

**Fig. 7.** Effect of sepsis on single muscle fiber contractility and stiffness. Maximal absolute force (A), specific force (B), and stiffness (C) in isolated fibers from EDL muscles from sham-operated and septic (CLP) rats are shown. Fibers were prepared from 3 sham-operated and 4 septic rats. An average of 10 fibers were examined from each muscle. A total number of 30 fibers were studied in muscles from sham-operated and 37 fibers were studied in muscles from septic rats. Results are means ± SE. *P < 0.05 vs. sham by Student’s t-test.
force was not caused by loss of muscle proteins and myofiber atrophy. To explore other potential mechanisms of reduced muscle fiber force, we next determined stiffness of EDL muscle fibers from sham-operated and septic rats. Muscle fiber stiffness was decreased by ~40% in EDL muscle fibers from septic rats (Fig. 7C) suggesting that the actomyosin cross-bridge function was reduced during sepsis.

Sepsis-induced muscle fiber dysfunction may be regulated in part by glucocorticoids. To test whether glucocorticoids were involved in the sepsis-induced changes in isolated muscle fibers, an additional experiment was performed in which septic rats were treated with RU38486. The results from this experiment are shown in Fig. 8. Of note, absolute maximal and specific force and stiffness in fibers from sham-operated and septic rats were almost identical to the corresponding values observed in our first experiment, suggesting that the measurements in isolated muscle fibers as well as the effects of sepsis are highly reproducible (compare with results in Fig. 7). Treatment of septic rats with RU38486 improved, but did not normalize, absolute maximal and specific force in isolated muscle fibers (Fig. 8, A and B). The differences between the CLP+RU and sham groups in absolute maximal and specific force were not statistically significant. Treatment of septic rats with RU38486 resulted in a statistically significant improvement of specific force (Fig. 8B), whereas the differences between the CLP and CLP+RU groups were not statistically significant for absolute maximal force and fiber stiffness (Fig. 8, A and C). Taken together, the results in Fig. 8 suggest that glucocorticoids may be involved in the sepsis-induced reduction of maximal and specific force and stiffness in isolated muscle fibers, although other factors probably play an important role as well.

DISCUSSION

The present study suggests that sepsis results in an early and pronounced loss of muscle strength and that this effect of sepsis is at least in part regulated by glucocorticoids. Our observations also indicate that muscle weakness during sepsis does not primarily reflect loss of muscle mass but may be caused by intrinsic molecular changes in the contractile apparatus, in particular, changes in the actomyosin cross-bridge function resulting in reduced fiber stiffness. The results are important from a clinical standpoint because an increased understanding of mechanisms causing muscle weakness during sepsis may help develop new treatment strategies to prevent this debilitating consequence of sepsis.

The present observation of sepsis-induced muscle weakness is in line with several previous reports demonstrating loss of muscle strength during sepsis and endotoxemia (8, 29, 31, 39). In particular, a number of important studies by Supinski and Callahan have provided detailed information about muscle weakness in critical illness and mechanisms involved in the regulation of muscle strength during sepsis and endotoxemia (3, 47–52) as was also reviewed recently (4). The present study adds novel information expanding some of the previous reports of sepsis-induced muscle weakness. Thus the role of glucocorticoids in sepsis-induced muscle weakness has not been reported previously. Although the influence of endotoxin on limb muscle strength has been reported (49, 50), most previous reports described the effects of sepsis and endotoxemia on respiratory muscle function. Reduced muscle fiber stiffness during sepsis is an additional novel observation. Interestingly, based on observations in isolated skinned fibers from diaphragm and lower extremity muscles, Supinski et al. (49) suggested already more than 10 years ago that endotoxin-induced muscle weakness may reflect modifications of the contractile apparatus that impair myosin-actin interactions, although muscle fiber stiffness was not determined at that point.

Fig. 8. Effect of RU38486 on single muscle fiber contractility and stiffness in septic rats. Maximal absolute force (A), specific force (B), and stiffness in isolated fibers (C) from extensor digitorum muscles from sham-operated and septic (CLP) rats treated with vehicle and septic rats treated with RU38486 (CLP + RU) as described in METHODS. Fibers were prepared from 3 sham-operated, 3 septic, and 4 septic rats treated with RU38486. A total number of 30 fibers were analyzed in the sham group, 32 fibers in the CLP group, and 40 fibers in the CLP + RU group. Results are means ± SE. *P < 0.05 vs. sham; †P < 0.05 vs. CLP by ANOVA.
point. Mechanisms of sepsis-induced muscle weakness were discussed in a recent comprehensive review (4).

Although we found in several previous reports that the same experimental septic model as used in the present study resulted in a rapid increase in ubiquitin-proteasome-dependent degradation of myofibrillar proteins (18, 53–55, 57), the present results suggest that decreased muscle strength during sepsis does not reflect loss of muscle mass (as suggested by decreased normalized peak twitch and tetanic strength in intact muscles and decreased specific force in isolated muscle fibers), at least not during the acute phase of sepsis. Of note, the influence of sepsis and endotoxemia on specific (normalized) muscle force varied in previous reports. For example, in a model of chronic septic peritonitis, normalized muscle force was actually increased in the presence of reduced absolute muscle strength suggesting that the relative loss of muscle mass was more pronounced than the decrease in absolute muscle strength during the protracted septic course (39). Indeed, the cross-sectional area of fast-twitch muscle fibers was reduced by ~40% in that study, whereas absolute EDL muscle force was reduced by ~20%. In another report, normalized muscle force was unchanged during sepsis induced by intraperitoneal injection of zymosan, suggesting that the decrease in absolute force in that study was accompanied by a similar loss of muscle mass (31). In several previous reports from the laboratory of Supinski and Callahan, normalized muscle force was reduced during sepsis and endotoxemia (4, 47, 48, 50–52), suggesting that in those experiments, the decrease in muscle strength was not primarily caused by loss of muscle mass (similar to the observations in the current study). Thus previous reports and the present study suggest that reduced muscle strength during sepsis and endotoxemia is not caused by loss of muscle mass alone and that the role of muscle atrophy for the development of muscle weakness may depend on the experimental model and duration of the septic course.

In a recent study examining an intensive care unit model in rats consisting of general anesthesia, neuromuscular blockade, and mechanical ventilation maintained for up to 14 days, reduced muscle fiber stiffness was primarily due to a decreased number of force-generating cross bridges rather than reduced stiffness of each cross bridge (34). Previous studies suggest that different conditions may reduce muscle fiber stiffness by different mechanisms (28, 34, 35). It will be important in future studies to determine the mechanism of reduced muscle fiber stiffness in septic muscle.

In the present study, we treated septic rats with RU38486 to test the role of glucocorticoids in sepsis-induced muscle weakness. Although this treatment resulted in improved muscle force during sepsis, both in intact muscles and in isolated muscle fibers, the muscle strength did not return to control levels but remained reduced. In previous studies, the increase in muscle proteolysis and changes in the expression of muscle wasting-associated transcription factors and nuclear cofactors during sepsis were more extensively prevented by RU38486 (1, 36, 45, 53, 57), supporting the concept that reduced muscle strength during early sepsis does not primarily reflect loss of muscle mass. Of note, we recently found that muscle calcium homeostasis, in particular store-operated calcium entry, is regulated by glucocorticoids (21), and it is possible that the effect of RU38486 observed in the present study reflected improved calcium homeostasis, although additional experiments are needed to test that hypothesis.

The present observations of only partial improvement of muscle strength in RU38486-treated rats and less pronounced muscle weakness in dexamethasone-treated rats than in septic rats indicate that other mediators than glucocorticoids may be involved in sepsis-induced muscle weakness. Previous studies suggest that proinflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor, may mediate some of the effects of sepsis and endotoxemia on muscle strength (7, 15, 29, 56), and it is likely that these factors played a role in the present experiments.

The present findings are important because they provide novel insight into the potential role of glucocorticoids in sepsis-induced muscle weakness and contribute to an increased understanding of cellular mechanisms of muscle dysfunction during sepsis. In particular, results suggest that muscle weakness during sepsis may in part be caused by reduced muscle fiber stiffness, at least during the early (acute) phase of sepsis.

**Perspectives and Significance**

Muscle weakness is a serious consequence of sepsis and other critical illness. Understanding mechanisms of sepsis-induced muscle weakness may help develop new treatment strategies to prevent loss of muscle strength in septic patients. Reducing the negative impact of sepsis on muscle strength will decrease the risk for respiratory failure, reduce the length of intensive care unit and hospital stay, and improve the rehabilitation of septic patients.

**GRANTS**

The study was supported in part by National Institutes of Health Grant R01 DK-37908 (to P.-O. Hasselgren), the Swedish Research Council (8651) (to L. Larsson), and the European Commission (MyoAge, EC Fp7 CT-223756 and COST CM1001) (to L. Larsson). Z. Aversa was supported in part by the Department of Clinical Medicine, Sapienza University of Rome, Rome, Italy. E. Castillero was supported in part by Gobierno Vasco, Spain (BF2010-240).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**

MUSCLE WEAKNESS IN SEPSIS

Larsson L, Li X, Frontera WR.

R1098

Larsson L, Li X, Edstrom L, Eriksson LI, Zackrisson H, Argentini C, R1098 MUSCLE WEAKNESS IN SEPSIS

Martyn DA, Smith L, Kreutziger KL, Xu S, Yu LC, Regnier M.

22.

Li X, Larsson L.

14.

Gordon AM, Homsher E, Regnier M.

17.

Hasselgren PO, James JH, Benson DW, Hall-Angerås M, Angerås U, Galler S, Hilber K.

11.

Higuchi H, Yanagida T, Goldman YE.

5.

El-Dwairi QA, Comtois A, Guo Y, Hussain SNA.

7.


5.

Chand S, Seto JT, Houwelting PJ, Yang N, North KN, Head SL.


28.


26.


23.

El-Dwairi QA, Comtois A, Guo Y, Hussain SNA. Endotoxin-induced skeletal muscle contractile dysfunction: contribution of nitric oxide syn-

25.

Eucken M, Arany ZP, Alamdari N, Aversa Z, Gonzella O, O’Neal P, Smith I, Tizio S, Hasselgren PO. Sepsis and glucocorticoids down-

21.

Fabiato A. Computer programs for calculating total from specified free or free from specified ionic concentrations in aqueous solutions con-

13.


16.

Hasselgren PO, James JH, Benson DW, Hall-Angerås M, Angerås U, Galler S, Hilber K.

11.

Higuchi H, Yanagida T, Goldman YE.

5.

El-Dwairi QA, Comtois A, Guo Y, Hussain SNA.

7.

Fabiato A. Computer programs for calculating total from specified free or free from specified ionic concentrations in aqueous solutions con-

13.


16.

Hasselgren PO, James JH, Benson DW, Hall-Angerås M, Angerås U, Galler S, Hilber K.

11.

Higuchi H, Yanagida T, Goldman YE.

5.

El-Dwairi QA, Comtois A, Guo Y, Hussain SNA.

7.

Fabiato A. Computer programs for calculating total from specified free or free from specified ionic concentrations in aqueous solutions con-

13.


16.

Hasselgren PO, James JH, Benson DW, Hall-Angerås M, Angerås U, Galler S, Hilber K.

11.

Higuchi H, Yanagida T, Goldman YE.

5.

El-Dwairi QA, Comtois A, Guo Y, Hussain SNA.

7.

Fabiato A. Computer programs for calculating total from specified free or free from specified ionic concentrations in aqueous solutions con-

13.


16.

Hasselgren PO, James JH, Benson DW, Hall-Angerås M, Angerås U, Galler S, Hilber K.

11.

Higuchi H, Yanagida T, Goldman YE.

5.

El-Dwairi QA, Comtois A, Guo Y, Hussain SNA.

7.

Fabiato A. Computer programs for calculating total from specified free or free from specified ionic concentrations in aqueous solutions con-