Aus der Klinik für Anästhesiologie mit Schwerpunkt operative Intensivmedizin
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DISSERTATION

Effect of protocol-based physical therapy on molecular mechanisms leading to muscle atrophy and intensive care unit acquired weakness in critically ill patients

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1. Abstrakt

1.1.Deutsch

Der Effekt von Protokoll-basierter Physiotherapie auf molekulare Mechanismen von Muskelatrophie und auf der Intensivstation erworbener Muskelschwäche bei kritisch kranken Patienten

Hintergrund: Kritisch kranke Patienten entwickeln regelhaft während der Therapie auf der Intensivstation eine ausgeprägte Muskelschwäche, welche zum einen mit einer erhöhten Mortalität und Morbidität, sowohl kurz- als auch langfristig, sowie zum anderen auch mit einer schnell fortschreitenden Muskelatrophie einhergeht. Die Muskelatrophie zeichnet sich durch eine Reduktion der Myosinsynthese und eine Induktion der Proteindegradation aus. Wir haben in dieser Arbeit untersucht, welchen Effekt therapeutische Ansätze, wie Protokoll-basierte Physiotherapie und Muskel aktivierende Maßnahmen auf die molekularen Mechanismen der Muskelatrophie bei der auf der Intensivstation erworbenen Muskelschwäche haben.

Methodik: 50 Patienten mit einem Sepsis-related Organ Failure Assessment Score ≥ 9 innerhalb der ersten 72 Stunden nach Aufnahme auf eine von zwei Intensivstationen der Charité – Universitätsmedizin Berlin wurden randomisiert Protokoll-basierte Physiotherapie alleine (Kontrollen) oder in Kombination mit frühen Muskel aktivierenden Maßnahmen (Intervention), wie neuromuskuläre Elektrostimulation oder Ganzkörpervibrationstherapie, zu erhalten. Am fünfzehnten Liegetag wurde eine offen-chirurgische Muskelbiopsie am M. vastus lateralis vorgenommen, um den Effekt der Protokoll-basierten Physiotherapie mit und ohne Muskel aktivierende Maßnahmen auf die Proteinsynthese und -degradation mittels quantitativer Echtzeit-Polymerase-Kettenreaktion und Western Blot zu untersuchen. Patienten aus einer vorangehenden Observationsstudie, welche Standardphysiotherapie erhalten haben und alle Einschlusskriterien erfüllten, wurden als Vergleichsgruppe mit einbezogen. Muskelbiopsien, welche gesunden Patienten im Rahmen einer elektiven orthopädischen Operation entnommen wurden, wurden für Referenzwerte mit eingeschlossen. Das Ethikvotum wurde von der Ethikkommission der Charité – Universitätsmedizin Berlin eingeholt (Charité EA 2/041/10).

Ergebnisse: Patienten in der Interventionsgruppe zeigten eine signifikant erhöhte mRNA-Expression für *MYH1*, *MYH2* und *MYH4* im Vergleich zu Referenzwerten sowie für *MYH1* und *MYH4* im Vergleich zur Standardphysiotherapie. Die relative mRNA-Expression für *FBXO32* und *TRIM62*, als Schlüsselkomponenten des Ubiquitin-Proteasom-Systems, waren über Referenzwerte für alle Gruppen von kritisch kranken Patienten erhöht. Im Gegensatz dazu war die *TRIM63* mRNA-Expression in der Interventions- und Kontrollgruppe gegenüber der Standardphysiotherapiegruppe sowie gegenüber den Referenzwerten erhöht. Der Anstieg der Myosingenexpression spiegelt sich auch auf Proteinebene in einem signifikant erhöhten Myosinproteingehalt für gesamtes, langsames und schnelles Myosin in der Interventionsgruppe im Vergleich zur Standardphysiotherapiegruppe wieder. Der Proteingehalt für Atrogin-1 und MuRF-1 war sowohl in der Interventions- als auch in der Kontrollgruppe oberhalb der Referenzwerte und der Werte der Standardphysiotherapiegruppe.

Schlussfolgerung: Protokoll-basierte Physiotherapie und additive Muskel aktivierende Maßnahmen erhöhen die Myosingenexpression sowie den Proteingehalt, ohne das Ubiquitin-Proteasom-System zu hemmen.

1.2. Englisch

Effect of protocol-based physical therapy on molecular mechanisms leading to muscle atrophy and intensive care unit acquired weakness in critically ill patients

Background: Up to 50% of critically ill patients develop a severe muscle weakness during their intensive care treatment which leads to increased mortality and morbidity. Characteristic for this intensive care unit acquired weakness is a rapidly progressing muscle atrophy, which is the results of a decrease muscle protein synthesis as well as an increased muscle protein degradation. Our aim was to investigate the effect of therapeutic and preventatives interventions such as protocol-based physiotherapy and muscle activating measures on molecular mechanisms leading muscle atrophy during intensive care unit acquired weakness are unknown.

Methods: 50 patients with a Sepsis-related Organ Failure Assessment ≥ 9 within the first 72 hours after admission to one of two ICUs within the Charité –

Universitätsmedizin Berlin were randomised to receive either protocol-based physiotherapy alone (control) or protocol-based physiotherapy with additional muscle activating measures, such as neuromuscular electrical stimulation or whole body vibration (intervention). 15 days after admission to the ICU an open surgical muscle biopsy of the m. vastus lateralis was performed to assess the effect of protocol-based physiotherapy and muscle activating measures on muscle protein synthesis and degradation via real-time PCR and Western Blot. Patients receiving common physiotherapeutic practice from an earlier observational trial fulfilling the same inclusion criteria were included as a comparison group. Healthy patients undergoing elective orthopaedic surgery were similarly included for reference values. Ethical approval was granted by the institutional review borad (Charité EA 2/041/10).

Results: Patients in the intervention group presented significantly increased mRNA expression values for *MYH1*, *MYH2* and *MYH4* in comparison to baseline values as well as for *MYH1* and *MYH4* in comparison to the common physiotherapeutic practice group. Relative mRNA expression for *FBXO32* and *TRIM62* as key components of the ubiquitine-proteasome system were increased above baseline values for all groups of critically ill patients. While *TRIM63* mRNA expression was increased above baseline values and common physiotherapeutic practice for both the intervention and control group. The increase in myosin heavy chain mRNA expression is accompanied by a significantly increased protein content for total, slow and fast myosin in the intervention group as opposed to the common physiotherapeutic practice group. Protein content for Atrogin-1 and MuRF-1 was higher in the control and intervention groups in comparison to baseline values and values from the common physiotherapeutic practice group.

Conclusion: Protocol-based physiotherapy and muscle activating measures increase myosin gene expression as well as protein content and do not suppress the ubiquitine-proteasome system.

2. Einleitung

Im Verlauf der letzten Jahrzehnte haben verbesserte therapeutische und diagnostische Möglichkeiten zu einem starken Anstieg der Anzahl an Intensivstationsüberlebenden geführt (1). Innerhalb dieser wachsenden Kohorte hat sich deutlich herauskristallisiert, dass diese Patienten mit schwerwiegenden Folgeerscheinungen der kritischen Erkrankung sowie Intensivstationstherapie zu kämpfen haben. Aktuell wird dieser Folgezustand aus körperlichen, seelischen und kognitiven Einschränkungen unter dem Begriff Post Intensive Care Syndrom (PICS) zusammengefasst (2-6).

Die körperlichen Einschränkungen manifestieren sich dabei bereits sehr früh während des Intensivstationsaufenthalts in Form einer auf der Intensivstation erworbenen Schwäche (Intensive Care Unit acquired Weakness (ICUAW)) (7). Diese hat sowohl kurzfristige Konsequenzen für den Patienten im Sinne einer reduzierten Überlebenswahrscheinlichkeit, Weaning-Wahrscheinlichkeit und Krankenhausentlassungwahrscheinlichkeit sowie auch dramatische langfristige Auswirkungen, wie eine reduzierte körperliche Leistungsfähigkeit, deutliche ökonomische Einschränkungen durch reduzierte Arbeitsfähigkeit und eine eingeschränkte körperliche gesundheitsbezogene Lebensqualität (2, 3, 8, 9). Es hat sich gezeigt, dass besonders Patienten mit Sepsis und den assoziierten Organdysfunktionen gemessen anhand des Sepsis-related Organ Failure Assessment (SOFA) ein besonders hohes Risiko für die Entstehung einer ICUAW haben (10-12).

Pathophysiologisch zeichnet sich die ICUAW durch einen sehr frühen Beginn und ein rasches Fortschreiten mit einem Muskelmassenverlust von circa 4% pro Tag aus (13, 14). Die Muskelatrophie resultiert aus einer Dysbalance zwischen Muskelauf- und -abbau hin zum Muskelabbau, bedingt durch Einschränkungen der Muskelsynthese und einer Verstärkung der muskelabbauenden Stoffwechselwege (15). Das Ubiquitin-Proteasom-System ist eines der wichtigsten Systeme im Rahmen des Muskelabbaus. Es konnte gezeigt werden, dass es zu einer vermehrten mRNA-Expression und einem erhöhten Proteingehalt von MuRF1 und Atrogin-1 als Schlüsselenzyme des Ubiquitin-Proteasom-Systems bereits innerhalb der ersten 5 Tage nach Beginn der kritischen Erkrankung kommt (15).

Neben der abschließenden Klärung des Mechanismus der Krankheitsentstehung sind auch definitive therapeutische beziehungsweise präventive Strategien ausstehend (15). Während der letzten Dekade lag der wissenschaftliche Fokus hinsichtlich präventiver und therapeutischer Strategien auf Protokoll-basierter Physiotherapie, wobei die Ergebnisse aufgrund heterogener Patientenkollektive und Outcomeparameter insgesamt unzufrieden

stellend zu vergleichen waren und daher eine definitive Aussage erschwert ist (16-20). Ein zentrales Problem mit klassischer Physiotherapie ist der sedierte und nicht-kooperationsfähige Patient in der Intensivmedizin, da dieser nicht aktiv an der Mobilisation teilnehmen kann und daher die Möglichkeiten zur Prävention von Muskelmassenverlust eingeschränkt sind.

Muskel aktivierende Maßnahmen, wie die neuromuskuläre Elektrostimulation (NMES) und die Ganzkörpervibrationstherapie sind auch bei sedierten und nicht-kooperationsfähigen Patienten vollumfänglich anwendbar, da für das Hervorrufen einer Muskelkontraktion keine aktive Partizipation des Patienten benötigt wird. Während die NMES bereits erfolgreich in der Muskelatrophieprävention bei anderen Krankheitsbildern Anwendung findet und auch erste Untersuchungen in kritisch kranken Patienten durchgeführt worden sind, ist die Ganzkörpervibrationstherapie ein neuartiges Therapieverfahren und bisher sind lediglich Machbarkeitsuntersuchungen erfolgreich abgeschlossen (21-27). Während die klinischen Effekte von NMES, kongruent zu den Mobilisationsstudien, geprägt sind durch heterogene Patientenkollektive und Outcomeparameter fehlen Untersuchungen hinsichtlich des Einflusses auf die Pathophysiologie komplett. Dies wiederum verhindert, dass eine klare therapeutische Empfehlung hinsichtlich des Nutzens der NMES bei kritisch kranken Patienten gegeben werden kann.

3. Fragestellung

Das Ziel meiner Arbeit war es daher im Rahmen einer randomisiert-kontrollierten Interventionsstudie den Effekt von Protokoll-basierter Physiotherapie in Kombination mit Muskel aktivierenden Maßnahmen auf Muskelsynthese und -degradation bei kritisch kranken Patienten auf der Basis folgender Hypothesen zu untersuchen:

- Frühe Mobilisation mittels Protokoll-basierter Physiotherapie und Muskel-aktivierenden Maßnahmen verhindert Muskelmassenverlust bei kritisch kranken Patienten.
- Frühe Mobilisation mittels Protokoll-basierter Physiotherapie und Muskel-aktivierenden Maßnahmen führt zu einer Steigerung der Myosinsynthese.
- Frühe Mobilisation mittels Protokoll-basierter Physiotherapie und Muskel-aktivierenden Maßnahmen führt zu einer Hemmung des Ubiquitin-Proteasom-Systems.

4. Material und Methodik

4.1. Studiendesign

Diese randomisiert kontrollierte Interventionsstudie (ISRCTN19392591) wurde auf zwei Intensivstationen der Charité – Universitätsmedizin Berlin durchgeführt. Die Patienten wurden nach schriftlicher Einwilligung durch den gesetzlichen Vertreter in die Studie eingeschlossen und in einem Verhältnis von 1:2 randomisiert entweder Protokoll-basierte Physiotherapie (Kontrolle) oder Protokoll-basierte Physiotherapie mit zusätzlichen Muskel-aktivierenden Maßnahmen (Intervention), wie NMES oder Ganzkörpervibrationstherapie, zu erhalten. Innerhalb der Interventionsgruppe wurde mittels sequentieller Allokation dann noch einmal hinsichtlich der unterschiedlichen Muskel-aktivierenden Maßnahmen randomisiert. Die Studie wurde von der Ethikkommission der Charité – Universitätsmedizin Berlin genehmigt (Charité EA 2/041/10).

In den Analysen wurden Patienten und Muskelbiopsien aus einer vorangegangenen Observationsstudie mit übereinstimmenden Einschlusskriterien berücksichtigt, um den Vergleich zu Standardphysiotherapie zu ermöglichen (Charité EA2/061/ 06; ISRCTN77569430) (15). Die Standardphysiotherapie reflektiert das aktuelle klinische Vorgehen auf den meisten Intensivstationen, welches nicht mit den aktuellen Leitlinienempfehlung übereinstimmt (28, 29). Wir konnten daher aus ethischen Gesichtspunkten unsere Kontrollgruppe nicht unterhalb der Leitlinienempfehlungen behandeln und haben die Standardphysiotherapie Gruppe hinzugenommen, um den Bezug zur aktuellen klinischen Praxis zu haben. Die Behandlung der Patient oblag den jeweiligen Intensivstationsärzten und wurde entsprechend der etablierten Standard Operating Procedures durchgeführt (30).

4.2. Patienten

Patienten ≥ 18. Lebensjahr mit einem SOFA ≥ 9 innerhalb der ersten 72 Stunden nach Aufnahme auf die Intensivstation und mechanischer Beatmung kamen für den Studieneinschluss in Frage. Die Ausschlusskriterien umfassten eine Adipositas 2. Grades (BMI > 35 kg/m_2), vorbestehende neuromuskuläre Erkrankungen, eine infauste Prognose, Insulin-abhängiger Diabetes mellitus, Teilnahme an einer anderen klinischen Studie, Schwangerschaft, Unfähigkeit vor Aufnahme auf die Intensivstation sich laufend fortzubewegen, Erkrankungen oder Therapien, welche die Durchführung der Interventionen verhindern würden und eine bereits erfolgte Behandlung im Krankenhaus von mehr als 7 Tagen. Referenzwerte für die molekularen Analysen

stammen aus Muskelbiopsien von 6 Patienten, welche sich einer elektiven orthopädischen Operation unterzogen.

4.3. Intervention

4.3.1. Protokoll-basierte Physiotherapie

Ab dem Tag des Studieneinschlusses erhielten die Patienten je nach Randomisierung entweder Protokoll-basierte Physiotherapie mit oder ohne Muskel-aktivierende Maßnahmen. In einer täglichen multiprofessionellen Fallbesprechung bestehend aus Ärzten/-innen, Krankenpflegern/-innen, Physiotherapeuten/innen und Atmungstherapeuten/-innen wurde anhand des Mobilisationsprotokolls (siehe Supplement Table S1 (31)) ein Mobilisationsziel definiert. Das Protokoll berücksichtigte dabei in besonderem Maße den klinischen Status (Bewusstseinszustand, Hämodynamik, Respiration) des Patienten. Das Mobilisationsziel wurde bei der täglichen Mobilisierung der Patienten durch den speziell geschulten Studienphysiotherapeuten dann erarbeitet. Die Mobilisation erfolgt täglich für 25 – 35 Minuten. Im Anschluss an die Mobilisation erfolgt wiederum ein multiprofessionelles Feedback-Gespräch, um Barrieren oder Problematiken bei der Mobilisation zu erörtern und zu beseitigen, damit eine optimale und individualisierte Mobilisation möglich war.

4.3.2. Neuromuskuläre Elektrostimulation

Patienten in der Interventionsgruppe erhielten zusätzlich täglich NMES und/oder Ganzkörpervibrationstherapie. Die NMES wurde dabei bilateral an 8 Muskelgruppen der oberen und unteren Extremitäten (M. tibialis anterior, M. triceps surae, M. vastus lateralis, ischiocrurale Muskulatur, M. biceps brachii, M. triceps brachii, Unterarmextensoren, Unterarmflexoren) durchgeführt. Die NMES dauerte 20 Minuten und wurde mit 50 Hz Impulsen bei einer Impulsbreite von 350 µs, einer Rampe von 1 Sekunde, einer Stimulationszeit von 6 bzw. 10 Sekunden und einer Pausenzeit von 10 bzw. 15 Sekunden je nach verwendetem Gerät (MUSKELaktiv 2-Kanal, schwa-medico®, Germany; Physiomed-Expert-2-Kanal, Physiomed®, Germany) appliziert. Die Stromstärke wurde erhöht bis eine Kontraktion sichtbar oder tastbar war. Sollte bis zu einer maximalen Stromstärke von 70 mA keine Kontraktion erfolgt sein, wurde die entsprechende Stimulationssitzung mit 40 mA durchgeführt. Sollte bei wachen Patienten vor

der Kontraktion die Schmerzschwelle erreicht sein wurde die Stromstärke auf den letzten Wert vor Erreichen der Schmerzschwelle reduziert und mit dieser Stromstärke dann die Stimulation durchgeführt.

4.3.3. Ganzkörpervibrationstherapie

Es wurden täglich 20 Zyklen der Ganzkörpervibrationstherapie mit dem Galileo® (Novotec®, Germany) durchgeführt. Die Stimulation erfolgte dabei alternierend, mit einer Frequenz von 26 Hz, einer Amplitude von 15 mm sowie einer Stimulationszeit und Pausenzeit von jeweils einer Minute. Der optimale Kontakt zur Vibrationsplatte wurde unter Berücksichtigung der hämodynamischen Situation sichergestellt, indem der Patient in eine nahezu aufrechte Position gebracht wurde. Patienten, die dies nicht toleriert haben, wurden mit einem 30° angehobenen Oberkörper sowie mit leichter Flexion in Hüfte und Knie stimuliert.

4.4. Analysen

4.4.1. Klinische Endpunkte

4.4.1.1. Erstes adäquates Erwachen

Alle Patienten wurden täglich durch die Studienärzte visitiert und dabei hinsichtlich des ersten adäquaten Erwachens untersucht. Dies war definiert als Richmond Agitation and Sedation Score zwischen +1 und -1 sowie einer adäquaten Antwort auf mindestens 3 der folgenden 5 verbalen Kommandos: „Öffnen/Schließen Sie ihre Augen“, „Schauen Sie mich an“, „Öffnen Sie ihren Mund und strecken Sie die Zunge heraus“, „Nicken Sie mit dem Kopf“ und „Heben Sie ihre Augenbrauen, wenn ich bis 5 gezählt habe“ an zwei aufeinanderfolgenden Tagen entsprechend der Publikation von DeJonghe et al. (10).

4.4.1.2. Medical Research Council score

Die Erhebung der Muskelkraft mittels Medical Research Council score wurde von speziell geschultem Studienpersonal durchgeführt. Die Kraft wurde in 8 verschiedenen Muskelgruppen (Extension/Flexion im Ellenbogengelenk, Extension/Flexion im Handgelenk, Abduktion in der Schulter, Flexion im Hüftgelenk, Extension im Kniegelenk, Extension/Flexion im Sprunggelenk) bilateral untersucht. Der Medical Research Council score umfasst eine 6 Punkte Skala mit folgender

Abstufung: 0 = keine Kontraktion vorhanden, 1 = Kontraktion des Muskels ohne Bewegung, 2 = Bewegung ohne Schwerkraft, 3 = Bewegung gegen die Schwerkraft, 4 Bewegung gegen leichten Widerstand und 5 = Bewegung gegen vollen Widerstand. Die Erhebung erfolgte beim ersten adäquaten Erwachen sowie bei Entlassung von der Intensivstation.

4.4.1.3. Minimal modified Functional Independence Measure

Der minimal modifed Functional Independence Measures (mmFIM) ist eine auf körperliche Fähigkeiten reduzierte Form des Functional Independence Measure. Er setzt sich aus den zwei Domänen Lokomotion und Transfer zusammen, welche auf einer Skala von 0 = keine Aktivität bis 4 = komplette Unabhängigkeit für die Aktivität beurteilt werden. Der mmFIM wurde zum Zeitpunkt der Entlassung von der Intensivstation erhoben.

4.4.2. Molekulare Endpunkte

4.4.2.1. Chirurgische Muskelbiopsien

Am Tag 15 nach Aufnahme auf die Intensivstation wurde bei den Patienten eine chirurgische Muskelbiopsie aus dem M. vastus lateralis gewonnen. Sollte aus klinischen oder organisatorischen Gründen eine Muskelbiopsie an Tag 15 nach Aufnahme auf die Intensivstation nicht möglich gewesen sein, wurde die Muskelbiopsie stattdessen am nächsten möglichen Tag gewonnen. Initial wurde die Haut durch eine Infiltration mit Lidocain anästhesiert. Anschließend wurde im distalen Drittel des M. vastus lateralis der Muskel frei präpariert und die chirurgische Muskelbiopsie gewonnen. Danach wurde die Wunde verschlossen und verbunden. Die Muskelbiopsien wurden bei -80°C gelagert.

4.4.2.2. Histologie

Ein Teil wurde für immunhistochemische sowie histologische Darstellungen nach der Einbettung in Tragacanth unter Kryoprotektion tiefgefroren. Alternative wurden die Präparate in 3,7% Paraformaldehyde fixiert und in Paraffin eingebettet. Die Präparate wurden dann mit einem Cryotome (Leica CM3050 S) geschnitten. Die histologischen Färbungen (Haemotoxylin-Eosin und Gomori Trichrome) sowie die metachromatische ATPase Färbung wurden entsprechend vorangegangener Publikationen durchgeführt (15, 32-36).

4.4.2.3. Quantitative Echtzeit-Polymerase-Kettenreaktion

Die gesamte RNA habe ich mittels des TRIzol® Reagenz (Invitrogen) aus den Muskelbiopsien entsprechend der Empfehlung des Herstellers gewonnen. Im Anschluss daran habe ich die Reverse-Transkription von 1 µg RNA mittels SuperScript® First-Strand Synthesis System (Invitrogen) zu cDNA ebenfalls entsprechend der Empfehlung des Herstellers durchgeführt. Die quantitative Echtzeit-Polymerase-Kettenreaktion habe ich unter Zuhilfenahme von TaqMan® Universal PCR Mastermix (Applied Biosystems), spezifischen TaqMan Primer Sets (Applied Biosystems)(siehe Supplement Table S2. (31)) und dem Step-One™ Plus Thermocycler (Applied Biosystems) durchgeführt. Grundsätzlich habe ich während meines Vorgehens alle Herstellerprotokolle eingehalten. Während der verschiedenen Prozessschritte, wie mRNA Extraktion und cDNA Synthese kommt es zu einer regulären Streuung der Effektivität von Durchlauf zu Durchlauf. Um die dadurch entstehende Ungenauigkeit zu beseitigen habe ich die gemessene Genexpression zu der Expression des sogenannten Housekeepers-Gens Glyceraldehyd-3-Phosphat Dehydrogenase (GAPDH) normalisiert. Weitergehend habe ich alle Werte der kritisch kranken Patienten, zu denen der gesunden Freiwilligen normalisiert, sodass die relative Abweichung beurteilt werden kann.

4.4.2.4. Western Blot

Initial habe ich die Muskelbiopsien homogenisiert für 30 Sekunden bei 2000 Umdrehungen pro Minute. Die Homogenisierung habe ich in eiskaltem Puffer bestehend aus 1:3 wt/vol (10 mM Tris HCl, pH 7.5, 140 mM NaCl, 1 mM EDTA, 25% Glycerol, 0.5% Natriumlaurylsulfat , 0.5% Nonident P-40, 0.1 mM 5 Dithiothreitol, 0.5 mM Phenylmethylsulfonylfluorid und 100 ng/ml Proteaseinhibitor (Roche)) durchgeführt. Anschließend habe ich, um die Gewinnung des Überstands zur ermöglichen, das Homogenisat für 10 Minuten bei 4°C und 14000 Umdrehungen pro Minute zentrifugiert. Den Überstand habe ich abgenommen und mittels des Bio-Rad Protein Assays (Bio Rad) auf Proteingehalt untersucht. Die Proteine habe ich durch die Natriumdodecylsulfat-Polyacrylamidgelelektrophorese anhand ihres

molekularen Gewichts aufgetrennt und im Anschluss auf Polyvinylidendifluorid oder Nitrocellulose Membranen (Amersham Pharmacia Biotech) geblottet. Zur Darstellung der verschiedenen Proteine habe ich die Membranen mit primärem und sekundärem Antikörper (siehe Supplement Table S3. (31)) inkubiert und dies im Anschluss durch Chemilumineszenz visualisiert

4.5. Statistik

Metrische Variablen habe ich als Median und Interquartilsabstand dargestellt. Kategorische Variablen habe ich als Anzahl und Prozentsatz dargestellt. Aufgrund der geringen Gruppengröße bin ich davon ausgegangen, dass keine Normalverteilung vorliegt. Zur Testung der statischen Signifikanz habe ich daher nicht-parametrische Tests angewendet. Den Gruppenunterschied für metrische Variablen habe ich mittels Kruskal-Wallis und Mann-Whitney U bei verbundenen Stichproben und Wilcoxon-Vorzeichen-Rang-Test bei unverbundenen Stichproben berechnet. Den Gruppenunterschied für kategorische Variablen habe ich mittels Chi-Square Test berechnet. Die Verschiebung der Myozyten-Querschnittsfläche habe ich mit dem ANOVA berechnet und durch den Welch- sowie Brown-Forsythe Test validiert, falls die Varianz im Levene's Test inhomogen war. Die Analysen habe ich mit SPSS IBM (Version 25) durchgeführt. Alle Grafiken habe ich mit GraphPad Prism (Version 7) und Sigma Plot (Version 12) erstellt.

5. Ergebnisse

3147 Patienten wurden insgesamt auf beide Intensivstationen während der zweijährigen Einschlussperiode aufgenommen, wobei nur 468 davon einen SOFA ≥ 9 innerhalb der ersten 72 Stunden hatten. Weitere 418 Patienten konnten aus verschiedensten Gründen nicht eingeschlossen werden, wie aus dem Einschlussdiagramm (siehe Figure 1(31)) ersichtlich ist, sodass am Ende 50 Patienten in die Kontroll- und Interventionsgruppe randomisiert wurden. Zusätzlich wurden 33 Patienten aus der vorangegangenen Beobachtungsstudie, welche Standardphysiotherapie erhalten haben inkludiert (15).

5.1. Klinisch

5.1.1. Gruppencharakteristika

Die drei berücksichtigten Gruppen zeigen insgesamt ausgeglichene Charakteristika. Lediglich die Krankheitsschwere gemessen als Tage im septischen Schock, APACHE oder SAPS2 ist in der Gruppe mit

Standardphysiotherapie geringer als in der Kontroll- und Interventionsgruppe (siehe Table 1 (31)). Patienten in der Kontroll- und Interventionsgruppe erhielten im Median für 22 Minuten pro Tag Physiotherapie, was signifikant mehr war als die 13 Minuten in der Standardphysiotherapie Gruppe. Darüber hinaus erhielt die Interventionsgruppe weitere 20 Minuten NMES und/oder Ganzkörpervibrationstherapie zusätzlich zu den 22 Minuten Protokoll-basierter Physiotherapie.

5.1.2. Muskelkraft

Zum Zeitpunkt des ersten adäquaten Erwachens präsentieren nahezu alle Patienten eine deutlich eingeschränkte Muskelkraft gemessen am MRC score. Dies spiegelt sich auch in einer ICUAW-Inzidenz von über 80% wider. Alle Gruppen zeigen unabhängig von der Intervention eine signifikante Zunahme der Muskelkraft vom ersten adäquaten Erwachen bis zur Entlassung von der Intensivstation. Nichts desto trotz bleibt der mediane MRC score unterhalb des Grenzwertes von 4 für die ICUAW Diagnose (siehe Figure 2 (31)).

5.1.3. Muskelfunktion

Zum Zeitpunkt der Entlassung von der Intensivstation zeigen alle Gruppen eine deutliche Einschränkung in der funktionellen Unabhängigkeit gemessen als mmFIM. Dies ist in beiden beurteilten Domänen Transfer und Lokomotion unabhängig voneinander zu beobachten. Ein signifikanter Unterschied zwischen den Gruppen ist nicht vorhanden (siehe Table 2 (31)).

5.2. Histologisch

In der Interventionsgruppe zeigt sich ein signifikant größerer Muskelfaserquerschnittsdurchmesser für Typ I, IIa und Ib Muskelfaser im Vergleich zur Kontrollgruppe (I = + 10%, IIa = +13%, IIb * +3%) aber auch zur Standardphysiotherapie Gruppe (I = +36%, IIa = +49%, IIb = +I65%). Ebenso zeigt sich in der Kontrollgruppe ein signifikanter Anstieg der Muskelfaserquerschnittsfläche im Vergleich zur Standardphysiotherapie Gruppe (I = +23%, IIa = + 33%, IIb = +60%) (siehe Figure 3 (31)).

5.3. Molekular

5.3.1. Geneexpression

5.3.1.1. Muskelsynthese

In der Interventionsgruppe ist eine signifikante Hochregulation der Genexpression für Myosinschwerketten, als integrales Struktur- und Funktionsprotein des Muskels, im Vergleich zur Standardphysiotherapie Gruppe (*MYH1*, *MYH2*, *MYH4*) und den Referenzwerten von gesunden Probanden (*MYH1*, *MYH4*) zu beobachten. Es zeigt sich in der Interventionsgruppe ebenfalls eine signifikante Hochregulation im Vergleich zur Kontrollgruppe für *MYH4*. Die Standardphysiotherapie Gruppe zeigt eine signifikante Reduktion der Genexpression für *MYH2* im Vergleich zu den Referenzwerten von gesunden Probanden (siehe Figure 4 (31)).

5.3.1.2. Muskeldegradation

Die Genexpression für *TRIM63* (kodiert für MuRF1) und *FBXO32* (kodiert für Atrogin-1) ist sowohl in der Interventions- als auch in der Kontrollgruppe signifikant hochreguliert im Vergleich zu Referenzwerten von gesunden Probanden. Zusätzliche zeigt sich für beide Gruppen eine signifikante Erhöhung der Genexpression für *TRIM63* im Vergleich zur Standardphysiotherapie Gruppe. Die Standardphysiotherapie Gruppe hingegen zeigt für *FBXO32* eine signifikant erhöhte Genexpression im Vergleich zu den Referenzwerten von gesunden Probanden, ohne einen Unterschied zur Kontroll- und Interventionsgruppe aufzuweisen. Deckungsgleich zu *FBXO32* zeigt *TRIM62* eine signifikant erhöhte Genexpression für alle drei Gruppen mit kritisch kranken Patienten im Vergleich zu Referenzwerten von gesunden Probanden (Figure 4 (31). *CAPN1* (kodiert für Calpain 1), *CASP3* (kodiert für Caspase 3) und *PSMB2* (kodiert für eine proteasomale Untereinheit) zeigen eine signifikante Erhöhung der Genexpression über Referenzwerte von gesunden Probanden in allen drei Gruppen. Weitergehend zeigt *PSMB2* einen signifikanten Anstieg in der Geneexpression für die Kontroll- und Interventionsgruppe gegenüber der Standardphysiotherapie Gruppe. *CAPN1* und *CASP3* zeigen keinen Gruppenunterschiede in der Geneexpression (Figure 5 (31)).

5.3.1.3. Inflammation

Eine signifikant erhöhte Genexpression über die Referenzwerte für gesunden Probanden zeigen *IL-6* (kodiert für Interleukin 6) und *SAA1/2*

(kodiert für Serum Amyloid A 1/2) in allen drei Gruppen, während *TNF* (kodiert für Tumornekrosefaktor) dies nur für Patienten in der Standardphysiotherapie Gruppe zeigt. Während die Genexpression für *SAA1/2* in der Interventionsgruppe signifikant höher ist als in der Standardphysiotherapie Gruppe, ist es für *TNF* umgekehrt und sie ist in der Interventionsgruppe signifikant niedriger als in der Standardphysiotherapie Gruppe. Es sind ansonsten keine signifikanten Unterschiede zwischen den Gruppen zu beobachten (siehe Figure 5 (31)).

5.3.2. Proteingehalt

5.3.2.1. Muskelproteingehalt

Der Proteingehalt des gesamten, schnellen und langsamen Myosins zeigt in Patienten aus der Kontroll- und Interventionsgruppe Werte die vergleichbar sind mit den Referenzwerten von gesunden Probanden. Die Interventionsgruppe zeigt allerdings einen signifikanten Anstieg im Proteingehalt für gesamtes, schnelles und langsames Myosin im Vergleich zur Standardphysiotherapie Gruppe (siehe Figure 4 (31)).

5.3.2.2. Muskeldegradation

Wir können zeigen, dass sich MuRF1 lediglich bei kritisch kranken Patienten nachweisen lässt, wobei der Gehalt in der Kontroll- und Interventionsgruppe höher war als in der Standardphysiotherapie Gruppe. Atrogin-1 hingegen zeigt einen deutlichen Anstieg nur in der Interventionsgruppe (siehe Figure 4 (31)).

6. Diskussion

Meine Arbeit diente der Untersuchung des Effekts von Protokoll-basierter Physiotherapie mit oder ohne frühe Muskel-aktivierende Maßnahmen auf molekulare Mechanismen von Muskelsynthese und -degradation bei kritisch kranken, septischen Patienten mit Multiorganversagen.

Ich konnten dabei zeigen, dass durch Protokoll-basierte Physiotherapie und frühe Muskel-aktivierende Maßnahmen Muskelatrophie verhindert werden konnte, wie sich in einer größeren Myozytenquerschnittsfläche und einem erhöhten Myosin Proteingehalt zeigt. Es kommt dabei sowohl zu einem Anstieg von Komponenten des Ubiquitin-Proteasom-Systems als auch der Muskelproteinsynthese, woraus sich schließen lässt, dass der

Muskelmassenerhalt primär über eine Induktion der Proteinsynthese und nicht über eine Suppression der Proteindegradation erreicht wird.

Unsere Ergebnisse eines fehlenden Effekts auf Muskelkraft und -funktion sind deckungsgleich zu den Ergebnissen der Studie von Fossat et al., die den Effekt von Mobilisation mittels Bettfahrrad und NMES bei kritisch kranken Patienten untersucht hat (37). Weitergehend können wir den Fund von Hickmann et al., dass frühe Physiotherapie zu einem Erhalt der Muskelmasse führt, bestätigen und ihn sogar erweitern, indem wir zeigen, dass frühe Muskel-aktivierende Maßnahmen den Effekt noch einmal verstärken (38).

Interessanterweise zeigt sich molekular nicht wie von uns vermutet eine Hochregulation der Synthese und eine Verminderung des Muskelabbaus, sondern eine Hochregulation von Synthese und Abbau. Aufgrund der vergrößerten Myozytenquerschnittsfläche sowie des vermehrten Muskelproteingehalts kann davon ausgegangen werden, dass die Hochregulation der Synthese im Vergleich zur Hochregulation des Abbaus überwiegt. MuRF1 und Atrogin-1 wurden bei kritisch kranken Patienten bis jetzt nur als Mediatoren für Muskelatrophie etabliert (15). Während dessen hat sich in gesunden Probanden gezeigt, dass es auch als Antwort auf Muskeltraining zu einer Hochregulation kommt. Dies zeigt, dass MuRF1 nicht nur im Rahmen der pathologischen Muskelatrophie sondern auch im Prozess von Muskelwachstum und -remodeling eine Rolle spielt (39, 40). Aufgrund der erhöhten TRIM63 Expression sowie des erhöhten MuRF1 Proteingehalts in der Kontroll- und Interventionsgruppe im Vergleich zur Standardphysiotherapie Gruppe gehen wir davon aus, dass diese Hochregulation ein Zeichen für ein Interventions-induziertes muskuläres Remodelling ist. Dies bestätigt sich durch die Tatsache, dass Muskelproteingehalt und die Myozytenquerschnittsfläche in diesen Patienten insgesamt erhöht sind. Die erhöhte Expression von *FBXO32* hingegen betrachten wir eher als einen residualen inflammatorischen Effekt, da sie in allen Gruppen mit kritisch kranken Patienten zu beobachten ist und mit der Expression von *IL-6* und *SAA1/2* übereinstimmt.

Die erhaltene Muskelmasse geht dabei nicht mit einer Verbesserung der muskulären Funktion einher. Es zeigt sich keine Verbesserung der Muskelkraft oder Muskelfunktion durch die Intervention beim ersten adäquaten Erwachen oder bei Entlassung von der Intensivstation.

Die hier beschriebene Diskrepanz zwischen Muskelkraft beziehungsweise -funktion und Muskelmasse wurde bereits bei kritisch kranken Patienten nach Entlassung von der Intensivstation beobachtet (41). Dos Santos et al. beobachteten einen fehlenden Zusammenhang zwischen Muskelmasse und Muskelmassenzunahme bis 6 Monate nach Entlassung von der Intensivstation und Muskelkraft (41). Unsere Daten zeigen ein kongruentes Phänomen während der Frühphase der kritischen Erkrankung.

Diese Ergebnisse bestätigen somit, dass neben der Muskelatrophie noch weitere Pathomechanismen zur Entstehung der ICUAW beitragen.

Der Muskel ist in seiner Funktion auf eine hohe Energiezufuhr angewiesen, die größtenteils aerob über die Atmungskette und die Mitochondrien gewonnen wird. Im Rahmen der schweren Sepsis, die einen wesentlichen Risikofaktor für ICUAW darstellt, konnte gezeigt werden, dass es im Rahmen von kritischer Erkrankung zu einer Einschränkung der mitochondrialen Funktion und ATP-Bereitstellung kommt (42). Es wurde dabei ein deutlicher Unterschied im muskulären ATP-Gehalt zwischen Überlebenden und Nicht-Überlebenden gefunden. Erste Untersuchungen zeigen, dass auch bei ICUAW Patienten eine Einschränkung der mitochondrialen Funktion vorhanden ist (43). Eine zukünftige Fragestellung ist daher die Rolle der Mitochondrien sowie des bioenergetischen Versagens bei ICUAW zu untersuchen.

Wir haben in unsere Studie nur schwerstkranke Patienten eingeschlossen, da diese aufgrund der Sepsis und des Multiorganversagens ein besonders hohes Risiko haben eine ICUAW zu entwickeln (10, 12, 44). Dies limitiert allerdings unsere Aussagekraft bezüglich weniger kranker Patienten auf der Intensivstation. Daraus ergibt sich die Fragestellung, ob Intensivstationspatienten unterschiedlicher Krankheitsschwere unterschiedlich auf die Intervention ansprechen.

Weitergehend stellt sich die Frage, ob die erhöhte muskuläre Masse das rehabilitative Potential des Patienten nach Entlassung von der Intensivstation verbessert. Weitere Untersuchungen über das gesamte Kontinuum des Krankheitsverlaufs von Intensivstationsaufnahme bis hin zum Abschluss der rehabilitativen Therapie sind notwendig, um diese Fragen zu klären.

Zusammenfassend verhindert Protokoll-basierte Physiotherapie mit Muskel-aktivierenden Maßnahmen Muskelatrophie in kritisch kranken Patienten mit Sepsis und Multiorganversagen durch eine Hochregulation der Proteinsynthese ohne Suppression der Proteindegradation.

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8. Eidesstattliche Versicherung

„Ich, Julius J. Grunow, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Effect of protocol-based physical therapy on molecular mechanisms leading to muscle atrophy and intensive care unit acquired awekaness in critically ill patients“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum _____ Unterschrift

9. Ausführliche Anteilserklärung an der erfolgten Publikation

Publikation:

Muscle wasting and function after muscle activation and early protocol-based physiotherapy: an explorative trial, Tobias Wollersheim, Julius J. Grunow, Niklas M. Carbon, Kurt Haas, Johannes Malleike, Sara F. Ramme, Joanna Schneider, Claudia D. Spies, Sven Märdian, Knut Mai, Simone Spuler, Jens Fielitz & Steffen Weber- Carstens, Journal of Cachexia Sarcopenia and Muscle, 23. April 2019

Tobias Wollersheim und Julius J. Grunow sind gleichberechtigte Erstautoren dieses Artikels.

Beitrag von Julius J. Grunow im Einzelnen:

Alle Arbeiten im Labor die notwendig waren zu Generierung der in der Publikation präsentierten Daten aus Genexpressions- und Proteingehaltanalysen habe ich selbstständig durchgeführt. Weitergehend habe ich an der Pflege der klinischen Datenbank mitgearbeitet und die klinischen Daten für die Aufarbeitung mittels SPSS aufbereitet. Die statistische Auswertung und grafische Aufbereitung aller der in der Publikation präsentiert Daten habe ich in Zusammenarbeit mit Herrn Dr. med. Tobias Wollersheim vorgenommen. Herr Dr. med. Tobias Wollersheim und ich habe auch in Zusammenarbeit die erste Version des Manuskripts verfasst. Ich habe in Zusammenarbeit mit Herrn Prof. Steffen Weber-Carstens und Herrn Dr. med. Tobias Wollerheim die Revision erstellt. Verantwortlich für die Einreichung waren Prof. Dr. med. Steffen Weber-Carstens und Dr. med. Tobias Wollersheim.

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Unterschrift, Datum und Stempel des betreuenden Hochschullehrers/der betreuenden Hochschullehrerin

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Unterschrift des Doktoranden/der Doktorandin

10. Auszug aus der Journal Summary List (ISI Web of KnowledgeSM)

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
Selected Categories: **"MEDICINE, GENERAL and INTERNAL"**
Selected Category Scheme: WoS
Gesamtanzahl: 154 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NEW ENGLAND JOURNAL OF MEDICINE	332,830	79.258	0.702000
2	LANCET	233,269	53.254	0.435740
3	JAMA-JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION	148,774	47.661	0.299960
4	BMJ-British Medical Journal	109,303	23.259	0.150320
5	JAMA Internal Medicine	11,840	19.989	0.076280
6	ANNALS OF INTERNAL MEDICINE	53,689	19.384	0.099140
7	Nature Reviews Disease Primers	1,559	16.071	0.007250
8	Journal of Cachexia Sarcopenia and Muscle	2,207	12.511	0.005180
9	PLOS MEDICINE	24,232	11.675	0.058710
10	BMC Medicine	12,000	9.088	0.041600
11	MAYO CLINIC PROCEEDINGS	13,828	7.199	0.025970
12	Cochrane Database of Systematic Reviews	62,332	6.754	0.167260
12	JOURNAL OF INTERNAL MEDICINE	10,327	6.754	0.016070
14	CANADIAN MEDICAL ASSOCIATION JOURNAL	14,191	6.210	0.016510
15	Journal of Clinical Medicine	1,673	5.583	0.005320
16	AMERICAN JOURNAL OF MEDICINE	25,399	5.117	0.026830
17	Translational Research	3,416	4.880	0.009000
18	ANNALS OF FAMILY MEDICINE	4,711	4.540	0.011480
19	MEDICAL JOURNAL OF AUSTRALIA	11,255	4.227	0.013820
20	AMERICAN JOURNAL OF PREVENTIVE MEDICINE	20,455	4.127	0.039330

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Muscle wasting and function after muscle activation and early protocol-based physiotherapy: an explorative trial

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Abstract

Background Early mobilization improves physical independency of critically ill patients at hospital discharge in a general intensive care unit (ICU)-cohort. We aimed to investigate clinical and molecular benefits or detriments of early mobilization and muscle activating measures in a high-risk ICU-acquired weakness cohort.

Methods Fifty patients with a SOFA score ≥9 within 72 h after ICU admission were randomized to muscle activating measures such as neuromuscular electrical stimulation or whole-body vibration in addition to early protocol-based physiotherapy (intervention) or early protocol-based physiotherapy alone (control). Muscle strength and function were assessed by Medical Research Council (MRC) score, handgrip strength and Functional Independence Measure at first awakening, ICU discharge, and 12 month follow-up. Patients underwent open surgical muscle biopsy on day 15. We investigated the impact of muscle activating measures in addition to early protocol-based physiotherapy on muscle strength and function as well as on muscle wasting, morphology, and homeostasis in patients with sepsis and ICU-acquired weakness. We compared the data with patients treated with common physiotherapeutic practice (CPP) earlier.

Results ICU-acquired weakness occurs within the entire cohort, and muscle activating measures did not improve muscle strength or function at first awakening (MRC median [IQR]: CPP 3.3 [3.0–4.3]; control 3.0 [2.7–3.4]; intervention 3.0 [2.1–3.8]; $P > 0.05$ for all), ICU discharge (MRC median [IQR]: CPP 3.8 [3.4–4.4]; control 3.9 [3.3–4.0]; intervention 3.6 [2.8–4.0]; $P > 0.05$ for all), and 12 month follow-up (MRC median [IQR]: control 5.0 [4.3–5.0]; intervention 4.8 [4.3–5.0]; $P = 0.342$ for all). No signs of necrosis or inflammatory infiltration were present in the histological analysis. Myocyte cross-sectional area in the intervention group was significantly larger in comparison with the control group (type I +10%; type IIa +13%; type IIb +3%; $P < 0.001$ for all) and CPP (type I +36%; type IIa +49%; type IIb +65%; $P < 0.001$ for all). This increase was accompanied by an up-regulated gene expression for myosin heavy chains (fold change median [IQR]: *MYH1* 2.3 [1.1–2.7]; *MYH2* 0.7 [0.2–1.8]; *MYH4* 5.1 [2.2–15.3]) and an unaffected gene expression for *TRIM63*, *TRIM62*, and *FBXO32*.

Conclusions In our patients with sepsis syndrome at high risk for ICU-acquired weakness muscle activating measures in addition to early protocol-based physiotherapy did not improve muscle strength or function at first awakening, ICU discharge, or 12 month follow-up. Yet it prevented muscle atrophy.

Keywords Sepsis; Early mobilization; ICU-acquired weakness; Neuromuscular electrical stimulation; Whole-body vibration; Protocol-based physiotherapy

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Introduction

Muscle wasting, as an acknowledged pathomechanism involved in the development of intensive care unit (ICU)-acquired weakness, results from impaired muscle protein homeostasis, with protein degradation outbalancing protein synthesis.[1,2] Systemic inflammation is a major risk factor considerably provoking impaired muscle protein homeostasis in most if not all patients suffering from sepsis and multiple organ dysfunction syndrome (MODS).[3] Until today, therapeutic and preventative measures for muscle atrophy and the accompanying ICU-acquired weakness remain vague and mostly confined to the general treatment of critical illness and reduction of risk factors.[4] Early mobilization has been shown to be clinically beneficial in general ICU patients, but with regard to severity of critical illness and MODS, it has overall yielded conflicting results.[5–11] Hodgson *et al*. even mentioned that early mobilization in these patients may be harmful.[12] Moreover, all of these studies did not investigate the impact of mobilization on prevention of muscle atrophy.

A small number of pilot studies investigating the effect of additional physiotherapeutic measures like neuromuscular electrical stimulation (NMES) show inconsistent results with respect to prevention of muscle atrophy and improvement of physical function as well as muscle strength.[13–16] A recent large scaled randomized controlled trial by Fossat *et al*. investigating the effects of in-bed leg cycling and electrical muscle stimulation in a general ICU-cohort described no effect on muscle strength but did not investigate muscle morphology.[17]

The aim of our exploratory trial was to investigate if an advanced protocol-based physiotherapy alone or combined with additional muscle activating measures, such as NMES, would prevent muscle atrophy, maintain protein homeostasis, and improve muscle strength and functional independency in patients with sepsis-related MODS at high risk for ICU-acquired weakness.

Methods

Study design

The exploratory randomized interventional single-centre trial (ISRCTN19392591) was conducted in two ICUs at the Charité – Universitätsmedizin Berlin, a tertiary care centre. In this trial, muscle activating measures in addition to protocol-based physiotherapy (intervention) compared with protocol-based physiotherapy alone (control) were investigated. Patients were enrolled and randomized after written informed consent by legal proxy. The institutional review board granted ethical approval (Charité EA 2/041/10). A sample size calculation was not performed because of insufficient published data on that topic.

For comparison to common physiotherapeutic practice, as it was performed before protocol-based physiotherapy was implemented as a clinical standard, we included clinical data and muscle samples from patients fulfilling the same inclusion criteria enrolled into an earlier observational trial into the analysis (Charité EA2/061/06; ISRCTN77569430).[1]

Participants

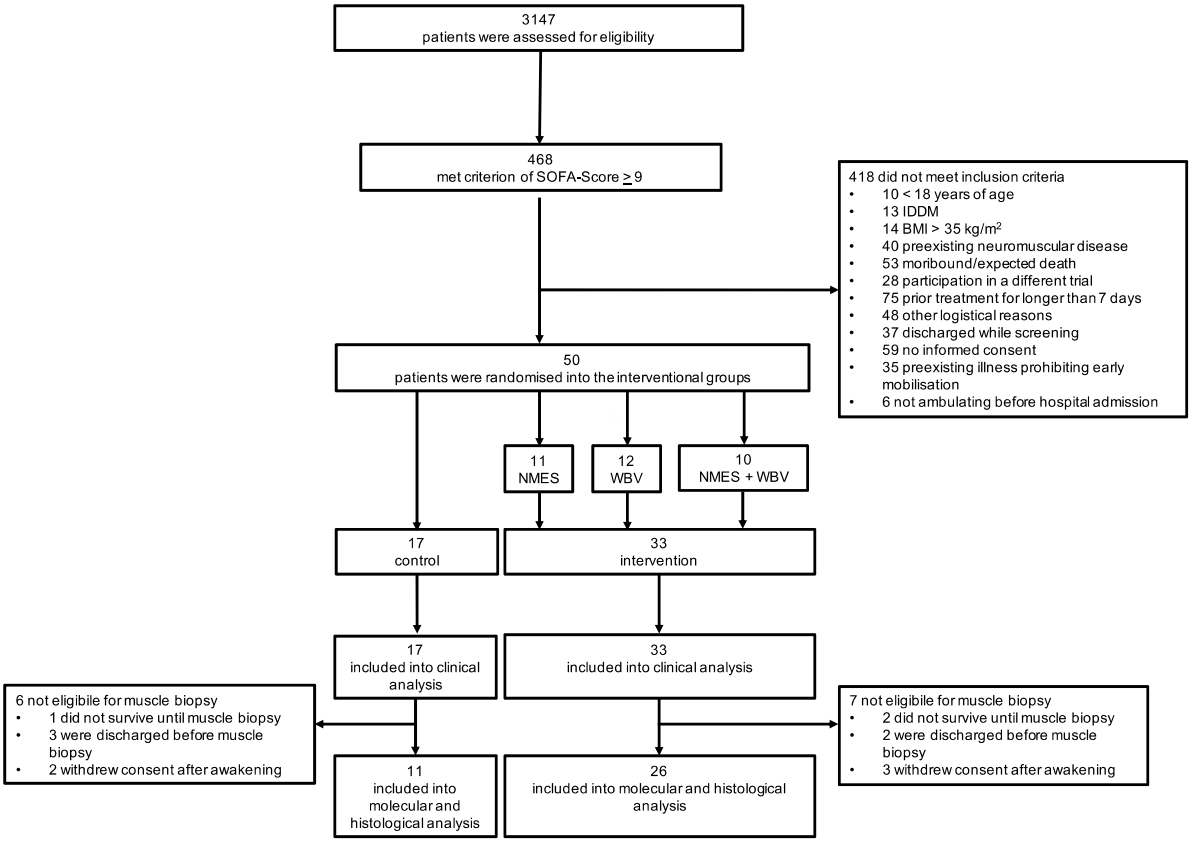
Mechanically ventilated patients ≥18 years of age with sepsis-related MODS indicated by a sepsis-related organ failure assessment (SOFA) score ≥9 within the first 72 h after ICU admission were eligible for enrolment (*Figure* 1). Patients with pre-existing neuromuscular disease, illness prohibiting early mobilization, insulin-dependent diabetes mellitus, prior treatment for longer than 7 days, body mass index > 35 kg/m^2, not ambulating before admission, or with a poor prognosis prone to die within the next hours were not considered for enrolment. Samples from six healthy volunteers undergoing elective orthopaedic surgery were used as reference for molecular analyses as well as plasma samples provided by 91 healthy volunteers for blood analysis.

Procedures

In the interventional part of the analysis, early mobilization, starting on the day of ICU admission, was performed in all patients in accordance to the physiotherapy protocol (Supporting Information, *Table* S1), which consists of an individualized approach with daily predefined goals, consented by an interdisciplinary staff including experienced physiotherapists, nurses, respiratory therapists, and physicians. The physiotherapy protocol included a daily closed-loop feedback system consisting of frequent reassessments and analysis of progress and barriers in the treatment of each patient, aiming to achieve the highest possible level of physiotherapeutic care under consideration of the patient's clinical status.

In the intervention group, muscle activating measures, such as NMES and/or whole-body vibration (WBV), were carried out daily throughout the ICU stay up to day 28 in addition to protocol-based physiotherapy. NMES was performed bilaterally on eight different muscle groups for 20 min, starting on the

Figure 1 Trial enrolment scheme. 'Other logistical reasons' indicates cases where a legal proxy could not be appointed within the screening timeframe or study personal was not available for logistical reasons. IDDM, insulin-dependent diabetes mellitus; NMES, neuromuscular electrical stimulation; WBV, whole-body vibration. Healthy patients were included for reference values (n = 6 for molecular and histological analysis of muscle biopsy specimens; n = 91 for myostatin analysis).



day of enrolment. Electrical current was increased to a maximum of 70 mA until visible or palpable muscle contraction took place. WBV was performed daily for 20 cycles (alternating stimulation, 26 Hz, amplitude 15 mm), with 1 min pause following each 1 min stimulation cycle. To ensure an appropriate patient-instrument coupling, patients were brought to an almost upright position using a tilt table whenever clinically possible. Otherwise, patients received WBV while in bed with head raised and legs lowered up to 30°. In patients receiving NMES and WBV, both measures were applied simultaneously. For detailed information, see Supporting Information.

Common physiotherapeutic practice consisted of a physician initiated mobilization that was performed only on weekdays without prespecified goals, multiprofessional feedback, and a clear protocol regarding type of mobilization. General ICU treatment in all patients adhered to published standard operating procedures.[18]

Outcomes

Clinical endpoints

Muscle strength was evaluated by Medical Research Council (MRC) score and handgrip dynamometry on the first day the patient became sufficiently awake, at ICU discharge, and at a 12 month in-hospital follow-up. Physical ability was evaluated by Functional Independence Measure (FIM) at ICU discharge and at a 12 month follow-up. Handgrip strength measurements were normalized to each individual's expected standard value, as published by Dodds *et al.*[19] A 6 min walking test was performed at the 12 month in-hospital follow-up, as for most patients, this was not yet feasible at ICU discharge. For comparison to the common physiotherapeutic practice group, MRC score and minimal modified FIM at first awakening and at ICU discharge were available.

Molecular analyses

On the 15th day after ICU admission, all patients received an open surgical muscle biopsy of the *M. vastus lateralis*. Stored muscle samples from the common physiotherapeutic practice group were reanalysed together with the muscle samples from the current trial for molecular data. Histological analyses included an ATPase and Gomori Trichrome staining to evaluate fibre type distribution, specific myocyte cross-sectional area (MCSA), and muscular infiltration with inflammatory cells. We additionally performed real-time polymerase chain reactions and western blot analyses to quantify gene expression and protein content, respectively, to investigate myosin content, pathways of protein synthesis, protein degradation, and local inflammation. Myostatin plasma levels from blood samples obtained at day 14 were evaluated via ELISA. All clinical and molecular measurements were performed by blinded study staff. For detailed information, see Supporting Information.

Statistical analysis

Categorical variables are presented as count and percentages, and metric variables as median and interquartile range. Non-parametric tests were used to analyse differences between groups, specifically Mann–Whitney *U* test for independent samples and Wilcoxon test for dependent samples. Group differences for categorical variables were analysed via χ^2 test. Differences in myocyte cross-sectional area were analysed by the Levene's test and ANOVA. Significance was accepted with $P < 0.05$. Statistical analyses were performed with SPSS IBM (version 25), and graphics were created with GraphPad Prism (version 7.0) and Sigma Plot (version 12.0).

Results

During the 2 year inclusion period, 3147 patients were admitted to two ICUs at the Charité – Universitätsmedizin Berlin and assessed for eligibility; 468 patients met the inclusion criterion of SOFA score ≥9 within the first 72 h after ICU admission, and 50 of those patients were successfully enrolled. We stopped enrolment in the interventional trial after 2 years because of difficult acceptance of open surgical muscle biopsy by legal proxies. An enrolment scheme displaying included and excluded patients is shown in *Figure* 1.

In our cohort selected by multiple organ dysfunction, median SOFA score at admission was 14 and incidence of sepsis was 100%. Overall, patients revealed a significant muscle weakness with median [IQR] MRC score of 3.0 [2.1/3.7] as they first became sufficiently awake. These characteristics are in line with the common physiotherapeutic practice group as shown in *Table* 1.

Table 1 Baseline characteristics

	Common physiotherapeutic practice	Control	Intervention	*P*-value
n	33	17	33	
Age (years)	49 [41/67]	45 [39/61]	54 [45/68]	(a) $P = 0.448$ (b) $P = 0.635$ (c) $P = 0.186$
Gender (m/f)	24/9 [72.7/27.3]	9/8 [52.9/47.1]	24/9 [72.7/27.3]	$P = 0.292$
Relationship status				$P = 0.313$
Married	17 [51.5]	5 [29.4]	19 [57.6]	
Divorced	4 [12.1]	3 [17.6]	0 [0.0]	
Widowed	2 [6.1]	1 [5.9]	1 [3.0]	
Single	6 [18.2]	3 [17.6]	5 [15.2]	
Unknown	4 [12.1]	5 [29.4]	8 [24.2]	
Employment status at admission				$P = 0.114$
Employee	4 [12.1]	5 [29.4]	3 [9.1]	
Unemployed	1 [3.0]	0 [0.0]	0 [0.0]	
Trainee	2 [6.1]	0 [0.0]	0 [0.0]	
Retiree	14 [42.4]	6 [35.3]	10 [30.3]	
Homemaker	2 [6.1]	0 [0.0]	0 [0.0]	
Unknown	10 [30.3]	6 [35.3]	[20/60.6]	
BMI (kg/m^2)	26.9 [23.2/30.3]	26.1 [22.7/27.7]	27.5 [25.2/30.9]	(a) $P = 0.326$ (b) $P = 0.352$ (c) $P = 0.071$
Body surface area (m^2)	2.01 [1.92/2.08]	1.96 [1.79/2.01]	2.03 [1.82/2.20]	(a) $P = 0.152$ (b) $P = 0.696$ (c) $P = 0.110$
Predicted body weight (kg)	71.36 [64.12/74.98]	65.96 [61.43/70.45]	70.45 [65.93/74.98]	(a) $P = 0.200$ (b) $P = 0.933$

(*Continues*)

Table 1 (continued)

	Common physiotherapeutic practice	Control	Intervention	*P*-value
				(c) $P = 0.245$
ICU length of stay (days)	26.0 [20.0/41.0]	26.0 [17.0/30.0]	32.0 [21.0/48.0]	(a) $P = 0.300$ (b) $P = 0.564$
				(c) $P = 0.106$
Time of first awakening (days after admission)	11.0 [8.0/16.5]	11.0 [10.0/23.0]	14.5 [9.0/25.0]	(a) $P = 0.448$ (b) $P = 0.155$
				(c) $P = 0.533$
Survival (non-survivors/survivors)	8/25 [24.2/75.8]	2/15 [11.8/88.2]	4/29 [12.1/87.9]	$P = 0.345$
Catastrophic event leading to ICU admission				$P = 0.952$
ARDS	13 [39.4]	5 [29.4]	10 [30.3]	
Sepsis	8 [24.2]	4 [23.5]	8 [24.2]	
Trauma	6 [18.2]	5 [29.4]	8 [24.2]	
CNS	6 [18.2]	3 [17.6]	6 [18.2]	
Miscellaneous	0 [0]	0 [0]	1 [3.0]	
Pre-existing co-morbidities				
Arterial hypertension	10 [30.3]	7 [41.2]	17 [51.5]	$P = 0.215$
Heart valve disease	6 [18.2]	5 [29.4]	13 [39.4]	$P = 0.164$
Atrial fibrillation	6 [18.2]	2 [11.8]	10 [30.3]	$P = 0.264$
Coronary artery disease	1 [3.0]	2 [11.8]	1 [3.0]	$P = 0.325$
Chronic heart failure	3 [9.1]	3 [23.5]	5 [15.2]	$P = 0.384$
Chronic obstructive lung disease	3 [9.1]	1 [5.9]	3 [9.1]	$P = 0.914$
ICU-acquired co-morbidities				
Pressure ulzera	14 [42.2]	4 [23.5]	14 [42.4]	$P = 0.268$
Acute renal failure	17 [51.5]	9 [52.9]	16 [48.5]	$P = 0.948$
Anaemia	30 [90.9]	13 [82.4]	26 [78.8]	$P = 0.387$
Survived reanimation	4 [12.1]	2 [11.8]	6 [18.2]	$P = 0.735$
Illness severity at ICU admission				
SOFA score	12 [10/14]	14 [12/17]	12 [11/14]	(a) $P = 0.120$ (b) $P = 0.506$
				(c) $P = 0.164$
APACHE	18 [15/23]	26 [19/31]	24 [20/28]	(a) $P = 0.019$ (b) $P = 0.002$
				(c) $P = 0.720$
SAPS2	43 [36/53]	62 [43/68]	57 [44/65]	(a) $P = 0.018$ (b) $P = 0.012$
				(c) $P = 0.448$
Time interval between ICU admission and muscle biopsy				
n	22	11	26	
Biopsy day (days after admission)	15.5 [14.0/20.0]	16.0 [13.5/16.0]	16.0 [13.0/19.0]	(a) $P = 0.396$ (b) $P = 0.454$
				(c) $P = 0.781$
RASS	−3.0 [−3.0/−1.0]	−4.0 [−4.5/−2.25]	−3.0 [−4.0/−1.0]	(a) $P = 0.063$ (b) $P = 0.736$
				(c) $P = 0.051$
Percent of days with RASS > -3	45.0 [33.3/66.7]	28.6 [9.2/47.3]	39.0 [5.6/70.6]	(a) $P = 0.069$ (b) $P = 0.367$
				(c) $P = 0.421$
Noradrenalin (μg/kg * min)	0.05 [0.03/0.10]	0.04 [0.02/0.10]	0.06 [0.03/0.10]	(a) $P = 0.510$ (b) $P = 0.869$
				(c) $P = 0.707$
Noradrenalin days (days noradrenalin was required to maintain blood pressure)	7.5 [6.0/12.0]	10.0 [6.0/11.5]	9.0 [5.0/12.0]	(a) $P = 0.778$ (b) $P = 0.992$
				(c) $P = 0.909$
Cortisone equivalent (mg/day)	52.8 [24.3/72.9]	26.7 [0/102.8]	15.7 [0/71.6]	(a) $P = 0.440$ (b) $P = 0.190$
				(c) $P = 0.961$
Caloric intake (kcal/kg PBW/day)	20.64 [16.76/21.97]	19.01 [13.93/27.44]	15.77 [12.67/20.92]	(a) $P = 0.909$ (b) $P = 0.74$
				(c) $P = 0.438$
Insulin administration (IE/m^2 BSA)	21.47 [15.92/33.4]	20.75 [7.26/32.17]	18.33 [10.29/31.35]	(a) $P = 0.597$ (b) $P = 0.420$
				(c) $P = 0.940$

(*Continues*)

Table 1 (continued)

	Common physiotherapeutic practice	Control	Intervention	*P*-value
Percent of days with septic shock (%)	14.3 [0/33.3]	33.3 [19.8/45.6]	23.6 [8.1/41.1]	(a) $P = 0.029$ (b) $P = 0.240$ (c) $P = 0.299$
Intervention quantity				
Net time patient received physiotherapy per day until muscle biopsy (min)[+]	11.8 [6.5/14.7]	20.4 [18.4/22.2]	21.6 [18.2/25.3]	(a) $P < 0.001$ (b) $P < 0.001$ (c) $P = 0.366$
Net time patient received physiotherapy per day until ICU discharge (min)[+]	13.2 [9.2/16.3]	22.3 [20.0/24.0]	22.2 [20.0/24.0]	(a) $P < 0.001$ (b) $P < 0.001$ (c) $P = 0.927$
Time of additional muscle activating measures per day	—	—	20 min of electrical muscle stimulation and/or 20 min of whole-body vibration as outlined in the protocol	

Values for metric variables are presented as median and interquartile range and for categorical variables as counts and percentages. Mann–Whitney *U* or χ^2 test were used to calculate statistical significance. ARDS, acute respiratory distress syndrome; BMI, body mass index; CNS, central nervous system; PBW, predicted body weight; RASS, Richmond Agitation-Sedation Scale; SAPS2, simplified acute physiology score; SOFA, sepsis-related organ failure assessment. a = common physiotherapeutic practice vs. control; b = common physiotherapeutic practice vs. intervention; c = control vs. intervention; [+]time shown is the time the patient received the actual physiotherapeutic intervention during which the muscle was stimulated not including preparation or documentation.

Treatment in the protocol-based physiotherapy group (control) resulted in a net daily median [IQR] mobilization time of 22.3 [20.0/24.0] minutes, excluding time for preparation and documentation. The intervention group received the same protocol-based physiotherapy with a daily median [IQR] mobilization time of 22.2 [20.0/24.0] minutes plus an additional 20 min of muscle activating measures, resulting in a net daily treatment time of 42 min (*Table* 1). Patients treated by common physiotherapeutic practice received a daily median net mobilization time of 13.2 [9.2/16.3] minutes per day. Patients in the intervention group reached a significantly higher level of mobilization (*Table* 2).

Muscle strength and function

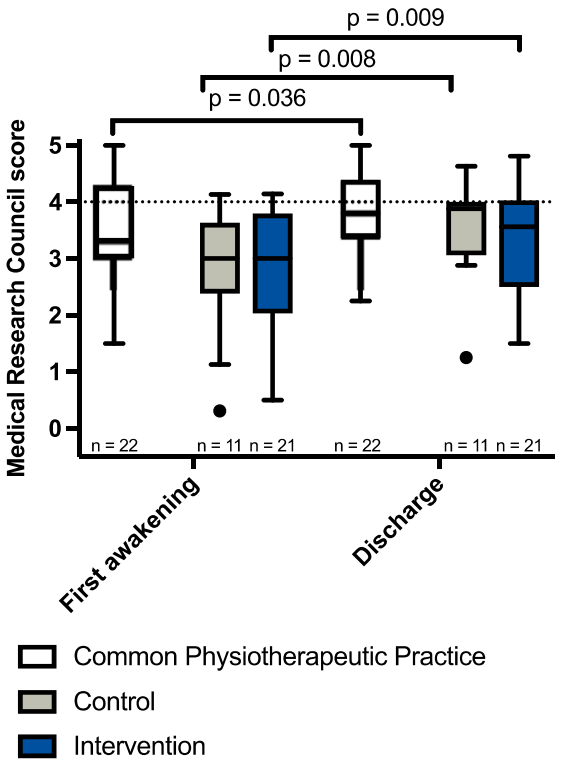
Muscle strength, as measured by MRC score and handgrip strength, or functional mobility assessed by the locomotive component of the FIM score at ICU discharge (*Figure* 2) did not present any significant differences between the intervention and control group. Muscle strength increased significantly from the first day the patients became sufficiently awake until ICU discharge regardless of the therapeutic regimen (*Figure* 2). Nevertheless, patients in both groups remained weak until ICU discharge, with a median MRC score below 4.0 and a median handgrip strength below 40% of

Table 2 Functional outcome at ICU discharge

		Common physiotherapeutic practice ($n = 33$)	Control ($n = 17$)	Intervention ($n = 33$)	*P*-value
mmFIM	Sum score	0.5 [0.5/1.5]	0.5 [0.5/2.0]	0.5 [0.25/2.0]	(a) $P = 0.372$ (b) $P = 0.467$ (c) $P = 0.842$
	Transfer	1 [1.0/2.0]	1.0 [1.0/2.5]	1.0 [0.5/2.0]	(a) $P = 0.269$ (b) $P = 0.495$ (c) $P = 0.657$
	Locomotion	0.0 [0.0/1.0]	0.0 [0.0/1.5]	0.0 [0.0/2.0]	(a) $P = 0.697$ (b) $P = 0.217$ (c) $P = 0.574$
Highest achieved level of mobilization during the ICU stay (*n*/%)	1	2 [6.06%]	1 [5.88%]	0.0 [0.0%]	(a) $P = 0.247$ (b) $P = 0.039$
	2	6 [18.18%]	3 [17.65%]	8 [24.24%]	(c) $P = 0.584$
	3	14 [42.42%]	3 [17.65%]	7 [21.21%]	
	4	10 [30.30%]	7 [41.18%]	10 [30.30%]	
	5	1 [3.03%]	3 [17.65%]	8 [24.24%]	

Values for metric variables are presented as median and interquartile range and for categorical variables as count and percentages. Statistical significance was calculated accordingly through Mann–Whitney *U* or χ^2 test. mmFIM, mini-modified Functional Independence Measure. a = common physiotherapeutic practice vs. control; b = common physiotherapeutic practice vs. intervention; c = control vs. intervention.

Figure 2 Muscle strength measured by Medial Research Council sum score. MRC score showed a significant increase for the control, intervention, and common physiotherapeutic practice group from first awakening until discharge, while no difference between the groups at either time point could be observed. Median values for all three groups stayed below the cut-off value for ICU-acquired weakness. The dotted black line indicates the MRC score cut-off value of 4 for ICU-acquired weakness diagnosis. Data are shown as box plots with median and interquartile range. Statistical significance between groups was tested with Mann–Whitney *U* test and between time points with Wilcoxon test. ● represent outliers that are more than 1.5 interquartile ranges above or below the first or third quartile. ICU, intensive care unit.



expected values (Supporting Information, *Figure* S1). Additionally, all patients presented poor functional mobility at ICU discharge (Supporting Information, *Figure* S1). Furthermore, muscle strength (MRC score) and function (minimal modified FIM) compared with common physiotherapeutic practice showed no significant improvement in the control or intervention group (*Figure* 2, *Table* 2).

At the 12 month follow-up visit, muscle strength and FIM returned to normal values in both groups independently of the study intervention. However, the 6 min walking test revealed significant muscle fatigue, with a median walking distance of 72% of expected reference values at that time, with no difference between the intervention and control group (Supporting Information, *Figure* S1). Long-term follow-up data from the common physiotherapeutic practice group are not available.

Muscle morphology

The surgical muscle biopsy specimen were obtained at median [IQR] day 16 [13/19]. Necrosis was not observed in the ATPase staining in either group. This result was reinforced by the gomori trichrome staining, where no signs of macrophage infiltration were seen (*Figure* 3A/B). In both groups and as earlier published for our common physiotherapeutic practice group, no shift in fibre type distribution was observed, with comparable results with the healthy references (Supporting Information, *Table* S4).

Myofibre size

Myocyte cross-sectional area of slow-twitch (type I, +10%) and fast-twitch (type IIa, +13%, and type IIb, +3%) myofibres as measured on histological cross sections were significantly larger in the intervention group compared with the control group ($P < 0.001$ for all). This finding is pronounced if comparing to the myocyte cross-sectional area of the patients treated with common physiotherapeutic practice. The median MCSA presented an increase of 23% for type I, 33% for type IIa, and 60% for type IIb myofibres in patients of the control group and 36% for type I, 49% for type IIa, and 65% for type IIb myofibres in patients of the intervention group when compared with the common practice group (*Figure* 3C/D/E).

Protein degradation and synthesis pathways

Gene expressions of key mediators of the protein-degradation pathway, such as *TRIM63* (encoding for MuRF-1), *FBXO32* (encoding Atrogin-1), *TRIM62*, *CAPN1* (encoding calpain 1), *CASP3 (*encoding caspase 3), and proteasome subunit *PSMB2* were significantly increased in the muscle of all critically ill patients in comparison with healthy references. No significant differences were observed between intervention and control group (*Figures* 4D/E/F and 5D/E/F). Remarkably, *MSTN* (encoding myostatin) gene expression and myostatin plasma levels, normally associated to sarcopenia, were significantly decreased in both groups and remained unaffected by the intervention (*Figure* 4J/K). The common physiotherapeutic practice group presented similar expression values for *FBXO32*, *TRIM62*, *CAPS3*, *CAPN1*, and *MSTN* as well as similar plasma levels for myostatin in comparison with the control and intervention group (*Figures* 4D/F and 5). Gene expression for TRIM63 and PSMB2 was significantly increased in the control and intervention group as opposed to the common physiotherapeutic practice group (*Figures* 4E and *Figure* 5F).

Myosin heavy chain genes encoding for contractile filaments of the skeletal muscle presented similar expression values in control patients and healthy references. In the intervention group, a significantly increased gene expression

Figure 3 Myocyte cross-sectional area. (A) Representative ATPase stainings for fibre type analysis. Black marker indicates 100 μm. (B) Representative Gomori trichrome stainings for detection of inflammatory infiltration. Black marker indicates 50 μm. (C) MCSA for type I myofibres was significantly increased for the intervention group in comparison with all others groups as well as reference values. Similarly, for the control group, MCSA was significantly increased in comparison with the common physiotherapeutic practice group as well as to reference values. The common physiotherapeutic practice group presented a significantly increased MCSA in comparison with reference values. (D) MCSA for type IIa myofibres in the intervention group showed no differences to reference values while it was significantly larger in comparison with the control group and common physiotherapeutic practice group. These two groups showed a significantly decreased MCSA in comparison with reference values. Nevertheless, the decrease was of a smaller magnitude for the control group with MCSA being significantly larger as opposed to the common physiotherapeutic practice group. (E) Similarly to type I myofibres, type IIb myofibres showed an increased MCSA in the intervention groups in comparison with all other groups as well as reference values. The same applies to the control group that presented a significantly increased MCSA in comparison with common physiotherapeutic practice and reference values. MCSA in the common physiotherapeutic practice presented values similar to reference. Data are shown as frequency of myofibres within the specific myocyte cross-sectional area range (left side of C–E) and box plots with median and interquartile range (right side of C–E). Solid lines represent distribution for groups. The dashed-dotted line refers to the blank bars of the common physiotherapeutic practice group. Statistical significance between groups was tested with Mann–Whitney *U* test or ANOVA. The dotted black line indicates myocyte cross-sectional area in healthy references. ● represent outliers that are more than 1.5 interquartile ranges above or below the first or third quartile.

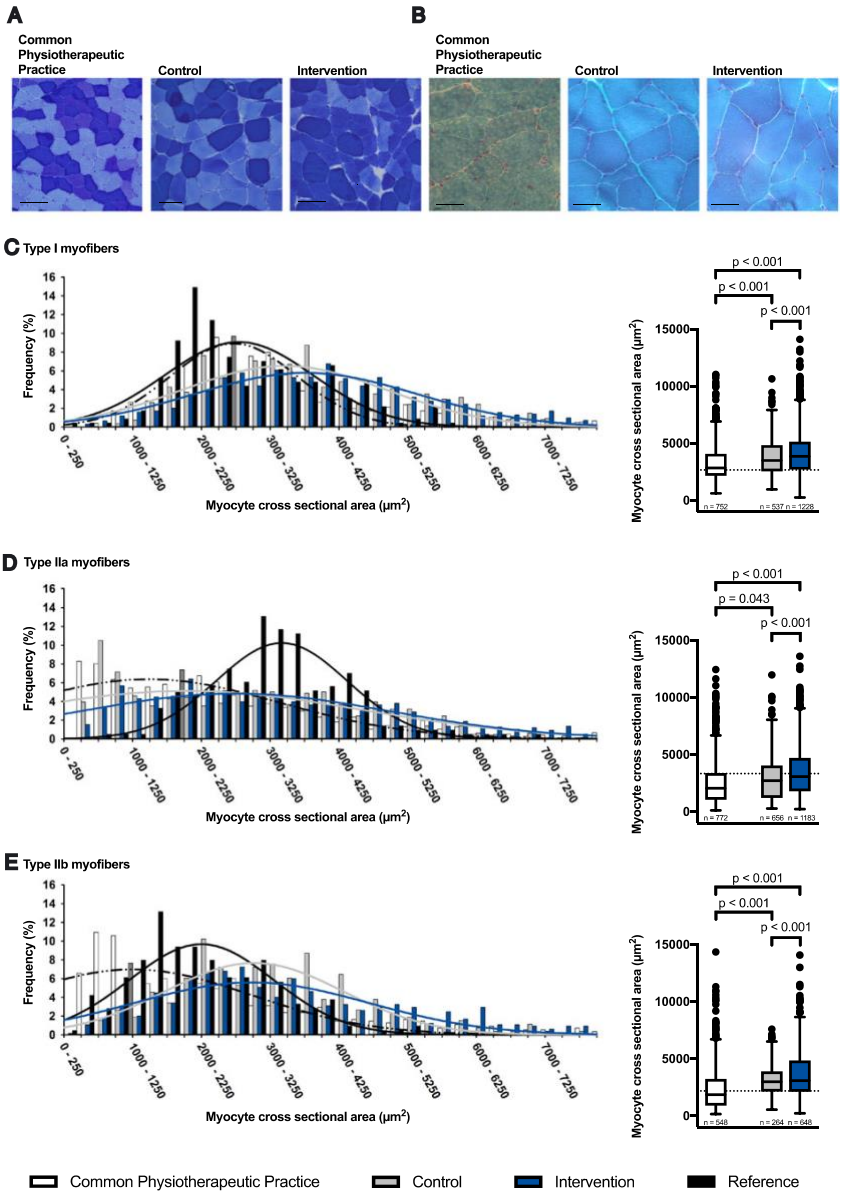
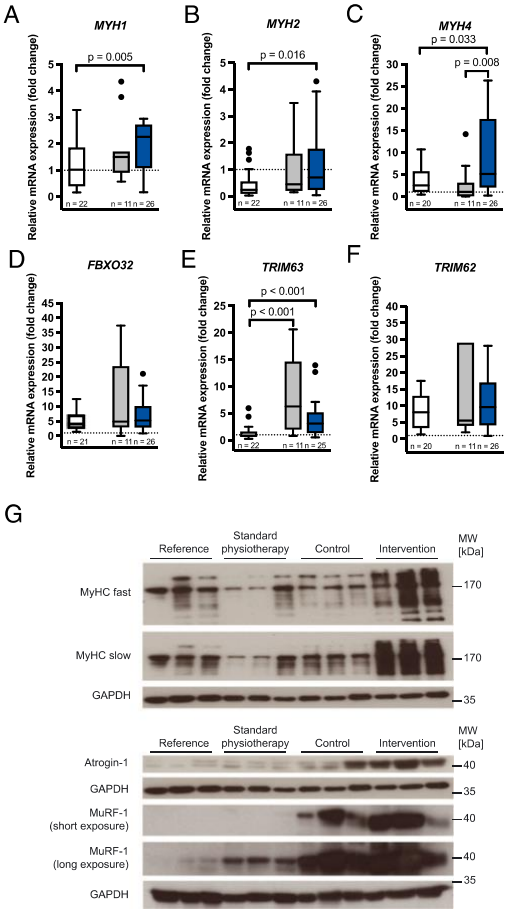


Figure 4 Gene expression for myosin heavy chains and atrogenes as well as protein content for myosin and key proteins of the ubiquitin proteasome system. (A) *MYH1* gene expression was significantly increased in the intervention group in comparison with the common physiotherapeutic practice group and reference values. (B) *MYH2* gene expression was significantly decreased in the common physiotherapeutic practice group as opposed to reference values. This decrease was mitigated through a significant increase in the intervention group. (C) *MYH4* gene expression was significantly increased in the intervention group in comparison with all other groups as well as reference values. Also for the common physiotherapeutic practice group, gene expression was significantly elevated over reference values. (D) *FBXO32* and (F) *TRIM62* show a significantly increased gene expression for all groups over reference values without between group differences. (E) *TRIM63* gene expression was significantly elevated over reference values and the common physiotherapeutic practice group in the control and intervention group. (G) Representative western blot for MyHC fast, MyHC slow, Atrogin-1, and MuRF-1. Protein content for (H) fast myosin and (I) slow myosin was significantly increased over reference values. No differences between groups could be observed for (J) *MSTN* gene expression or for (k) Myostatin relative serums concentration, while all groups presented values significantly lower than reference values. mRNA expression and protein content were normalized to GAPDH (*MYH1*, *MYH2*, *MYH3*, *FBXO32*, *TRIM63*, and *TRIM62*) and HPRT1 (*MSTN*) with mean set as 1 and expressed as fold change. The dotted black line indicates mean reference values from healthy controls. Data are shown as box plots with median and interquartile range. Statistical significance between groups was tested with Mann–Whitney *U* test. ● represent outliers that are more than 1.5 interquartile ranges above or below the first or third quartile.

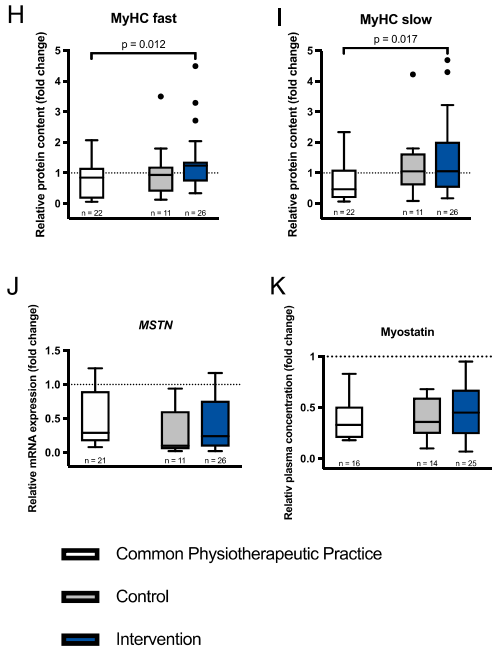


for *MYH1* (encoding for type IIX/D muscle fibres) and *MYH4* (encoding for type IIb muscle fibres) was observed in comparison with healthy references, while only *MYH4* expression increased significantly over the control group (*Figure* 4A/C). *MYH2* gene expression (encoding for type IIx muscle fibres) was not affected by the intervention, and expression levels were similar to levels in healthy references for both groups (*Figure* 4B). The intervention group showed a significantly higher *MYH1*, *MYH2*, and *MYH4* expression compared with the common physiotherapeutic practice group (*Figure* 4A/B/C).

Protein content

Myosin protein content presented values similar to healthy references in both groups without a difference between the control and intervention group. When comparing the

Figure 4 Continued



intervention with common physiotherapeutic practice group, we observed a significantly increased myosin protein content for both slow-twitch and fast-twitch myosin heavy chain protein (*Figure* 4H/I), more specifically, MyHC fast increased by 46% and MyHC slow by 130%.

Inflammation

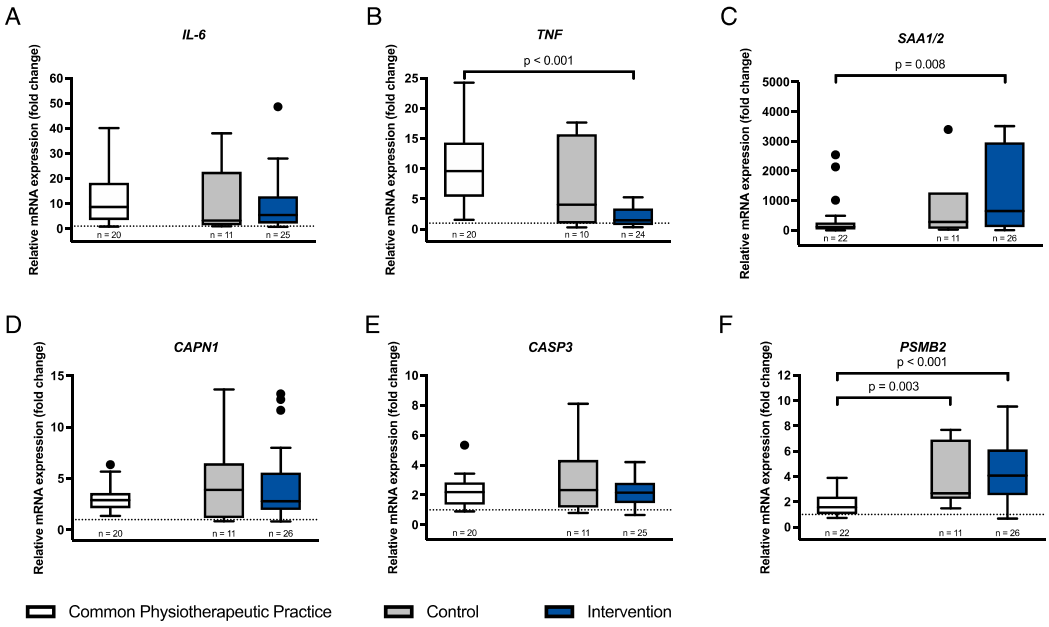
The inflammatory cytokines *IL-6* (encoding for interleukin 6) and *SAA1/2* (encoding for serum amyloid a1/2) were both significantly increased above values for healthy references while *TNF* (encoding for tumor necrosis factor alpha) presented values similar to healthy references for the intervention and control group (*Figure* 5A/B/C). No difference between these two groups was observed (*Figure* 5A/B/C). When comparing common physiotherapeutic practice with both these groups, we observed a significantly increased gene expression for *TNF* and a significantly decreased gene expression for *SAA1/2* as opposed to the intervention group but no differences in comparison with the control group (*Figure* 5B/C). *TNF* gene expression was also increased above healthy references for the common physiotherapeutic practice group (*Figure* 5B).

Discussion

In our study, we investigated the impact of muscle activating measures in addition to protocol-based physiotherapy on muscle wasting, protein homeostasis, and muscle function in a selected cohort of patients with MODS and sepsis. Myocyte cross-sectional area in light microscopy was larger in patients receiving additional muscle activating measures as opposed to the control group. Interestingly, the application of protocol-based physiotherapy alone had a significant impact as opposed to common physiotherapeutic practice as it lead to a prevention of muscle atrophy and significantly larger myocyte cross-sectional area. Despite preserving myocyte cross-sectional area, the intervention did neither prevent muscle weakness at first awakening nor did it enhance muscle strength and function at ICU discharge or at the 12 month follow-up. Matching the histological results, myosin gene expression was increased, whereas indicators of protein degradation were equally induced in all patients, regardless of the therapeutic regimen. Hence, the difference in muscle fibre size is likely attributed to an exercise induced improvement in myosin synthesis, rather than to a suppression of protein degradation.

Early mobilization of critically ill patients is generally recommended in international guidelines, whereas additional muscle activating measures are not recommended because of lack of evidence.[20,21] Implementation of mobilization protocols during early critical illness improves safety, intensity, and degree of mobilization as also shown in our data.[22] However, in regard to functional outcome, the effectiveness of early mobilization remains inconsistent, which is corroborated by our findings.[8,10,11] Moreover, the large scaled randomized controlled interventional trial by Fossat *et al*. could

Figure 5 Gene expression of markers for muscle inflammation and muscle protein degradation. Gene expression for (A) *IL-6* and (C) *SAA1/2* was significantly increased over reference values for all three groups, while in contrast, gene expression for (B) *TNF-α* was only increased above reference values for the common physiotherapeutic practice group. (A) *IL-6* did not show differences between the three groups. Meanwhile, the intervention group had a significantly decreased gene expression for (B) *TNF-α* and an increased gene expression for *SAA1/2* in comparison with the common physiotherapeutic practice group. Gene expression for (D) *CAPN1*, (E) *CASP3*, and (F) *PSMB2* was significantly increased over reference values for the control, intervention, and common physiotherapeutic practice group. (D) *CAPN1* and (E) *CASP3* did not show any further differences between the groups while for (F) *PSMB2*, gene expression in the control and intervention group was significantly increased in comparison with the common physiotherapeutic practice group. The dotted black line indicates reference values from healthy controls. Statistical significance between groups was tested with Mann–Whitney *U* test. ● represent outliers that are more than 1.5 interquartile ranges above or below the first or third quartile.



show that application of in-bed cycling and NMES has no effect on clinical outcome, what is in agreement with our clinical results regarding muscle strength and function.[17] Factors likely to influence the effect of physiotherapy on muscle strength and functional outcome are time point of initiation of early mobilization, the scope of protocols, and, crucially, the patient cohort investigated. Significant differences are present in the different studies with respect to severity of illness by MODS and incidence of sepsis as major risk factors predisposing patients to ICU-acquired weakness.[9,11,23] In this special patient cohort, there is no evidence regarding the molecular effect of early mobilization except a pilot trial by Hickmann *et al*. lacking clinical data.[24] Our randomized trial is unique because it is the first that enables the interpretation of a broad molecular characterization in the light of clinical outcome data. Additionally, the high standard of early protocol-based physiotherapy utilized in the intervention and control group as well as the retrieval of open surgical muscle biopsies in patients with MODS distinguish our trial from previous investigations. In our molecular analyses, we found no evidence that muscle activating measures are harmful, as discussed by Hodgson *et al*., but rather preserve myocyte cross-sectional area when applied early in patients with MODS and sepsis. These findings are in line with recently published data by Hickmann *et al*. presenting a pilot trial where very early mobilization including bed cycling of septic patients led to preservation of myocyte cross-sectional area.[24]

Interestingly, our finding cannot be attributed to an intervention-associated suppression of muscle protein degradation, because MuRF-1 and Atrogin-1 gene expression and protein content were increased in the control and intervention group. It rather can be attributed to an increase in myosin heavy chain gene expression indicating that the muscle protein synthesis pathway was activated. Importantly, in light of the effect the intervention had on myocyte cross-sectional area and myosin content, the up-regulation of MuRF-1 and Atrogin-1, which are known key mediators of protein degradation, appears to be counterintuitive.[1,2] We published data on *TRIM63*/MuRF-1 and *FBXO32*/Atrogin-1 expression in muscle of critically ill patients showing their role during muscle atrophy.[1] However, both MuRF-1 and Atrogin-1 are not exclusively involved in pathological muscle atrophy. They also play an important role in muscle remodelling and hypertrophy especially during resistance exercise training as shown in healthy volunteers.[25,26] In our cohort of critically ill septic

patients, we found an up-regulation of MuRF-1 in muscle of patients of the control and intervention group in contrast to those patients who received common physiotherapeutic practice. We therefore hypothesize that up-regulation of MuRF-1 was caused by muscle activation and is reflective for muscular remodelling caused by protocol-based physiotherapy with and without muscle activating measures in comparison with common physiotherapeutic practice rather than representing a pathological process. This remodelling hypothesis is corroborated by an up-regulation of the muscle synthesis mRNA expression *MYH1*, *MYH2*, and *MYH4* encoding for slow and fast type myosin. Because *FBXO32*/Atrogin-1 was increased in all patients, we think that this is a residual effect of inflammation. This view is supported by increased gene expressions of *IL-6*, *SAA1/2*, and *TNF* in skeletal muscle tissue of both groups, which was not affected by muscle activating measures on top of high-quality protocol-based physiotherapy at this stage of the disease severity. These findings are in line with the observation of Kayambu *et al*., who found a time dependent and pronounced reduction of IL-6 levels over time in patients receiving early mobilization, but no significant group specific differences in IL-6 plasma concentrations at the individual time points. Because IL-6 was shown to play a major role in muscle protein synthesis, increased *IL-6* mRNA levels support the hypothesis of an induced muscle remodelling.

Overall, these findings suggest that muscle remodelling with a net positive effect on preservation of muscle fibre size was induced by protocol-based physiotherapy and pronounced by additional muscle activating measures. Decreased gene expression and plasma levels of myostatin can be understood as a general compensatory regulation to reduce further protein degradation without a response to the intervention. We suspect myostatin neither to be a key regulator responsible for ICUAW nor a promising target for future interventions.

A discrepancy between muscle atrophy and muscle function has already been noticed by Dos Santos and colleagues.[27] They showed that the contractile capacity of skeletal muscle is only inconsistently related to muscle atrophy and muscle regain in long-term outcome of critically ill patients. Our data extend their findings indicating that even if muscle atrophy is prevented, it does not inevitably enhance muscle strength and functional independency in patients with MODS.

When comparing the group receiving additional muscle activating measures with the common physiotherapeutic practice group, we observed a remarkable improvement in muscle mass via muscle remodelling, astonishingly the improvement does not reflect clinically.

In conclusion, the application of muscle activating measures in addition to early protocol-based physiotherapy in critically ill patients with MODS and sepsis syndrome did not cause any harm and prevented muscle atrophy. We therefore see a role for muscle activating measures as part of early mobilization of critically ill patients in the future. Nevertheless, an improvement in muscle strength or function – attributable to the prevention of atrophy – could neither be observed at ICU discharge nor at 12 month follow-up. Long-term outcome is influenced by the mode and quality of rehabilitation therapy performed between ICU discharge and follow-up visit. We could unfortunately not evaluate this factor. The hypothesis that the clinical improvement during rehabilitation would be greater in patients with integer muscle morphology can be discussed. Studies investigating the clinical pathway from ICU admission to the end of the rehabilitation process are therefore needed.

Limitations

Our exploratory trial has limitations. The sample size is as a result of inclusion difficulties because of the open surgical muscle biopsy, relatively small and therefore prone to type I as well as type II error. An inherent limitation of clinical trials in a critical care setting is the fact that patients are usually admitted unplanned. In our trial, that was the case for all patients. It was therefore not possible to perform a specific pre-admission evaluation to establish a baseline regarding, for example, nutritional status, functional status, and cognitive performance. Moreover, 13 patients that were randomized could not be included into the molecular analysis because of withdrawal of consent or discharge respectively death before the biopsy date. Further, the nature of the intervention prevented blinding of the treating physician, which must be respected as a bias. Current real word practice regarding mobilization is as previously shown not meeting guideline recommendations.[28,29] We considered it would nevertheless be unethical to perform anything less than protocol-based physiotherapy, which is our clinical standard, in the control group. We therefore had to include a common physiotherapeutic practice group, as an historic comparison, closely resembling the real world mobilization practice.

Long-term outcome is likely influenced by the mode and quality of rehabilitation therapy performed between ICU discharge and follow-up visit. We could unfortunately not evaluate this factor. The hypothesis that a high quality rehabilitation programme would have a greater benefit in patients with integer muscle morphology can be discussed.

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The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle.[30]

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Physiotherapy protocol
Table S2 Specifications of gene expression assays from Applied Biosystems
Table S3 Specification for antibodies used for Western Blots
Fig. S1 Muscle strength and functional independence. **a** Medical Research Council score (MRC) increased significantly between first awakening and discharge in both groups. A further increase between discharge and 12-month follo-up could only be observed for the intervention group. The dotted black line indicates an MRC score cut-off value of 4 for ICUAW diagnosis. **b** Relative hand grip strength also increased significantly between first awakening and discharge in both groups while a further increase until 12-month could only be observed for the intervention group. The dotted black line indicates reference values for age and gender matched references. **c** 6 minute walking distance was reduced in both groups at 12-month follow-up. The dotted black line indicates reference values for age and gender matched references. Data are shown as box plots with median and interquartile range. Statistical significance between groups was tested with Mann-Whitney U Test and between timepoints with Wilcoxon-Test. ● represent outliers which are more than 1.5 interquartile ranges above or below the first or third quartile.
Table S4 Fiber type distribution

Conflict of interest

T.W., J.J.G., N.M.C., K.H., J.M., S.F.R., J.S., C.D.S., S.M., K.M., S.S., J.F., and S.W.-C. declare that they do not have a conflict of interest.

ESICM Best abstract award

1 2016 Best abstract award European Society of Intensive Care Medicine (ESICM), Mailand 2016: 'Randomized controlled trial using daily protocol-based physiotherapy or protocol-based physiotherapy with additional electrical muscle stimulation (EMS) in critically ill patients to prevent intensive care unit (ICU) acquired weakness (ICUAW)' T. Wollersheim, J. Malleike, K. Haas, N. Carbon, J. Schneider, C. Birchmeier, J. Fielitz, S. Spuler, S. Weber-Carstens in Sivakumar S, Taccone FS, Desai KA, Lazaridis C, Skarzynski M, Sekhon M, et al. ESICM LIVES 2016: Part two: Milan, Italy. 1–5 October 2016. Intensive Care Med Exp 2016, Sep;4(Suppl 1):30.

2 2017 Best abstract award European Society of Intensive Care Medicine (ESICM), Vienna 2017: 'Effect of protocol-based physiotherapy and muscle activating measures on muscle synthesis and degradation balance in intensive care unit acquired weakness' J. Grunow, T. Wollersheim, N.M. Carbon, M. Kny, M. Giesecke, C. Birchmeier, J. Fielitz, S. Weber-Carstens; ESICM LIVES 2017: 30th ESICM Annual Congress. September 23–27, 2017. Intensive Care Medicine Experimental 2017, 5(Suppl 2):0403.

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Online Data Supplement

Muscle wasting and function after muscle activation and early protocol-based physiotherapy: an explorative trial

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Study Design

The trial is a prospective randomised controlled interventional trial (ISRCTN19392591). Enrolment of patients took place in two intensive care units within the Department of Anesthesiology and Operative Intensive Care Medicine (CCM, CVK) at Charité - Universitätsmedizin Berlin, Germany. The Charité institutional review board granted ethical approval (Charité EA 2/041/10).

Participants

Inclusion criteria:

- Mechanical ventilation
- SOFA ≥ 9 within the first 72 hours after ICU admission

Exclusion criteria:

- age < 18 years
- insulin-dependent diabetes mellitus
- body-mass-index > 35 kg/m^2
- preexisting neuromuscular disease
- moribund health status
- participation in another clinical trial
- prior ICU treatment or mechanical ventilation for more than 72 hours before inclusion
- pregnancy
- not ambulating before admission

Enrolment process

Patients were screened daily on ward rounds through participating ICUs by study physicians for eligibility to be enrolled into the trial. In case of fulfillment of all inclusion criteria without applicability of an exclusion criterion the legal proxy was approached for enrolment into the trial.

Muscle biopsy samples (n = 6) from patients undergoing elective orthopedic surgery but who were otherwise healthy were included as references to determine baseline values. Similarly, plasma sampls from healthy volunteers were included to determine baseline values for myostatin plasma concentrations (n = 91).

Randomisation

Primary randomisation with a 1:2 ratio into two groups of 30 [control group and intervention group] was done with sealed opaque envelopes. Sequential allocation of patients to first NMES + WBV, second NMES and, third

WBV within the intervention group was done for further subrandomisation. Study staff was blinded during the assessment of all outcome parameters and had no influence on treatment decisions.

Procedures

General ICU treatment adhered to published standard operating procedures [1-3].

Protocols for performed interventions

Protocol-based physiotherapy

Protocol-based physiotherapy was performed twice daily for 25/35 minutes by a trained and dedicated study physiotherapist seven days a week. Further muscle activating measures were performed daily by trained study staff as described below. Every morning the mobilisation goal was defined by a multiprofessional case conference considering patients ability to participate in terms of consciousness, haemodynamic stability and respiratory stability as outlined in Supplement Table S1.

1 **Table S1** Physiotherapy protocol

Level of mobilisation	**Level 1**	**Level 2**	**Level 3**	**Level 4**	**Level 5**
Description of the level of mobilisation according to patient's status	RASS: -5 • haemodynamically unstable • respiratorically unstable • ICP not compensable • "minimal-handling"	RASS: -5 to -4 • haemodynamically stable • respiratorically stable • ICP stable	RASS: -3 to -2 • haemodynamically stable • respiratorically stable • ICP stable • active participation	RASS: ≥-2 • haemodynamically stable • respiratorically stable • ICP stable • active participation	RASS: ≥-1 • no intensive care monitoring necessary • transfer to general ward planned
Mobilisation	Ø	• passive mobilisation of upper and lower extremities	• passive mobilisation • assistive mobilisation • active mobilisation	• passive mobilisation • assistive mobilisation • active mobilisation • activities of daily living	• passive mobilisation • assistive mobilisation • active mobilisation • activities of daily living • intensified therapy
Respiratory therapy	Ø	• as medically indicated • pneumonia and atelectasis prophylaxis	• as medically indicated • pneumonia and atelectasis prophylaxis	as medically indicated • pneumonia and atelectasis prophylaxis	as medically indicated • pneumonia and atelectasis prophylaxis
Active transfer	Ø	Ø	• increasing mobility within the bed	• supine → lateral → sitting → standing (assistive	•• supine → lateral → sitting → standing (assistive

(Mobilisation with participation of the patient)				devices are permitted)	devices are permitted)
Mobility training	Ø	Ø	Ø	yes	yes
Passive transfer (Mobilisation without participation of the patient)	Ø	• sitting in the Thekla®* • standing in the Thekla® *	• sitting in the Thekla®* • standing in the Thekla®*	If necessary • sitting in the Thekla®* • standing in the Thekla®*	If necessary • sitting in the Thekla®* • standing in the Thekla®*
Positioning therapy	• Prophylaxis • specific positioning	• Prophylaxis • specific positioning	• Prophylaxis • specific positioning	• Prophylaxis • specific positioning	• Prophylaxis as required • specific positioning
Frequency	NA	• 2x daily	• 2x daily	• 2x daily	• 2x daily
Duration per session	NA	• 25-35 minutes	25-35 minutes	25-35 minutes	25-35 minutes

**Thekla® refers to a chair produced by Hanse-Medizintechnik, which was developed as an assistive device that enables passively transferring an unconscious patient from supine into upright standing position.*

Protocol for neuromuscular electrical stimulation

Neuromuscular electrical stimulation (NMES) was performed bilaterally on 8 different muscle groups (M. tibialis anterior, M. triceps surae, M. vastus lateralis, posterior thigh, M. biceps brachii, M. triceps brachii, wrist extensors, wrist flexors) daily for 20 minutes starting on the day of enrolment (MUSKELaktiv 2-Kanal, schwa-medico®, Germany; Physiomed-Expert-2-Kanal, Physiomed®, Germany). Electrical impulses of 350 µs at 50 Hs with a ramp of 1 second and an on-time of 6/10 seconds as well as an off-time of 10/15 seconds. Electrical current was increased to maximal 70 mA until visible or palpable muscle contraction in unconscious respectively contraction or discomfort in awake patients occurred. If no contraction could be observed NMES was performed with 40 mA.

Protocol for Whole-body vibration

Whole-body vibration was performed daily for 20 cycles (alternating stimulation, 26 Hs, amplitude 15 mm) with one minute stimulation and a one minute break using the Galileo® (Novotec®, Germany) instrument. To assure a complete patient-instrument coupling haemodynamically stable patients were brought into an almost upright

position (80-90° with 90° meaning upper body was perpendicular to the floor) with the use of a Thekla® (Hanse-Medizintechnik, Germany) while lightly flexing their knees (0/10°). Haemodynamically unstable patients received whole-body vibration within the bed with head raised and legs lowered up to 30°. Furthermore, to ensure patient-instrument coupling knees and hips were flexed lightly (knees: 0/10° and hip: 10/30°).

Protocol for first adequate awakening and MRC

Screening for adequate awakening was performed daily by study physicians. In order to be classified as adequately awake a patient had to have a Richmond Agitation and Sedation Score between -1 and +1 as well as an adequate response to three out of the five following verbal commands: "Open/close your eyes," "Look at me," "Open your mouth and put out your tongue," "Nod your head," and "Raise your eyebrows when I have counted up to 5." on two consecutive days as previously published by DeJonghe et al.[4]. Medical Research Council score was assessed by trained study staff on a 6 point scale (0 no visible or palpable contraction; 1 visible or palpable contraction without limb movement; 2 movement without gravity; 3 movement against gravity; 4 movement against resistance; 5 full force) in 8 different muscle groups bilaterally (wrist extension, wrist flexion, elbow extension, elbow flexion, should aberduction, hip flexion, knee extension, ankle extension, ankle flexion). The sum score was divided by the number of muscle examined.

Protocols for performed analyses

Protocol for muscle biopsy and histological analyses

Muscle biopsy specimen were obtained 15 days after onset of critical illness respectively the closest day to this date in case of any circumstances not allowing muscle biopsy on the predefined date (Ethical approval: Charité EA 2/041/10). Afterwards Lidocain was administered as a local anaesthetic to the incision site located in the distal third of the *Vastus lateralis* muscle. A surgical incision through skin and fascia was done to expose muscle tissue and retrieve the muscle biopsy specimen. If necessary bleeding was stopped and the wound was closed as well as dressed. We obtained biopsy specimens from 11 patients in the control group and 26 patients in the intervention group. Furthermore 6 control biopsy specimens were obtained from volunteers undergoing elective orthopaedic surgery.

For gene expression and protein analyses biopsy specimens were directly snap frosen in liquid nitrogen and for immunohistochemistry and metachromatic ATPase staining they were mounted and frozen under cryoprotection. Specimens were stored at -80°C.

Biopsy specimens for histological analyses (Haematoxylin & Eosin or Gomori trichrome) were fixed in 3.7% paraformaldehyde, embedded into paraffin and sectioned using a cryotome (Leica CM3050 S) into 10 µm thick sections. Haematoxylin and Eosin and Gomori trichrome staining was performed as recently published [5-9]. For

metachromatic ATPase staining muscle biopsy specimens were embedded in tissue-freezing medium (Triangle Biomedical Sciences, Durham, NC) supplemented with gum tragacanth (Sigma, St. Louis, MO). Subsequently the samples were immediately frozen in liquid nitrogen-cooled isopentane (2-Methylbutan; Fa. Carl Roth) and stored afterwards in liquid nitrogen until sectioning. Mounted tissue samples were sectioned with a cryotome (Leica CM3050 S, 10 µm) for metachromatic ATPase staining which was performed as previously published [5, 8, 10]. Myocyte cross sectional area was determined on pictures of histological sections of in average 115.5 (IQR: 106 - 136) myofibers per patient from 6 control patients, 19 patients in the standard physiotherapy group, 11 patients in the control group and 25 patients in the intervention group with Image J (Version 1.47v).

GLUT4 immunohistochemical stains were performed with a rabbit anti-GLUT4 antiserum (1154p) provided by Hoffmann-La Roche.

Protocol for quantification of gene expression

TRIzol® Reagent (Invitrogen) was used to extract total RNA from muscle biopsy specimens according to the manufacturer's protocol and as recently published [[5, 6, 9]. Reverse-transcription of 1 µg RNA into cDNA was performed by using the SuperScript® First-Strand Synthesis System (Invitrogen) according to the manufacturers protocol and as recently published [5, 6, 8, 9]. TaqMan® Universal PCR Mastermix (Applied Biosystems) was used for real-time polymerase chain reaction (RT-PCR) together with commercially available primer and probe sets (Applied Biosystems) (see Table S2). Step-One™ Plus thermocycler (Applied Biosystems) was used for all PCR reactions. All experiments were conducted as manufacturer's instructions stated. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene expression was used to normalise gene expression, in order to correct for a variance in mRNA extraction and cDNA synthesis efficiency between samples. For normalisation values of volunteers undergoing elective orthopaedic surgery were set as one and patient values were expressed as fold change [5, 8, 10].

Table S2 Specifications of gene expression assays from Applied Biosystems

Gene name	**Catalogue number**
MYH1	Hs00428600_m1
MYH2	Hs00430042_m1
MYH4	Hs00757977_m1
TRIM63	Hs00822397_m1
TRIM62	Hs00217089_m1
FBXO32	Hs00369714_m1

MYH indicates myosin heavy chain; TRIM, tripartite motif containing protein and FBXO, F-box containing protein.

Protocol for protein analyses

All protein analyses were performed as previously published [5, 8, 10]. Skeletal muscle biopsy specimen were homogenised (30s, 2000 rpm) in ice-cold extraction buffer 1:3 wt/vol (10 mM Tris HCl, pH 7.5, 140 mM NaCl, 1 mM EDTA, 25% glycerol, 0.5% sodium dodecyl sulfate (SDS), 0.5% Nonident P-40) supplemented with 0.1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 100 ng/ml protease inhibitor cocktail (Roche). Clearing was done through centrifugation (4°C, 10 min, 14000 rpm). The supernatant was assayed for protein concentration using Bio-Rad Protein Assay and stored at -80°C until analyses. SDS polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins according to their molecular weight. Afterwards proteins were blotted onto nitrocellulose or PVDF membranes (Amersham Pharmacia Biotech). Primary and secondary antibodies used are shown in Table S3. Visualisation was performed by enhanced chemiluminescence (ECL) detection reagent (Amersham Pharmacia Biotech).

Table S3 Specification for antibodies used for Western Blots

Antibody	Clone	Manufacturer	Concentration	Membrane
anti-total Myosin heavy chain	MF20	Sigma	1:3000	nitrocellulose
anti-fast Myosin heavy chain	MY32	Sigma	1:3000	nitrocellulose
anti-slow Myosin heavy chain	NOQ7	Sigma	1:3000	nitrocellulose
anti-MuRF1		R&D	1:200	PVDF
anti-Atrogin1		Abcam	1:500	PVDF
anti-mouse IgG HRP		CellSignaling	1:3000	
anti-goat IgG HRP		Abcam	1:3000	

Plasma analysis

Myostatin plasma concentration were analysed with R&D Systems GDF-8/Myostatin Quantikine ELISA Kit (Catalogue number DGF80)

Statistics

Counts and percentages are used to present categorical variables and median and interquartile range to present metric variables. Due to small group size non-normal distribution was assumed. Statistic test were selected accordingly. Specifically, non-parametric tests for metric variables and differences between groups. Kruskal-

Wallis and Mann-Whitney U tests were used for independent samples and Wilcoxon signed-rank test test for dependent samples. Chi-Square test was used for group differences and categorical variables. $P < 0.05$ was accepted as significant. Myocyte cross sectional area shift was analysed through ANOVA and validated through Welch- and Brown-Forsythe test in case of inhomogenous variance tested by the Levene's Test. Statistical analyses were performed with SPSS IBM (version 25), and graphics were created with GraphPad Prism (version 7.0) and Sigma Plot (version 12.0).

1 Supplement results:

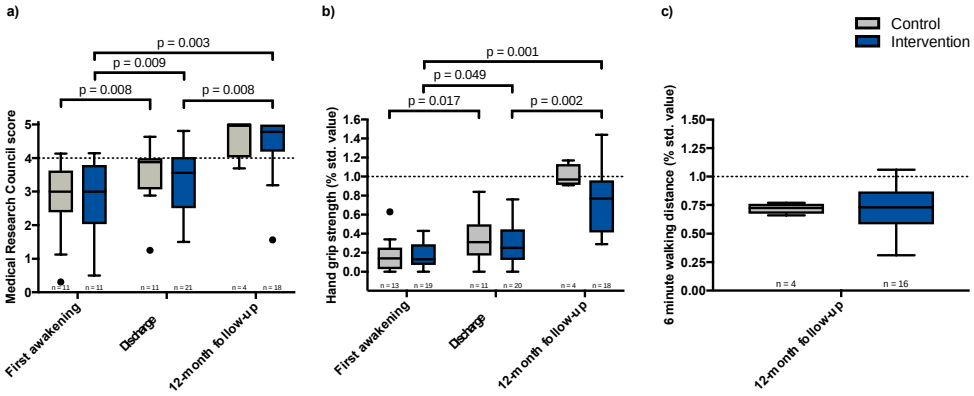

Fig. S1 Muscle strength and functional independence. **a** Medical Research Council score (MRC) increased significantly between first awakening and discharge in both groups. A further increase between discharge and 12-month follo-up could only be observed for the intervention group. The dotted black line indicates an MRC score cut-of f value of 4 for ICUAW diagnosis. **b** Relative hand grip strength also increased significantly between first awakening and discharge in both groups while a further increase until 12-month could only be observed for the intervention group. The dotted black line indicates reference values for age and gender matched references. **c** 6 minute walking distance was reduced in both groups at 12-month follow-up. The dotted black line indicates reference values for age and gender matched references. Data are shown as box plots with median and interquartile range. Statistical significance between groups was tested with Mann-Whitney U Test and between timepoints with Wilcoxon-T est.
represent outliers which are more than 1.5 interquartile ranges above or below the first or third quartile.

Table S4 Fiber type distribution

	Control	Intervention	p-value
Type I	20.5 [16.0/25.3]	23.6 [17.5/26.7]	0.399
Type IIa	46.4 [39.1/63.4]	44.3 [31.7/54.8]	0.140
Type IIb	26.5 [16.7/45.2]	31.7 [19.6/41.6]	0.124

Values represent frequency of fiber types and are presented as median and interquartile range. Statistical significance was tested with Kruskal-Wallis-Test

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12.Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

13. Publikationsliste

1. Wollersheim T, **Grunow JJ**, Carbon NM, Haas K, Malleike J, Ramme SF, et al. Muscle wasting and function after muscle activation and early protocol-based physiotherapy: an explorative trial. J Cachexia Sarcopenia Muscle. 2019.
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