OPEN

Impact of Very Early Physical Therapy During Septic Shock on Skeletal Muscle: A Randomized Controlled Trial

Cheryl E. Hickmann, PT, PhD[1]; Diego Castanares-Zapatero, MD, PhD[1]; Louise Deldicque, PhD[2]; Peter Van den Bergh, MD, PhD[3]; Gilles Caty, MD, PhD[4]; Annie Robert, PhD[5]; Jean Roeseler, PT, PhD[1]; Marc Francaux, PhD[2]; Pierre-François Laterre, MD[1]

Objectives: As the catabolic state induced by septic shock together with the physical inactivity of patients lead to the rapid loss of muscle mass and impaired function, the purpose of this study was to test whether an early physical therapy during the onset of septic shock regulates catabolic signals and preserves skeletal muscle mass.

Design: Randomized controlled trial.

Setting: Tertiary mixed ICU.

Patients: Adult patients admitted for septic shock within the first 72 hours.

Interventions: Patients were assigned randomly into two groups. The control group benefited from manual mobilization once a day. The intervention group had twice daily sessions of both manual mobilization and 30-minute passive/active cycling therapy.

Measurements and Main Results: Skeletal muscle biopsies and electrophysiology testing were performed at day 1 and day 7. Muscle biopsies were analyzed for histology and molecular components of signaling pathways regulating protein synthesis and degradation as well as inflammation markers. Hemodynamic values and patient perception were collected during each session. Twenty-one patients were included. Three died before the second muscle biopsy. Ten patients in the control and eight in the intervention group were analyzed. Markers of the catabolic ubiquitin-proteasome pathway, muscle atrophy F-box and muscle ring finger-1 messenger RNA, were reduced at day 7 only in the intervention group, but without difference between groups (muscle atrophy F-box: –7.3% ± 138.4% in control vs –56.4% ± 37.4% in intervention group; $p = 0.23$ and muscle ring finger-1: –30.8% ± 66.9% in control vs –62.7% ± 45.5% in intervention group; $p = 0.15$). Muscle fiber cross-sectional area (μm²) was preserved by exercise (–25.8% ± 21.6% in control vs 12.4% ± 22.5% in intervention group; $p = 0.005$). Molecular regulations suggest that the excessive activation of autophagy due to septic shock was lower in the intervention group, without being suppressed. Markers of anabolism and inflammation were not modified by the intervention, which was well tolerated by the patients.

Conclusions: Early physical therapy during the first week of septic shock is safe and preserves muscle fiber cross-sectional area. (*Crit Care Med* 2018; 46:1436–1443)

Key Words: autophagy; catabolism; critically ill; early mobilization; muscle atrophy; septic shock

[1]Department of Critical Care Medicine, Saint Luc University hospital, Université catholique de Louvain (UCL), Brussels, Belgium.

[2]Institute of Neuroscience, Pôle Cellulaire et moléculaire, Université catholique de Louvain (UCL), Louvain-la-Neuve 1348, Belgium.

[3]Neuromuscular Reference Centre, Pôle Cellulaire et moléculaire, Saint Luc University hospital, Université catholique de Louvain (UCL), Brussels, Belgium.

[4]Department of Physical Medicine and Rehabilitation, Saint Luc University hospital, Université catholique de Louvain (UCL), Brussels, Belgium.

[5]Institut de Recherche Expérimentale et Clinique, Pôle Epidémiologie et Biostatistique, Université catholique de Louvain (UCL), Brussels, Belgium.

This work was performed at ICU, Saint Luc university hospital, Université catholique de Louvain (UCL), Brussels, Belgium.

Clinical trial registered with www.clinicaltrials.gov (NCT01787045).

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccmjournal).

Supported, in part, by funds from the "Department of Critical Care Medicine of Saint Luc University Hospital." During study inclusions, the ICU received a lending from RECK-Technik GmbH & Co. (KG, 88422 Betzenweiler, Germany) of the motorized cycling device MOTOmed Viva2 with MOTOmed sam2 training analysis program.

Drs. Hickman and Laterre disclosed that, during study inclusions, the ICU received a lending from RECK-Technik GmbH & Co. (KG, 88422 Betzenweiler, Germany) of the motorized cycling device MOTOmed Viva2 with MOTOmed sam2 training analysis program (the company RECK-Technik GmbH & Co had no role in the study design, conduction of the study, analyses, and article preparation). The remaining authors have disclosed that they do not have any potential conflicts of interest.

Address requests for reprints to: Pierre-François Laterre, MD, ICU, Department of Critical Care Medicine, Saint Luc University Hospital, Université catholique de Louvain (UCL), Avenue Hippocrate 10, 1200 Brussels, Belgium. E-mail: pierre-francois.laterre@uclouvain.be

DOI: 10.1097/CCM.0000000000003263

Despite an improvement in outcome after critical illness, survivors frequently undergo long-term impairment in physical function, impacting their quality of life (1). Sepsis, systemic inflammation (2), hyperglycemia (3), inadequate nutritional delivery/absorption (4), prolonged/deep sedation, and immobilization (5) are factors contributing to the development of ICU-acquired weakness (ICUAW).

ICUAW is considered as an organ failure and is associated with prolonged mechanical ventilation, higher length of stay, and mortality (6, 7). It includes disturbances of peripheral nerves, membrane inexcitability, and accelerated skeletal muscle atrophy (8). During sepsis, electrophysiology abnormalities have been reported within the first days of ICU stay (9, 10). In this population, the unbalanced protein turnover is primarily attributed to an increased protein breakdown rather than a decrease in protein synthesis (11). Muscle proteins regulation is regulated by several anabolic (Akt-mammalian target of rapamycin [mTOR]) and catabolic pathways, including autophagy along with proteolytic lysosomal pathways. Dysregulation of those processes may promote the wasting of proteins (12).

As no pharmacologic treatment exists to restrict skeletal muscle wasting and neuromuscular dysfunction, a preventive approach is the only current treatment. Interventions include a prompt treatment of sepsis and a reduction of immobilization as soon as possible (13). Considering the rapid progression of ICUAW, early mobilization/physical therapy should be initiated during the first days after ICU admission.

Providing early mobilization/physical therapy during critical illness has been demonstrated to be a safe approach limiting bed rest–induced morbidity (14, 15). It could represent a potential treatment to counteract sepsis-induced catabolism (16). Since sepsis is consistently associated with inflammation leading to multiple organ failure, septic patients frequently experienced ICUAW (17). Nevertheless, such interventions are often delayed as no evidence supports their safety and benefits in this high-risk population.

We hypothesized that very early physical therapy at the onset of septic shock may preserve skeletal muscle mass, through catabolic/synthesis signaling modifications.

METHODS

Participants

The study was a randomized controlled trial performed in a tertiary 14-bed mixed ICU at Saint-Luc University Hospital in Brussels. The local Ethics Committee approved the study protocol (B403201214359), and consent was signed by the subjects or next of kin.

Adults with septic shock were included within the 72 hours after ICU admission and randomized in two groups assigned in a 1:1 ratio. Exclusion criteria were preexisting cognitive abnormalities, malnutrition or cachexia, inability to walk independently, leg amputation, fractures, ongoing chemotherapy, long-term corticoid treatment, cardiorespiratory arrest, expected ICU stay less than 7 days, therapy withdrawal, imminent death, and consent refusal. Additionally, as skeletal muscle wasting is more pronounced during the first days of illness (18), patients with hospital stay greater than 5 days were not eligible.

Intervention

To promote early physiotherapy and routine chair transfer, sedation was limited in order to keep patients calm and comfortable whenever possible and was combined with adequate analgesia, as previously described (19, 20). The control group underwent a daily physiotherapy session through manual passive/active limbs mobilization (5/7 d). The intervention group had two physiotherapy sessions per day (7/7 d) including 30 minutes (1 hr/d) of continuous passive/active leg chair/bed cycling followed by manual passive/active limbs mobilization (for an explicative video of interventions, see **Supplemental Video File**, Supplemental Digital Content 1, http://links.lww.com/CCM/D818; **legend**, Supplemental Digital Content 2, http://links.lww.com/CCM/D819). Physiotherapy sessions started at the inclusion day, if inclusion occurred before 1.00 PM, otherwise the consecutive day. Passive/active activities were performed according to the patient capacity. Cycling therapy power output was recorded using MOTOmed-Sam2 software on motorized cycling devices, MOTOmed Letto2 (bed-position) (RECK-Technik GmbH&Co. KG, Betzenweiler, Germany) and Viva2 (chair-position) (RECK-Technik GmbH&Co. KG).

Outcomes

Primary outcome was the regulation of protein degradation/synthesis pathways during the first week following the onset of septic shock.

Secondary outcomes were preservation of the muscle fiber cross-sectional area (CSA), presence of exercise-induced muscle inflammation, restoration of neuromuscular function by measuring electrophysiology values and muscle strength, safety and tolerance of the intervention by monitoring hemodynamic/respiratory values, safety events, and patient perception.

Data Collection

At the study inclusion day (day 1) and after 7 ± 1 day (day 7), microbiopsies were performed in vastus lateralis (**Supplemental Fig. 1**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820). The following analyses were performed in the muscle samples in a blinded procedure: muscle fiber CSA by adenosine triphosphatase stain (pH 4.50) and assessment of signaling pathway intermediates by immunohistochemistry, immunoblotting, and quantitative real-time polymerase chain reaction (**Supplemental Table 1**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820). Individual values from muscle biochemical markers were compared with values of four healthy subject samples in postprandial state, simultaneously analyzed and obtained from another study (21). Data of these healthy subjects were used to define a physiologic baseline.

A blinded physician performed electrophysiologic tests at day 1 + 1 and day 7 ± 1, including direct muscle stimulation. If patients were alert, a blinded physiotherapist measured muscle strength by using Medical Research Council (MRC) sum score (22) at day 1 + 1 and day 7 ± 1 (for detailed methods, see

Supplemental Digital Content 3, http://links.lww.com/CCM/D820). Due to the intervention nature, patients and physiotherapists were not blinded to group allocation.

In order to ensure a similar nutritional support between groups, indirect calorimetry was carried during the first week. Energy and protein intakes, insulin dose, glycemia, and nitrogen balance were recorded daily. Predefined safety events (23) and hemodynamic/respiratory monitoring values were also recorded. Exertion and enjoyment were evaluated from communicative patients with a 10-point score (19).

Statistical Analyses

Analyses were conducted using SPSS software (IBM, Released 2011. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp). Details for sample size calculation and analysis of the results are given in Supplemental Digital Content 3 (http://links.lww.com/CCM/D820).

RESULTS

Patient Characteristics

Strict inclusion criteria, especially the short previous hospital stay limited the enrollment, allowing to include 21 patients over the 2-year period (**Supplemental Fig. 2**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820). Ten patients were randomized in the control group and nine in the intervention group as two died before group allocation. Day 1/day 7 analyses were possible for 18 patients as one died before day 7. Main characteristics of patients are presented in **Table 1** and **Supplemental Table 2** (Supplemental Digital Content 3, http://links.lww.com/CCM/D820).

Nutritional Delivery

Indirect calorimetry was performed during the first week for 12 patients as the high F_{IO_2} impeded measurement in the remaining patients, in which Harris-Benedict prediction was applied (24). The amount of nutrition effectively delivered was similar in both groups (**Supplemental Table 3**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820). Insulin doses and glycemia were similar in both groups at the time of muscle biopsy.

Early Mobilization

The first mobilization session was performed 46 ± 25 hours after ICU admission in the control group and 28 ± 9 hours in intervention group ($p = 0.05$). During the first week, patients in the control and intervention groups performed respectively a total of 85 and 163 activities ($p = 0.01$) including 36 and 29 chair sitting ($p = 0.75$), 49 and 65 manual mobilization ($p = 0.14$). Manual mobilization sessions were actively performed in 61% of control group and 59% of intervention group ($p = 0.93$) (**Fig. 1**). Only for the intervention group, 69 continuous 30-minute cycling sessions were performed and actively achieved during 59% of sessions by seven of nine patients with a mean power of 3 ± 2 Watts.

In general, activities were well tolerated by the patients. Seven percent of the cycling sessions (5/69) were prematurely stopped, due to a request of the patients on three occasions, one agitation episode and one reversible hypotension. The latter was the sole safety event, representing 0.4% of total activities.

Patient perception was obtained from eight patients in the control group (24 sessions) and seven in the intervention group (29 sessions). Exertion was similar in both groups (control group: 6 ± 4 and intervention group: 5 ± 2; $p = 0.43$) as well as enjoyment (control group: 8 ± 3 and intervention group: 7 ± 1; $p = 0.25$).

Before ICU discharge, most patients were able to be transferred to a chair and to walk with assistance of physiotherapists (control group: 83% and intervention group: 100%).

Primary Outcome

As previously reported by Klaude et al (11), our baseline measures confirmed a clear increase of both major catabolic mechanisms, namely the ubiquitin-proteasome pathway (UPP) and autophagy, together with unchanged anabolic signals observed by measuring key elements of the Akt-mTOR pathway (**Supplemental Fig. 3**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820).

UPP. The dominant catabolic system UPP was studied by investigating the transcription factors and the muscle ubiquitin ligases (E3-ligases) muscle atrophy F-box (MARbx) and muscle ring finger-1 (MURF-1) responsible for the increased protein degradation (25). The phosphorylation state of central catabolic transcription factors FoxO1 and FoxO3a remained unchanged after 7 days. The expression of MAFbx and MURF-1 did not showed statistically significant difference in both groups (MAFbx: –7.3% ± 138.4% in control group vs –56.4% ± 37.4% in intervention group; $p = 0.23$ and MURF-1: –30.8% ± 66.9% in control group vs –62.7% ± 45.5% in intervention group; $p = 0.15$). Nevertheless, both ligases expression tended to be lower at day 7 in the intervention group (**Supplemental Figs. 4–6**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820).

Autophagy-Lysosomal System. Autophagy involves a complex chain of interconnected biochemical signals aiming at delivering intracellular substrates to lysosomes (12, 26). Its regulation was studied through the phosphorylation of a central key protein controlling its activation (Unc-51 like kinase [ULK] 1) and the expression of proteins involved in autophagosome formation (microtubule-associated protein 1 light chain 3 beta [LC3b], GabarapL1). Besides, proteins targeting the delivery of organelles to lysosome (p62, Bnip3) and catabolic enzymes present in lysosomes (Cathepsin-L) were investigated. The phosphorylation of ULK1, on its autophagy inhibitory site (ULK1 Ser^{757}), was decreased in the control group (–16% ± 33%) and increased in the intervention group (30% ± 59%; $p = 0.01$), whereas its activatory site (ULK1 Ser^{317}) was increased only in the control group (control group: 311% ± 703% vs intervention group: 20% ± 148%; $p = 0.03$). Other markers followed the same trend: LC3b messenger RNA (mRNA) (control group: 5% ± 47% vs intervention group: –21% ± 18%; $p = 0.16$), Bnip3 mRNA (control group: 27% ± 198% vs intervention group: –59% ± 23%; $p = 0.003$), and GabarapL1 mRNA (control group: 73% ± 174% vs intervention group:

TABLE 1. Clinical and Demographic Characteristics

Variables	Control Group, *n* = 10	Intervention Group, *n* = 9	*p*
Age (yr), mean ± SD	57 ± 20	59 ± 19	0.81
Male, % (*n*)	60 (6)	56 (5)	1.00
Body mass index (kg/m^2), mean ± SD	27 ± 5	29 ± 7	0.44
Sequential Organ Failure Assessment score, mean ± SD	8 ± 3	10 ± 4	0.27
Acute Physiology and Chronic Health Evaluation II score, mean ± SD	17 ± 7	20 ± 6	0.43
28-d mortality, % (*n*)	40 (4)	22 (2)	0.63
ICU length of stay (d), median (IQR)	7.3 (6.8–15.3)	6.7 (4.8–21.0)	0.27
ICU stay at inclusion (hr), mean ± SD	23 ± 8	35 ± 22	0.11
Hospital stay at ICU admission (d)	2.0 ± 1.6	2.7 ± 1.9	0.42
MV, % (*n*)	100 (10)	89 (8)	0.47
MV duration (d), median (IQR)	4.7 (1.9–10.8)	5.5 (1.8–16.9)	0.99
Admission Pao_2/Fio_2, mean ± SD	144 ± 63	97 ± 37	0.06
Extracorporeal membrane oxygenation	0 (0)	11 (1)	1.00
Renal replacement therapy, % (*n*)	40 (4)	22 (2)	0.63
Vasoactive drug use, % (*n*)	100 (10)	100 (9)	1.00
Sedative drug use, % (*n*)	100 (10)	89 (8)	0.47
Paralyzing agents, % (*n*)	0 (0)	11 (1)	0.47
Richmond Agitation-Sedation Scale, median (IQR)	−1 (0 to −2)	0 (0 to −3)	0.99
Urgency surgery, % (*n*)	40 (4)	56 (5)	0.66

IQR = interquartile range, MV = mechanical ventilation.

−16% ± 85%; $p = 0.09$). Cathepsin-L and p62 mRNA, LC3bII/I ratio, and p62 protein levels remained unchanged (Supplemental Figs. 4–6, Supplemental Digital Content 3, http://links.lww.com/CCM/D820).

Autophagy markers were also assessed by double immunologic staining of the colocalization of the protein p62 with LC3b and lysosomal-associated membrane protein (LAMP) 2. LAMP2/p62 colocalization was decreased at day 7 in intervention group and increased in control group ($p = 0.007$) (**Fig. 2**, ***A*** and ***C***). No difference was found for LC3b/p62 (**Fig. 2**, ***B*** and *C*).

These results together suggest that autophagy is better controlled after 7 days of intervention.

Anabolic Akt-mTOR Pathway. Regarding the central anabolic pathway Akt-mTOR, the phosphorylation of its upstream activator Akt(Ser^{473}) was increased at day 7 only in the intervention group ($p = 0.04$) (Supplemental Figs. 4 and 6, Supplemental Digital Content 3, http://links.lww.com/CCM/D820). However, the phosphorylation of both downstream proteins of mTOR, 4E-BP1(Thr$^{37/46}$), and S6K(Thr^{389}) remained unchanged. This indicates that protein synthesis was not modified. The expression of the mTOR inhibitor and autophagy activator, regulated in development and DNA damage responses 1 (REDD1) (27) increased in the control group (33% ± 57%) and decreased (−10% ± 80%) in the intervention group ($p = 0.05$).

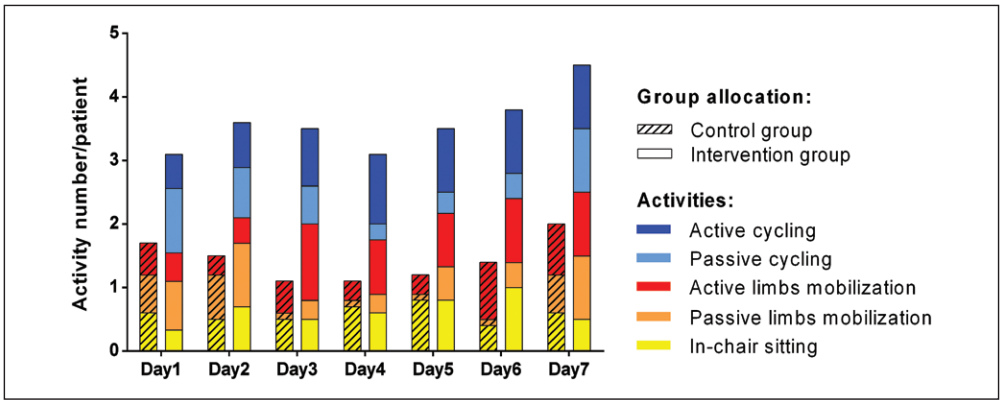

Figure 1. Amount of mobility activities performed per patient during the first week.

Secondary Outcomes

Muscle Fiber CSA. Structural analyses were performed in 17 patients, as the quality of one muscle sample in the control group was unsatisfactory.

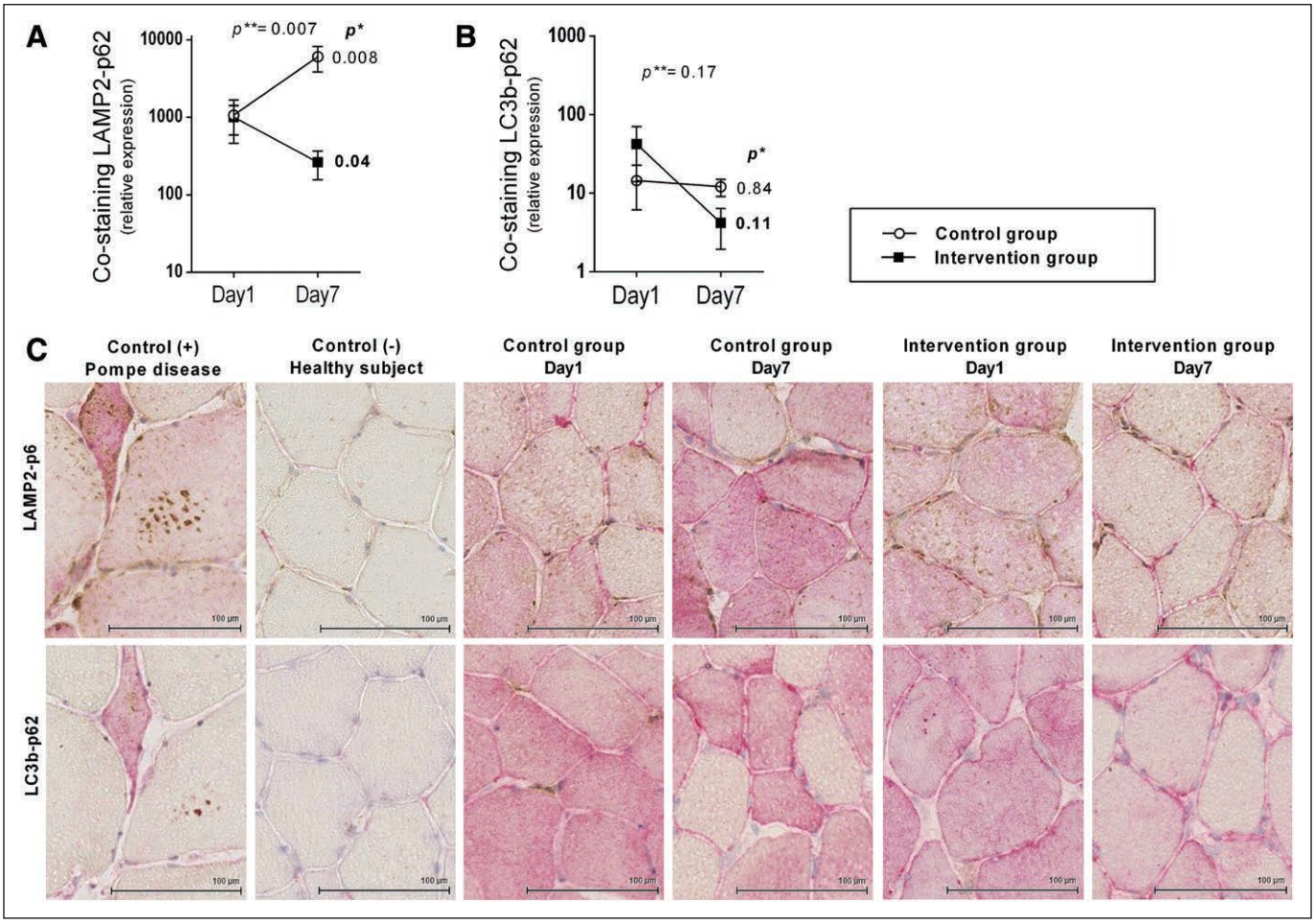

Figure 2. A, Quantitative analysis of double immunologic stain LAMP2-p62 by groups. **B**, Quantitative analysis of double immunologic stain LC3b-p62 by groups. Double immunologic stain. p^* represents p values from difference between day 1 and day 7 for each group; p^{**} represents p values from difference between changes of control and intervention groups. **C**, Representative images of double immunological stain. Pompe disease sample was used as positive control of elevated autophagy. LAMP2 = lysosomal-associated membrane protein 2, LC3b = microtubule-associated protein 1 light chain 3 beta, p62 = sequestosome 1. LAMP2 or LC3b positive areas are *brown* and p62 positive areas are *red*.

We found that muscle fiber CSA was preserved by the intervention between day 1 and day 7 in each type of fibers (**Fig. 3** and **Table 2**).

Furthermore, muscle fiber CSA changes were positively correlated with the amount of daily activities ($r = 0.64$; $p = 0.006$) (**Supplemental Fig. 7**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820). No correlation was detected between muscle fiber CSA changes and nitrogen balance, the amount of energy or protein intake.

Inflammation and Other Markers. Skeletal muscle mRNA expression of both proinflammatory, antiinflammatory cytokines and oxidative stress (nitrogene oxides) or leucocytes infiltration markers (CD68, CD64) were not modified by intervention (**Supplemental Fig. 8**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820). Similarly, phosphorylations of AMP-activated kinase α and p38, as well as the mRNA levels of myostatin, were unchanged. Caspase3 expression was similarly increased in both groups (Supplemental Figs. 5 and 6, Supplemental Digital Content 3, http://links.lww.com/CCM/D820).

Electrophysiology Evaluation. Electrophysiology evaluation was performed in 13 of 19 patients on day 1 (**Supplemental Table 4**, Supplemental Digital Content 3, http://links.lww.com/CCM/D820). Electromyography abnormalities (8) were present at day 1 in 10 of 13 patients (77%). Nerve conduction for both sensory and motor components were abnormal in eight of 13 patients (62%) and spontaneous activity was found in one of 13 patient (8%). Direct muscle stimulation was achieved in 12 patients. Abnormal values for direct muscle compound motor action potential amplitude (< 3 mV), suggesting muscle membrane inexcitability (28, 29), were observed in nine of 12 patients (75%). Since only a few patients could be reassessed at day 7 (five in the control group and four in the intervention group), comparison between groups was not performed due to the small sample size.

Muscle Strength

On account of the lack of consciousness/cooperation or unavailability of blinded evaluator, data from five patients have been collected at day 1 and day 7, three from the control group (mean at day 1: 54 ± 5 and day 7: 53 ± 3) and two from the intervention group (60, 58 [day 1] and 60, 59 [day 7]). Paucity of data did not allow any comparison between the two time points by groups.

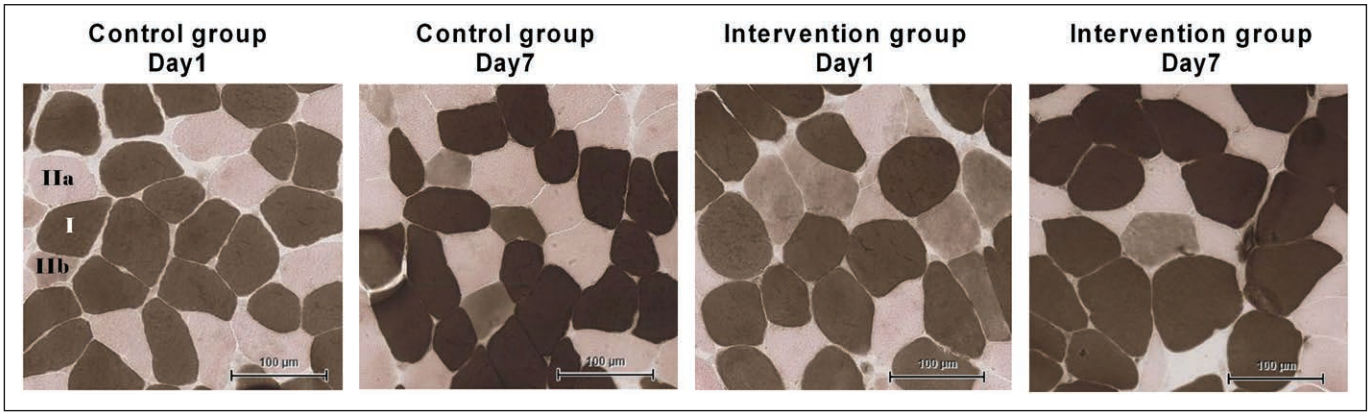

Figure 3. Muscle fiber cross-sectional area changes by group. Skeletal muscle sections stained with adenosine triphosphatase pH 4.50; *black* fibers correspond to type-I fibers; *gray* fibers are type-IIb fibers and; *pink* fibers correspond to type-IIa.

TABLE 2. Changes in Cross-Sectional Area by Groups

	Control Group ($n = 9$), Mean ± SD		Intervention Group ($n = 8$), Mean ± SD		
Fiber Type	Day 1	Day 7	Day 1	Day 7	p^b
All fibers types (μm^2)	3,603 ± 1,284	2,629 ± 1,174[a]	3,448 ± 1,993	3,770 ± 1,473	0.01
Type I fibers (μm^2)	4,236 ± 1,379	3,135 ± 1,103[a]	4,250 ± 1,977	4,678 ± 1,189	0.02
Type-IIa fibers (μm^2)	3,949 ± 1,447	2,744 ± 1,260[a]	2,574 ± 856	2,920 ± 745	0.003
Type-IIb fibers (μm^2)	2,624 ± 1,243	2,006 ± 1,286[a]	2,082 ± 1,083	2,576 ± 948	0.04

[a]Different than day 1 ($p < 0.05$).

[b]p of the difference between groups changes, no differences were detected between groups at day 1 in any fibers type.

DISCUSSION

In this translational research, the achievement of an intensive physiotherapy including cycling therapy during the first week of septic shock preserved muscle fiber CSA without reduction of atrogens regulating the UPP. However, based on our results, this preservation of fiber CSA seems to be related to a better regulation, but not suppression, of skeletal muscle autophagy markers. This intervention did not enhance septic shock–induced inflammation. No safety events were observed, with exception of one reversible hypotension.

A rising interest on early mobilization has been observed over the last years. Most studies promoting this approach have shown an improvement of the outcome when interventions begin in the early course of illness (20, 30). However, most severe patients are often excluded from trials due to instability or multiple supports. Yet, these patients and particularly those suffering from sepsis are more susceptible to precociously develop ICUAW (31) with pronounced skeletal muscle catabolism (11). In this report, we showed for the first time that this approach enables to limit the skeletal muscle atrophy when intervention is applied during the early phase of septic shock.

Baseline measurements evidenced a rapid increase of inflammatory and catabolic markers in skeletal muscle together with a high frequency of inexcitability of muscle membrane. That confirms the rapid involvement of nerves and muscle membrane previously observed at 72 hours of sepsis or multiple organ failure (9, 10).

The majority of patients in both groups performed manual active mobilization although only the intervention group received cycling sessions. Since the transcription of proinflammatory cytokines in muscles was not higher in the intervention group than in the control group, we suggested that sepsis-induced muscle inflammation was not enhanced by cycling exercise. This contrasts with Callahan and Supinski (32), who suggested that the exercise in patients with damaged muscles could delay the recovery or propagate muscle inflammation and injury (33).

A muscle fiber CSA loss of 17.5% was observed in critically ill patients of whom 50 percent had sepsis (34). In patients with septic shock, a loss of 16–20% in muscle volume of the quadriceps at 7 days has been previously evidenced by tomography (35). In the former report, the use of neuromuscular electrical stimulation was not able to prevent muscle atrophy. In contrast, a recent trial on comatose patients reports that a loss of 16% and 24% (type I and II muscle fiber CSA, respectively) can be prevented by neuromuscular electrical stimulation (36). Differences can be explained by the unexcitable nature of skeletal muscle during sepsis (37), also confirmed in our work. To our best knowledge, no data on muscle fiber CSA loss and on its prevention exist during the early course of septic shock. In this report, a loss of 26% of muscle fiber CSA was evidenced during the first 7 days in control group patients. This loss was reduced by an intensive physiotherapy including cycling therapy 7/7 days. Furthermore, our data support the thought that the amount of activity is associated with a better muscle mass maintenance.

The main catabolic pathways in skeletal muscle were upregulated by septic shock and seemed to be better regulated by the intervention. Indeed, the excessive activation of autophagy can induce accelerated skeletal muscle wasting (12, 26). However, autophagy is also a crucial repair process necessary during critical illness and should not be completely abolished (12, 38). In our trial, we showed a better regulation of autophagy by the intervention, but it was far to be suppressed. In an animal model of sepsis, REDD1 has been shown to play a central role in regulating both synthesis and autophagy in skeletal muscle (25). In a previous report during recovery from sepsis, mTOR activity was increased together with lower REDD1 expression and autophagy, without changes in UPP E3-ligases (39). Here, we confirmed those observations on REDD1 and several autophagy markers in the intervention group. The limitation of the excessive autophagy activation was corroborated by immunohistochemistry by the costaining of LAMP2/p62 confirming a better control, rather than its complete suppression. On the other hand, UPP E3-ligases tended to be better controlled with the intervention.

No effect was observed on the anabolic pathway, probably as a result of a sustained activation of Akt-mTOR pathway in critically ill patients receiving continuous insulin infusion (40). In addition, the nature of the intervention, that is, endurance exercise when cycling was actively performed, was not supposed to induce a substantial activation of the mTOR pathway.

The strength of this work is conferred by the early intervention in a critically ill population with septic shock undergoing mechanical ventilation. We effectively managed to perform the first mobilization within 28 ± 9 hours in intervention group, whereas patients in the control group were mobilized later (46 ± 25 hr). This difference lays in the 7/7 days mobilization sessions in the intervention group, whereas the control group received mobilization five times a week.

Some limitations of our study should be considered. First, our study has a small number of patients, and data on electrophysiologic analysis and muscle strength are limited. In this line, we could not draw any conclusion about muscle function and the relationship between muscle fiber CSA changes and strength. Since we used the MRC score, particularly challenging in the critically ill, our study would have benefited from recent standardized and reproductible techniques to evaluate the quadriceps force (41). Besides, although it is difficult to perform our protocol with a blindness approach, the fact that physiotherapists were not blinded to the allocation should be considered.

Second, the impact of this approach in long-term outcomes was not evaluated. We were not able to distinguish the respective benefits of active and/or passive interventions since both types of activity were performed in the vast majority of our patients.

Last, as almost two thirds of the physiotherapy sessions were active, generalization of our approach supports the need for a rapid tapering of sedation to promote patients' active participation. This low sedation and early mobilization approach is nevertheless not highly generalizable and requires sufficient staffing to ensure appropriate safety. As such, extrapolation of our results to all centers should be moderated.

In conclusion, this study demonstrates that exercising during the first week of septic shock preserves muscle fiber CSA, possibly by a limitation of the excessive activation of autophagy, without its suppression. This approach does not increase muscle inflammation and is well tolerated. These results should be confirmed in a larger population, including functional measurements.

ACKNOWLEDGMENTS

We acknowledge every patient and family member who accepted study participation.

REFERENCES

1. Needham DM, Davidson J, Cohen H, et al: Improving long-term outcomes after discharge from intensive care unit: Report from a stakeholders' conference. *Crit Care Med* 2012; 40:502–509
2. Witteveen E, Wieske L, van der Poll T, et al; Molecular Diagnosis and Risk Stratification of Sepsis (MARS) Consortium: Increased early systemic inflammation in ICU-acquired weakness; a prospective observational cohort study. *Crit Care Med* 2017; 45:972–979
3. Nanas S, Kritikos K, Angelopoulos E, et al: Predisposing factors for critical illness polyneuromyopathy in a multidisciplinary intensive care unit. *Acta Neurol Scand* 2008; 118:175–181
4. Biolo G: Protein metabolism and requirements. *World Rev Nutr Diet* 2013; 105:12–20
5. Kress JP, Hall JB: ICU-acquired weakness and recovery from critical illness. *N Engl J Med* 2014; 370:1626–1635
6. Weijs PJ, Looijaard WG, Dekker IM, et al: Low skeletal muscle area is a risk factor for mortality in mechanically ventilated critically ill patients. *Crit Care* 2014; 18:R12
7. Ali NA, O'Brien JM Jr, Hoffmann SP, et al; Midwest Critical Care Consortium: Acquired weakness, handgrip strength, and mortality in critically ill patients. *Am J Respir Crit Care Med* 2008; 178:261–268
8. Stevens RD, Marshall SA, Cornblath DR, et al: A framework for diagnosing and classifying intensive care unit-acquired weakness. *Crit Care Med* 2009; 37:S299–S308
9. Tennilä A, Salmi T, Pettilä V, et al: Early signs of critical illness polyneuropathy in ICU patients with systemic inflammatory response syndrome or sepsis. *Intensive Care Med* 2000; 26:1360–1363
10. Tepper M, Rakic S, Haas JA, et al: Incidence and onset of critical illness polyneuropathy in patients with septic shock. *Neth J Med* 2000; 56:211–214
11. Klaude M, Mori M, Tjäder I, et al: Protein metabolism and gene expression in skeletal muscle of critically ill patients with sepsis. *Clin Sci (Lond)* 2012; 122:133–142
12. Sandri M: Autophagy in health and disease. 3. Involvement of autophagy in muscle atrophy. *Am J Physiol Cell Physiol* 2010; 298:C1291–C1297
13. Hermans G, De Jonghe B, Bruyninckx F, et al: Interventions for preventing critical illness polyneuropathy and critical illness myopathy. *Cochrane Database Syst Rev* 2014; (1):CD006832
14. Kayambu G, Boots R, Paratz J: Physical therapy for the critically ill in the ICU: A systematic review and meta-analysis. *Crit Care Med* 2013; 41:1543–1554
15. Stiller K: Physiotherapy in intensive care: An updated systematic review. *Chest* 2013; 144:825–847
16. Kayambu G, Boots R, Paratz J: Early physical rehabilitation in intensive care patients with sepsis syndromes: A pilot randomised controlled trial. *Intensive Care Med* 2015; 41:865–874
17. Hermans G, Van den Berghe G: Clinical review: Intensive care unit acquired weakness. *Crit Care* 2015; 19:274
18. Helliwell TR, Wilkinson A, Griffiths RD, et al: Muscle fibre atrophy in critically ill patients is associated with the loss of myosin filaments and

the presence of lysosomal enzymes and ubiquitin. *Neuropathol Appl Neurobiol* 1998; 24:507–517

19. Hickmann CE, Castanares-Zapatero D, Bialais E, et al: Teamwork enables high level of early mobilization in critically ill patients. *Ann Intensive Care* 2016; 6:80
20. Pohlman MC, Schweickert WD, Pohlman AS, et al: Feasibility of physical and occupational therapy beginning from initiation of mechanical ventilation. *Crit Care Med* 2010; 38:2089–2094
21. D'Hulst G, Jamart C, Van Thienen R, et al: Effect of acute environmental hypoxia on protein metabolism in human skeletal muscle. *Acta Physiol (Oxf)* 2013; 208:251–264
22. Kleyweg RP, van der Meché FG, Schmitz PI: Interobserver agreement in the assessment of muscle strength and functional abilities in Guillain-Barré syndrome. *Muscle Nerve* 1991; 14:1103–1109
23. Lee H, Ko YJ, Suh GY, et al: Safety profile and feasibility of early physical therapy and mobility for critically ill patients in the medical intensive care unit: Beginning experiences in Korea. *J Crit Care* 2015; 30:673–677
24. McClave SA, Martindale RG, Vanek VW, et al; A.S.P.E.N. Board of Directors; American College of Critical Care Medicine; Society of Critical Care Medicine: Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). *JPEN J Parenter Enteral Nutr* 2009; 33:277–316
25. Sandri M, Sandri C, Gilbert A, et al: Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 2004; 117:399–412
26. Penna F, Baccino FM, Costelli P: Coming back: Autophagy in cachexia. *Curr Opin Clin Nutr Metab Care* 2014; 17:241–246
27. Steiner JL, Crowell KT, Kimball SR, et al: Disruption of REDD1 gene ameliorates sepsis-induced decrease in mTORC1 signaling but has divergent effects on proteolytic signaling in skeletal muscle. *Am J Physiol Endocrinol Metab* 2015; 309:E981–E994
28. Trojaborg W, Weimer LH, Hays AP: Electrophysiologic studies in critical illness associated weakness: Myopathy or neuropathy–a reappraisal. *Clin Neurophysiol* 2001; 112:1586–1593
29. Weber-Carstens S, Koch S, Spuler S, et al: Nonexcitable muscle membrane predicts intensive care unit-acquired paresis in mechanically ventilated, sedated patients. *Crit Care Med* 2009; 37:2632–2637
30. Schweickert WD, Pohlman MC, Pohlman AS, et al: Early physical and occupational therapy in mechanically ventilated, critically ill patients: A randomised controlled trial. *Lancet* 2009; 373:1874–1882
31. Fan E, Cheek F, Chlan L, et al; ATS Committee on ICU-acquired Weakness in Adults; American Thoracic Society: An official American Thoracic Society Clinical Practice guideline: The diagnosis of intensive care unit-acquired weakness in adults. *Am J Respir Crit Care Med* 2014; 190:1437–1446
32. Callahan LA, Supinski GS: Sepsis-induced myopathy. *Crit Care Med* 2009; 37:S354–S367
33. Dumont N, Bouchard P, Frenette J: Neutrophil-induced skeletal muscle damage: A calculated and controlled response following hindlimb unloading and reloading. *Am J Physiol Regul Integr Comp Physiol* 2008; 295:R1831–R1838
34. Puthucheary Z, Montgomery H, Moxham J, et al: Structure to function: Muscle failure in critically ill patients. *J Physiol* 2010; 588: 4641–4648
35. Poulsen JB, Møller K, Jensen CV, et al: Effect of transcutaneous electrical muscle stimulation on muscle volume in patients with septic shock. *Crit Care Med* 2011; 39:456–461
36. Dirks ML, Hansen D, Van Assche A, et al: Neuromuscular electrical stimulation prevents muscle wasting in critically ill comatose patients. *Clin Sci (Lond)* 2015; 128:357–365
37. Segers J, Hermans G, Bruyninckx F, et al: Feasibility of neuromuscular electrical stimulation in critically ill patients. *J Crit Care* 2014; 29:1082–1088
38. Hermans G, Casaer MP, Clerckx B, et al: Effect of tolerating macronutrient deficit on the development of intensive-care unit acquired weakness: A subanalysis of the EPaNIC trial. *Lancet Respir Med* 2013; 1:621–629
39. Crowell KT, Soybel DI, Lang CH: Restorative mechanisms regulating protein balance in skeletal muscle during recovery from sepsis. *Shock* 2017; 47:463–473
40. Jespersen JG, Nedergaard A, Reitelseder S, et al: Activated protein synthesis and suppressed protein breakdown signaling in skeletal muscle of critically ill patients. *PLoS One* 2011; 6:e18090
41. Laghi F, Khan N, Schnell T, et al: New device for nonvolitional evaluation of quadriceps force in ventilated patients. *Muscle Nerve* 2018; 57:784–791