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Direktorin: Univ.-Prof. Dr. med. Claudia Spies

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Neuromuskuläres Organversagen auf der Intensivstation und perioperativ

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Dr. med. Tobias Wollersheim

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Dekan:	Prof. Dr. med. Axel R. Pries
1. Gutachter/in:	Prof. Dr. med. Heidrun Lewald, München
2. Gutachter/in:	Prof. Dr. med. Bettina Jungwirth, Ulm

Inhaltsverzeichnis

Abkürzungsverzeichnis

ICUAW = Intensive Care Unit acquired weakness
SIRS = Systemix Inflammatory Response Syndrome
CIP = Critical Illness Polyneuropathy (CIP)
GLUT4 = Glukosetransporter 4
MuRF1 = muscle-specifc ring finger 1
UPS = Ubiquitin-Proteasom-System
PICS = Post Intensive Care Syndrom
TRIM62 = Tripartite Motif Containing 62
mRNA = messenger Ribonukleinsäure
POAW = Perioperative Acquired weakness
SOFA = Sepsis-related Organ Failure Assessment (SOFA
APACHE II = Acute Physiology And Chronic Health Evaluation II
SAPS = Simplified Acute Physiology Score
IL-6 = Interleukin 6
SAA1 = Serum Amyloid A1
SAA2 = Serum Amyloid A2
FoxO1 =Forkhead-Box-Protein O1
FoxO3 = Forkhead-Box-Protein O3
NFκB = nuclear factor kappa-light-chain-enhancer of activated B
IKKβ = inhibitor of nuclear factor kappa-B kinase subunit beta
TNF-α = Tumornekrosefaktor-α
IGF-1 = insulin growth factor-1
PI3K = Phosphoinositide 3-Kinase
PKB = Proteinkinase B
mTOR = mammalian target of rapamycin
FoxO = Frokhed-Box-Protein O (FoxO)
FIM = Functional Independence Measures

1. Einleitung

1.1 Klinisches Bild des erworben neuromuskulären Organversagens

Die erworbene Muskelschwäche während intensivmedizinischer Behandlung ist eine relevante Komplikation, welche die quergestreifte Skeletmuskulatur betrifft (6, 7). Die Inzidenz ist abhängig vom Schweregrad der Grunderkrankung und variiert in Untersuchungen von 25% aller mechanisch beatmeten Intensivpatienten bis hin zu mehr als 70% bei Patienten mit Sepsis und Multiorganversagen (8). Das sind alleine in Deutschland mindestens 400.000 Fälle pro Jahr. Ein akuter Muskelmassen- und Muskelfunktionsverlust führt zu einer körperlichen funktionellen Einschränkung, kann bereits am ersten Behandlungstag beginnen und persistiert als Langzeitfolge auch noch Jahre nach Entlassung von der Intensivstation (9-13). Akute körperlich funktionelle Einschränkungen, sowie Funktionseinschränkungen im Langzeitverlauf sind die Folgen (10, 14). Neben der Muskulatur der Extremitäten ist auch das Diaphragma anfällig für einen Muskelmassenverlust und Funktionseinschränkungen, was zur eingeschränkten Leistungsfähigkeit der Atemmechanik führt (15-19). Eine prolongierte oder unmögliche Entwöhnung von der Beatmung können die Folge sein (20, 21). Die Mimische- und Kaumuskulatur bleibt selbst bei ausgeprägter Muskelatrophie der Extremitäten normalerweise unbeschadet (22). Ebenso scheint die Herzmuskulatur von der erworbenen Muskelschwäche auf der Intensivstation nicht unmittelbar betroffen (23).

Die Ausprägung der Schwäche kann sehr variable sein, ist jedoch in den meisten Fällen distale betont und betrifft die untere Extremität häufiger und ausgeprägter als die obere Extremität (14).

Muskelmassen- und Muskelfunktionsverlust kennzeichnen die erworbene muskuläre Organdysfunktion (14, 24, 25). Gesunde Individuen zeigen eine positive Korrelation zwischen Muskelkraft und Muskelmasse (26, 27). Dieser Zusammenhang geht im Kontext der neuromuskulären Organfunktion bei Intensivpatienten verloren, da zumeist der Muskelkraftverlust deutlich ausgeprägter ist als der Muskelmassenverlust (3, 28). Dieser fehlende Zusammenhang persistiert auch nach Krankenhausentlassung in der Rehabilitation, sodass ein Wiederaufbau der Muskelmasse, falls das gelingt, trotzdem nicht zwingend zu einem Wiederaufbau der Muskelkraft führt (28). Das klinische Symptom der auf der Intensivstation erworbenen Muskelschwäche, welches

international und auch in der deutschen Fachsprache als „Intensive Care Unit acquired weakness“ (ICUAW) bezeichnet wird, ist dabei nur durch die Muskelschwäche, unabhängig vom Muskelmassenverlust definiert (29). Dem entgegen stehen die Begriffe Atrophie und Muskelschwund, welche durch Bildgebung oder Biopsie einen Muskelmassenverlust beschreiben, jedoch ohne Rückschlüsse auf die Funktion. Bei der Intensive Care Unit acquired weakness kommt es zu einem Funktionsverlust, der nahezu immer mit einem Muskelmassenverlust einhergeht, sich jedoch nicht oder zumindest nicht ausschließlich durch diesen erklären lässt (25, 28).

1.2 Pathophysiologie

Der menschliche Skelettmuskel unterliegt einem ständigen Auf- und Abbau. Training oder Inaktivität führen dabei zur geregelten Anpassung der Muskelmasse an ihre Bedürfnisse (30). Intensivpatienten leiden häufig an einer systemischen Inflammation im Sinne eines Systemic Inflammatory Response Syndrome (SIRS) oder einer Sepsis. Eine in diesem Zusammenhang auftretende Multiorgandysfunktion, zusammen mit Immobilisation, sind die wichtigsten bekannten Risikofaktoren für das Erleiden eines neuromuskulären Organversagens (14, 31-34). Schwerwiegende Erkrankungen initiieren eine katabole Stoffwechsellage, welche die Homöostase der Skelettmuskulatur verschiebt und zum Muskelmassenverlust führt, und so die Muskelkraft negativ beeinflusst (25).
Neben diesem als physiologisch zu bezeichnendem Prozess der Muskelmassenanpassung, gibt es weitere Faktoren, die zur erworbenen Muskelschwäche beitragen können (35, 36). Dabei kann man anatomisch Schäden des Muskels selbst von Schäden der versorgenden Nerven trennen (37-39). Die Elektrophysiologie kann hier zwischen einer Myopathie oder einer Neuropathie differenzieren (37, 40, 41). Häufig tritt jedoch eine Kombination beider pathologischen Veränderungen auf (37). Sowohl am Nerven als auch auf der Muskelzellmembran führen Veränderungen der Ionenkanäle zur verringerten Membranerregbarkeit, die unabhängig von erhaltener Muskelmasse zu Funktionseinschränkungen führen können (35). Die Kraftentwicklung pro Querschnittesfläche der Muskelfasern ist dabei messbar reduziert (28). Jedoch folgt einer Denervierung und einer Myopathie ebenso ein Muskelmassenverlust, was zur regelmäßigen Koexistenz von Muskelmassenverlust und einem darüber hinaus

gehenden Muskelfunktionsverlust führt. Ob es sich hierbei um eine primäre Ionenkanalstörung handelt oder, ob die verminderte Erregbarkeit vor allem einem verminderten Energieangebot der Muskelzellen geschuldet ist, ist unklar (42, 43). Die Beteiligung des Glukosestoffwechsels an der Entstehung eines neuromuskulären Organversagens liegt nahe, da Glukosedysregulation bei Intensivpatienten klar mit dem Auftreten einer erworbenen Muskelschwäche assoziiert ist (44, 45). Im speziellen konnten wir finden, dass Patienten, die elektrophysiologische Hinweise auf eine Myopathie zeigen, eine besonders ausgeprägte Störung der Glukosehomöostase vorweisen im Vergleich zu Intensivpatienten ohne Anhalt für eine Myopathie (42). Dabei führt eine verminderte Translokation des Glukosetransporter 4 (GLUT4) in die Zellmembran der Skelettmuskulatur zur verminderten Aufnahme von Glukose in die Muskelzellen und so zu einem verringerten Substratangebot für die Glykolyse, die primäre Energiequelle der Skeletmuskulatur. Dies konnten wir mittels interstitieller Mikrodialysetechnik nachweisen (42). Die Arbeitsgruppe um Gret van den Berghe konnte im Patientenkollektiv einer intensivierten Insulintherapiestudie eine verminderte Inzidenz einer Critical Illness Polyneuropathy (CIP) bei den Patienten mit Blutzuckerspiegeln von 80-110mg/dl im Vergleich zu höheren Blutzuckerwerten zeigen (46). Nach aktuellem Wissenstand wird jedoch dem intramuskulären Proteinstoffwechsel die größte Bedeutung bei der Entwicklung einer ICUAW zugesprochen (47). An der Energiebereitstellung sind Proteine, besonders während kataboler Stoffwechselsituationen, maßgeblich beteiligt. Während die Actin-Myosin-Homöostase, als kontraktiles Element der Skelettmuskulatur für die Funktion entscheidend ist, können die Proteine auch der Energiegewinnung oder dem systemischen Aminosäurepool zur Verfügung gestellt werden (48, 49). Wir konnten in einer früheren Arbeit zeigen, dass schon innerhalb von wenigen Tagen ein sehr ausgeprägter Abbau des Muskelproteins Myosin erfolgt (25). In unseren Daten wird der Abbau über die E3-Ligasen muscle-specifc ring finger 1 (MuRF1) und Atrogin vermittelt und durch das Ubiquitin-Proteasom-System (UPS) umgesetzt (25). Zudem beschreiben wir eine reduzierte Syntheserate von Muskelproteinen in der Frühphase der intensivmedizinischen Behandlung, auf die eine reduzierte mRNA Expression der muskelspezifischen Myosin Subtypen hinweist (25). Diese Ergebnisse decken sich mit den Erkenntnissen anderer Arbeitsgruppen (7, 18, 24, 28). Dieser Verlust an Muskelprotein geht mit einer lichtmikroskopischen Atrophie einher, die jedoch erst im weiteren Verlauf der Behandlung sichtbar wird. In unserer Arbeit sieht man

elektronenmikroskopisch an Median Tag 5 erhaltene Ultrastrukturen, aus denen das Myosin ausgelöst ist. Erst an Median Tag 15 zeigen sich die Ultrastrukturen der Muskelfasern zusammengesintert und weisen somit auch lichtmikroskopisch eine verringerte Querschnittsfläche auf (25).
Neben dem regulierten Abbau durch das UPS gibt es im Skelettmuskel auch den Mechanismus der Autophagie. Als Autophagie bezeichnet man den regulierten Selbstabbau, der im Prozess der Muskelhomöostase eine wichtige Rolle spielt (50). Der Einfluss von Ernährungs- und Insulintherapie auf den Skelettmuskel wurde auch bezüglich Autophagie untersucht. Aus einer Subgruppenanalyse einer großen Ernährungsstudie geht hervor, dass frühe hochkalorische Ernährung und entsprechender Insulinbedarf zu einer pathologisch reduzierten Autophagieaktivität im Vergleich zu niedriger kalorisch ernährten Patienten führt (51). Diese supprimierte Aktivität der Autophagie scheint für die Muskulatur schädlich und ist mit reduzierter Muskelkraft assoziiert (51).

1.3 Klinische Relevanz

Die erworbene Muskelschwäche ist eine gravierende Begleitkomplikation. Sowohl für das akute Überleben auf der Intensivstation als auch für das Langzeitüberleben konnte gezeigt werden, dass Muskelschwäche mit reduzierter Überlebenswahrscheinlichkeit assoziiert ist (12, 13). Dabei ist sogar der Grad der Schwäche diskriminierend (12, 13). Neben dem Überleben per se ist die Lebensqualität und funktionelle Unabhängigkeit zum wichtigen Parameter geworden, um den Erfolg einer Intensivtherapie zu bewerten (52). Langzeitschäden sind für den einzelnen Patienten als auch aus ökonomischen Gesichtspunkten für das gesamte Gesundheitssystem von immenser Bedeutung (53-58). Lange Rehabilitationszeiten mit eingeschränkten Erfolgsraten, langfristige Hilfebedürftigkeit zur Bewältigung des Alltages, eingeschränkte Arbeitsfähigkeit und psychologische Folgeerkrankungen machen die Erarbeitung von Therapieoptionen und Präventionsansätzen im Interesse des individuellen Patienten und des Gesundheitssystems unabdingbar (10, 13, 54, 59). Der Begriff des „Post Intensive Care Syndrom (PICS)“ fast diese Langzeitkomplikationen nach intensivmedizinischer Behandlung zusammen (60).

1.4 Therapie und Prävention

Bisher gibt es keine gezielte Therapie, die beim Erleiden einer ICUAW angewendet werden kann, wie zwei Cochrane Reviews zeigen (61, 62). Im Vordergrund steht die konsequente Therapie der Grunderkrankung (61, 62). Gerade die Sepsis mit Organdysfunktionen sind maßgebliche Risikofaktoren einer ICUAW und gehören schnell und zielgerichtet therapiert (32-34, 51). Daneben hat die frühe Mobilisation auf der Intensivstation, um der Immobilisation gezielt entgegenzuwirken, Einzug in die Leitlinien gefunden (63). Durch physiotherapeutische Interventionen konnten teilweise Vorteile im funktionellen Status der Patienten bei Entlassung von der Intensivstation gezeigt werden. So empfiehlt die aktuelle S2e Leitlinie für Lagerungstherapie und Frühmobilisation, zweimal täglich, 20 Minuten Frühmobilisation und zudem einen Beginn innerhalb von 72 Stunden nach Aufnahme auf die Intensivstation. Darüber hinaus gibt es muskelstimulierende physiotherapeutische Möglichkeiten, wie die Anwendung von Bettfahrrädern, elektrischer Muskelstimulation oder Vibrationstherapien, die bisher nur vereinzelt in klinischen Studien zur Verbesserung des funktionellen Outcomes oder dem Muskelmassenerhalt beitragen konnten und somit keinen Evidenzgrad für eine routinemäßige Anwendung erlangt haben (3, 64-71). Die Sicherheit solcher zusätzlichen Verfahren konnte jedoch mehrfach gezeigt werden und es obliegt der Einzelfallentscheidungen nach klinischem Ermessen, ob ein Patient gegebenenfalls von solchen Interventionen profitieren kann (2).

1.5 Zielsetzungen der Veröffentlichungen

Seit 2010 beschäftige ich mich mit dem Themengebiet der erworbenen Muskelschwäche. Meinen wissenschaftlichen Arbeiten liegen klinische Observations- und Interventionsstudien zu Grunde, die sich klinisch und molekular mit der Entstehung eines erworbenen muskulären Organversagens beschäftigen. In den letzten Jahren stehen die Untersuchungen von Interventionsmöglichkeiten als mögliche Therapieoptionen und Präventionsstrategien im Fokus meiner Arbeit. Die in dieser Schrift eingebrachten wissenschaftlichen Originalarbeiten untersuchen die Anwendbarkeit und Wirksamkeit von Physiotherapie und erweiterten physiotherapeutischen Verfahren als Präventionsoption zur Vermeidung oder

zumindest Reduktion der Ausprägung einer erworbenen Muskelschwäche auf dem Hintergrund pathophysiologischer Observationsstudien. Eigene und andere Arbeiten der letzten Jahre haben den sehr frühen Beginn eines neuromuskulären Organverfahrens gezeigt und widersprechen dem ursprünglich angenommen Erleiden einer Muskelschwäche erst bei mehrwöchigem Aufenthalt auf der Intensivstation (24, 25). Mittlerweile sind Mechanismen bekannt, die innerhalb von Tagen, wenn nicht sogar Stunden, den übermäßigen Muskelabbaus initiieren (24, 25). Gerade deshalb kann Prävention nur möglich sein, wenn Interventionen zügig nach Aufnahme auf die Intensivstation beginnen. Um dem Beginn einer muskulären Schwäche noch näher zu kommen haben wir erstmalig auch elektiv operative Patienten untersucht, um die Auswirkungen des perioperativen Ablaufs auf die Muskulatur zu beobachtet. Hier konnten wir erstmalig das Auftreten einer erworbenen Muskelschwäche im unmittelbaren perioperativen Verlauf sogar ganz ohne intensivmedizinische Behandlung zeigen (5).

2. Zusammenfassung der Ergebnisse eigener Arbeiten

2.1 Inflammationsgetriggerte Muskelatrophie wird auch durch die E3 Ligase TRIM62 vermittelt

Schmidt F, Kny M, Zhu X, **Wollersheim T**, Persicke K, Langhans C, Lodka D, Kleber C, Weber-Carstens S, Fielitz J. The E3 ubiquitin ligase TRIM62 and inflammation-induced skeletal muscle atrophy. Crit Care. 2014;18(5):545. doi: 10.1186/s13054-014-0545-6

Wir konnten in einer klinischen Studie zeigen, dass bereits zu einem sehr frühen Zeitpunkt ein massiver Myosinverlust der Skelettmuskulatur resultiert. Dabei haben wir molekulare Mechanismen identifiziert, die für den massiven Abbau und eine verminderte Muskelsynthese verantwortlich waren. Das UPS ist maßgeblich am Proteinabbau beteiligt. MuRF1 und Atrogin1 sind E3-Ligasen, die Proteine ubiquitinieren und so dem Abbau durch Proteasomen zugänglich machen. Eine frühe Hochregulation schon an Tag 5 der kritischen Erkrankung markiert dabei den frühzeitigen Myosinverlust bei Patienetn mit ICUAW. Neben den schon untersuchten E3-Ligasen MuRF1 und Atrogin-1 gibt es weitere E3-Ligasen, die möglicherweise am Proteinabbau der Skelettmuskulatur beteiligt sind. In der hier vorliegenden Arbeit untersuchten wir in den Muskelbiopsien unserer Patienten den Tripartite Motif Containing 62 (*TRIM62*) Signalweg. *TRIM62* ist eine bekannte E3-Ligase die durch systemische Inflammation induziert werden kann. Dazu untersuchten wir im Maus Model parallel den Einfluss von Inflammation, Denervierung und Fasten auf den Skelettmuskelabbau, um die zeitliche Dynamik besser abbilden zu können.

In dieser Observationsstudie konnten wir in 26 Intensivpatienten, die eine ICUAW erlitten haben, zweizeitige Skelettmuskelproben, an Tag 5 und 15, des musculus vastus lateralis gewinnen. In diesen Proben bestimmten wir die messenger Ribonukleinsäure (mRNA) Expression und den Proteingehalt von *TRIM62*. In einem etablierten Maus Modell schauten wir uns parallel die unterschiedlichen Einflüsse von Inflammation, Denervierung und Fasten auf die E3-Ligasen *TRIM62*, MuRF1 und Atrogin-1 vermittelte Skelettmuskelatrophie an.

Unsere vorherigen Arbeiten wurden hier bestätigt und es kann ein klarer Zusammenhang zwischen Inflammation und die über MuRF1, Atrogin-1 und *TRIM62* induzierte Muskelatrophie gefunden werden. Wir sehen hier einen Ansatzpunkt zur Intervention, um den Proteinabbau zu reduzieren. Um die Aktivität der durch Inflammation induzierte E3-Ligasen abzuschwächen, besteht theoretisch die Möglichkeit den gesamten Inflammationsweg oder die E3-Ligasen selektiv zu hemmen. Auch Muskelaktivität kann zur Reduktion von E3-Ligasen vermittelter Muskelatrophie beitragen.

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The E3 ubiquitin ligase TRIM62 and inflammation-induced skeletal muscle atrophy

Franziska Schmidt[1†], Melanie Kny[1†], Xiaoxi Zhu[1], Tobias Wollersheim[2], Kathleen Persicke[1], Claudia Langhans[1], Doerte Lodka[1], Christian Kleber[3], Steffen Weber-Carstens[2] and Jens Fielitz[1,4*]

Abstract

Introduction: ICU-acquired weakness (ICUAW) complicates the disease course of critically ill patients. Inflammation and acute-phase response occur directly within myocytes and contribute to ICUAW. We observed that tripartite motif–containing 62 (TRIM62), an E3 ubiquitin ligase and modifier of inflammation, is increased in the skeletal muscle of ICUAW patients. We investigated the regulation and function of muscular TRIM62 in critical illness.

Methods: Twenty-six critically ill patients with Sequential Organ Failure Assessment scores ≥8 underwent two skeletal muscle biopsies from the vastus lateralis at median days 5 and 15 in the ICU. Four patients undergoing elective orthopedic surgery served as controls. TRIM62 expression and protein content were analyzed in these biopsies. The kinetics of *Trim62*, *Atrogin1* and *MuRF1* expression were determined in the gastrocnemius/plantaris and tibialis anterior muscles from mouse models of inflammation-, denervation- and starvation-induced muscle atrophy to differentiate between these contributors to ICUAW. Cultured myocytes were used for mechanistic analyses.

Results: TRIM62 expression and protein content were increased early and remained elevated in muscles from critically ill patients. In all three animal models, muscular *Trim62* expression was early and continuously increased. Trim62 was expressed in myocytes, and its overexpression activated the atrophy-inducing activator protein 1 signal transduction pathway. Knockdown of Trim62 by small interfering RNA inhibited lipopolysaccharide-induced interleukin 6 expression.

Conclusions: TRIM62 is activated in the muscles of critically ill patients. It could play a role in the pathogenesis of ICUAW by activating and maintaining inflammation in myocytes.

Trial registration: Current Controlled Trials ID: ISRCTN77569430 (registered 13 February 2008)

Introduction

ICU-acquired weakness (ICUAW) is a devastating complication of critical illness characterized by loss of muscle mass [1], preferential atrophy of fast-twitch myofibers and weakness [2-4]. Affected patients face a prolonged hospital stay and mechanical ventilation, increased hospital mortality and chronic physical disability [5,6]. The pathophysiology of ICUAW is poorly understood [7]. However, we [8] and others [1] have shown that dysbalanced muscular protein homeostasis due to increased protein degradation and reduced protein synthesis occurs in muscle of critically ill patients and may contribute to ICUAW [1,2,8,9]. Breakdown of muscular proteins such as myosin heavy chain (MyHC) is mediated by the ubiquitin-proteasome system (UPS) [10], which is activated in muscle of critically ill patients [1,8,11] and involves the F-box adaptor protein FBXO32/Atrogin1 [12] and the E3 ubiquitin ligase muscle RING (really interesting new gene) finger–containing protein 1 (MuRF1). Atrogin1 and MuRF1 are rapidly and transiently increased in the skeletal muscle of critically ill patients [8]. However, muscle atrophy and regulation of *Atrogin1* and *MuRF1* expression are not synchronized, because atrophy occurs later in the disease process, when *Atrogin1* and *MuRF1* have already returned to baseline [8]. This discrepancy argues for additional continuously activated atrophy pathways. Chronic and persistent inflammation and acute-phase response directly occurring in the skeletal muscle of critically ill patients might be one of these

* Correspondence: jens.fielitz@charite.de
† Equal contributors
[1]Experimental and Clinical Research Center (ECRC), a Cooperation between Max-Delbrück-Centrum and Charité-Universitätsmedizin Berlin, Campus Buch, Lindenberger Weg 80, 13125 Berlin, Germany
[4]Cardiology, Charité-Universitätsmedizin Berlin, Campus Virchow, Augustenburger Platz 1, 13353 Berlin, Germany
Full list of author information is available at the end of the article

mechanisms [13]. Recently, we have shown that interleukin 6 (IL-6) and the acute-phase response proteins serum amyloid A1 (SAA1) and SAA4 are continuously elevated in the muscle of critically ill patients [13]. Both IL-6 [14,15] and SAA1 [16,17] are known to induce atrophy by increasing protein degradation in the skeletal muscle of both patients and rodents. We performed a gene expression array and found the modifier of inflammation tripartite motif–containing 62 (TRIM62) to be increased in the muscle of critically ill patients [13]. TRIM62 belongs to the family of RING finger E3 ubiquitin ligases [18,19] and was identified as a dominant regulator of acinar morphogenesis in the mammary gland [20]. Strong evidence exists that TRIM62 plays a role in Toll-like receptor 4 (TLR4) signaling. More specifically, TRIM62 activates the Toll/interleukin 1 receptor domain–containing adapter inducing interferon β (TRIF) branch of the TLR4 signaling pathway, leading to increased activity of the activator protein 1 (AP-1) transcription factor in primary macrophages [21]. Because AP-1 signaling is essential for denervation-induced atrophy [22], we hypothesized that TRIM62-mediated activation of AP-1 signaling in myocytes contributes to inflammation-induced atrophy in critically ill patients. To specifically focus on early time points of muscle atrophy and to differentiate between the major contributors of ICUAW, we relied on three mouse atrophy models described elsewhere: cecal ligation and puncture (CLP) mimicking sepsis, denervation-induced atrophy and food deprivation [13]. These models were used to compare the kinetics of *Trim62* with *Atrogin1* and *MuRF1* gene expression in muscle. Cultured myocytes and reporter gene assays were used for mechanistic analyses.

Material and methods

Patients

The institutional review board of the Charité approved the study, and written informed consent was obtained from the patient or the patient's legal proxy (Charité EA2/061/06). We recently reported clinical data and molecular analyses in the biopsy specimens of the same patients [2,8,13,23]. We specifically included patients at high risk of developing ICU-acquired muscle wasting and weakness [24]. Open muscle biopsies from the vastus lateralis were performed at median day 5 in 26 ICU patients (early time point). Of these 26 patients, 14 remained at least to median day 15 in the ICU (late time point), when a second biopsy specimen from the vastus lateralis was obtained. Four age- and gender-matched patients undergoing elective orthopedic surgery, otherwise healthy, permitted a biopsy from the vastus lateralis at the time point of elective surgery. For further details, refer to Additional file 1.

Animal models of muscle atrophy

To focus on early time points of muscle atrophy, we relied on three mouse models described elsewhere: CLP surgery that mimics sepsis, denervation-induced atrophy and food deprivation [13,25,26]. All animal procedures were performed in accordance with the guidelines of the Max-Delbrück Center for Molecular Medicine and the Charité-Universitätsmedizin Berlin and were approved by the Landesamt für Gesundheit und Soziales (LaGeSo, Berlin, Germany) for the use of laboratory animals (permit number G 0129/12). They followed the principles of laboratory animal care set forth by the National Institutes of Health (NIH) in the *Guide for the Care and Use of Laboratory Animals* (NIH Publication 86-23, revised 1985), as well as the current version of the German Law on the Protection of Animals. Briefly, 6- to 8-week-old male C57BL/6 N mice were used for all experiments. CLP surgery was performed to induce polymicrobial sepsis according to a published protocol [25] and as recently reported [13]. Sham mice were treated identically, except for the ligation and puncture of the cecum. CLP (n =4 or 5) and sham-treated (n =4 or 5) mice were sacrificed 24 hours, 48 hours, 72 hours or 96 hours after surgery. Neurogenic atrophy was induced by dissection of the left sciatic nerve (denervation). The sciatic nerve of the right leg was cut, and a 3-mm piece was excised (denervated). The right leg remained innervated and was used as the control (innervated). Mice were sacrificed at baseline or 7 days, 14 days or 21 days postsurgery (n =6 each). Food deprivation was performed for 0 hours (control), 24 hours or 48 hours (n =6 each).

For detailed information about animal experiments, quantitative RT-PCR (qRT-PCR), immunohistology, immunoblotting, myoblast culture and immunocytology, small interfering RNA (siRNA) transfection and luciferase reporter assay, please refer to Additional files 1.

Statistical tests

A two-sided Mann-Whitney U test was used to determine statistical differences. Data shown are mean ± SEM. Statistical tests were calculated using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). Box plots showing medians with 25th and 75th percentiles were made using GraphPad Prism 5. P <0.05 was considered statistically significant.

Results

TRIM62 mRNA was upregulated in muscle of critically ill patients

The study protocol (Additional file 2: Figure S1), data on patients' characteristics (Additional file 1: Table S1) and treatment (Additional file 1: Table S2) are provided. The data from our microarray have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database [GEO:GSE53702] (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53702). We found a 3.9-fold

upregulation of *TRIM62* [13]. We performed qRT-PCR in a subset of our recently published ICU patients [8] at the early (day 5, n =26) and late (day 15, n =14) time points and orthopedic controls (n =4) to confirm these data. Continuous upregulation of muscular *TRIM62* mRNA was confirmed for both time points of critical illness (P <0.01) (Figure 1A). Immunoblot analysis showed that muscular TRIM62 protein was increased at the early time point and remained elevated until the late time point in critically ill patients (Figure 1B). In contrast, *Atrogin1* and *MuRF1* expression was significantly increased in the early and returned to control levels in the late biopsy specimens in these patients as recently reported [8].

Trim62 was persistently elevated during skeletal muscle atrophy in mice

Because Trim62 has been shown to be involved in inflammatory response of immune cells [21], we reasoned that Trim62 could play a role in the development of ICUAW. We used three standard animal models of muscle atrophy—CLP [13,25], denervation [26] and food deprivation—to investigate regulation of *Trim62* and to compare it with the expression of the standard atrophy markers *Atrogin1* and *MuRF1*.

Muscular *Trim62* expression was increased during inflammation-induced skeletal muscle atrophy in mice

Inflammation-induced muscle atrophy was induced by the CLP method of polymicrobial sepsis as previously described [13,25]. Recently, we reported that increased expression of *Il-6* and *Saa1* in muscles of CLP mice might contribute to inflammation-induced atrophy [13,25]. In the present study, general inflammation was confirmed by an early and persistent increase in gene expression of the inflammatory cytokines *Il-6* and tumor necrosis factor α (*Tnf-α*), as well as of the acute-phase protein Saa1, in the liver of CLP mice (Additional file 3: Figure S2). Septic mice showed a significant decrease in body weight as soon as 24 hours after surgery. CLP mice continued their weight loss, which reached its maximum after 96 hours at a reduction of 19% of body weight (P <0.05) (Additional file 4: Figure S3A). A significant decrease in muscle weights was found after 72 hours and 96 hours of sepsis, indicative of muscular atrophy (Figure 2A). Using qRT-PCR, we found a significant increase in *Atrogin1* and *MuRF1* expression after 24 hours of sepsis in gastrocnemius/plantaris and tibialis anterior muscles. *Atrogin1* and *MuRF1* expression followed a time course with early induction and a subsequent decrease (Figures 2B and 2C). Whereas *MuRF1* expression was highest at the 24-hour time point, the induction of *Atrogin1* peaked at 48 hours of sepsis (Figures 2B and 2C). Immunoblot analysis confirmed that muscular MuRF1 protein content went in parallel with its expression, showing an early induction and a subsequent decrease in gastrocnemius/plantaris and tibialis anterior muscles of CLP mice (Figure 2D).

Muscular *Trim62* expression was measured by qRT-PCR. It was significantly increased in gastrocnemius/plantaris and tibialis anterior muscles at 48 hours and 72 hours after CLP surgery. Throughout the experiment, *Trim62* expression levels were persistently elevated in both muscle types (Figure 3A).

Muscular Trim62 expression was increased in denervation-induced skeletal muscle atrophy in mice

We investigated if *Trim62* was regulated in neurogenic muscle atrophy. A progressive loss of muscle weight was

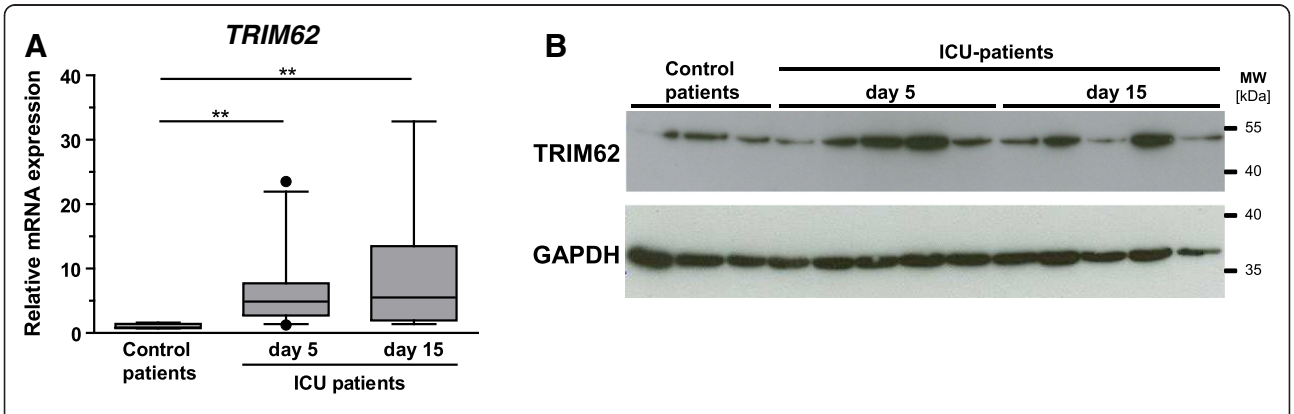

Figure 1 Muscular *TRIM62* expression in critically ill patients. (A) Quantitative RT-PCR analyses of tripartite motif–containing 62 (*TRIM62*) expression in vastus lateralis muscles of orthopedic control patients (n =4) and patients with ICU-acquired weakness (ICUAW) at early (day 5, n =26) and late (day 15, n =14) time points. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) expression was used as the reference. Data shown are relative expression and fold changes compared to control patients. Data presented in box plots are medians and 25th and 75th percentiles. **P <0.01. **(B)** Immunoblots of proteins from vastus lateralis muscles of orthopedic control and critically ill patients at early (day 5) and late (day 15) time points using anti-TRIM62 antibody. GAPDH was used as loading control.

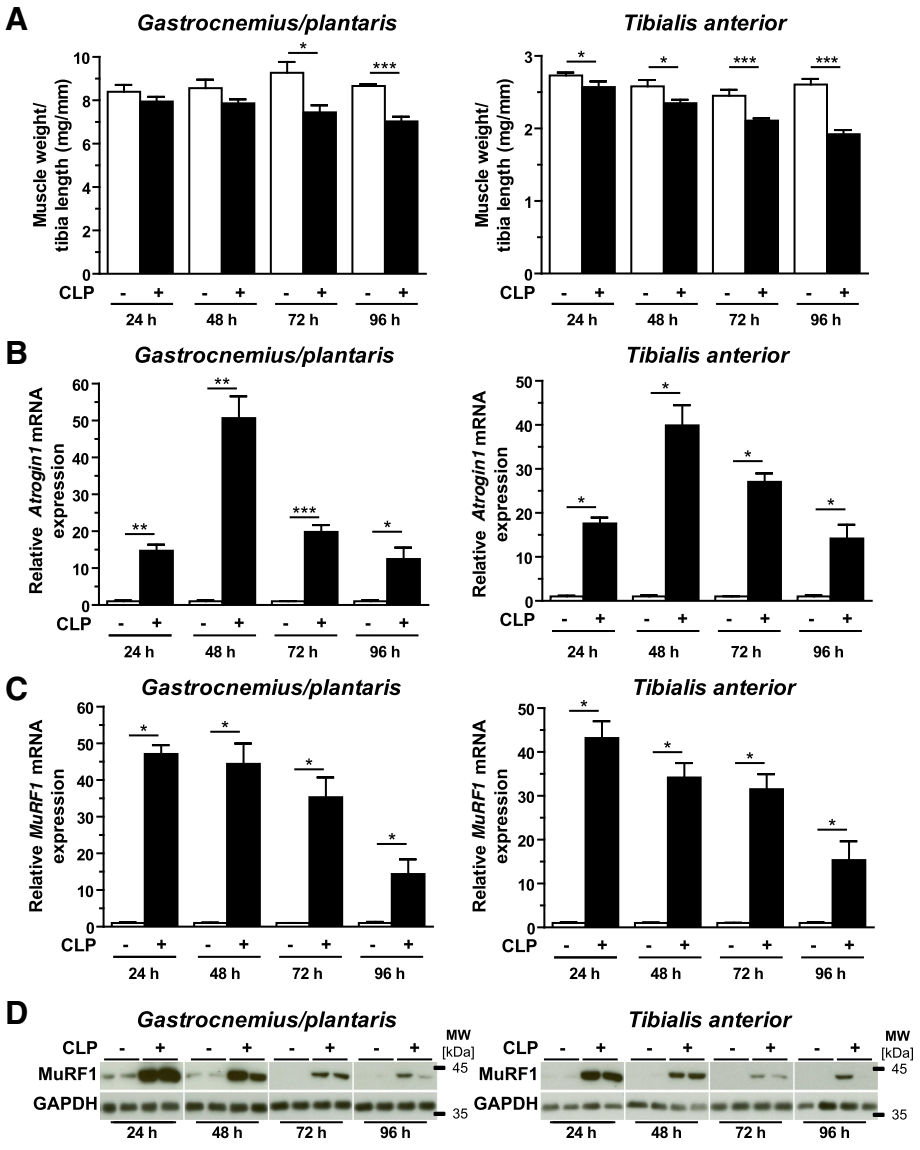

Figure 2 Inflammation leads to skeletal muscle atrophy *in vivo*. Six- to eight-week-old male C57BL/6 N mice were subjected to sham operations (sham, *n* =5) or cecal ligation and puncture (CLP) surgery (*n* =5), as indicated, to induce polymicrobial sepsis. **(A)** Weights of the gastrocnemius/plantaris and tibialis anterior muscles were determined after 24 hours, 48 hours, 72 hours or 96 hours and normalized to tibia length. Data are presented as mean ± SEM. ***P <0.001, *P <0.05. Quantitative RT-PCR analyses of *Atrogin1* **(B)** and *MuRF1* **(C)** expression, and immunoblotting of proteins using anti-MuRF1 antibody **(D)**, in gastrocnemius/plantaris and tibialis anterior muscles at 24 hours, 48 hours, 72 hours or 96 hours after surgery, as indicated. Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) expression and protein content were used as reference values, and data shown are relative changes. Data are presented as mean ± SEM. ***P <0.001, **P <0.01, *P <0.05. MuRF1, Muscle RING (really interesting new gene) finger–containing protein 1.

observed for gastrocnemius/plantaris and tibialis anterior muscles. Weights of gastrocnemius/plantaris and tibialis anterior muscles were significantly decreased after 7 days of denervation (Figure 4A). A continuous decrease in muscle mass was observed until 21 days of denervation, reaching 54% and 49% (P <0.01 for both) of muscle weight for gastrocnemius/plantaris and tibialis anterior muscles, respectively (Figure 4A). *Atrogin1* (Figure 4B) and *MuRF1* (Figure 4C) expression was significantly increased at both time points in gastrocnemius/plantaris and tibialis anterior muscles. More specifically, *Atrogin1* and *MuRF1* expression was highest after 7 days of denervation. *Atrogin1* expression was 8.3- and 4.2-fold upregulated in gastrocnemius/plantaris and tibialis anterior muscles, respectively, after 7 days of denervation (P <0.01 for both) (Figure 4B). At the same time point, *MuRF1* expression was increased four- and threefold in gastrocnemius/plantaris and tibialis anterior muscles,

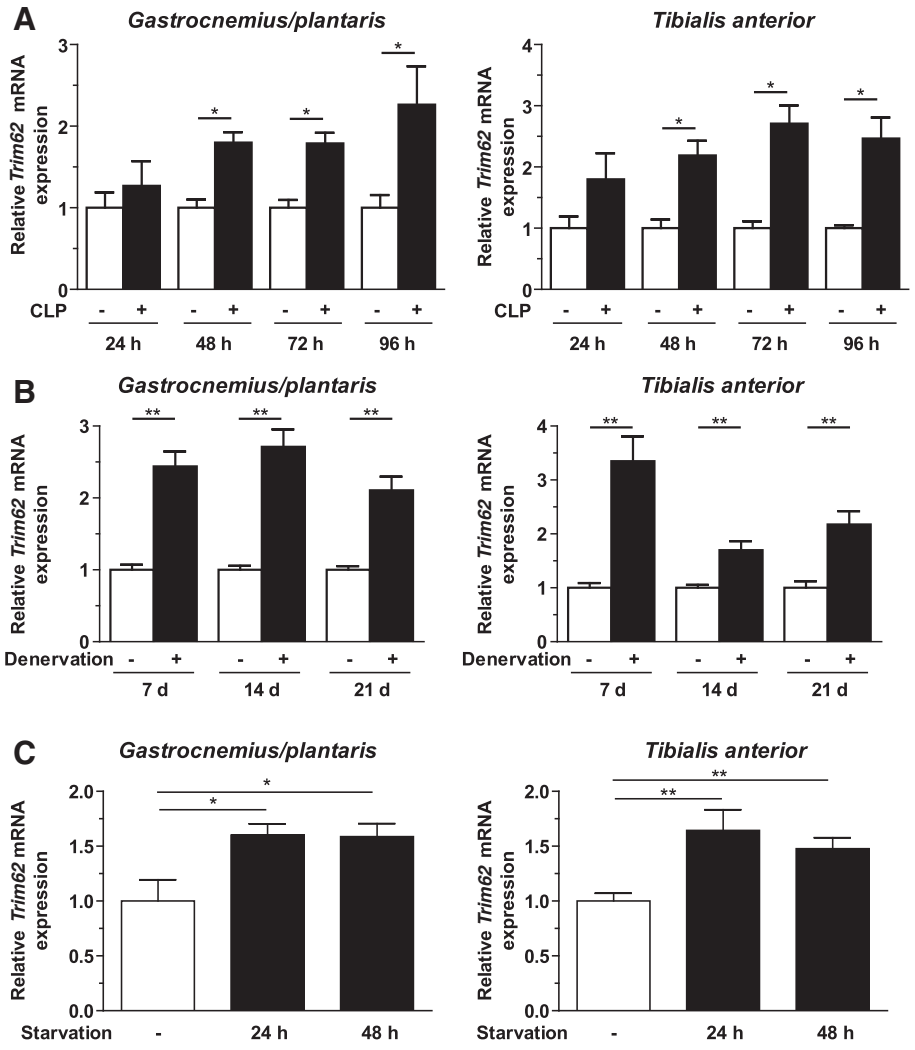

Figure 3 *Trim62* expression is increased during inflammation-, denervation- and starvation-induced muscle atrophy. (A) Quantitative RT-PCR (qRT-PCR) analyses of *Trim62* expression in gastrocnemius/plantaris and tibialis anterior muscles at 24 hours, 48 hours, 72 hours and 96 hours after surgery, as indicated. CLP, Cecal ligation and puncture surgery. **(B)** qRT-PCR analyses of *Trim62* expression in gastrocnemius/plantaris and tibialis anterior muscles 7 days, 14 days and 21 days after denervation or sham surgery. **(C)** qRT-PCR analyses of *Trim62* expression in gastrocnemius/plantaris and tibialis anterior muscles of control mice (–, n =6) and mice deprived of food for 24 hours (n =6) or 48 hours (n =6), as indicated. Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) expression was used as the reference in all assays. Data shown are fold changes of expression in sham-operated and untreated mice. Data are presented as mean ± SEM. **P <0.01, *P <0.05.

respectively (P <0.01 for both) (Figure 4C). The MuRF1 protein content showed a comparable time course during denervation in gastrocnemius/plantaris and tibialis anterior muscles (Figure 4D).

Trim62 expression was also significantly increased at both time points in denervated gastrocnemius/plantaris and tibialis anterior muscles (Figure 3B). In gastrocnemius/plantaris muscles, a 2.4-fold upregulation of *Trim62* was measured 7 days after denervation, and it remained at this level at 14 days and 21 days (P <0.01 for all). In tibialis anterior muscle, *Trim62* expression reached its maximum at 7 days (3.3-fold induction) and decreased thereafter (1.7-fold after 14 days and 2.2-fold after 21 days) (P <0.01 for both) (Figure 3B).

Starvation-induced atrophy was accompanied by increased *Trim62* expression in mouse skeletal muscle

To investigate the impact of starvation on muscle atrophy, mice were food-deprived for 24 hours and 48 hours. Food deprivation led to decreases in body weight (14% after 24 hours (P <0.01) and 20% after 48 hours (P <0.01)) (Additional file 4: Figure S3B). Following food deprivation, a reduction in muscle weight was found for the gastrocnemius/plantaris muscles (15% after 48 hours (P <0.001) of starvation), but not tibialis anterior muscle

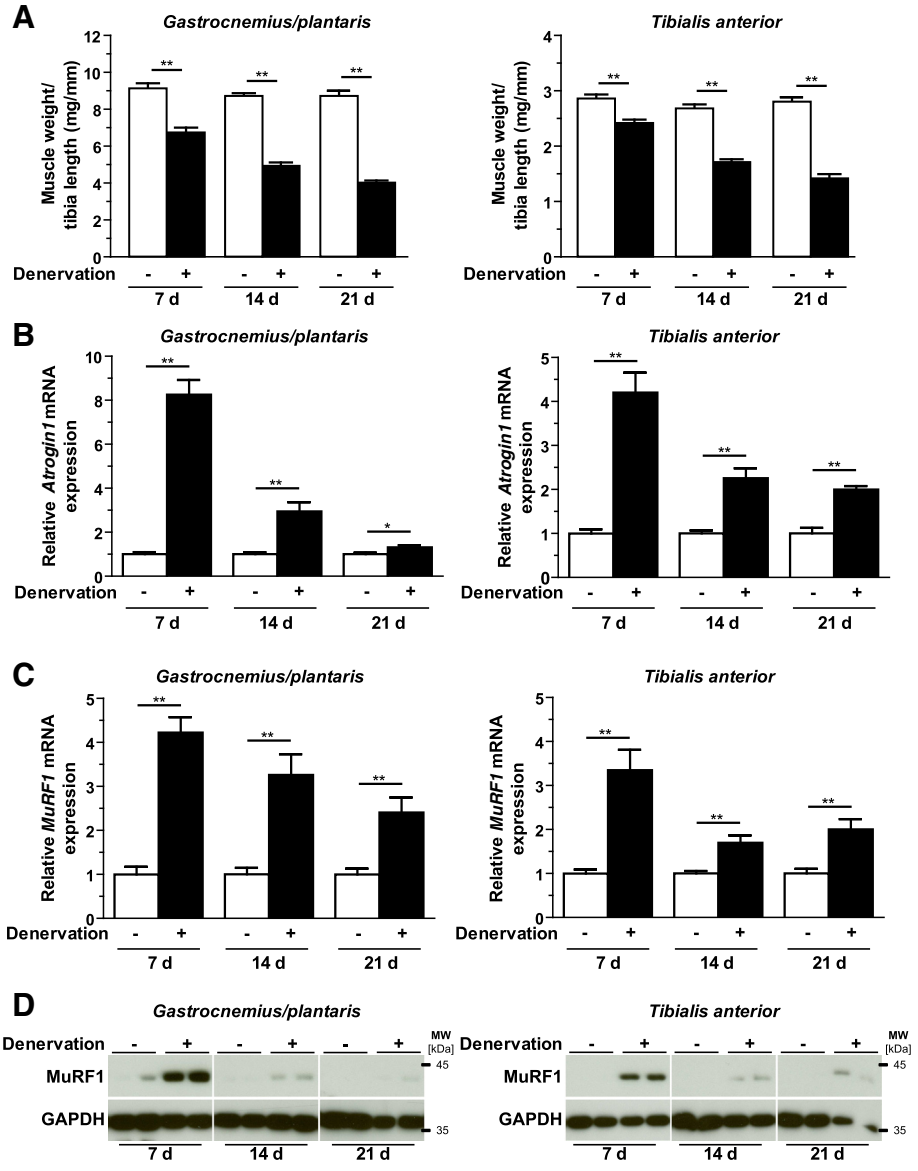

Figure 4 Denervation-induced atrophy. Six- to eight-week-old male C57BL/6 N mice were subjected to surgery. The sciatic nerve of the left hindlimb was dissected (denervated, *n* =6), and a sham procedure was performed at the right side (innervated, *n* =6), as indicated. **(A)** The weights of gastrocnemius/plantaris and tibialis anterior muscles normalized to tibia length were determined at baseline (–) and after 7 days, 14 days and 21 days. Data are presented as mean ± SEM. ***P* <0.05. Quantitative RT-PCR analyses of *Atrogin1* **(B)** and *MuRF1* **(C)** expression and immunoblotting of proteins using anti-MuRF1 antibody **(D)** in gastrocnemius/plantaris and tibialis anterior muscles at 7 days, 14 days and 21 days after surgery, as indicated. Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) expression and protein content were used as reference values, and data shown are the fold changes of the respective innervated sites. Data are presented as mean ± SEM. ***P* <0.01, **P* <0.05. MuRF1, Muscle RING (really interesting new gene) finger–containing protein 1.

(Figure 5A). qRT-PCR experiments showed an upregulation of muscular *Atrogin1* and *MuRF1* expression during starvation (Figures 5B and 5C). *Atrogin1* expression increased 21.6-fold and 29.4-fold after 24 hours and 48 hours of starvation, respectively, in gastrocnemius/plantaris muscles (*P* <0.01 for both). *Atrogin1* expression was also increased in tibialis anterior muscle (Figure 5B). In gastrocnemius/plantaris muscles, *MuRF1* expression increased 21-fold and 24-fold after 24 hours and 48 hours of starvation, respectively (*P* <0.01 for both). *MuRF1* expression was also increased in tibialis anterior muscle (Figure 5C). The MuRF1 protein content was increased in gastrocnemius/plantaris and tibialis anterior muscles of mice subjected to starvation (Figure 5D).

Fasting resulted in a significant increase in muscular *Trim62* expression after 24 hours and 48 hours of

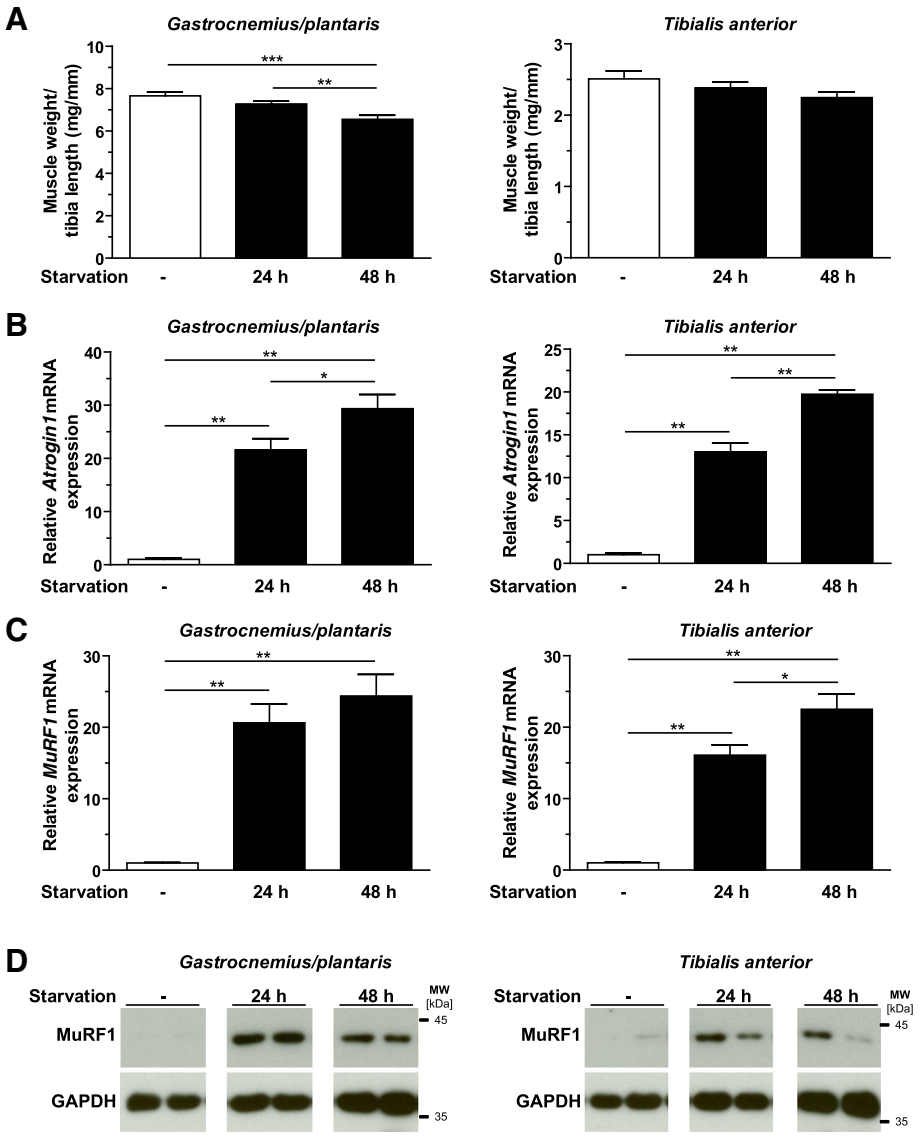

Figure 5 Starvation leads to skeletal muscle atrophy *in vivo*. Six- to eight-week-old male C57BL/6 N mice were subjected to food deprivation for 24 hours (n =6) or 48 hours (n =6), as indicated. Mice fed standard chow (controls, –; n =6) were used as controls. **(A)** Weights of gastrocnemius/plantaris and tibialis anterior muscles normalized to tibia length are shown. Data are presented as mean ± SEM. ***P <0.001, **P <0.01. Quantitative RT-PCR analyses of *Atrogin1* **(B)** and *MuRF1* **(C)** expression, and immunoblotting of proteins using anti-MuRF1 antibody **(D)**, in gastrocnemius/plantaris and tibialis anterior muscles of control animals (–) and mice deprived of food for 24 hours and 48 hours are shown, as indicated. Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) expression and protein content were used as reference values, and data shown are fold changes compared to controls. Data are presented as mean ± SEM. **P <0.01, *P <0.05. MuRF1, Muscle RING (really interesting new gene) finger–containing protein 1.

starvation (Figure 3C). *Trim62* expression increased 1.6-fold after 24 hours (P <0.05) and remained at this level at 48 hours (P <0.05) of starvation in gastrocnemius/plantaris and tibialis anterior muscles (P <0.01 for both).

Trim62 contributes to inflammatory response in muscle cells

Immunocytochemistry was performed to elucidate whether endogenous Trim62 protein was contained in myocytes. As expected, differentiated C2C12 myotubes contained Trim62 endogenously. Trim62 was exclusively localized in the cytoplasm of these cells (Figure 6A). The localization of Trim62 in the cytoplasm was confirmed when we transfected expression plasmids encoding Trim62 with either an amino-terminal FLAG tag or a carboxy-terminal myc/His-6 tag in C2C12 myoblasts (Figure 6B). These data indicate that Trim62 is contained in myocytes, where it is localized mainly in the cytoplasm.

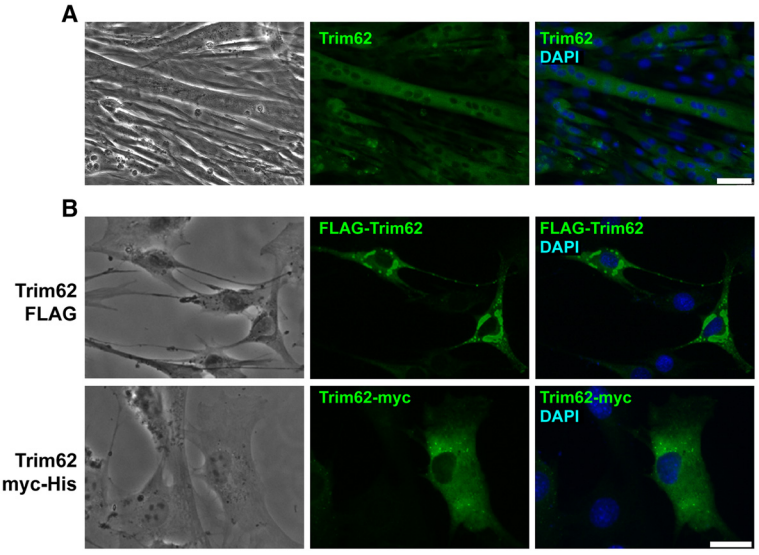

Figure 6 Trim62 is expressed in myocytes. (A) Immunocytochemistry of differentiated C2C12 myotubes was performed using an anti-Trim62 antibody. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, blue). Scale bar, 50 μm. **(B)** C2C12 myoblasts were transfected with expression plasmids encoding Trim62-FLAG (top panel), Trim62 myc/His-6 (bottom panel) or an empty vector control plasmid. Immunofluorescence analysis was performed using anti-FLAG and anti-Myc antibodies, respectively, as well as an Alexa Fluor 488–coupled secondary antibody. Nuclei were stained with DAPI (blue). Scale bar, 25 μm.

Trim62 was implicated in the inflammatory response of immune cells by regulating the TLR4 signaling pathway, leading to activation of AP-1 [21]. Therefore, we analyzed whether overexpressed Trim62 affects a reporter construct harboring three consecutive AP-1 consensus sites. Indeed, overexpression of Trim62 induced the AP-1-dependent promoter construct, indicating that Trim62 activates AP-1-dependent signaling events (Figure 7A). We also tested whether deletion of Trim62 in differentiated C2C12 myotubes by siRNA has an effect on TLR4-mediated activation of AP-1. Following knockdown of Trim62 in C2C12 myotubes (Figure 7B), we treated these cells with the TLR4 agonist lipopolysaccharide (LPS) and analyzed *Il-6* expression by qRT-PCR. As expected, LPS treatment led to increased *Il-6* expression in myocytes (Figure 7C). When Trim62 was downregulated, the LPS-mediated increase in *Il-6* expression was diminished. These data indicate that Trim62 is involved in LPS-induced *Il-6* expression and thus in the inflammatory response in myocytes.

Discussion

Increased *TRIM62* gene expression and protein content were found in skeletal muscle of critically ill patients. This finding was confirmed by increased *Trim62* expression in skeletal muscle of three muscle atrophy mouse models. In contrast to decreasing levels of *Atrogin1* and *MuRF1* during the late phase of muscle atrophy, *Trim62* levels remained continuously elevated in all atrophy models. These data implicate a role of Trim62 for the later phase of muscle atrophy. Because Trim62 activates the TRIF branch of TLR4 signaling which leads to increased LPS-induced *IL-6* expression [21], we propose that continuous Trim62 activation contributes to persistent inflammation in muscle, promoting atrophy and development of ICUAW in critically ill patients. To our knowledge, the present study is the first in which TRIM62 regulation has been investigated in human and mouse skeletal muscle during atrophy.

TRIM62 belongs to the TRIM family of RING finger E3 ubiquitin ligases [18,19] that is involved in the regulation of differentiation, immunity, development and apoptosis [18]. This protein family is also involved in muscular protein homeostasis. In muscle, TRIM mutations lead to primary myopathies. For instance, mutated *TRIM32* leads to limb-girdle muscular dystrophy type 2H [27]; mutations in *TRIM18* (MID1) cause Opitz G/BBB syndrome [28]; and *TRIM63*/MuRF1 mutations result in hypertrophic cardiomyopathy [29]. The MuRF family (MuRF1/*TRIM63*, MuRF2/*TRIM55* and MuRF3/*TRIM54*) of TRIM proteins has essential functions for protein homeostasis in striated muscle responsible for myogenesis [30] and maintenance [10,31-33]. Our data indicate that TRIM62 is also involved in muscular protein homeostasis, especially during inflammation-induced atrophy. We have established TRIM62 as novel atrophy marker. However, further studies are needed to understand its importance in muscle atrophy.

Critically ill patients often develop ICUAW as a severe complication of critical illness. It affects more than half

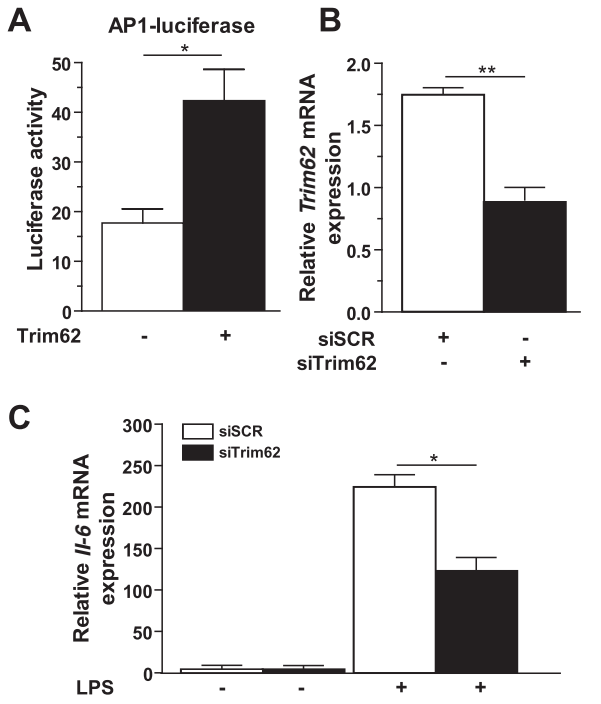

Figure 7 Trim62 is involved in atrophy signaling. (A) Luciferase assays were performed on cell extracts of HEK293 cells transfected with the activator protein 1 (AP-1) luciferase reporter plasmid, along with expression plasmids encoding the Trim62 protein (+) or empty (–) expression plasmid. Values were normalized to expression of CMV-LacZ and are expressed as the relative luciferase activity to CMV-LacZ ratio with the Trim62 expression plasmid compared to the reporter alone. Data are shown as mean ± SEM. *P <0.05. **(B)** Differentiated C2C12 myotubes were transfected with control scrambled small interfering RNA (siSCR) or siRNA against Trim62 (siTrim62) (25 nM each), as indicated. Cells were harvested 24 hours after transfection, and *Trim62* expression was quantified using quantitative RT-PCR analysis. Glyceraldehyde-3 phosphate dehydrogenase (*Gapdh*) expression was used as the reference value. Data are presented as mean ± SEM. **P <0.01. **(C)** Differentiated C2C12 myotubes were transfected with siSCR or siTrim62 (25 nM each) and treated with vehicle (–) or lipopolysaccharide (LPS, +, 100 μM, for 2 hours), as indicated. RT-PCR was used to quantitate *Trim62* expression. *Gapdh* expression was used as the reference value. Data are presented as mean ± SEM. *P <0.05. IL-6, Interleukin 6.

of all ICU patients [34]. ICUAW is characterized by skeletal muscle atrophy and weakness [2-4], is associated with elevated morbidity and mortality and impairs short- and long-term clinical outcomes [6,35]. Recently, we found persistently increased *IL-6* expression and elevated SAA1 content in muscles of critically ill patients [13,24]. On the basis of these data, we hypothesized that continuous inflammation and acute-phase response in muscle play an important role in ICUAW [13,24]. Our observation that TRIM62 is persistently increased in muscles of critically ill patients at high risk of developing ICUAW and in all atrophy mouse models strengthens this hypothesis. Knockdown of TRIM62 in primary macrophages abolished TRIF-mediated AP-1 signal transduction following LPS treatment [21]. We show that this pathway is also active in myocytes in our finding that knockdown of Trim62 inhibited LPS-induced *Il-6* expression in C2C12 cells. These proinflammatory actions of TRIM62 might explain our recent finding of persistently increased *IL-6* expression in muscles of ICUAW patients [13,24]. Because AP-1 signaling is essential in denervation-induced atrophy [22], TRIM62-mediated AP-1 activation is particularly important. We confirm that Trim62 is involved in this cascade, because its overexpression increased AP-1 activity in myocytes. We propose that persistent activation of TRIM62 in muscle could lead to chronically increased AP-1 activity, promoting atrophy even when Atrogin1 and MuRF1 expression have normalized.

An imbalanced muscular protein homeostasis with increased UPS-mediated protein degradation and reduced protein synthesis plays a dominant role in critically ill patients [1,8]. Atrogin1 and MuRF1 are key factors in this pathway and are consistently used as atrophy markers [12]. Atrogin1 and MuRF1 are rapidly, but only transiently, increased in muscles of critically ill patients [8] and in atrophy mouse models [12]. This time course of *Atrogin1* and *MuRF1* expression is consistent with our findings reported here. In contrast, muscular *Trim62* expression remained increased throughout the disease course in ICUAW patients and in all mouse models. These findings support the assumption that Atrogin1 and MuRF1 are predominantly involved in the early phase of atrophy. We suggest that early and sustained muscular *Trim62* expression continuously activate atrophy signaling pathways involved in later phases of the disease process. Increased muscular *Trim62* expression in all atrophy models implicates that *Trim62* upregulation is a general and nonspecific feature in muscle atrophy.

Limitations

We used CLP to investigate inflammation-induced muscle atrophy. This method causes polymicrobial sepsis and systemic inflammation in mice. Therefore, we cannot define a precise pathomechanism (that is, specific inflammatory cytokines, bacteria or fungi) responsible for inflammation-induced muscle atrophy. However, CLP is considered a gold standard in sepsis research [25,36,37]. The pathomechanisms and cytokine release following CLP are close to the human situation in sepsis [38-40]. Further studies are needed to investigate specific pathways in inflammation-induced atrophy.

The balance between protein synthesis and degradation responsible for maintenance of muscular protein homeostasis is disturbed in critically ill patients [1]. In our present study, we found an increased atrogene gene expression indicative for enhanced UPS-dependent protein degradation and muscle atrophy [12,41,42]. However, we did not

investigate whether protein synthesis was reduced in our models. Therefore, we cannot draw any conclusions about whether muscle atrophy occurred because of increased protein degradation alone or whether an accompanying decrease in protein synthesis contributed to this phenotype.

Conclusions

Chronic inflammation and acute-phase response in muscle plays an important role in ICUAW pathogenesis [13,24]. TRIM62 might be a new factor responsible for persistent inflammation in muscle that promotes muscle atrophy and ICUAW. *TRIM62* gene expression and protein content were increased in muscles of critically ill patients and three standard murine muscle atrophy models. Continuously increased muscular *TRIM62* expression throughout the disease course in ICUAW patients and in all mouse models was in clear contrast to the time course of the commonly used atrophy markers Atrogin1 and MuRF1, which are rapidly, but only transiently, increased in muscles of critically ill patients [8] and murine muscle atrophy models [12]. Sustained muscular *TRIM62* expression might point toward its role in later phases of muscle atrophy, whereas Atrogin1 and MuRF1 are involved predominantly in the early disease phase. With these data, we establish TRIM62 as a novel marker for muscle atrophy. In summary, we think that Trim62 contributes to inflammation-induced muscle atrophy at least in part by activation of the AP-1 signal transduction pathway.

Key messages

- Persistent inflammation and acute-phase response in muscle contributes to the pathogenesis of ICUAW.
- The E3 ubiquitin ligase and activator of inflammation TRIM62 is continuously increased in skeletal muscle of ICUAW patients and mouse models of inflammation-induced atrophy.
- In contrast to bona fide atrogenes, TRIM62 expression remains increased in atrophic muscle throughout the disease process.
- Overexpression of Trim62 activated the atrophy-inducing AP-1 signal transduction pathway in myocytes, and its knockdown inhibited LPS-induced *Il-6* expression.
- TRIM62 could play a role in ICUAW pathogenesis by activating and maintaining inflammation in muscle.

Additional files

Additional file 1: Table S1. Patient characteristics. ALI/ARDS, Acute lung injury/acute respiratory distress syndrome; BMI, Body mass index; CNS, Central nervous system; ICU, Intensive care unit; MRC, Medical Research Council; NA, Not applicable; ND, Not determined; SOFA, Sequential Organ Failure Assessment score; SAPS-II, Simplified Acute Physiology Score II. **Table S2.** Treatment of ICU patients during study period. RASS, Richmond Agitation Sedation Scale; PBW, Predicted body weight. Data shown are median (IQR) or number and percentage. **Table S3.** Primer pairs for quantitative RT-PCR are shown. TRIM62, Tripartite motif–containing 62; Saa1, Serum amyloid A1; MuRF1, Muscle RING (really interesting new gene) finger–containing protein; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; IL-6, Interleukin 6; Tnfα, Tumor necrosis factor α; Hs, Homo sapiens; Mm, *Mus musculus*.

Additional file 2: Figure S1. Study protocol.

Additional file 3: Figure S2. CLP-induced inflammation and acute-phase response in liver. Quantitative RT-PCR analyses of interleukin 6 (*Il-6*), tumor necrosis factor α (*Tnfa*) and serum amyloid A1 (*Saa1*) expression in the liver 24 hours, 48 hours, 72 hours or 96 hours after surgery, as indicated. Glyceraldehyde 3-phosphate dehydrogenase expression was used as a reference, and data are shown as relative expression. Data presented are mean ± SEM. $^{**}P$ <0.01, $^{*}P$ <0.05.

Additional file 4: Figure S3. Body weight during skeletal muscle atrophy. Body weight normalized to tibia length is shown for skeletal muscle atrophy induced by CLP **(A)**, starvation **(B)** and denervation **(C)**. Data presented are mean ± SEM. $^{**}P$ <0.01, $^{*}P$ <0.05.

Abbreviations

AP-1: Activator protein 1; CLP: Cecal ligation and puncture; ICUAW: ICU-acquired weakness; Il-6: Interleukin 6; MuRF: Muscle RING (really interesting new gene) finger–containing protein; MyHC: Myosin heavy chain; qRT-PCR: Quantitative real-time polymerase chain reaction; SAA: Serum amyloid A; TLR4: Toll-like receptor 4; TRIF: Toll/interleukin 1 receptor domain–containing adapter inducing interferon β; Trim: Tripartite motif–containing.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FS, MK, XZ, CL, DL and JF designed and analyzed the experiments and prepared the manuscript. KP performed and analyzed the experiments. TW, CK and SWC conducted and analyzed the clinical trial. All authors read and approved the final manuscript.

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Author details

[1]Experimental and Clinical Research Center (ECRC), a Cooperation between Max-Delbrück-Centrum and Charité-Universitätsmedizin Berlin, Campus Buch, Lindenberger Weg 80, 13125 Berlin, Germany. [2]Charité-Universitätsmedizin Berlin, Campus Virchow and Campus Mitte, Anesthesiology and Operative Intensive Care Medicine, Augustenburger Platz 1, 13353 Berlin, Germany. [3]Charité-Universitätsmedizin Berlin, Campus Virchow, Center for Musculoskeletal Surgery, Augustenburger Platz 1, 13353 Berlin, Germany. [4]Cardiology, Charité-Universitätsmedizin Berlin, Campus Virchow, Augustenburger Platz 1, 13353 Berlin, Germany.

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2.2 Anwendbarkeit einer Ganzkörpervibrationstherapie bei Intensivpatienten zur Prävention einer auf der Intensivstation erworbenen Muskelschwäche

Wollersheim T, Haas K, Wolf S, Mai K, Spies C, Steinhagen-Thiessen E, Wernecke KD, Spranger J, Weber-Carstens S. Whole-body vibration to prevent intensive care unit-acquired weakness: safety, feasibility, and metabolic response. Crit Care. 2017;21(1):9. doi: 10.1186/s13054-016-1576-y

Wir führten in unserer Arbeitsgruppe eine Pilotstudie zur intra-induviduellen elektrischen Muskelstimulation durch, die auf molekularer Ebene einen Erhalt von Muskelmasse durch frühe, tägliche Intervention zeigte. Jedoch ist die elektrische Muskelstimulation sehr zeitaufwendig und nicht bei allen Patienten anwendbar. Als alternative Maßnahme zur frühen Muskelaktivierung stießen wir auf die Ganzkörpervibrationstherapie. Diese wurde vor unserer Studie bei neurologischen Patienten, in der Raumfahrt und im Leistungssport eingesetzt. Untersuchungen an Intensivpatienten waren nicht beschrieben. Erstmalig führten wir eine Untersuchung zur Anwendbarkeit einer Ganzkörpervibrationstherapie bei Intensivpatienten mit den Gesichtspunkten Durchführbarkeit, Patientensicherheit und metabolische Detektion einer tatsächlichen Muskelaktivierung durch.

In der Studie untersuchten wir den direkten Einfluss von passiver Physiotherapie und einer anschließenden 20-minütiger Ganzkörpervibrationstherapie auf die Vitalparameter und den Energiestoffwechsel während und bis 1 Stunde nach der Therapie. Wir verglichen unsere Messungen mit den jeweiligen Ausgangswerten unmittelbar vor dem Interventionsbeginn der 19 Intensivpatienten.
Als Ergebnis zeigte sich, dass eine Ganzkörpervibrationstherapie nur geringe Änderungen der Vitalparameter bewirkt und dabei den Energieumsatz im Sinne einer Muskelaktivierung steigert.
Zusammenfassend konnten wir beschreiben, dass eine Ganzkörpervibrationstherapie auch bei Intensivpatienten einfach und sicher anwendbar ist. Diese Daten waren die Grundlage für die folgende randomisiert-kontrollierte Interventionsstudie mit elektrischer Muskelstimulation und Ganzkörpervibrationstherapie mit den Zielparametern von Muskelkraft- und Muskelmassenerhalt bei Intensivpatienten.

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Critical Care

RESEARCH Open Access

Whole-body vibration to prevent intensive care unit-acquired weakness: safety, feasibility, and metabolic response

Tobias Wollersheim[1,2†], Kurt Haas[1†], Stefan Wolf[3], Knut Mai[2,4], Claudia Spies[1], Elisabeth Steinhagen-Thiessen[5], Klaus-D. Wernecke[1,6], Joachim Spranger[2,4,7] and Steffen Weber-Carstens[1,2*]

Abstract

Background: Intensive care unit (ICU)-acquired weakness in critically ill patients is a common and significant complication affecting the course of critical illness. Whole-body vibration is known to be effective muscle training and may be an option in diminishing weakness and muscle wasting. Especially, patients who are immobilized and not available for active physiotherapy may benefit. Until now whole-body vibration was not investigated in mechanically ventilated ICU patients. We investigated the safety, feasibility, and metabolic response of whole-body vibration in critically ill patients.

Methods: We investigated 19 mechanically ventilated, immobilized ICU patients. Passive range of motion was performed prior to whole-body vibration therapy held in the supine position for 15 minutes. Continuous monitoring of vital signs, hemodynamics, and energy metabolism, as well as intermittent blood sampling, took place from the start of baseline measurements up to 1 hour post intervention. We performed comparative longitudinal analysis of the phases before, during, and after intervention.

Results: Vital signs and hemodynamic parameters remained stable with only minor changes resulting from the intervention. No application had to be interrupted. We did not observe any adverse event. Whole-body vibration did not significantly and/or clinically change vital signs and hemodynamics. A significant increase in energy expenditure during whole-body vibration could be observed.

Conclusions: In our study the application of whole-body vibration was safe and feasible. The technique leads to increased energy expenditure. This may offer the chance to treat patients in the ICU with whole-body vibration. Further investigations should focus on the efficacy of whole-body vibration in the prevention of ICU-acquired weakness.

Trial registration: Applicability and Safety of Vibration Therapy in Intensive Care Unit (ICU) Patients. ClinicalTrials.gov NCT01286610. Registered 28 January 2011.

Keywords: Intensive care unit-acquired weakness, Physiotherapy, Whole-body vibration, Mobilization, Muscle wasting, Metabolism

* Correspondence: steffen.weber-carstens@charite.de
†Equal contributors
[1]Department of Anesthesiology and Operative Intensive Care Medicine, Campus Virchow Klinikum and Campus Mitte, Charité—Universitätsmedizin Berlin, Augustenburger Platz 1, Berlin 13353, Germany
[2]Berlin Institute of Health (BIH), Berlin 13353, Germany
Full list of author information is available at the end of the article

Background

Muscle wasting and intensive care unit-acquired weakness (ICU-AW) are common complications in ICU patients, leading to longer ICU and hospital stay, higher morbidity and mortality, as well as a poor long-term prognosis [1–3]. Sepsis, multiple organ failure, muscle inactivity, hyperglycemia, as well as the use of corticosteroids and neuromuscular blocking agents were identified as risk factors [1, 4, 5]. ICU-AW diagnosis is often delayed during the ICU stay, usually after a reduction of analgesics and anxiolytics, as the patients first become fully alert. Decreased muscle protein synthesis and increased protein degradation are involved in the pathomechanism, and occur very early during critical illness [6, 7]. Early mobilization of alert patients reduces the length of mechanical ventilation and ICU and hospital stay [8, 9], and leads to better functional independence at hospital discharge [8]. These results only relate to patients who are able to participate in active physiotherapy. Hence follows the idea of closing the gap between onset of critical illness and active muscle training, using external devices during immobilization and sedation phases to evoke muscle contractions [10–13]. During this time course of disease there are further options for intensified passive mobilization by physiotherapists, such as passive cycling or motorized continuous passive motion for different conditions, which we separate from treatment options for active muscle training indicated by patients initiating muscle contraction or from external evoked ones. A series of investigations with electrical muscle stimulation (EMS) in critically ill patients therefore commenced, and while some EMS studies showed promising results [11, 14], others could not [13]. From our own experience we know that application of EMS is time consuming, if feasible at all, and effectiveness is inconsistent [15]. As an alternative, we propose the use of whole-body vibration (WBV) for muscle activation in the ICU. First investigations of human tolerance when exposed to vibration date back to the 1960s [16], and to this day the use of vibration has become more and more interesting in many different approaches and popular in the fitness world. Companies offer devices starting at around €1000. WBV is used as a countermeasure to muscle atrophy and bone loss during the absence of gravity in space, as well as a training option for professional athletes [17, 18] and patients with various underlying diseases [19]. The spinal cord reflex function means that WBV may be suitable for unconscious patients, because muscle contraction occurs at a spinal level and not at a cerebral level [20–22]. There is evidence that prolonged application of WBV helps to maintain muscular mass and strength, increases bone density, improves outcome, and increases glucose metabolism, as shown in healthy volunteers, athletes, older people, or non-ICU patients in the short term [17, 18, 23–30]. These benefits correspond to the needs of critically ill patients and may support ICU patient recovery, although thus far there are no WBV investigations in mechanically ventilated ICU patients. Our aim is to transfer the application of WBV to the ICU.

We hypothesize that the use of WBV in mechanically ventilated ICU patients is safe, feasible, and effective in inducing skeletal muscle activation.

Methods

Design

During a 12-month period, we recruited patients in a mixed ICU and a neurosurgical ICU at a university hospital. In our pilot interventional study, we enrolled critically ill patients who were mechanically ventilated for more than 48 hours with an estimated ICU stay of at least 7 days. Our primary outcome was to show safety and tolerability of WBV by stability of vital parameters (see Additional file 1). Criteria for noninclusion were: lack of informed consent, age < 18 years, preexisting neuromuscular diseases, implanted pacemaker or defibrillator, pregnancy, acute venous thrombosis, unhealed fractures or recently attached implants in body region to be stimulated, recent eye surgery, history of acute herniated discs with acute symptoms, participant in another study, as well as terminal cases. Informed consent was obtained from a legal proxy. The local ethics committee of the Charité (Charité—Universitätsmedizin Berlin, Ethics Commission, Charitéplatz 1, 10117 Berlin, Germany) gave their consent (EA1/017/11). Following a predefined protocol, enrolled patients received passive physiotherapy followed by a single session of WBV. Continuous monitoring of vital signs, hemodynamics, and energy metabolism, as well as intermitted blood sampling (Fig. 1a), took place from the start of baseline measurements up to 1 hour post intervention (for detailed data processing see Additional file 1). The patients were in the supine position during the entire intervention, and no changes in body position took place to avoid any influence on hemodynamic parameters and vital signs. Following baseline measurements, patients were mobilized passively by a physiotherapist for 6 minutes as a warm-up. WBV treatment was then initiated, consisting of a vibration device placed under the patient's feet, with resistance to the end of the bed. The patient's hips and knees were flexed at about 20°. An elastic strip provided pressure on the knees, pushing the patient's feet against the vibration device (Fig. 1b). WBV sessions took 15 minutes, with 9 minutes of clear vibration time. We used two different devices following the manufacturers' instructions for WBV: one device with synchronous vibration (Promedi, Vibrosphere®, 26 Hz, nine times for

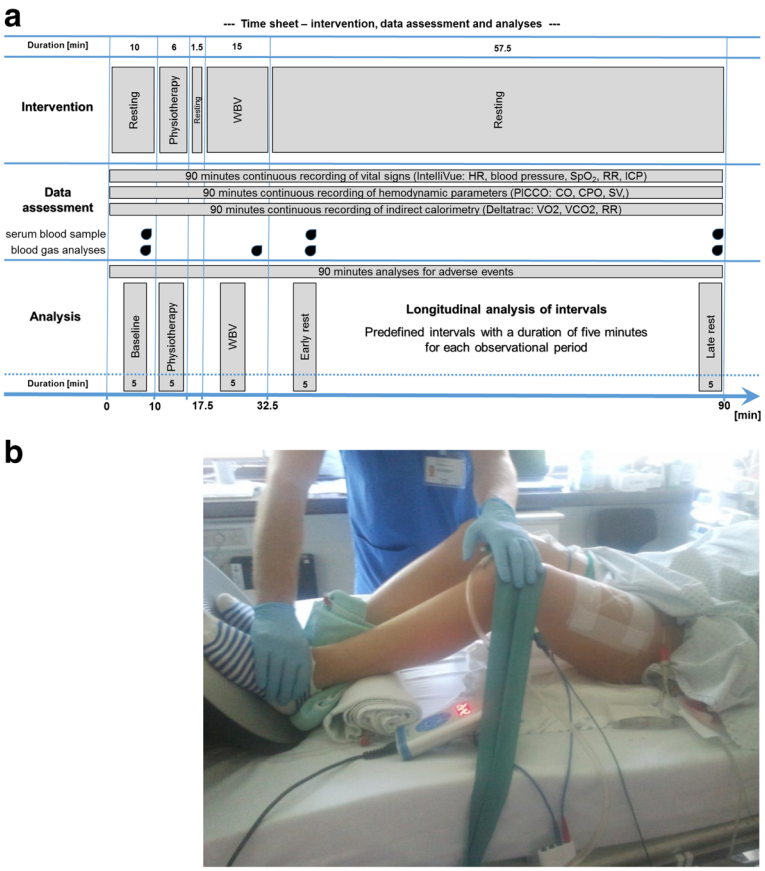

Fig. 1 Study protocol and visual presentation of study execution. **a** Visualization of study protocol. Intervention started with 10 minutes of resting, followed by 6 minutes of physiotherapy (passive range of motion of upper and lower extremity). After physiotherapy there was a short resting time, followed by WBV. After WBV, a long resting period took place. Serum blood samples and blood gas analyses were performed at different time points, as shown. Longitudinal analysis of intervals was performed at five different time segments. Analysis was performed at baseline, at physiotherapy, during WBV, and at early and late rest periods. **b** Female patient in a supine position. Vibration device positioned at the end of the bed, with the patient's feet placed on the middle of the device. An elastic strap is placed around the knee joint to generate pressure on the vibration device. The aim was to flex the knee joint about 20°. The physiotherapist assisted in the stabilization of the lower extremities if necessary. *WBV* whole-body vibration

1 minute), and the other with side alternating vibration (Galileo, home-ICU®, 24 Hz, three times for 3 minutes).

Termination criteria for WBV sessions were predefined as follows: heart rate < 40 or > 180 beats per minute; systolic blood pressure < 80 mmHg or > 200 mmHg; mean arterial blood pressure < 60 mmHg or > 120 mmHg; increase in intracerebral pressure > 20 mmHg; SpO_2 < 88%; or potassium levels < 3.0 mmol/l or > 5.5 mmol/l.

Data assessment

Data collection was performed using ICM+ software (University of Cambridge) with a recording rate of 50 Hz, where vital signs were monitored using Intellivue (MP30; Phillips) and hemodynamic parameters using $PiCCO_2$ (Pulsion Medical Systems, Germany). Indirect calorimetry was performed using Deltatrac (Datex Ohmeda, Finland), and was recorded with Datex Collect with a frequency of one mean per minute. Thermodilution for the $PiCCO_2$ system and calibration of all devices took place before each individual session.

We obtained blood gas analyses (BGA) at four time points (Fig. 1a), and measured levels of pO_2, pCO_2, pH, sodium, potassium, and blood glucose concentration using a Radiometer ABL 800. Values were used to describe steady-state conditions during the observation, and to observe metabolic response to the intervention. We additionally investigated serum levels of insulin-like growth factor I (IGF-I) and cortisol before and twice after the intervention, because they represent systemic anabolic and catabolic hormones with major influence on the skeletal muscle. Both hormones had been investigated previously within a WBV setting and showed significant changes in healthy controls [31, 32].

Data analyses

Besides evaluating the continuous recordings to exclude adverse events, we focused our analyses on comparable time intervals for different parts during the observation. Furthermore, we selected similar predefined time intervals of 5-minute recordings, so as to have coherent and comparable longitudinal data for these observations (Fig. 1a). Testing for equivalence of the multiple primary endpoint (heart rate and systolic blood pressure) was performed for the first observations from baseline and WBV therapy as well as for the mean values of the respective phases. Longitudinal analysis examined data in phases from the baseline, physiotherapy, WBV therapy, early resting period (10 minutes after intervention), and late resting period (50 minutes after intervention).

Statistical analyses

Results are expressed as medians with interquartile range, or as indicated in the legend. After proof of the multiple primary endpoint for equivalence using the confidence interval method and Schuirman's OST/TOST for means-paired design [33], we analyzed our time-dependent data in a multivariate nonparametric analysis of longitudinal data in a two-factorial design (first factor (dependent): phases, second factor (dependent): time) [34]. Blood analyses over phases were tested by paired Wilcoxon rank tests for depending samples. A two-tailed p value < 0.05 was considered statistically significant. All tests of secondary endpoints were conducted in the area of exploratory data analysis. Therefore, no adjustments for multiple testing have been made. Statistical analyses and graphs were performed using R i386 software, version 2.15.3, IBM SPSS statistics, version 22, and SigmaPlot, version 12.

Results

Patients

Patients' baseline characteristics and medical status on the intervention day are presented in Table 1. All 19 study participants completed the intervention. During the entire observation, no patient reached predefined termination criteria or suffered from related adverse events. No endotracheal tube, tracheal cannula, drain, infusion line, ECMO-cannula central venous catheter, or dialyses catheter was dislocated. The application procedure was simple for a physiotherapist and did not influence the clinical routine more than standard physiotherapy. Preparation for WBV is simple and takes less than 3 minutes.

Table 1 Characterization of study participants

Study participants, *n*	19
Subgroup Vibrosphere	12
Subgroup Galileo	7
Age, years	54 (52/59)
Gender, male/female	11/7 (57.9%/42.1%)
BMI (kg/m^2)	28 (24/31)
Diagnosis	
ARDS	9 (47.4%)
Trauma	2 (10.5%)
CNS	8 (42.1%)
Days between ICU admission and intervention	15 (8/18)
Illness severity at ICU admission	
SOFA score	10 (9/13)
SAPS-II	53 (35/78)
Illness severity at intervention day	
SOFA score	9 (6/10)
SAPS-II	48 (38/52)
GCS at intervention day	5 (3/11)
Sedation, RASS at intervention day	−4 (−4/0)
Selective medication during intervention, number of patients received and rate in those	
Norepinephrine, 12 of 19 patients (rate μg/kg/min)	0.100 (0.048/0.140)
Propofol, 3 of 19 patients (rate mg/kg/min)	0.033 (0.031/0.033)
Midazoloam, 3 of 19 patients (rate mg/kg/min)	0.002 (0.001/0.003)
Sufentanil, 10 of 19 patients (rate μg/kg/min)	0.011 (0.003/0.020)
Clonidin, 6 of 19 patients (rate μg/kg/min)	0.013 (0.007/0.014)

Results expressed as medians with interquartile range (median (25th/75th), or as absolute numbers with percentages (%)
BMI body mass index, *ARDS* acute respiratory distress syndrome, *CNS* central nervous system, *ICU* intensive care unit, *SOFA* Sequential Organ Failure Assessment, *SAPS-II* Simplified Acute Physiology Score-II, *GCS* Glasgow Coma Scale, *RASS* Richmond Agitation Sedation Scale

Multiple primary endpoint

Equivalence testing for baseline against WBV therapy of the multiple primary endpoint consisting of heart rate and systolic blood pressure in a means-paired design (equivalence margins: ±20% (mean baseline) each) resulted in significant equivalence ($p < 0.0001$), adjusted for multiple testing, both using first observations and mean values of the respective phases.

Longitudinal analyses

Vital signs

Measurements of vital signs did not significantly change during and after intervention, when compared with baseline (Fig. 2). Minor changes were observed, but were never critical for the patients' safety. Although the baseline values varied between patients (Fig. 2, gray dots and lines), individual changes were in a small range (Fig. 2, black triangles and lines). Diastolic blood pressure was significantly elevated during the physiotherapy period as compared with baseline ($p = 0.014$), which did not occur

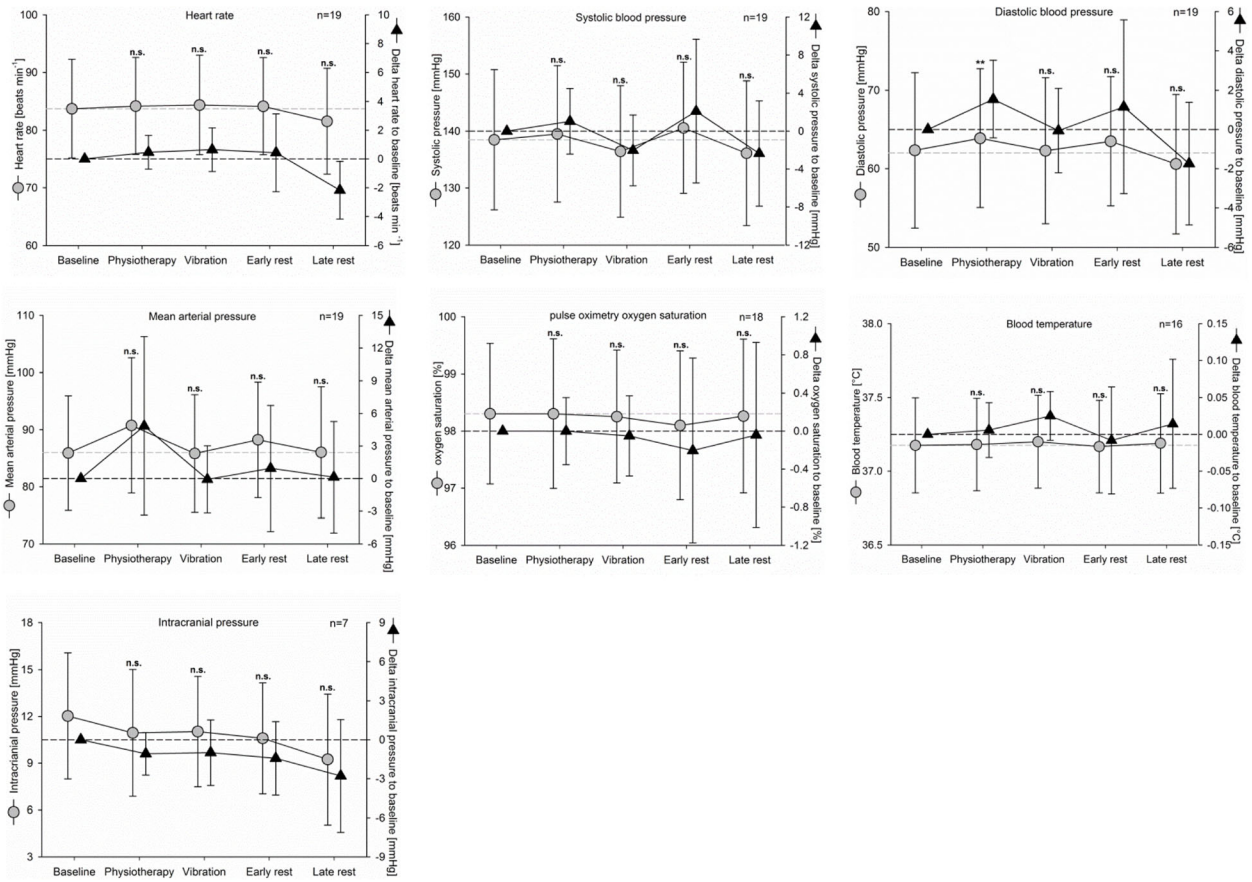

Fig. 2 Vital signs for longitudinal observation. *Gray dots and lines*, absolute values; *black triangles and lines*, changes compared with baseline values, mean and 95% CI. *n.s.* not significant to baseline, $^{**}p < 0.01$

during the WBV, early, or late resting periods. Heart rate, mean arterial pressure, systolic blood pressure, and oxygen saturation did not differ significantly from baseline during physiotherapy, WBV, or the resting periods.

Intracranial pressure

Out of 19 patients, seven had an extraventricular liquor drain to measure intracranial pressure (Fig. 2). Neither the physiotherapy intervention, in line with previous investigations [35], nor the WBV significantly influenced intracranial pressure levels.

Hemodynamics

Hemodynamic parameters were measured using the $PiCCO_2$ Medical-System in a total of 15 patients (Fig. 3). Cardiac output (CO), stroke volume (SV), and stroke volume range (SV minimum, SV maximum) were not significantly influenced by the interventions and remained stable during resting time. Cardiac power output (CPO) showed a significant, but clinically irrelevant decrease during the WBV period compared with baseline ($p = 0.047$), without significant changes in CO and blood pressure. SV variability increased significantly during the physiotherapy period in comparison with the baseline ($p < 0.001$), but was not significantly influenced by WBV or during resting periods when compared with baseline.

Energy metabolism

We measured indirect calorimetry for 16 patients, and found increased energy expenditure (EE) only during WBV (Fig. 4). Comparing the WBV period with the baseline, oxygen uptake levels were significantly increased ($p = 0.012$) and carbon dioxide production was enhanced ($p < 0.001$), showing increased energy expenditure ($p = 0.007$). In contrast, physiotherapy led to increased elimination of carbon dioxide ($p = 0.041$) but not to increased oxygen uptake or increased energy expenditures. During the early and late resting periods, oxygen uptake and energy expenditure did return to baseline values. Carbon dioxide elimination values remained increased during the early resting period ($p < 0.01$), and achieved baseline levels only during the late resting

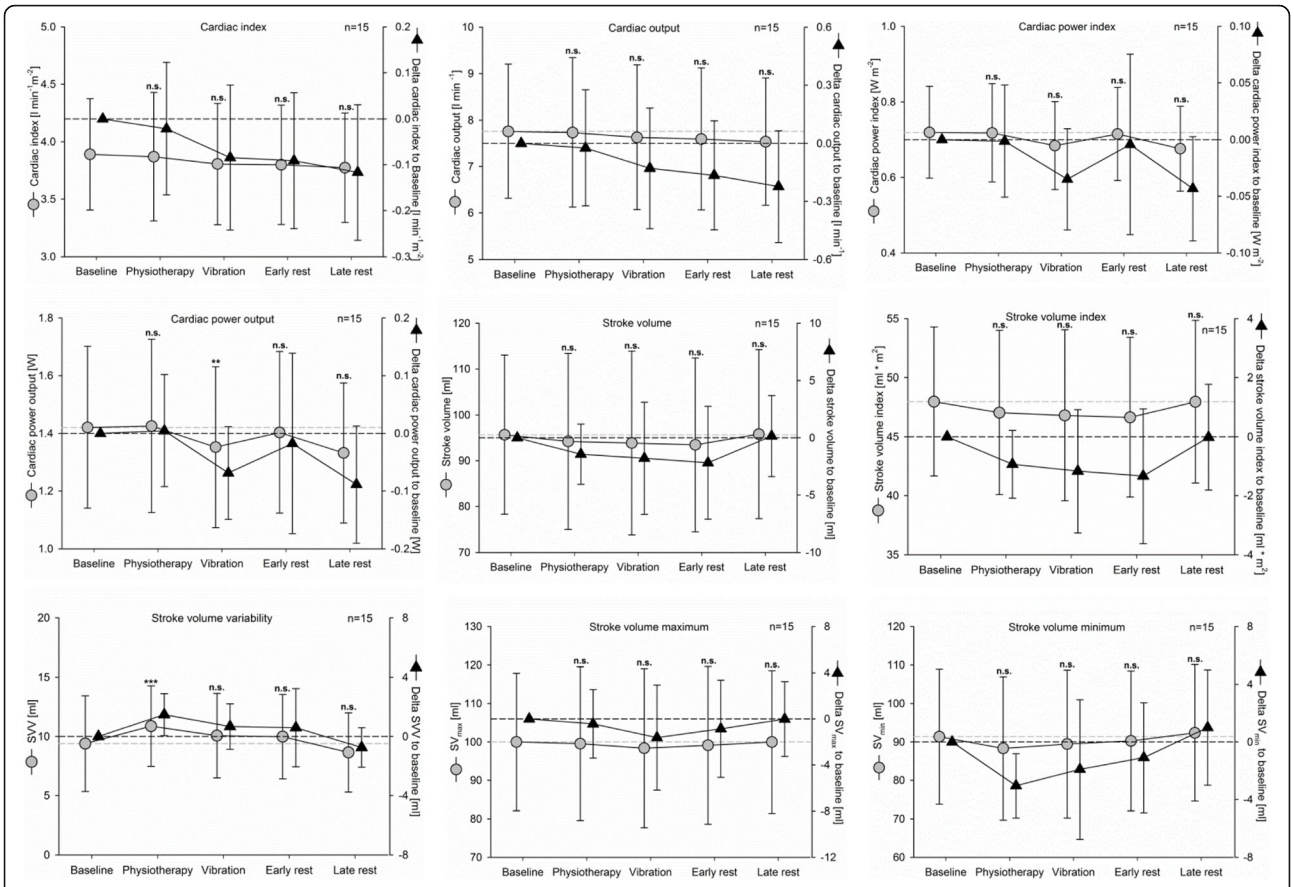

Fig. 3 Hemodynamic parameters for longitudinal observation. *Gray dots and lines*, absolute values; *black triangles and lines*, changes compared with baseline values, mean and 95% CI. *n.s.* not significant to baseline, $^{**}p < 0.01$, $^{***}p < 0.01$

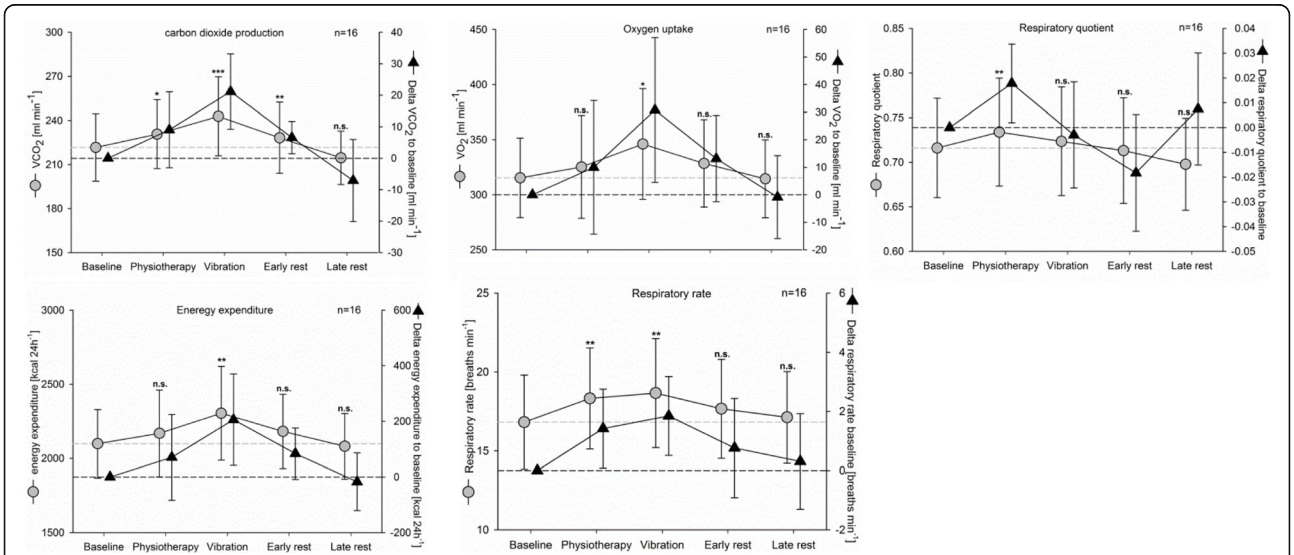

Fig. 4 Energy metabolism measurements for longitudinal observation. *Gray dots and lines*, absolute values; *black triangles and lines*, changes compared with baseline values, mean and 95% CI. *n.s.* not significant to baseline, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$

period. Physiotherapy ($p < 0.01$) and WBV ($p < 0.001$) increased the respiratory rate significantly compared with baseline. The respiratory quotient (RQ) increase significant during physiotherapy ($p = 0.033$), which is caused by increased carbon dioxide elimination.

Blood analyses

The BGA ($n = 19$) show a stable ventilation state for the patients, indicated by unchanged pO_2 and pCO_2, acid–base state (pH, bicarbonate (HCO_3^-), base excess), and oximetry during the entire examination (Fig. 5). WBV

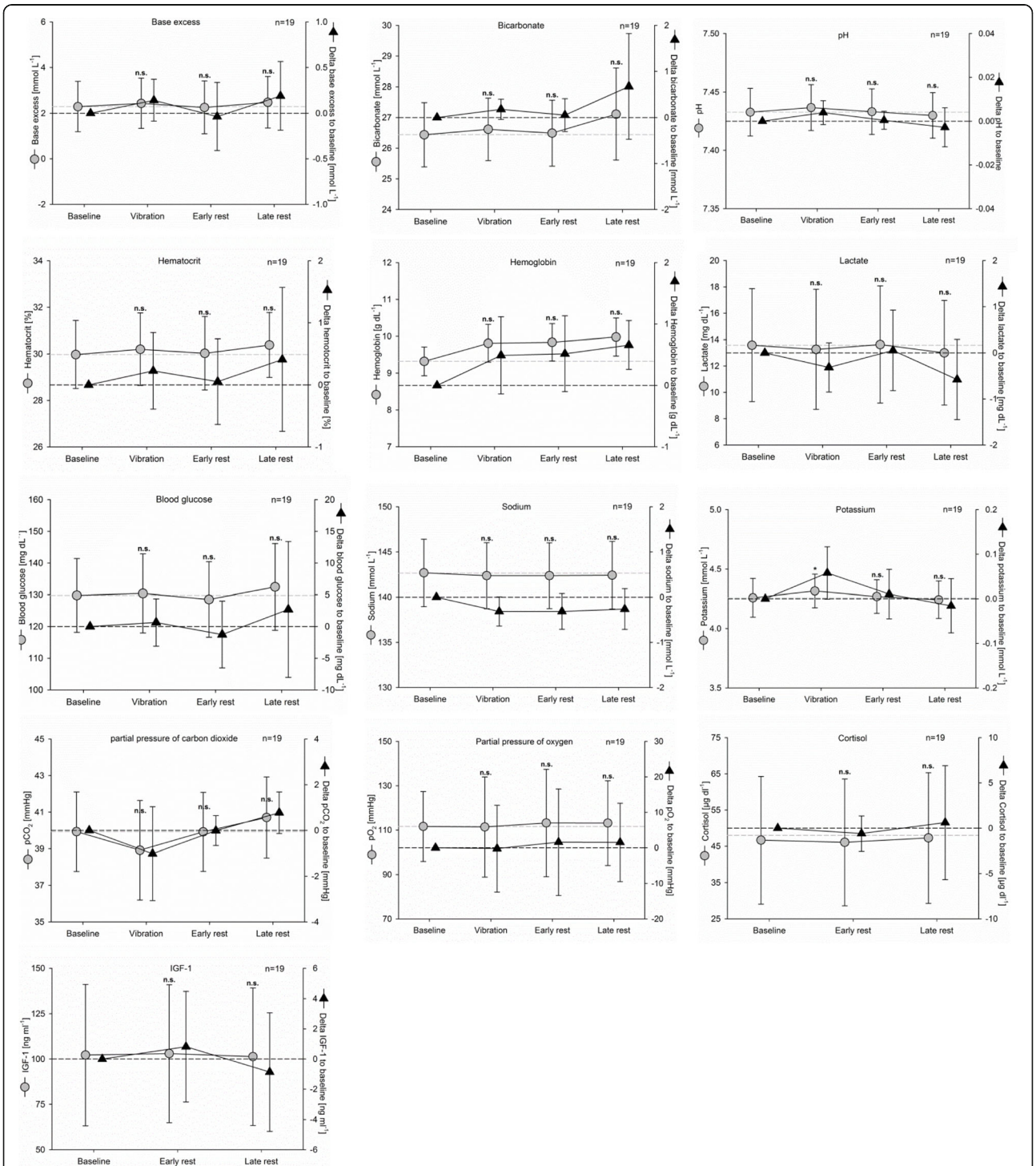

Fig. 5 Laboratory blood measurements for longitudinal observation. *Gray dots and lines*, absolute values; *black triangles and lines*, changes compared with baseline values, mean and 95% CI. *n.s.* not significant to baseline, *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$. *IGF-I* insulin-like growth factor I

was associated with a significant increase of potassium serum levels compared with baseline ($p = 0.048$). This effect was not observed during physiotherapy only. The sodium concentrations within the same blood samples remained unchanged, indicating no errors in the sampling. Furthermore, expected changes for glucose and lactate levels could not be observed. Measuring IGF-1 and cortisol levels resulted in a large range of baseline values, which may have contributed to the fact that no significant changes could be observed.

Discussion

To the best of our knowledge, this is the first report about safety and feasibility of WBV in critically ill, mechanically ventilated patients. We found that WBV is safely applicable even to critically ill patients in severe condition, as indicated by high SOFA and SAPS-II scores in addition to mechanical ventilation.

Our approach is to induce muscle activation during early critical illness, when patients are unable to participate in active physiotherapy due to sedation or unconsciousness due to neurological reasons. WBV might be an option to evoke muscle activation within a protocol-based physiotherapy and mobilization plan during the course of disease. Additionally, WBV may be a treatment option throughout the ICU stay; that is, may be continued when patients are awake.

The beneficial effect of physiotherapy and early mobilization, which has been shown to be safe and feasible, has been shown in several clinical studies [8, 9, 36, 37]. There are still phases in which patients are not available for active physiotherapy, and these intervals often coincide with intervals of severe illness, acute systemic inflammation, or dependency on norepinephrine for hemodynamic stability. These early periods of critical illness and inflammation are particularly significant in the development of muscle wasting and ICU-AW, as we [6, 14] and others [7] could recently show. Evoked muscle training to avoid immobilization due to EMS can be an option [10–12, 14], but application is labored, often not feasible [15], and in general EMS therapy for ICU patients remains controversial [38]. Alternatively WBV may be able to close the gap between immobilization and active physiotherapy, hypothesizing that frequently applied early muscle activation evoked by WBV may support patient recovery.

WBV represents a strong stimulus to the skeletal muscle, leading to physiological growth adaption in bone and muscle [39, 40]. Clinically, it was shown that WBV improves average velocity, average force, and average power [41] in volunteers and not critically ill patients. The activation on spinal linkage by WBV is evident, as published in a recent investigation showing increased EMG activity on the paretic and nonparetic sides of stroke patients, independent of the intensity of the stimulus [19].

The physiological principal behind WBV is a mechanical stretch and reflex mechanism by the peripheral nerve [20]. Dependent on the frequency of the vibration stimulus, WBV leads to much more than 1000 muscle contractions per minute, leading to increased muscle strength and mass, seen as muscle hypertrophy. This principle of muscle activation agrees with the metabolic findings and expected benefits for ICU patients. Our data show that passive range of motion via physiotherapy increases carbon dioxide elimination, which can be explained by the mobilization of resting blood in the capacity vessels. Absence of active muscle contraction in passive mobilization is reflected by a missing increase in oxygen uptake. In contrast, WBV in critically ill patients increases both carbon dioxide elimination and oxygen uptake in our patients. This has been shown by others in overweight and obese women [42]. The physiotherapist had the subjective impression that, in single cases, patients had an arousal reaction due to the intervention, which was not measurable by RASS scoring but may have an impact on their energy expenditure. We interpret this increased energy turnover as the result of muscular activation. That the increased energy expenditure is caused by actual muscle activation, and not by metabolic dysregulation, is confirmed by steady-state levels for pO2, pCO2, pH, HCO_3^-, and base excess. Time delay between intervention and measurement of the indirect calorimetry may occur but is improbable due to the selected time frame and no significant changes over time within each phase (see Additional file 1). Serum potassium levels were significantly increased only during WBV, probably due to muscle contraction, and unchanged serum sodium levels underline our interpretation.

Besides the mechanical stretch and reflex mechanism by the peripheral nerve caused by the vibration stimuli, there is evidence for an additional, direct impact on different tissues. This could be demonstrated by molecular findings showing beneficial effects of vibration in vivo and in vitro on separated stem cells, myoblasts, and muscle tissue [40, 43, 44]. Ceccarelli et al. [40] showed an increased synthesis and decreased activation of the ubiquitin–proteasome pathway with myostatin and Atrogin-1 suppression in vitro due to vibration. These findings imply that vibration could have a significant impact on maintaining muscle in ICU patients because decreased myosin synthesis and increased myosin degradation is an established mechanism in the development of ICU-AW [6].

Repetitive WBV was shown to have a positive effect on glucose metabolism in type II diabetes patients [27, 28]. We showed recently that EMS has an impact on maintaining muscular mass by improving glucose metabolism in the critically ill [14]. Future studies could investigate whether a similarly positive effect can be achieved by WBV.

We also did not find a serum lactate elevation, which might be expected during extensive muscle training. Thus, WBV does not result in substantial anaerobic muscle activity, which would presumably not be favorable in critically ill patients. Small changes were probably not measurable in an intervention of this scale. Small changes would also explain why we could not find any significant changes in the hormonal regulation of IGF-1 and cortisol, which were shown earlier for both hormones [31, 32].

This pilot study was limited to investigate safety, feasibility, and metabolic response of WBV in critically ill patients, focusing on hemodynamic stability. Thus it was outside the scope of the study to evaluate aspects such as patient comfort, staff workload, and staff acceptance. Further investigations are also needed to assess the most favorable type, intensity, frequency, and duration of WBV in ICU treatment. For the first time in critically ill patients, we could show a safe feasibility of WBV, as well as measure indicators for muscle activation and induced metabolism. These results could be further improved by measuring the muscle activity by electromyography. The next step would be an investigation to determine whether WBV could improve short-term and long-term outcome for ICU patients, by prevention or treatment, as already shown for non-ICU patients.

Conclusion

We conclude—under consideration of the absolute contraindications—that the application of WBV is safe and feasible in critically ill patients. Our results support the principle that WBV stimulates muscle and improves muscle metabolism, and therefore may have the potential to prevent and/or treat muscle weakness in critically ill patients. Further clinical trials are needed to investigate beneficial effects.

Additional file

Additional file 1: Supplement Extended methods: From study execution to the results. Statistical methods. Outcome parameters. Definition of passive and active physiotherapy. Extened results: Detailed results of two-factorial design. Results of three-factorial design. Subgroup analyses between differnet WBV devices. (PDF 2922 kb)

Abbreviations

BGA: Blood gas analyses; CO: Cardiac output; CPO: Cardiac power output; EE: Energy expenditure; EMG: Electromyography; EMS: Electrical muscle stimulation; ICU-AW: Intensive care unit-acquired weakness; IGF-I: Insulin-like growth factor I; pCO2: Partial pressure of carbon dioxide; pO2: Partial pressure of oxygen; SAPS-II: Simplified Acute Physiology Score-II; SOFA: Sepsis-related Organ Failure Assessment; SV: Stroke volume; WBV: whole-body vibration

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Authors' contributions

TW conceived the study, participated in its conception, design, and coordination, acquired the data, performed the statistical analysis and interpretation, and drafted and revised the manuscript. KH participated in the conception, design, and coordination of the study, acquired the data, performed the physiotherapy and WBV, performed statistical analysis and interpretation, coordinated laboratory matters, and drafted and revised the manuscript. SW acquired the data, performed the statistical analysis and interpretation, and revised the manuscript. KM performed laboratory analysis and coordinated laboratory matters and revised the manuscript. CS was responsible for laboratory matters and analysis, provided technical support, and revised the manuscript. ES-T participated in the conception and design of the study, provided technical support, and revised the manuscript. K-DW performed the statistical analysis and interpretation and revised the manuscript. JS was responsible for laboratory matters and analysis, provided technical support, and revised the manuscript. SW-C conceived the study, participated in its conception, design, and coordination, acquired the data, supervised laboratory and statistical analysis and interpretation, supervised laboratory matters, and drafted and revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests. ProMedVi® and Galileo® provided the vibration devices but had no influence on study design and data analysis.

Ethics approval and consent to participate

The local ethics committee of the Charité gave their consent (EA1/017/11). Charité—Universitätsmedizin Berlin; Campus Charité Mitte; Ethics Commission; Committee's Office, Charitéplatz 1, 10117 Berlin; Germany. Informed consent for participation and publication was obtained from each patient or legal proxy.

E-poster presentation

Preliminary data for this manuscript were presented at ESICM Lives 2012 as an e-poster: 0321—Safety and Efficacy of Whole-body-vibration in Critically Ill Patients. T. Wollersheim, *et al. Intensive Care Med* 2012;38(Suppl 1):0321.

Author details

[1]Department of Anesthesiology and Operative Intensive Care Medicine, Campus Virchow Klinikum and Campus Mitte, Charité—Universitätsmedizin Berlin, Augustenburger Platz 1, Berlin 13353, Germany. [2]Berlin Institute of Health (BIH), Berlin 13353, Germany. [3]Department of Neurosurgery, Charité—Universitätsmedizin Berlin, Berlin 13353, Germany. [4]Department of Endocrinology, Diabetes and Nutrition, Charité—Universitätsmedizin Berlin, Berlin 10177, Germany. [5]Research Group on Geriatrics, Charité—Universitätsmedizin Berlin, Berlin 13353, Germany. [6]CRO SOSTANA GmbH Berlin, Berlin 10318, Germany. [7]DZHK (German Centre for Cardiovascular Research), partner site Charité Berlin, Berlin 10177, Germany.

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2.3 Protokollbasierte Physiotherapie und Muskelaktivierung zur Prävention einer auf der Intensivstation erworbenen Muskelschwäche

Wollersheim T, Grunow JJ, Carbon NM, Haas K, Malleike J, Ramme SF, Schneider J, Spies CD, Mardian S, Mai K, Spuler S, Fielitz J, Weber-Carstens S. Muscle wasting and function after muscle activation and early protocol-based physiotherapy: an explorative trial. J Cachexia Sarcopenia Muscle. 2019.
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Frühmobilisation konnte mehrfach als erfolgreiche Maßnahme zur Reduktion von Muskelmassenverlust und Muskelfunktionsverlust gezeigt werden. Jedoch gibt es auch Studien ohne positiven Effekt. Es scheinen Art, Umfang und Zeitpunkt der Intervention von entscheidender Bedeutung zu sein. Unsere Studie beschäftigt sich mit der Frage, ob zusätzliche physiotherapeutische Verfahren ergänzend zur protokollbasierten Physiotherapie einen positiven Effekt auf Muskelmasse und Muskelfunktion haben können. Ein Vorteil der muskelaktivierenden Verfahren ist, dass sie auch ohne aktive Teilnahme der Patienten eine aktive Muskelkontraktion erwirken können.

Wir initiierten unsere Studie mit dem höchsten Maß an Physiotherapie, täglich und protokollbasiert, als Grundtherapie aller 50 eingeschlossenen Patienten. Wir randomisierten die Patienten in die Gruppen „nur Physiotherapie“ oder „Physiotherapie mit erweiterten muskelaktivierende Maßnahmen“. Es erfolgten die zusätzlichen Interventionen elektrische Muskelstimulation, Ganzkörpervibrationstherapie oder elektrische Muskelstimulation und Ganzkörpervibrationstherapie. Wir charakterisierten die Patienten klinisch und nahmen offen chirurgische Muskelbiopsien an Median Tag 15. Vor unserer Studie gab es keine Untersuchung, die klinische Muskelkraft- und Muskelfunktionsmessungen mit molekularen Untersuchungen übereinanderlegen konnte. Molekular untersuchten wir Proteindegradation und Myosinsynthese, wie in der Observationsstudie aus 2014 mit der wir die neuen Ergebnisse zusätzlich als historische Kontrolle mit Standardphysiotherapie in Relation setzten. Darüber hinaus erfolgte eine Langzeit-Follow-up Untersuchung nach einem Jahr.

Wir fanden in der Interventionsgruppe unserer Patienten mit zusätzlichen muskelaktivierenden Verfahren einen Erhalt der Muskelfaserquerschnittsfläche in den Muskelbiopsien des musculus vastus lateralis verglichen zur Kontrollgruppe mit der protokollbasierten Physiotherapie, die eine Atrophie zeigten. Jedoch zeigten beide Gruppen eine deutliche Überlegenheit gemessen an der Muskelfaserquerschnittsfläche gegenüber den historischen Kontrollpatienten, die lediglich eine Standardphysiotherapie erhalten hatten und eine massive Muskelatrophie aufwiesen. Überraschend dabei ist, dass die Intervention trotz erhaltender Muskelmasse scheinbar keinen Einfluss auf die klinisch gemessenen Parameter Muskelkraft- und Muskelfunktion hatte. Zudem zeigt die molekulare Aufbereitung, dass unsere Intervention nicht wie erwartet den Proteinabbau hemmte, sondern vielmehr die Synthese induzierte. Letztlich führt diese Induktion zu einem Muskelmassenerhalt in der Interventionsgruppe verglichen mit der physiotherapeutischen Kontrollgruppe und noch ausgeprägter gegenüber den historischen Kontrollen.

Zusammenfassend muss man sagen, dass unsere Intervention zwar Muskelmasse, jedoch weder Muskelkraft noch Muskelfunktion schützen konnte. Nicht im intensivmedizinischen Verlauf und auch nicht bis zur Nachsorgeuntersuchung nach einem Jahr.

Bereits während der Applikation der elektrischen Muskelstimulation war auffällig, dass viele unserer Patienten nicht immer mit einer Muskelkontraktion auf einen suffizienten elektrischen Muskelstimulationsreiz reagierten. Das führte uns zu einer erweiterten Analyse der Kontraktionsfähigkeit in der folgenden Publikation.

ORIGINAL ARTICLE

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

Tobias Wollersheim[1,2†], Julius J. Grunow[1,3†], Niklas M. Carbon[1], Kurt Haas[1], Johannes Malleike[1], Sara F. Ramme[1], Joanna Schneider[2,3], Claudia D. Spies[1], Sven Märdian[4], Knut Mai[2,5,6], Simone Spuler[3,7], Jens Fielitz[2,3,8,9†] & Steffen Weber-Carstens[1,2*†]

[1]*Department of Anesthesiology and Operative Intensive Care Medicine (CCM, CVK), Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany,* [2]*Berlin Institute of Health (BIH), Berlin, Germany,* [3]*Charité-Universitätsmedizin Berlin and Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Experimental and Clinical Research Center (ECRC), Berlin, Germany,* [4]*Center for Musculoskeletal Surgery, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany,* [5]*Department of Endocrinology and Metabolism, Charité – Universitätsmedizin Berlin, corporate member of Freie, Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany,* [6]*Charité-Center for Cardiovascular Research (CCR), Berlin, Germany,* [7]*Max-Delbrück Center for Molecular Medicine in the Helmholtz Society, Berlin, Germany,* [8]*DZHK (German Centre for Cardiovascular Research), Greifswald, Germany,* [9]*Department of Internal Medicine B, Cardiology, University Medicine Greifswald, Greifswald, Germany*

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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**Correspondence to: Professor Steffen Weber-Carstens, MD, Department of Anesthesiology and Operative Intensive Care Medicine (CCM, CVK), Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Augustenburger Platz 1, Berlin 13353, Germany. Email: steffen.weber-carstens@charite.de*
†These authors contributed equally to this work.
[Correction added on 24 May 2019 after first online publication: The heading "Fanova outcomes" has been corrrected to "Outcomes" in this current version.]

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

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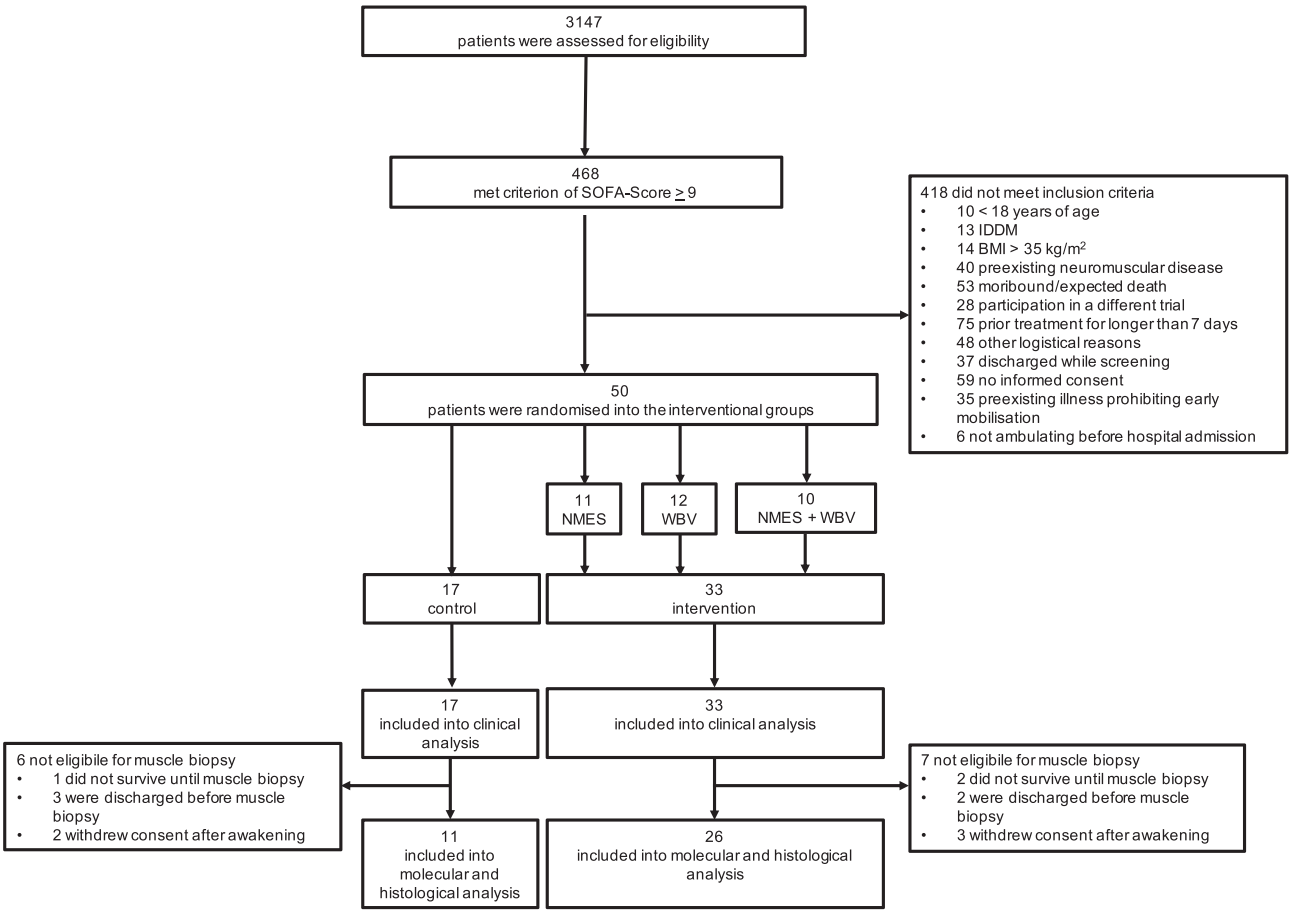
Figure 1 Trial enrolment scheme. 'Other logistical reasons' indicates cases where a legal proxy could not be appointed within the screening timeframe or study personal was not available for logistical reasons. IDDM, insulin-dependent diabetes mellitus; NMES, neuromuscular electrical stimulation; WBV, 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*)

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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.

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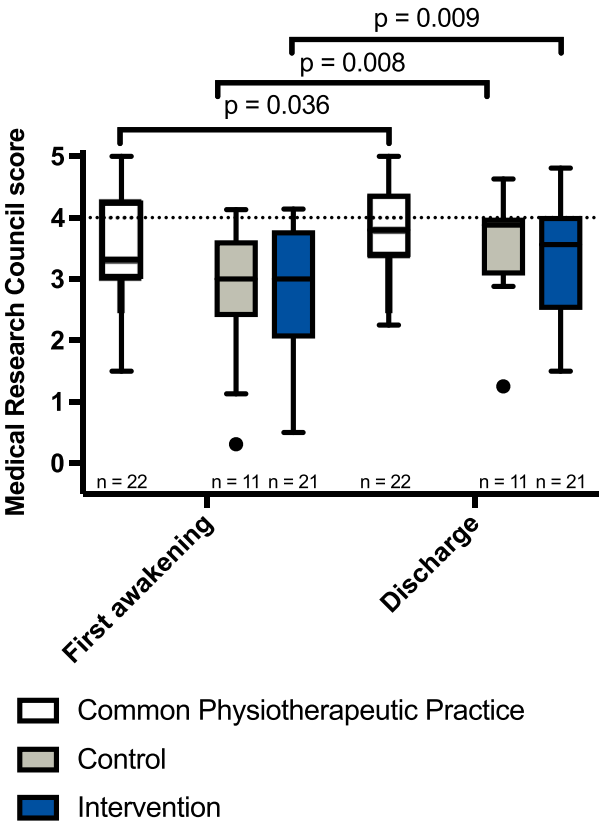
Figure 2 Muscle strength measured by Medial Research Council sum score. MRC score showed a significant increase for the control, intervention, and common physiotherapeutic practice group from first awakening until discharge, while no difference between the groups at either time point could be observed. 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.

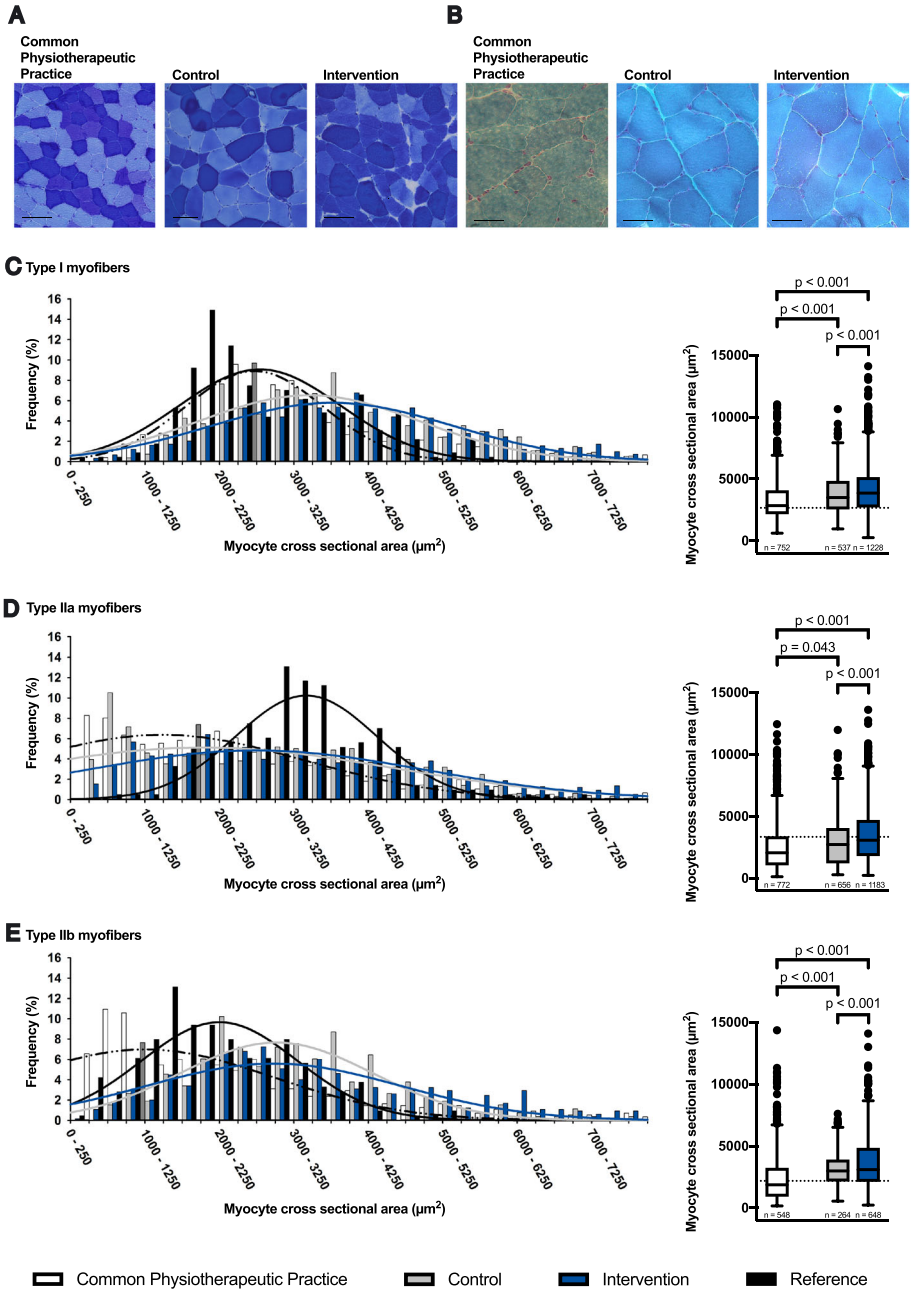


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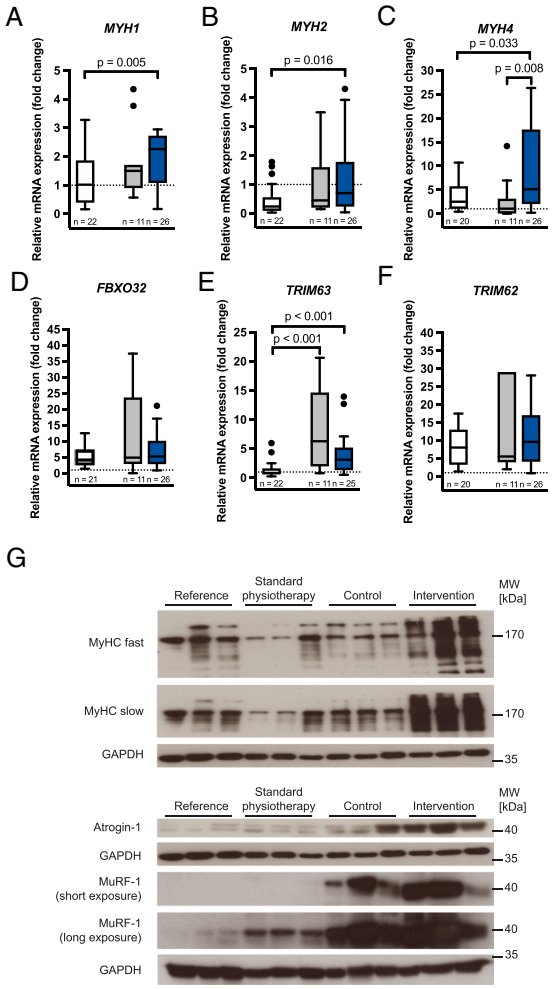
Figure 4 Gene expression for myosin heavy chains and atrogenes as well as protein content for myosin and key proteins of the ubiquitin proteasome system. (A) *MYH1* gene expression was significantly increased in the intervention group in comparison with the common physiotherapeutic practice group and 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

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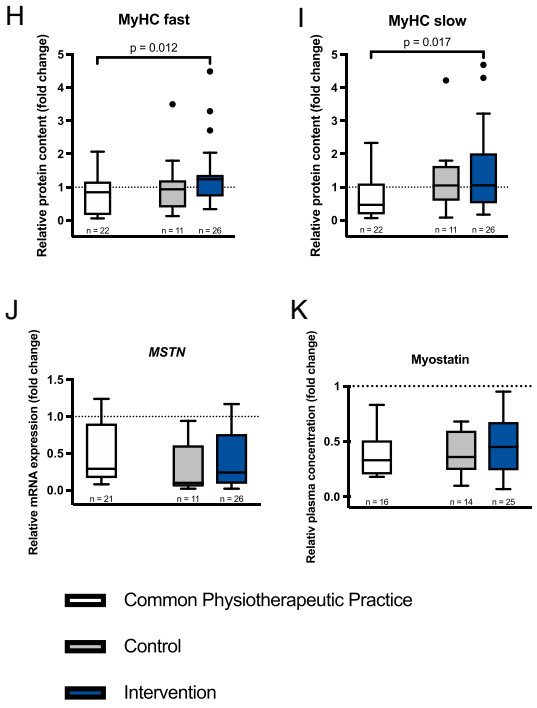
Figure 4 Continued



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

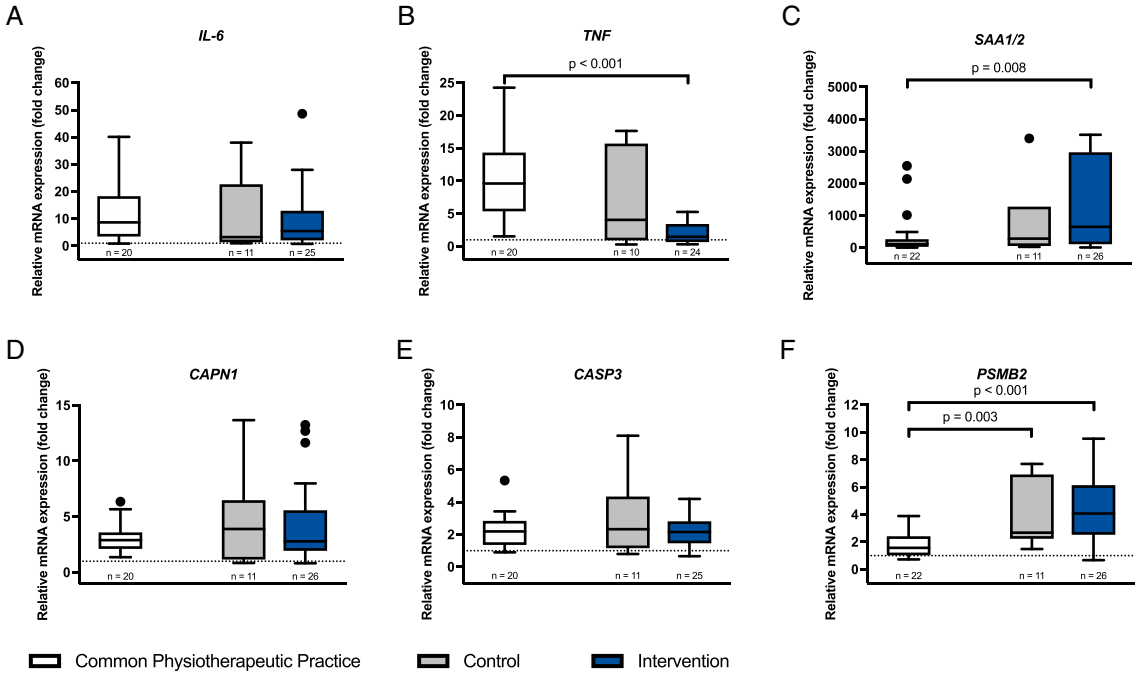
The inflammatory cytokines *IL-6* (encoding for interleukin 6) and *SAA1/2* (encoding for serum amyloid a1/2) were both significantly increased above values for healthy references while *TNF* (encoding for tumor necrosis factor alpha) presented values similar to healthy references for the intervention 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

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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.

Acknowledgements

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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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2.4 Unterschiedliches Ansprechen auf eine elektrische Muskelstimulation

Grunow JJ, Goll M, Carbon NM, Liebl ME, Weber-Carstens S, **Wollersheim T**. Differential contractile response of critically ill patients to neuromuscular electrical stimulation. Crit Care. 2019;23(1):308.
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Bei der Durchführung der randomisiert-kontrollierten Interventionsstudie fiel auf, dass unsere Interventionspatienten, die eine elektrische Muskelstimulation erhalten haben sehr unterschiedlich darauf reagierten. An Gesunden führt ein Elektrostimulationsgerät mit einer Stromstärke von etwa 15mA zu einer Muskelkontraktion. Es fiel auf, dass wir bei den Intensivpatienten deutlich höhere Stromstärken benötigten, um eine Kontraktion auslösen zu können. Darüber hinaus war bei einer Vielzahl von Stimulationen trotz sehr hoher Stromstärken bis zu 70 mA es überhaupt nicht möglich, eine Kontraktion zu erwirken. Bereits 2000 hatten Segers et al in Zusammenhang mit elektrischen Muskelstimulation Probleme mit der Anwendung beschrieben (126). Das führte uns dazu eine Subgruppen post hoc Analyse an unseren Pateinten durchzuführen, die eine elektrische Muskelstimulation erhalten hatten.

Methodisch fokussierten wir uns auf die Kontraktionsantwort der ersten 7 Tage unserer Intervention und untersuchten den Einfluss von Lokalisation, Dauer der Erkrankung und weiteren möglichen klinischen Parametern mit Einfluss auf die Kontraktionsfähigkeit in 21 Intensivpatienten, die in Summe 1824 Anwendungen elektrischer Muskelstimulationen erhalten hatten.
Wir fanden, dass eine Muskelkontraktion nur in etwa der Hälfte der Stimulationen erreicht werden konnte. Dabei waren die Lokalisation untere Extremität, Dauer zwischen Beginn der Erkrankung und Stimulationstag, als auch steigende Krankheitsschwere Einflussfaktoren, die eine Kontraktion erschwerten. Um die Bedeutung der Kontraktion zu beurteilen, haben wir die Patienten mit einer Kontraktionsrate größer und gleich 50%, den mit einer Kontraktionsrate kleiner 50% gegenübergestellt. Und tatsächlich ist die erfolgreich erwirkte Kontraktion mit dem positiven Effekt der elektrischen Muskelstimulation assoziiert. Ob man nun durch eine Individualisierung der Stimulationsparameter die Intervention mit einer höheren

Erfolgsrate anwenden kann, oder ob diese Therapieoption nur für einen Teil unserer Patienten überhaupt hilfreich ist, bleibt derzeit noch unklar.

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Critical Care

RESEARCH Open Access

Differential contractile response of critically ill patients to neuromuscular electrical stimulation

Julius J. Grunow[1], Moritz Goll[1], Niklas M. Carbon[1], Max E. Liebl[2], Steffen Weber-Carstens[1,3] and Tobias Wollersheim[1,3*]

Abstract

Background: Neuromuscular electrical stimulation (NMES) has been investigated as a preventative measure for intensive care unit-acquired weakness. Trial results remain contradictory and therefore inconclusive. As it has been shown that NMES does not necessarily lead to a contractile response, our aim was to characterise the response of critically ill patients to NMES and investigate potential outcome benefits of an adequate contractile response.

Methods: This is a sub-analysis of a randomised controlled trial investigating early muscle activating measures together with protocol-based physiotherapy in patients with a SOFA score ≥ 9 within the first 72 h after admission. Included patients received protocol-based physiotherapy twice daily for 20 min and NMES once daily for 20 min, bilaterally on eight muscle groups. Electrical current was increased up to 70 mA or until a contraction was detected visually or on palpation. Muscle strength was measured by a blinded assessor at the first adequate awakening and ICU discharge.

Results: One thousand eight hundred twenty-four neuromuscular electrical stimulations in 21 patients starting on day 3.0 (2.0/6.0) after ICU admission were included in this sub-analysis. Contractile response decreased from 64.4% on day 1 to 25.0% on day 7 with a significantly lower response rate in the lower extremities and proximal muscle groups. The electrical current required to elicit a contraction did not change over time (day 1, 50.2 [31.3/58.8] mA; day 7, 45.3 [38.0/57.5] mA). The electrical current necessary for a contractile response was higher in the lower extremities. At the first awakening, patients presented with significant weakness (3.2 [2.5/3.8] MRC score). When dividing the cohort into responders and non-responders (> 50% vs. ≤ 50% contractile response), we observed a significantly higher SOFA score in non-responders. The electrical current necessary for a muscle contraction in responders was significantly lower (38.0 [32.8/42.9] vs. 54.7 [51.3/56.0] mA, $p < 0.001$). Muscle strength showed higher values in the upper extremities of responders at ICU discharge (4.4 [4.1/4.6] vs. 3.3 [2.8/3.8] MRC score, $p = 0.036$).

Conclusion: Patients show a differential contractile response to NMES, which appears to be dependent on the severity of illness and also relevant for potential outcome benefits.

Trial registration: ISRCTN ISRCTN19392591, registered 17 February 2011

Keywords: Neuromuscular electrical stimulation, Intensive care unit-acquired weakness, Critical illness, Critical illness myopathy, Early mobilisation

* Correspondence: tobias.wollersheim@charite.de
[1]Department of Anesthesiology and Operative Intensive Care Medicine (CCM, CVK), Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt Universität zu Berlin and Berlin Institute of Health, Augustenburger Platz 1, 13353 Berlin, Germany
[3]Berlin Institute of Health, Berlin (BIH), Anna-Louisa-Karsch-Str. 2, 10178 Berlin, Germany
Full list of author information is available at the end of the article

Introduction

Critical illness and intensive care treatment can lead to severe impairments of physical function, cognitive capabilities and mental health, which directly affect the individuals' daily living, e.g. return to work [1–8]. The physical impairments, in terms of muscular weakness and reduced walking distance, directly affect short-term outcome, as patients suffering from weakness are less likely to be weaned from mechanical ventilation, discharged from the hospital or survive [4]. These impairments can persist at least up to 5 years after discharge from the intensive care unit (ICU) [9]. Therapeutic options that have been the focus of investigations throughout the last decade are early mobilisation and neuromuscular electrical stimulation (NMES). Both of these options have yielded positive as well as negative results in recent randomized controlled trials [10–14]. NMES is able to elicit muscle contractions in unconscious or sedated and therefore uncooperative patients. As it is possible to initiate this form of treatment in the early phase of critical illness, even if the patient has to remain heavily sedated, it was reasoned to be a promising therapeutic option to target muscular pathophysiological processes in their beginning stages [15, 16]. While some trials were able to corroborate this hypothesis, others yielded negative results [13, 14, 17]. Fossat and colleagues found no improvement in muscle strength or mobility in 314 patients receiving NMES of the M. quadriceps femoris in conjunction with in-bed-cycling [13]. An underrecognised aspect throughout recent NMES trials is the fact that not every electrical impulse necessarily elicits an appropriate muscle contraction, as Segers et al. showed [18]. Sepsis, use of vasopressors and lower limb edema were independent predictors for a missing contractile response to NMES [18]. It remains unclear whether neuromuscular electrical stimulation per se has a beneficial effect on the patient or if the beneficial effect is a causal effect of the evoked muscle contraction, which would be in line with current knowledge about muscle physiology. In this case, the lack of contraction from the electrical stimulation would leave study results prone to bias. Furthermore, it remains unclear whether the electrical stimulus during NMES has harmful effects when not properly translated into a muscle contraction. Our aim was, therefore, to characterise the response of critically ill patients to NMES, determine predictors for an adequate or inadequate therapeutic response and investigate potential benefits or detriments of an adequate respectively inadequate muscular response to NMES.

Methods

Study design

This is a prospectively planned sub-analysis of a randomised controlled exploratory interventional trial (ISRCTN19392591) performed in two ICUs at the Charité – Universitätsmedizin Berlin, a tertiary referral centre. Ethical approval was granted by the institutional review board (Charité EA 2/041/10).

Patients

Mechanically ventilated patients ≥ 18 years of age were included on the basis of a Sepsis-related Organ Failure Assessment (SOFA) score ≥ 9 within the first 72 h after ICU admission as well as written informed consent by a legal proxy and excluded if any of the following applied: prior hospital treatment for longer than 7 days, illness prohibiting early mobilisation, pre-existing neuromuscular disease, insulin-dependent diabetes mellitus, Body Mass Index > 35 kg/m^2, not ambulating before admission or poor prognosis with a high likelihood of death within the next hours. Only patients randomised to receive NMES were considered in this sub-analysis (for detailed study enrolment criteria, see [19]). Study staff had no influence on treatment decisions and patients were treated according to published standard operating procedures [20]. In order to investigate if an adequate response to the NMES had an influence on outcome and to define predictors for an adequate response, we separated the population into responders, defined as > 50% contractile response to NMES, and non-responders, with ≤ 50% contractile response to NMES during the first 7 days of the study intervention.

Intervention

NMES was applied daily, beginning on the day of enrolment up to day 28, for 20 min per muscle group bilaterally on eight different muscle groups (M. tibialis anterior, M. triceps surae, M. vastus lateralis, posterior thigh, M. biceps brachii, M. triceps brachii, wrist extensors, wrist flexors). We utilised two devices of whom each could stimulate four muscle groups simultaneously. We never stimulated counteracting muscle groups at the same time. Electrical impulses of 350 μs duration were applied at a frequency of 50 Hz. On-time was 6/10 s and off-time was 10/15 s with a ramp of 1 s (MUSKELaktiv 2-Kanal, schwa-medico®, Germany/ Physiomed-Expert-2-Kanal, Physiomed®, Germany). Electrical current was increased until muscle contraction was visible or palpable and then maintained at this level for the remainder of the session. In case pain threshold was reached in awake patients, electrical current was reduced to the last level below the pain threshold and stimulation was performed at that level. If a current of 70 mA failed to evoke a sufficient response, electrical current was not increased further, and the stimulation level was set to 40 mA for the session (Additional file 1: Table S2a). Data on contraction as well as electrical current were documented separately for every NMES session as well as muscle stimulated. For our analysis, only electrical

currents from stimulations that lead to a contractile response were respected.

Outcome

Muscle strength was assessed via Medical Research Council (MRC) score on the first day patients were sufficiently awake as well as at discharge from the ICU. Only patients with an MRC assessment at both timepoints were included in MRC analysis. Mean MRC scores were calculated for each patient. We excluded specific muscle groups from mean MRC score calculation if valid muscle strength assessment was not feasible due to clinical reasons (e.g. external fixators or casts). The day patients were sufficiently awake was determined by fulfilment of the following criteria on two consecutive days: first, Richmond Agitation and Sedation Score between − 1 and + 1, and second, an adequate response to three or more out of the following five 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." Contractile response to the electrical muscle stimulation was confirmed if a muscle contraction was either visible or palpable, or in the case patients were sufficiently awake, if a contraction was reported.

Non-excitable muscle membrane as an electrophysiological predictor for Critical Illness Myopathy was diagnosed via a direct muscle stimulation compound muscle action potential cut-off value of < 3.0 mV [21].

We included the first 7 days of the ICU stay into our investigations as it has been shown that pathophysiological processes already enormously influence the patient during that time and our intervention, specifically NMES, is predestined for the early phase of the disease trajectory as it can be properly applied in sedated, unconscious and uncooperative patients.

Statistical analysis

Values for categorical variables are shown as count and percentage, while values for metric variables are shown as median and interquartile range (IQR). Statistical difference for metric variables was tested with the Mann-Whitney *U* test for independent samples and Wilcoxon test for dependent samples. Chi-square test was used to test for statistical difference for categorical variables. A receiver operating characteristics (ROC) analysis was performed to determine a SOFA score cut-off value that predicts non-responders to NMES as well as a mA value that is sufficient to stimulate all responders. Sensitivity, specificity and area under the curve (AUC) are reported for the receiver operating characteristics analysis. The Pearson correlation coefficient (r^2) was calculated to determine correlations between variables. A priori, a p value < 0.05 was defined to indicate statistical significance.

Results

During the 2-year inclusion period, 468 out of 3147 patients that were admitted to two ICUs within the Charité – Universitätsmedizin Berlin fulfilled the inclusion criteria. Fifty of these patients were successfully enrolled in the trial and randomised. Further information regarding the enrolment process has been published before [19]. For this sub-analysis, only patients receiving NMES ($n = 21$) were considered (Table 1).

In the 21 patients treated with NMES, a total of 1824 muscle groups were stimulated during the first 7 days of treatment. These stimulations were equally distributed between upper ($n = 886$) and lower ($n = 938$) extremities ($p = 0.522$). On day 1 of NMES, only 64.4% of stimulations led to a contractile response with a significant difference between upper (100%) and lower (41.7%) extremities ($p = 0.001$). This difference could consistently

Table 1 Baseline characteristics

n		21
Sex (m/f)		16/76.2% / 5/23.8%
Age (years)		53.0 (45.0/70.0)
Weight (kg)		85.0 (75.0/100.0)
Height (m)		1.78 (1.74/1.80)
BMI (kg/m^2)		27.1 (24.2/30.9)
Diagnosis responsible for ICU admission	ARDS	8/38.1%
	Sepsis	3/14.3%
	Multiple trauma	7/33.3%
	Neurologic	2/9.5%
	Miscellaneous	1/4.8%
SOFA at ICU admission		13.0 (11.0/15.0)
APACHE II at ICU admission		25.0 (20.0/28.0)
SAPS2 at ICU admission		58.0 (47.0/65.0)
GCS at ICU admission		5.0 (3.0/6.0)
Time until first awakening (days)		17.0 (10.0/25.0)
ICU length of stay (days)		32.0 (21.0/43.0)
Percent of days with RASS > − 3 during ICU stay		64.3 (37.5/79.3)
Noradrenalin (μg/kg min)		0.07 (0.05/0.11)
Time requiring noradrenalin (days)		12.0 (9.0/18.0)
Survivors/non-survivors		18/85.7% / 3/14.3%
Non-excitable muscle membrane/excitable muscle membrane		7/50% / 7/50%
Start of NMES treatment after ICU admission (days)		3.0 (2.0/6.0)

Values for metric variables are presented as median and interquartile range and for categorical variables as count and percentages. BMI = Body Mass Index; SOFA = Sepsis-related Organ Failure Assessment; ICU = intensive care unit; APACHE II = Acute Physiology and Chronic Health Evaluation II; SAPS 2 = Simplified Acute Physiology Score 2; GCS = Glasgow Coma Scale; RASS = Richmond Agitation Sedation Scale; NMES = Neurmuscular electrical stimulation

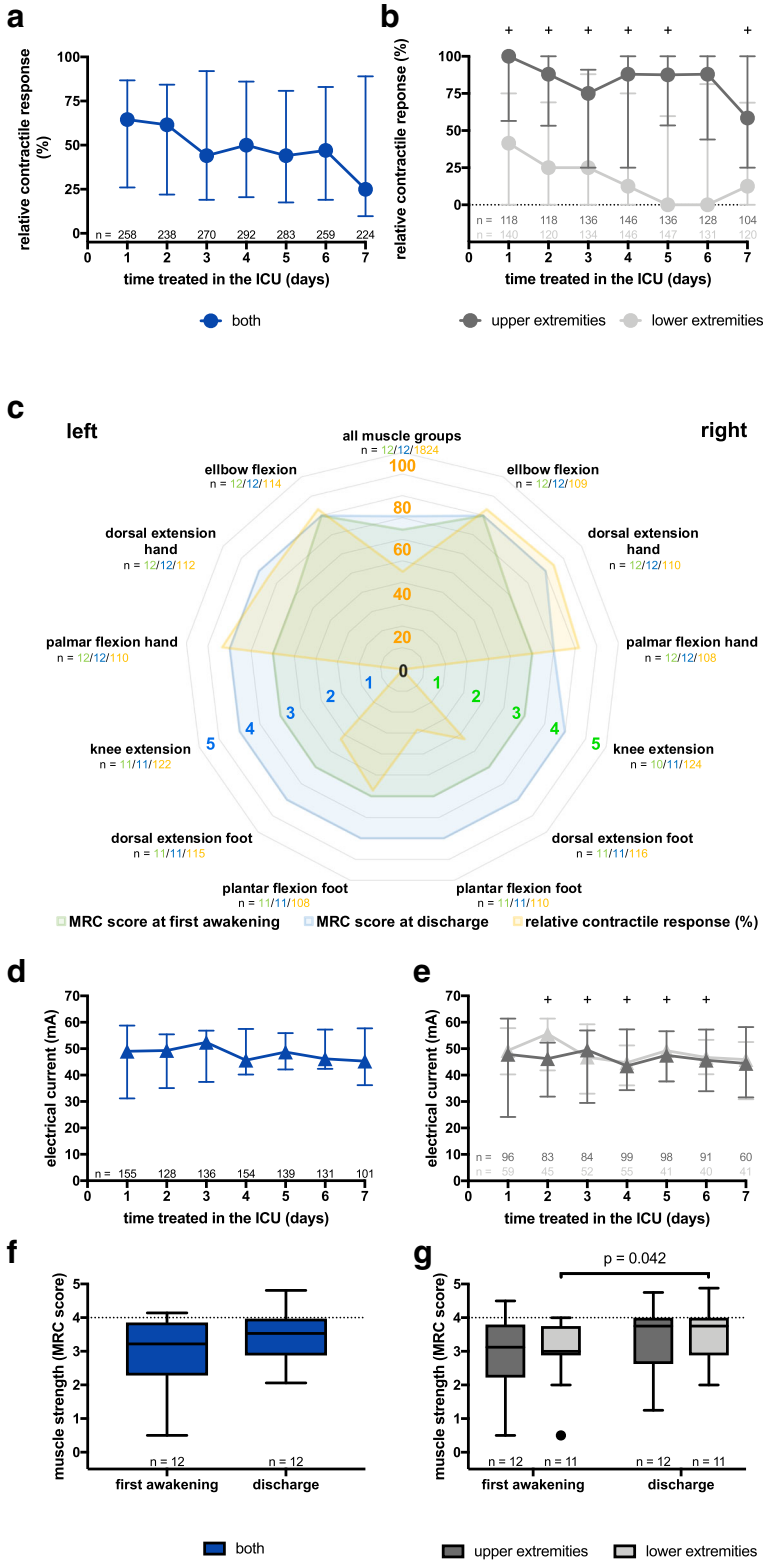

Fig. 1 (See legend on next page.)

(See figure on previous page.)
Fig. 1 Relative contractile response, electrical current and muscle strength during NMES. **a** Relative contractile response decreases between day 1 and day 7 without reaching statistical significance. **b** Upper extremities show a significantly higher response rate to NMES on days 1, 2, 3, 4, 5 and 7 in comparison to lower extremities. **c** Muscle strength and contractile response for all muscle groups separately. M. vastus lateralis shows the lowest response to NMES. **d** Electrical current required to elicit a muscle contraction remains unchanged between day 1 and day 7. **e** A significant difference in electrical current required to elicit a muscle contraction can be observed on days 2, 3, 4, 5 and 6 when comparing upper and lower extremities. **f** Muscle strength increase for both extremities between first awakening and ICU discharge does not reach statistical significance. **g** The increase in muscle strength between first awakening and ICU discharge reaches statistical significance for lower extremities. All values are shown as median and interquartile range. Statistical significance was calculated via Mann-Whitney *U* test or Wilcoxon signed-rank test as appropriate. A $p < 0.05$ is indicated by "+" in **b** and **e**. ICU = intensive care unit; MRC = Medical research council

be observed until day 7 (Fig. 1a + b). Nevertheless, a significant correlation for contractile response between upper and lower extremities was observed ($k = 0.687$, $r^2 = 0.472$, $p = 0.001$). Contractile response declined throughout the 7-day observation period from 64.4 to 25.0% overall. We similarly observed a decrease in contractile response for upper (100.0 to 58.3%) and lower (41.7 to 12.5%) extremities (Fig. 1a + b) (Additional file 1: Table S2b).

Furthermore, the results showed that the proximal muscle groups overall are less likely to respond to NMES than the distal muscle groups (median [IQR] proximal 40.7 [22.5/60.4] vs. distal 75.0 [38.0/91.7] %, $p < 0.001$). This finding is evident in both upper (median [IQR] proximal 73.2 [42.1/88.7] vs. distal 79.1 [72.5/94.6] %, $p = 0.047$) as well as the lower extremities (median [IQR] proximal 8.3 [0.0/45.8] vs. distal 52.5 [3.6/87.9] %, $p = 0.003$). The M. vastus lateralis was the least likely to respond to neuromuscular electrical stimulation (Fig. 1c) (Additional file 1: Table S2b).

The threshold of electrical current that led to a contractile response did not significantly change between day 1 and 7 for all muscle groups taken together (median [IQR] day 1, 50.2 [31.3/58.8] mA; day 7, 45.3 [38.0/57.5] mA) as well as for upper (median [IQR] day 1, 47.9 [24.4/60.5] mA; day 7, 44.4 [32.1/57.9] mA) and lower extremities (median [IQR] day 1, 49.3 [41.8/57.3] mA; day 7, 45.9 [37.1/49.8] mA) separately (Fig. 1d + e). Nevertheless, there was a significant difference between the upper and lower extremities observable on days 2, 3, 4, 5 and 6 with a higher electrical current mostly necessary in the lower extremities. The applied electrical current between upper and lower extremities correlated significantly ($k = 0.710$, $r^2 = 0.505$, $p = 0.002$) (Additional file 1: Table S2b).

At first adequate awakening, all patients presented with significant weakness overall (median [IQR] 3.2 [2.5/3.8] MRC score) as well as for upper (median [IQR] 3.1 [2.4/3.8] MRC score) and lower extremities (median [IQR] 3.0 [2.9/3.8] MRC score) individually (Fig. 1c, f, g). Until discharge, an increase was observable overall (median [IQR] 3.5 [3.0/3.9] MRC score), for upper (median [IQR] 3.8 [2.9/4.0] MRC score) and for lower extremities (median [IQR] 3.8 [3.1/4.0] MRC score), while statistical significance was only reached for the lower extremities ($p = 0.042$) (Fig. 1f, g). Muscle strength between upper and lower extremities at first awakening as well as at discharge showed a significant correlation (first awakening $k = 0.864$, $r^2 = 0.746$, $p < 0.001$; ICU discharge $k = 0.751$, $r^2 = 0.564$, $p < 0.001$).

Using the contractile response cut-off value, 8 patients were classified as responders (> 50% contractile response during the first 7 days) and 13 were classified as non-responders (≤ 50% contractile response during the first 7 days). Univariate analysis revealed a significantly higher SOFA score in non-responders at admission to the ICU. Baseline characteristics were otherwise balanced including norepinephrine treatment (Table 2).

When comparing the contractile response from responders to non-responders, we see a significantly greater proportion of stimulations leading to an adequate contractile response in responders (responders vs. non-responders median [IQR] both, 83.7 [73.4/93.5] vs. 35.0 [20.2/44.2] %, $p < 0.001$; upper extremities, 91.1 [86.6/99.1] vs. 67.0 [40.9/74.1] %, $p < 0.001$; lower extremities, 77.7 [62.5/90.2] vs. 7.1 [1.8/23.2] %, $p = 0.002$), indicating that our cut-off value was sufficient in distinguishing between patients that respond well to those who do not (Fig. 2a–d). Interestingly, no decrease in contractile response over time was observed in responders as opposed to non-responders (Additional file 2: Figure S1). When evaluating contractile response for all muscle groups separately, we also observed a consistent, remarkable and significant difference between responders and non-responders. Furthermore, it became evident that except for the dorsal thigh and M. vastus lateralis, the difference in response between upper and lower extremities is only significant for non-responders (Fig. 2d). This difference in contractile response is also reflected by the electrical current that was necessary to elicit a contraction if possible at all, as it was significantly higher in non-responders overall (responders vs. non-responders median [IQR] 38.0 [32.8/42.9] vs. 54.7 [51.3/56.0] mA, $p < 0.001$) and for upper extremities (responders vs. non-responders median [IQR] 32.6 [30.6/37.3] vs. 54.7 [45.4/56.0] mA, $p < 0.001$). Interestingly, no difference in electrical current was observed for lower extremities (responders vs. non-

Table 2 Univariate analysis

		Responder	Non-responder	*p* value
Patients/stimulations (*n*)		8/702	13/1122	
Sex (m/f)		7/87.5% / 1/12.5%	9/69.2% / 4/30.8%	0.340
Age (years)		56.0 [36.5/71.0]	53.0 [47.0/70.0]	0.645
Weight (kg)		80.0 [70.0/92.5]	92.0 [75.0/109.0]	0.301
Height (m)		1.80 [1.77/1.83]	1.76 [1.70/1.80]	0.500
BMI (kg/m^2)		26.5 [22.6/29.0]	27.8 [25.5/33.6]	0.210
Diagnosis responsible for ICU admission	ARDS	2/25.0%	6/46.2%	0.118
	Sepsis	0/0.0%	3/23.1%	
	Multiple trauma	4/50.0%	3/23.1%	
	Neurologic	2/25.0%	0/0.0%	
	Miscellaneous	0/0.0%	1/7.7%	
SOFA at ICU admission		12.0 [9.5/13.5]	14 [12.0/16.0]	*0.030*
APACHE II at ICU admission		24.0 [17.0/27.0]	25.0 [23.0/29.0]	0.414
SAPS2 at ICU admission		43.0 [33.0/61.5]	61.0 [57.0/66.0]	0.089
GCS at ICU admission		5.5 [3.0/7.5]	3.0 [3.0/6.0]	0.456
Time until first awakening (days)		12.0 [7.5/15.5]	20.5 [10.0/42.0]	0.287
ICU length of stay (days)		28.0 [19.0/36.0]	39.0 [25.0/49.0]	0.185
Percent of days with RASS > − 3 during ICU stay		50.2 [26.9/94.6]	71.4 [50.0/79.2]	0.750
Noradrenalin (μg/kg min)		0.08 [0.03/0.10]	0.07 [0.06/0.11]	0.414
Time requiring noradrenalin (days)		12.0 [3.5/15.5]	12.0 [11.0/25.0]	0.595
Survivors/non-survivors		7/87.5% / 1/12.5%	11/84.6% / 2/15.4%	0.854
Non-excitable muscle membrane/excitable muscle membrane		2/33.3% / 4/66.7%	5/62.5% / 3/37.5%	0.280
Start of NMES treatment after ICU admission (days)		3.0 (2.0/6.0)	4.0 (2.0/6.0)	0.750

Values for metric variables are presented as median and interquartile range and for categorical variables as count and percentages. Mann-Whitney *U* or chi-square test were used to calculate statistical significance. The statistically significant *p*-value ($p < 0.05$) is italicised to highlight it. BMI = Body Mass Index; SOFA = Sepsis-related Organ Failure Assessment; ICU = intensive care unit; APACHE II = Acute Physiology and Chronic Health Evaluation II; SAPS 2 = Simplified Acute Physiology Score 2; GCS = Glasgow Coma Scale; RASS = Richmond Aggitation Sedation Scale; NMES = Neurmuscular electrical stimulation

responders median [IQR] 44.6 [31.1/46.1] vs. 54.4 [33.0/58.3] mA, $p = 0.140$) (Fig. 2e–g) (Additional file 3: Table S1). Muscle strength at first awakening showed no statistically significant differences between responders and non-responders in the upper extremities (responders vs. non-responders median [IQR] 3.8 [3.4/4.2] vs. 3.0 [2.0/3.4] MRC score, $p = 0.145$), lower extremities (responders vs. non-responders median [IQR] 3.7 [3.3/3.7] vs. 2.9 [2.4/3.9] MRC score, $p = 0.630$) and overall (responders vs. non-responders median [IQR] 3.8 [3.4/4.0] vs. 3.1 [2.1/3.5] MRC score, $p = 0.282$) (Figs. 2h–j and 3 a). At ICU discharge, a statistically significant difference in muscle strength can be observed with higher muscle strength in the upper extremities of responders (responders vs. non-responders median [IQR] 4.4 [4.1/4.6] vs. 3.3 [2.8/3.8] MRC score, $p = 0.036$), while in lower extremities (responders vs. non-responders median [IQR] 4.3 [3.5/4.6] vs. 3.6 [3.1/4.0] MRC score, $p = 0.376$) and overall (responders vs. non-responders median [IQR] 4.3 [3.8/4.6] vs. 3.5 [2.8/3.9] MRC score, $p = 0.145$) statistical significance was not reached (Fig. 2h–j and 3b).

The proportion of contractile responses to NMES correlated inversely with the applied electrical current overall ($k = -0.746$, $r^2 = 0.556$, $p < 0.001$) as well as for upper extremities ($k = -0.653$, $r^2 = 0.427$, $p = 0.002$). No correlation between electrical current and contractile response was found for the lower extremities.

The receiver operating characteristics analysis (AUC 0.962; $p = 0.001$) revealed that an electrical current of 50.1 mA elicits a contraction in responders with a sensitivity of 100.0% and a specificity of 84.6%.

Contractile response furthermore correlates inversely with the SOFA score at admission ($k = -0.544$, $r^2 = 0.296$, $p = 0.011$). A SOFA score ≤ 13.5 has a sensitivity of 75% and a specificity of 61.5% to identify responders (AUC 0.788; $p = 0.03$) (Fig. 4a, b).

Contractile response to NMES presented no statistically significant difference in patients with electrophysiologically diagnosed non-excitable muscle membrane in comparison to patients with an excitable muscle membrane (non-excitable muscle membrane vs. excitable

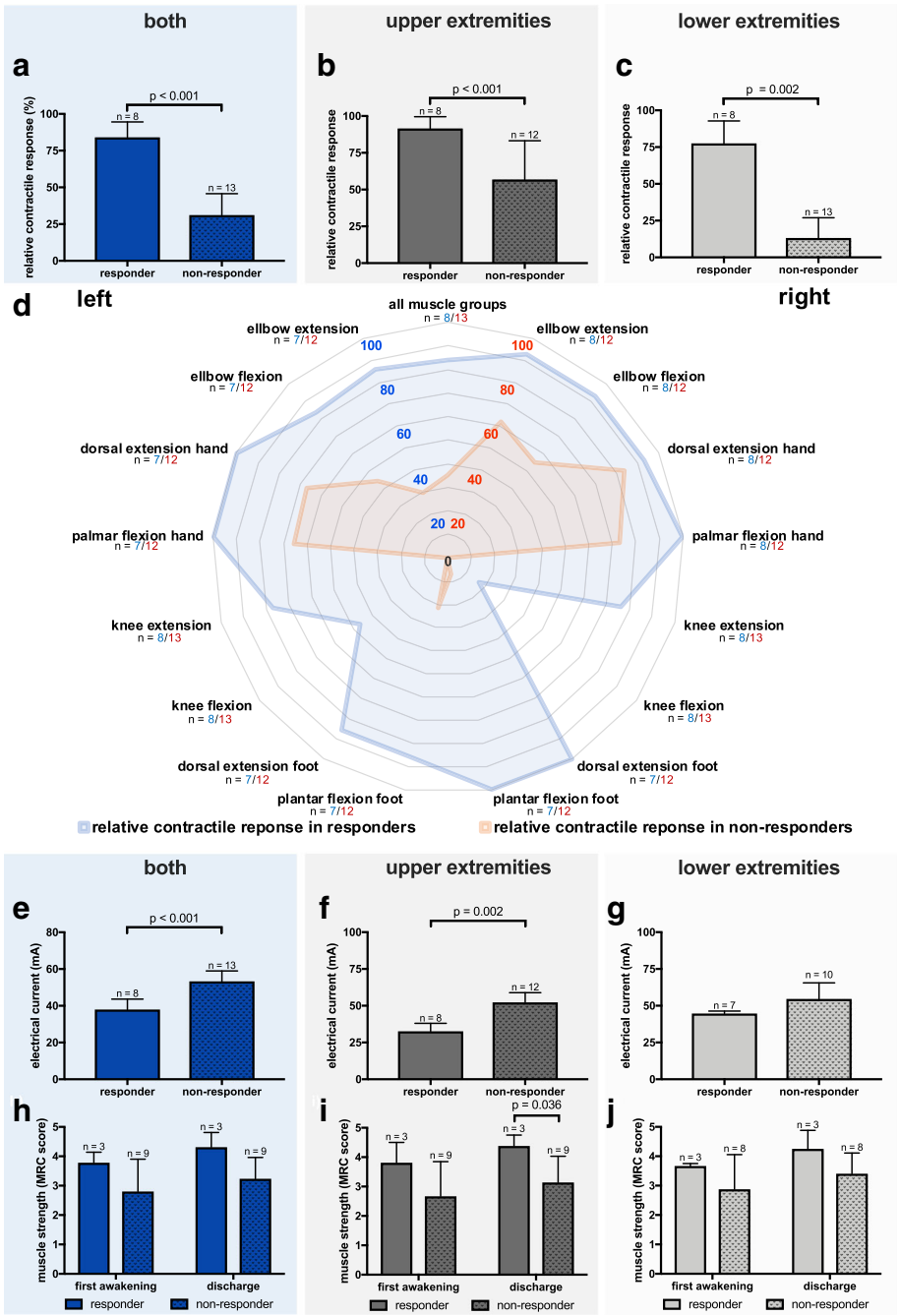

Fig. 2 Relative contractile response, electrical current and muscle strength in comparison between responders and non-responders. Relative contractile response is significantly higher in responders as opposed to non-responders during the first 7 days after ICU admission **a** for both extremities, **b** for upper extremities, **c** for lower extremities and **d** for all muscle groups separately. Electrical current required to elicit a contractile response is significantly higher in non-responders as opposed to responders during the first 7 days after ICU admission **e** for both extremities and **f** for upper extremities, while no difference can be observed for **g** lower extremities. Muscle strength measured via MRC scored at ICU discharge shows significantly higher values in responders as opposed non-responders for **i** upper extremities, while not reaching statistical difference for **h** both extremities as well as **j** lower extremities at first adequate awakening as well as ICU discharge and **h** upper extremities at first adequate awakening. All values are shown as median and interquartile range. Statistical significance was calculated via Mann-Whitney *U* test or Wilcoxon signed-rank test as appropriate. MRC = Medical research council

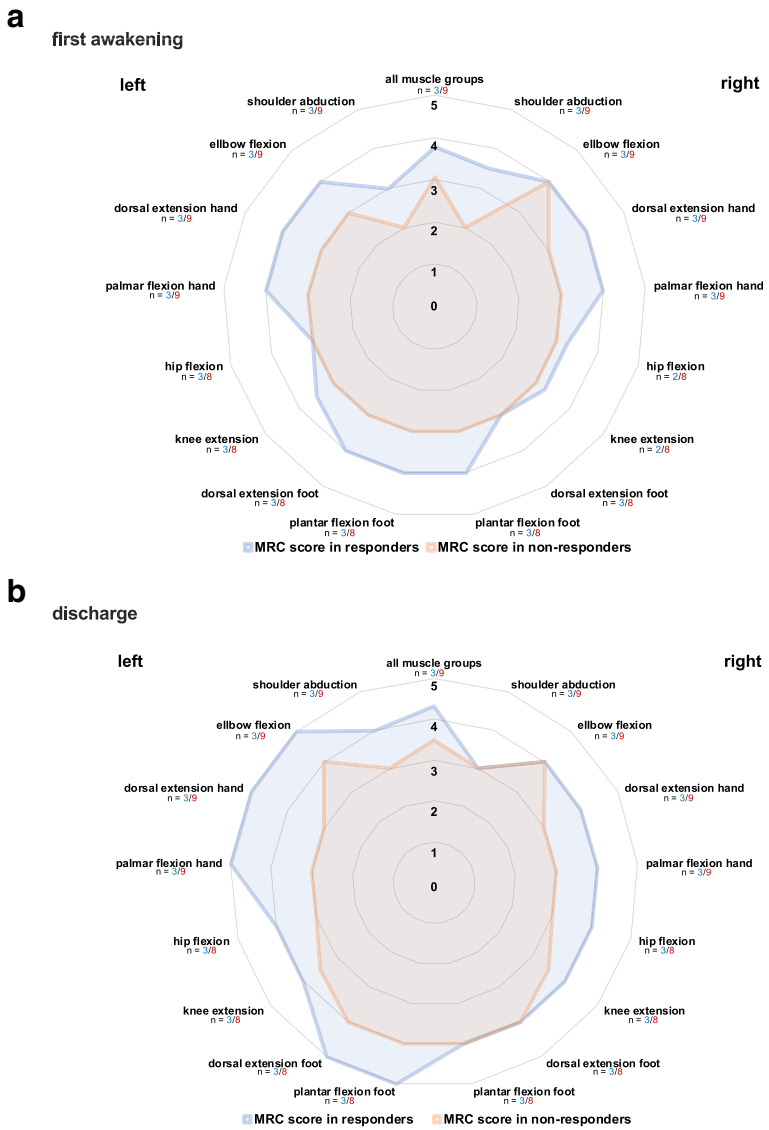

Fig. 3 Muscle strength at first adequate awakening and ICU discharge for all muscle groups separately. Muscle strength difference measured via MRC score at **a** first awakening and **b** ICU discharge in responders and non-responders. MRC = Medical research council

muscle membrane median [IQR] 44.2 [36.1/53.3] vs. 78.0 [42.5/88.7] %, $p < 0.128$).

Discussion

This retrospective sub-analysis aimed to outline the characteristics of, as well as predictors for, a contractile response to NMES, and also potential clinical benefits resulting from an adequate response to NMES. We were able to show that at the initiation of NMES, only two thirds of stimulations led to a contractile response in our cohort and that this number declined to one third throughout the first 7 days of stimulation, which we think is due to progressing pathophysiological processes. Established mechanisms that would hypothetically contribute to this effect are channelopathy, decreasing metabolic flexibility, advanced muscle atrophy and edema. Interestingly, the upper extremities proved to respond to NMES more frequently than the lower extremities and the distal extremities more frequently than the proximal extremities. If contractile response was present, the required electrical current for a muscle contraction did not change within the first 7 days, while in the lower extremities higher currents were usually required to elicit a contractile response. Patients classified as responders to NMES were shown to have a lower SOFA score, to require a lower electrical current and to have a significantly improved upper extremity muscle strength at discharge. An electrical current of 50.1 mA was sufficient

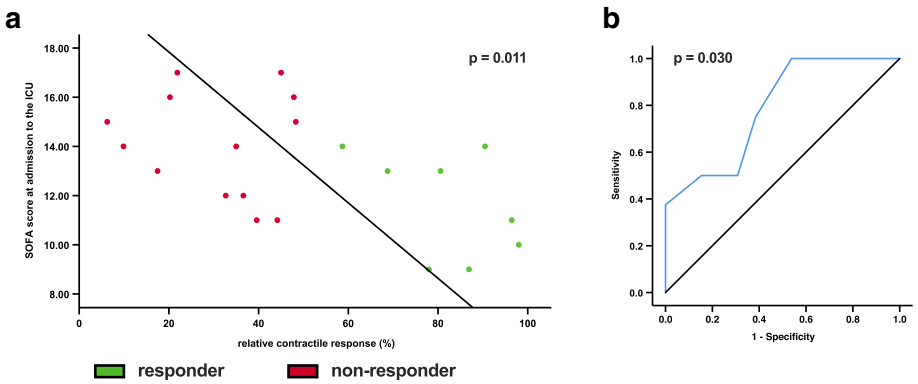

Fig. 4 Correlation and ROC curve for contractile response and SOFA. Relative contractile response between days 1 and 7 correlates with **a** SOFA score. **b** ROC curve for SOFA score in patients with adequate contractile response to neuromuscular electrical stimulation. SOFA = Sepsis-related Organ Failure Assessment; ROC = receiver operating characteristics

to adequately stimulate responders to NMES, which can be identified by a SOFA score ≤ 13.5.

NMES has been investigated in the context of critical illness and ICU-acquired Weakness for almost a decade without a clear conclusion regarding its effectiveness mainly due to controversial and incoherent study results [13, 14, 17]. As an example, an improvement in muscle strength using NMES was shown by Karatzanos and colleagues [22]. On the other hand, Kho et al. were not able to reproduce these findings [23]. An aspect that is critical when reporting interventional trials is whether the intervention was delivered as planned. Segers et al. first suggested that not every electrical impulse during NMES is converted into a muscle contraction [18]. Nevertheless, most trials on NMES published to date merely report the number or total duration of NMES sessions without mentioning the relative amount of stimulations successfully translated into a muscular contraction, a potentially useful surrogate indicating successful delivery of NMES. A lack of contractile response in the participants could be one cause for the incoherent and contradictory study results in NMES interventional trials throughout the past years. Contractile response was only reported in one outcome-focused interventional trial by Rodriguez and colleagues, who conducted an intraindividual comparison in 16 patients [17]. The muscular response rate of 77% was relatively high compared to our observations as well as to the numbers presented by Segers et al. [17, 18]. Interestingly, they also report an increase in muscle strength at ICU discharge, which is similar to the increased muscle strength we found in the upper extremities of our responder group at ICU discharge [17]. When looking at markers for muscle mass—extremity circumference or muscle thickness via ultrasound—Rodriguez et al. found no effect due to NMES. Interestingly, the dissociation between muscle mass and function has been repeatedly observed in critically ill patients shortly after ICU admission as well as 6 months after ICU discharge [24, 25]. Nevertheless could muscle strength only be assessed via a nonvolitional method based on electrical stimulation of a muscle contraction in the trial investigating the timeframe shortly after ICU admission. The effect of an impaired contractile response has on this form of muscle strength measurement in critically ill patients is unknown [24]. An impaired bioenergetic status has been shown to be of relevance for the development of muscle weakness during the ICU stay and could be a plausible culprit for the difference between muscle mass and muscle function [26]. Future trials are required to elucidate the exact mechanisms.

Our investigation into multiple muscle groups revealed that the M. vastus lateralis has the lowest response rate to NMES and that there is a significant difference between upper and lower extremities in the response to NMES. Based on these facts, we think another factor introducing significant bias into most investigations of NMES is the fact that almost all of them only investigated the lower extremities or solely the quadriceps femoris, which, according to our findings, would carry a high risk of yielding negative study results due to lack of contractile response [13, 14, 23, 27, 28]. A standardised NMES reporting sheet (Additional file 4: Standardised NMES Reporting Sheet) was developed by us in order to improve reporting of NMES protocols and responder rates in future trials and through that enable an adequate comparison of patient cohorts, protocols and muscle groups.

We have shown that a maximum electrical current of 50 mA is sufficient to stimulate all patients that respond adequately to NMES. This number was doubled and almost tripled in some studies, and while we did not observe any harm from NMES with electrical currents up to 70 mA in our cohort, further investigations are needed to rule out any harm from electrical currents above our defined threshold [29].

ICU-acquired weakness has been shown to develop early during critical illness, while the likelihood of developing it increases with the severity of critical illness [15, 16, 30]. In agreement with our results, Segers and colleagues have found that patients with higher disease severity, in their case differentiated by sepsis or no sepsis, are less likely to respond to NMES [18]. Furthermore, we were able to show that distal muscle groups respond better to NMES than proximal muscle groups, which is in accordance with DeJonghe et al., who showed that proximal muscle groups are more severely affected by ICU-acquired weakness than distal muscle groups [31]. Due to the overlap between characteristics of, and risk factors for, ICU-acquired weakness and insufficient response to NMES, it appears likely and plausible that pathophysiological mechanisms involved in the development of ICU-acquired weakness are also responsible for hampering contractile response to NMES [30]. Furthermore, could differences in impedance due to edema or fat mass, a different electrode to muscle ratio or differing adaptive reactions to therapy have an important impact on contractile response [18]. Segers et al. were not able to find an association between electrophysiological characteristics of critical illness myopathy and the lack of contractile response, within our data such an association could not be shown as well but as our analysis is of exploratory nature a large sample size in future trials might yield different results [18]. As such, future investigations specifically dedicated to this question are necessary, in order to further elucidate the connection between electrophysiologic abnormalities and lack of contractile response.

ICU-acquired weakness is a clinical syndrome defined by an average MRC score < 4 [32]. Multiple pathophysiological mechanisms, e.g. muscle wasting, bioenergetic failure and membrane inexcitability, have been described in the context of ICU-acquired weakness [15, 16, 21, 33]. We therefore hypothesise that depending on the aetiology, different pathophysiological mechanisms could be predominantly responsible for the development of ICU-acquired weakness and also be the reason patients respond differently to interventions such as NMES. Future studies should further evaluate these underlying mechanisms in the development of ICU-acquired weakness in order to more closely define different types of ICU-acquired weakness and possibly identify sub-cohorts that respond to and consequently benefit from the different interventions.

Our pilot analysis is limited by the number of patients as multivariate analysis to investigate independency of association between, for example, SOFA score and responder status could not be performed. Furthermore, as our ICU admissions were not planned, we were not able to obtain pre-admission muscle strength values. Our muscle strength values at first awakening will therefore already be affected by the intervention, and correction for potential baseline differences is not possible. We did not included patient with pre-existing neuromuscular disease in order to keep bias due to missing pre-admission values to a minimum. Since our protocol did not include an electrical current above 70 mA, we are not able to reach any conclusion—neither beneficial nor harmful effects—regarding NMES with higher electrical currents. We see that 50.1 mA is a good cut-off to differentiate responders and non-responders on this basis we do not believe increasing electrical current further will increase responder rates.

Conclusion

Different muscle groups as well as different patients show a varying response to NMES, which appears to be dependent on multiple factors that are linked to the respective pathomechanisms behind the development of the ICU-acquired weakness. Since we observed a varying degree of clinical improvement when comparing patients that responded adequately to NMES to those who did not, it is strongly suggested that response rates to NMES should be reported in future trials (Additional file 4: Standardised NMES Reporting Sheet) and evaluated in relation to clinical outcomes. The described difference in response rates to NMES may explain the controversial findings of previous trials and improve the quality of evidence in future critical care NMES trials.

Additional files

Additional file 1: Table S2. a Neuromuscular electrical stimulation reporting: protocol specifications. Specifications of the developed NMES protocol. **b** Neuromuscular electrical stimulation reporting: stimulation and contraction specifications. Specification of the applied NMES. Electrical current shows the median electrical current applied to patients with a positive contractile response per muscle group and day. Contractile response shows the percentage of patients with a positive contractile response per muscle group and day. (XLSX 19 kb)

Additional file 2: Figure S1. Contractile response dynamics between day 1 and day 7 in responders and non-responders. (PDF 30 kb)

Additional file 3: Table S1. Electrical current. Electrical current necessary to elicit a contractile response for all muscle groups separately for all patients as well as responders and non-responders. (PDF 266 kb)

Additional file 4: Standardised NMES Reporting Sheet. Standardised NMES reporting sheet for NMES protocol and stimulation specifications. (XLSX 17 kb)

Abbreviations

APACHE II: Acute physiology and chronic health evaluation II; AUC: Area under the curve; BMI: Body Mass Index; GCS: Glasgow Coma Scale; ICU: Intensive care unit; IQR: Interquartile range; MRC: Medical research council; NMES: Neuromuscular electrical stimulation; RASS: Richmond Aggitation Sedation Scale; ROC: Receiver operating characteristics; SAPS2: Simplified Acute Physiology Score 2; SOFA: Sepsis-related Organ Failure Assessment

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Authors' contributions
TW and SWC designed the study. TW and SWC developed the intervention protocol. SWC acquired the utilised funding. TW and SWC enrolled patients in the trial. TW, NMC and MG performed the intervention and acquired clinical data. JJG, MG, NMC and TW managed the database. JJG, SWC and TW analysed and interpreted the obtained data. JJG and TW prepared the first draft of the manuscript. ML, MG, NMC and SWC reviewed the manuscript. All authors participated in the revision process of the manuscript and therefore vouch for the integrity and accuracy of the presented data as well as the process, which lead to the presented data. All authors read and approved the final manuscript.

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Availability of data and materials
All collected and analysed data can be made available by the corresponding author upon reasonable request.

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The study was approved by the institutional review board of the Charité – Universitätsmedizin Berlin. Written informed consent was provided by study participants or legal proxies (Charité EA 2/041/10).

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The authors declare that they have no competing interests.

Author details
[1]Department of Anesthesiology and Operative Intensive Care Medicine (CCM, CVK), Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt Universität zu Berlin and Berlin Institute of Health, Augustenburger Platz 1, 13353 Berlin, Germany. [2]Department of Physical Medicine and Rehabilitation, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt Universität zu Berlin and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany. [3]Berlin Institute of Health, Berlin (BIH), Anna-Louisa-Karsch-Str. 2, 10178 Berlin, Germany.

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Publisher's Note

2.5 Perioperativ erworbene Muskelschwäche

Lachmann G, Morgeli R, Kuenz S, Piper SK, Spies C, Kurpanik M, Weber-Carstens S, **Wollersheim T**, Biocog Consortium. Perioperatively Acquired Weakness. Anesth Analg. 2020;130(2):341-51. doi: 10.1213/ANE.0000000000004068

Schon im Namen der Intensive Care Unit acquired weakness versteckt sich die ursprüngliche Patientengruppe. Jedoch haben die letzten Jahre dazu geführt, dass das Problem der Muskelschwäche nicht nur Intensivpatienten mit langem Aufenthalt betrifft, sondern eine Muskelreaktion der Frühphase ist. Tage, wenn nicht nur Stunden reichen aus, um einen verehrenden Muskelschaden zu induzieren. Dazu ist bekannt, dass auch perioperativ Komplikationen auftreten können, die den Krankheitsverlauf von Patienten negativ beeinflussen können, auch wenn sie nicht intensivmedizinisch behandelt werden. Es stellte sich für uns die Frage, ob sich bereits während einer elektiven perioperativen Versorgung ein messbarer Muskelkraftverlust einstellt. Die beschriebenen Risikofaktoren der ICUAW, wie Immobilisation, inflammatorische Reaktionen des Immunsystems, Applikation von Muskelrelaxantien, künstliche Beatmung und Nahrungskarenz treffen auch auf die perioperative Phase zu. Wir führten eine Observationsstudie zur objektiven Erhebung von perioperativ erworbener Muskelschwäche und deren Langzeitverlauf durch.

Wir schlossen während der präoperativen Visite 89 Patienten in unsere Untersuchung ein. Davon wurden 59 Patienten nicht intensivmedizinisch betreut und waren für unsere Auswertung relevant. Es zeigte sich ein Muskelkraftverlust der Extremitäten bereits am ersten postoperativen Tag von im Median 13,1%. Dieser Kraftverlust ging zusätzlich mit einer eingeschränkten Lungenfunktion einher. Erstaunlicherweise wiesen die Patienten bis zur 3-monatigen Nachuntersuchung noch funktionell messbare muskuläre Einschränkungen auf, auch wenn sie augenscheinlich einen komplikationslosen Verlauf genommen haben. Wir prägten so erstmalig den Begriff der Perioperative Acquired Weakness (POAW) und erweiterten damit das Kollektiv von Patienten, die eine muskuläre Schwäche entwickeln von den Intensivpatienten auch auf perioperative Patienten. Ob und welchen tatsächlichen Einfluss das auf den gesamten Erholungsprozess der Patienten hat bleibt aktuell noch zu untersuchen.

3. Diskussion

3.1 Bedeutung des neuromuskulären Organversagens

Das neuromuskuläre Organversagen wurde viele Jahre verkannt, da es im intensivmedizinischen Verlauf erst sichtbar wurde, wenn Patienten aus einer klinisch induzierten Sedierung erwachten. Im Vordergrund stand lange Zeit das Überleben der Patienten per se. Mit zunehmender Entwicklung in der Intensivmedizin schafften es immer mehr Patienten ihre schwere Erkrankung zu überstehen (72, 73). Dadurch gewannen erworbene Komorbiditäten in der letzten 20 Jahren zunehmend an Bedeutung. Schwierige oder frustrane Entwöhnung von der Beatmung und eingeschränkte körperliche Funktionen nach an sich überwundener Grunderkrankung rückten in den Fokus, um erfolgreiche Behandlung patientengerechter evaluieren zu können (12, 13, 21). Das war auch der Anstoß zur wissenschaftlichen Untersuchung. Ergänzend dazu zeigten Fried et al 2012, dass den meisten Patienten eine gute geistige und körperliche Verfassung nach einer schweren Erkrankung sogar wichtiger ist als das Überleben per se (52). Wichtige wissenschaftliche Arbeiten aus den Jahren 2009 und 2011 von Margret Herridge zeigten erstmalig massive Einschränkungen der gesundheitsbezogenen Lebensqualität und körperliche Einschränkungen nach erfolgreich überwundenem akutem Lungenversagen (10, 11). Eine zentrale Arbeit der letzten Jahre, die einen klaren Zusammenhang zwischen Muskelschwäche und Mortalität aufzeigt ist von Great Herrmanns, die nicht nur Muskelschwäche und Versterben in Zusammenhang bringt, sondern sogar steigenden Schweregrad der Muskelschwäche mit steigendem Risiko akut oder im Laufe eines Jahres zu versterben in Relation setzten konnte (12). Die Inzidenz einer erworbenen Muskelschwäche wird zwischen 25% und mehr als 70%, je nach Patientenkollektiv angegeben (8). So erleiden etwa 25% aller beatmeten Intensivpatienten eine messbare Muskelschwäche während sogar bis zu 100% der Patienten mit Sepsis und Multiorganversagen elekrophysiologische Hinweise auf einen neuromuskulären Schaden zeigen können (8). Die langjährigen Einschränkungen der muskulären Funktion, die auch zur reduzierten gesundheitsbezogenen Lebensqualität beiträgt sind eine erdrückende Folge für unsere Patienten (10). Darüber hinaus führen eine reduzierte oder aufgehobene Arbeitsfähigkeit zusammen mit langfristigen Rehabilitationsmaßnahmen und Hilfebedürftigkeit im Alltag zu immensen Kosten für das Gesundheitssystem (54,

58). Aus diesem Grunde sind Präventionsstrategien und Therapieoptionen für den Patienten selbst und das Gesundheitssystem von großer Bedeutung.

3.2 Risikofaktoren

Grundsätzlich können alle Intensivpatienten von der erworbenen Muskelschwäche betroffen sein. Jedoch konnte gezeigt werden, dass ein höherer Schweregrad der akuten Erkrankung mit Organdysfunktionen, gemessen als Sepsis-related Organ Failure Assessment (SOFA) Score, in Kombination mit einem schlechteren Gesundheitszustand vor der akuten Erkrankung, gemessen als Acute Physiology And Chronic Health Evaluation II (APACHE II)- oder Simplified Acute Physiology Score (SAPS), die Wahrscheinlichkeit für das Auftreten einer ICUAW erhöht (31-33, 51, 74, 75). Dabei sind besonders Patienten mit SIRS, Bakteriämie oder Sepsis, bei denen somit eine systemische Inflammation vorliegt einem deutlich erhöhten Risiko ausgesetzt (33, 51, 75). Die Entzündungszustände während der Sepsis führen dabei zu einem Anstieg von Interleukin-6 (IL-6), was nachweislich das Risiko zur Entwicklung einer ICUAW erhöht (32). Mit unseren Kooperationspartnern konnten auch wir den Einfluss von IL6, Serum Amyloid A1 (SAA1) und Serum Amyloid A2 (SAA2) auf die Inflammation induzierte Muskelatrophie der Muskelzellen in unseren Patienten und einem Mausmodel zeigen (76, 77). Der Einfluss von Blutglukosespiegeln und Insulintherapie wird kontrovers diskutiert. Es wurde jedoch gezeigt, dass Hyperglykämien und Muskelschwäche miteinander assoziiert sind und eine intensive Insulintherapie mit niedrignormalen Blutglukosespiegeln die Inzidenz einer ICUAW verringern konnte (31, 44, 45, 78). Ebenso ist die Rolle von Kortikosteroiden nicht abschließend geklärt (79, 80). Es gibt Hinweise auf eine Kortisol vermittelte Muskelatrophie (81, 82). Unterstütz wird diese Idee durch eine kürzlich erschienene Metaanalyse, die einen signifikanten Zusammenhang zwischen der Applikation von Kortikosteroiden und der Entwicklung einer ICUAW zeigt (80). Interessanterweise konnte für die Untergruppe der kritisch kranken septischen Patienten kein Zusammenhang gefunden werden, was sich mit Daten unserer Observationsstudie deckt, bei der Hydrokortison bei septischen Patienten keinen Einfluss auf Muskelkraft und Muskelfaserquerschnittsfläche hat (25, 80). Aktuell gilt die Empfehlung, die Anwendung von Kortikosteroiden auf möglichst niedrige Dosen und kurzfristige Anwendungen zu beschränken (80). Inaktivität geht nachgewiesener Weise mit einer

Muskelatrophie einher. Insbesondere die Immobilisation von Intensivpatienten ist hier ein entscheidender Risikofaktor für die Entwicklung einer ICUAW (31). Dieser Zusammenhang wird unterstütze durch den belegten Zusammenhang zwischen Sedierung und Muskelschwäche (83). Analgosedierung, wie wir sie nach aktuellen Leitlinien nicht mehr praktizieren wollen, führt zur Immobilisation und hindert den Patienten zusätzlich an der Partizipation aktiver Physiotherapie (84). In diesem Zusammenhang ist auch der Einfluss von Muskelrelaxantien umstritten, die sicherlich zu einer absoluten Muskelatonie führt (79). Deshalb wird im klinische Alltag unserer Intensivstation so weit als möglich auf die Verwendung von Muskelrelaxantien verzichtet und bleibt nur kurzzeitiger Anwendungen zur Durchführung bestimmter Interventionen vorbehalten.

3.3 Pathomechanismen des neuromuskulären Organversagens

3.3.1 Proteindegradation

Muskelproteinverlust und Muskelatrophie sind zwar nicht die definierenden, jedoch charakteristischen Merkmale der ICUAW. Vor allem der Myosinverlust aus dem kontraktilen Anteils der Skelettmuskulatur, sowohl aus den langsamen als auch den schnellen Aktin-Myosin-Komplexen, geht im Verlauf mit einer reduzierten Myozytenquerschnittsfläche, der Atrophie einher (25, 85). Wir konnten das in Muskelbiopsien von ICUAW Patienten an Tag 5 und 15 nach der Aufnahme auf die Intensivstation zeigen (25). Der Myosingehalt ist bereits an Tag 5 massiv reduziert und ändert sich nur wenig bis Tag 15. Lichtmikroskopisch findet man allerdings in der ersten Biopsie kaum Atrophie, da diese sich erst an Tag 15 deutlich demaskiert (25). Unsere elektronenmikroskopischen Bilder geben hierzu eine Erklärung. An Tag 5 zeigt sich eine erhaltene Ultrastruktur mit bereits massiv ausgelöstem Myosin (25, 85, 86). An Tag 15 zeigen sich die Ultrastrukturen der Muskulatur dann zusammengesintert und präsentieren dann auch lichtmikroskopisch kleinere Muskelfaserquerschnittsflächen im Sinne einer Atrophie (25). Dieser frühe Mechanismus des Muskelproteinabbaus konnte auch von Puthucheary et al. gezeigt werden (24). Wie auch wir zeigen konnten, spielt das UPS eine zentrale Rolle bei der ICUAW-

assoziierten Muskelatrophie (25). Dabei kommt es nach Aufschlüsselung der Proteine durch substratspezifische E3-Ligasen zur Ubiquitinierung. Die mit Ubiquitin markierten Proteine werden durch das Proteasom in die Aminosäuren zerlegt und dann der Proteinsysthese oder dem Energiestoffwechsel bereitgestellt. Als zentrale Regulatoren dieses Prozesses konnten zu Beginn einer kritischen Erkrankung die Induktion von Forkhead-Box-Protein O1 (FoxO1) und Forkhead-Box-Protein O3 FoxO3 auf Transkriptionsebene identifiziert werden (25, 42). Zusätzlich ist FoxO3 ein direkter Induktor der E3-Ligase Atrogin-1 (87, 88). Die Induktion der beiden Fox O-Transkriptionsfaktoren führt zur Hochregulation der MuRF1- und Atrogin-1-mRNA-Expression und im Western Blot nachweisbaren Proteingehalt an MURF-1 (18, 25). MuRF1 und Atrogin-1 wurden schon zuvor als E3-Ligasen etabliert, die eine Schlüsselrolle bei der Muskelatrophie im Allgemeinen und bei kritisch kranken Patienten spielen (18, 25, 89). In der vorliegenden Arbeit konnten wir zusätzlich *TRIM62* als E3-Ligase identifizieren, die in den Muskelabbau bei ICUAW involviert scheint (1). Auch die Aktivität der Proteasomen wird gesteigert, was sich in einer gesteigerten mRNA-Expression von Proteasom-Untereinheiten und einer vermehrten Ubiquitinierung von Proteinen bei Patienten mit ICUAW zeigt (7, 16, 18, 25). Die gesteigerte Expression der 20S-Proteasom Untereinheit konnte von Dos Santos et al. auch 7 Tage und 6 Monate nach der Entlassung von der Intensivstation noch beobachtet werden, obwohl zu diesem Zeitraum die Aktivität der Proteasomen bereits wieder signifikant abgenommen hatte (28). Parallel werden die E3-Ligasen nicht nur durch FoxO induziert. Es konnte gezeigt werden, dass der durch MuRF1 vermittelte Muskelproteinabbau auch durch den nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) aktiviert wird, der seinerseits über den inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) durch Tumornekrosefaktor alpha (TNF-α) inhibiert wird (90). Die systemische Inflammation führt bei kritisch kranken Patienten oftmals zu stark gesteigerten TNF-α -Plasmaspiegel. Interessanterweise konnten wir weder an Tag 5 noch an Tag 15 einen Anstieg der TNF-α -mRNA-Expression in

unseren Muskelbiopsien finden (76, 91). Einschränkend muss man anmerken, dass durch Muskelbiopsien nur einzelne Zeitpunkt abgebildet werden können, und die Konzentrationen von Zytokinen, wie TNF-α aufgrund der kurzen Halbwertszeit und starker Regulierung großen Schwankungen unterliegen können. Somit ist derzeit keine definitive Aussage über die Beteiligung von TNF-α an der Entwicklung einer ICUAW-assoziierten Muskelatrophie möglich (92-94). Myostatin ist ein wichtiger Regulator der Skelettmuskelmasse (95). Ein Mangel an Myostatin führt zu Muskelhypertrophie und Hyperplasie, während eine Überexpression den Verlust von Muskelmasse und Kachexie durch das UPS fördert, insbesondere durch die Induktion von FoxO1, Atrogin-1, MuRF-1 und *TRIM62*, die an der ICUAW-assoziierten Muskelatrophie beteiligt sind (96-101). Eigene präliminiere Daten von unseren Intensivpatienten können diese Hypothese jedoch nicht stützen und zeigen eine verminderte *MSTN*-Genexpression und niedrigere Myostatin-Plasmaspiegel im Vergleich zu gesunden Kontrollen (102).

3.3.2 Proteinsynthese

ICUAW-assoziierte Muskelatrophie ist nicht nur das Ergebnis eines induzierten Proteinabbaus, sondern auch einer reduzierten Proteinsynthese. Puthucheary et al zeigten bereits am ersten Tag einer intensivmedizinischen Behandlung eine reduzierte Proteinsyntheserate durch die Untersuchung markierter Aminosäuren (24). Dieser Befund wird durch eine erniedrigte mRNA-Expression für schwere Ketten von Myosin von uns und anderen Arbeitsgruppen unterstrichen (25, 51, 103). Maßgeblich an der Muskelproteinsynthese beteiligt ist der IGF1-PI3K-Akt / PKB-mTOR-Signalweg (insulin growth factor-1 (IGF-1); Phosphoinositide 3-Kinase (PI3K); Proteinkinase B (PKB); mammalian target of rapamycin (mTOR)) (104). Obwohl wir zeigen konnten, dass Komponenten des Signalwegs bei Patienten mit erworbener Muskelschwäche herunterreguliert sind, war Akt als zentrales Stellenzym sowohl auf Transkriptions- als auch auf Translationsebene hochreguliert. In physiologischem Maße lag es in der aktivierten, phosphorylierten Form in den Muskelbiopsien vor (42). Dies lässt vermuten, dass der Signalweg

bis zum Stellenzym Akt intakt ist. Somit sollte Akt in der Lage sein, den Abbau von Muskelproteinen durch Phosphorylierung von Forkhed-Box-Protein O (FoxO) zu unterdrücken, indem es den Transkriptionsfaktor an der Passage in den Nukleus hindert (87, 105, 106). Außerdem sollte über mTOR die Proteinsyntheseaktivität hochreguliert sein, was offensichtlich nicht erfolgt (25, 42).

3.3.3 Glukosestoffwechsel

Hyperglykämie tritt häufig bei schwerwiegenden Erkrankungen auf und ist das Ergebnis einer erhöhten Glukosefreisetzung in der Leber sowie einer verringerten peripheren Glukoseaufnahme durch die Skelettmuskulatur aufgrund einer schweren Insulinresistenz. Der Skelettmuskel ist für bis zu 95% der insulinabhängigen Glukoseaufnahme und damit die Glukosehomöostase verantwortlich (107-109). Wir konnten bei Intensivpatienten einen deutlich reduzierten Insulinsensitivitätsindex messen, der ein Maß für die aufgenommene Glukose bei maximalem Insulinstimulus über die Zeit darstellt (42). Diese verminderte Kapazität war bei Patienten mit einer erworbenen Muskelschwäche deutlich ausgeprägter als bei Patienten ohne Muskelschwäche, auch wenn alle Intensivpatienten eine reduzierte Glukoseaufnahme gegenüber gesunden Kontrollpatienten zeigten (42). Bei Typ II Diabetikern ist eine verminderte Insulinwirkung bzw. verminderte Insulinrezeptordichte für diese Insulinresistenz verantwortlich (110). Im Gegensatz dazu konnten wir in unseren Intensivpatienten einen funktionstüchtigen Insulinsignalweg, jedoch mit insuffizienter Endstrecke, der GLUT4 Translokation in die Zellmembran, zeigen (42). Die verminderte GLUT4 Translokation führt dann zu einer verminderten Glukoseaufnahmemöglichkeit (42). Eine insuffiziente Translokation findet sich in allen von uns untersuchten Intensivpatienten, ist aber in den Pateinten mit ausgeprägter Schwäche deutlich markanter und kann so die unterschiedliche Glukoseaufnahmekapazität erklären (42). Die Bedeutung der GLUT4-Translokation für die Insulinsensitivität wurde zuvor nur bei GLUT4-Knock-out-Mäusen gezeigt, die durch die fehlenden GLUT4 Transporter in der Zellmembran Hyperglykämien und Insulinresistenzen aufweisen (111). Die genaue Ursache für die verminderte

Translokation ist bisher nicht geklärt. Der Insulinsignalweg scheint bis zum phosphorylierten Akt intakt. Weitere nachgeschaltete Komponenten des insulinabhängigen GLUT4-Translokationswegs wie AS160 und verschiedene Rab-Proteine wurden bei Intensivpatienten bisher nicht untersucht (112).

3.4 Präventionsstrategien des neuromuskulären Organversagens

Zur Vermeidung der ICUAW steht die Behandlung der Grunderkrankung im Vordergrund, um den Risikofaktoren systemische Inflammation und Sepsis mit Multiorganversagen entgegenzuwirken (61, 62). In den letzten Jahren haben darüber hinaus verschiedene Arbeitsgruppen versucht dem Risikofaktor Immobilisation durch gezielte frühe Mobilisation entgegenzuwirken (83, 113-116). Das insgesamt nur unter der Maßgabe, dass Patienten weniger sediert werden und somit einer aktiven Physiotherapie zugänglich sind (83). Girard et al legten hier einen Meilenstein, indem sie zeigten, dass weniger Sedierung mit einem verbesserten Outcome assoziiert ist (117).

2009 publizierten Schweikert et al die Landmarkstudie zur frühen Physiotherapie auf der Intensivstation (83). Sie konnten zeigen, dass aktive Physiotherapie, durchgeführt während einer täglichen Sedierungspause zu funktionellen Verbesserungen der Patienten bei Entlassung von der Intensivstation führt (83). Seither kamen immer wieder Studien zur Frühmobilisation auf (113, 115, 116). Dabei fällt auf, dass es sowohl positive als auch null Ergebnisse gibt (113, 115, 116). Hier scheinen die Designs der Studien, die Patientenkollektive und der Zeitpunkt der ersten Intervention nach Beginn der Erkrankung von Bedeutung.

Zusätzlich wurden in den letzten Jahren muskelaktivierende Verfahren, wie die elektrische Muskelstimulation oder Bettfahrräder genutzt, um in der sehr frühen Phase der kritischen Erkrankung, wenn eine Sedierung unumgänglich ist oder die Patienten aus klinischen Gründen nicht an einer aktiven Physiotherapie teilhaben kann, trotzdem eine muskelaktivierende Stimulation durchführen zu können (64, 70, 71, 118). Zumeist werden dann aktivierende Verfahren in Ergänzung zur passiven Physiotherapie durchgeführt.

3.4.1 Frühmobilisation

In der Studie von Schweikert et al wurde bei 104 Patienten in einer täglichen Sedierungspause Physiotherapie und Egotherapie durchgeführt. Das primäre Outcome war die muskuläre Funktionalität bei Krankenhausentlassung, die einen klaren Vorteil in der Interventionsgruppe zeigte (83). Es folgte im Jahre 2015 die Studie von Kayambu, der in 50 Intensivpatienten 30-60 Minuten Physiotherapie pro Tag absolvierte. Nicht bei Krankhausentlassung, jedoch im 6 Monats Follow-Up zeigte sich eine verbesserte Funktionalität gemessen als Functional Independence Measure (FIM) (119). Auf diese zunächst vielversprechenden Studie folgte 2016 eine Untersuchung von Moss et al., der in 120 Intensivpatienten trotz intensiver Physiotherapie für 28 Tage keinen Vorteil finden konnte (116). Hierbei ist anzumerken, dass die erste Intervention innerhalb von 7 Tagen initiiert wurde und damit die Frage aufwirft, ob es sich hier tatsächlich um eine frühe Mobilisation handelt, wenn man das Zeitfenster der pathologischen Mechanismen, die innerhalb von wenigen Tagen beginnen, berücksichtigt. Im gleichen Jahr publizierten Morris et al. ihr Untersuchung an 300 Intensivpatienten, die innerhalb von 72 Stunden initiiert wurde und durch Physiotherapie eine funktionelle Verbesserung nach 6 Monaten zeigen konnten (113). Es begannen Bestrebungen die mögliche Intervention in einem Protokoll zu definieren, um ein patientenadaptierte, ehrgeizige Physiotherapie zu etablieren. So konnten Schaller et al. 2016 zeigen, dass eine Frühmobilisation, die entsprechend einem 4 stufigen Protokoll durchgeführt wurde, in der Interventionsgruppe bei Krankenhausentlassung zu einem verbesserten Mobilisationsgrad und verbesserter Funktionalität führte (115). Auch diese Intervention wurde innerhalb von 72 Stunden begonnen. 2018 erschien die bis dato größte Patientenkohorte einer Untersuchung zur Frühmobilisation. Wright zeigte, dass tägliche, 90 minütige Physiotherapie in 308 Patienten keinen funktionellen Vorteil erbrachte (120). Auch hier begann die erste Intervention erst nach mehr als 72 Stunden. In diese Reihe der Untersuchungen zur Frühmobilisation reiht sich auch unsere 2019 publizierte Studie ein (3). Unsere Studie verfolgte eine protokollbasierte Physiotherapie, die innerhalb von 72 begonnen wurde. Da es aus unserer

Sicht unethisch war eine Kontrollgruppe ohne Physiotherapie einzuschließen verglichen wir unsere Daten mit einer historischen Kontrolle, die lediglich eine Standardphysiotherapie erhalten hatte. Wir randomisierten Patienten in die Gruppe der protokollbasierten Physiotherapie und in die Gruppe einer protokollbasierten Physiotherapie plus muskelaktivierender Maßnahmen, wie elektrische Muskelstimulation und Ganzkörpervibrationstherapie, wie oben beschrieben. Einzigartig an unserer Studie ist die gemeinsame Untersuchung klinisch gemessener Funktion und molekularer Analysen aus chirurgischen Muskelbiopsien. In unseren Daten fanden wir keinen Vorteil der Interventionsgruppe bezüglich Muskelkraft oder –funktion. Jedoch waren die Muskelfaserquerschnittsflächen, im Sinne erhaltener Muskelmasse, in der Interventionsgruppe für Typ I und Typ II Muskelfasern signifikant größer. Spannend ist weiterhin, dass in keiner der hier aufgeführten Studien, der die ICUAW definierende Parameter Muskelkraft, einen Vorteil in der Interventionsgruppe zeigen kann.

3.4.2 Erweiterte physiotherapeutische Maßnahmen

Es gibt Bestrebungen, den Muskelabbau in der Frühphase intensivmedizinischer Behandlung durch zusätzliche muskelaktivierende Verfahren zu reduzieren. So zeigten Geovasilli et al 2009 erstmals, dass eine elektrische Muskelstimulation der Oberschenkel zu einem Krafterhalt bei Entlassung von der Intensivstation führen kann (69, 121). Aus der gleichen Arbeitsgruppe beschrieben Routsi et al. auch eine frühere Entwöhnung von der künstlichen Beatmung, was man auf positive systemische Effekte zurückführen müsste (68). Auch wir konnten in einer Pilotstudie an 5 Patienten in einem intra-individuellen Design zeigen, dass Muskelmasse und metabolischer Status im Oberschenkelmuskel eines täglich elektrostimulierten Beines, gegenüber dem unstimulierten Bein erhalten werden konnte (42). Jedoch gibt es auch Studien zur elektrischen Muskelstimulation bei Intensivpatienten, die keinen Vorteil zeigen (70). Die größte Untersuchung dazu ist von Fossat et al durchgeführt worden, die keinen Unterschied zwischen Intensivpatienten, die mit Bettfahrrad und zusätzlicher funktioneller Elektrostimulation behandelt wurden im

Vergleich zu Intensivpatienten ohne diese Intervention finden konnten (64). Da die Anwendung einer elektrischen Muskelstimulation sehr zeitaufwendig und nicht eindeutig vorteilhaft ist suchten wir nach alternativen muskelstimulierenden Verfahren. In der Rehabilitationsmedizin, der Neurologie, dem Leistungssport und der Weltraumforschung findet die Ganzkörpervibrationstherapie Anwendung als sehr effektives Muskeltraining in einem kurzen Zeitintervall ohne große Ansprüche an den Trainierenden (122-124). Über einen spinalen Reflex kann die Vibration in hoher Frequenz unwillkürlich Muskelkontraktionen auslösen (125). Zu diesem Zeitpunkt war eine Ganzkörpervibrationstherapie bei Intensivpatienten bisher nicht untersucht worden weshalb wir unsere Studie „Anwendbarkeit einer Ganzkörpervibrationstherapie bei Intensivpatienten zur Prävention einer auf der Intensivstation erworbenen Muskelschwäche“ initiierten. Zusammenfassend können wir sagen, dass eine Ganzkörpervibrationstherapie auch bei Intensivpatienten sicher anwendbar ist und es durch gesteigerten Energieumsatz einen klaren Hinweis auf eine tatsächliche Muskelaktivierung gibt (2). Wir nahmen damit die Ganzkörpervibrationstherapie als Intervention in unsere damals in Planung befindliche randomisierte Interventionsstudie auf. Wir konnten in unserer Studie zeigen, dass zusätzliche muskelaktivierenden Verfahren, unabhängig von Elektrostimulation, Ganzkörpervibrationstherapie, oder einer Kombination aus beidem, einen positiven Effekt auf den Erhalt von Muskelmasse hat (3).

Im Rahmen der elektrischen Muskelstimulation gibt es neben der aufwendigen Applikation auch mögliche Therapieversager. So berichtete Segers et al. 2014, dass nicht in allen Patienten durch elektrische Muskelstimulation eine Muskelkontraktion hervorgerufen werden kann (126). Deshalb haben wir bereits während der Studie erhoben mit welchem Strom an welcher Muskelgruppe eine Kontraktion erzielt werden konnte (4). In unsere post-hoc Subgruppenanalyse werten wir aus, welche Muskelgruppen wie kontrahieren. Man muss festhalten, dass nur in einem kleinen Teil der Stimulationen eine physiologische Stromstärke ausreichend ist, um eine Kontraktion zu erreichen. Zudem ist auffällig,

dass der am häufigsten untersuchte Muskel im Rahmen der erworbenen Muskelschwäche, der musculus vastus lateralis, der Muskel mit der geringsten Kontraktionsantwortrate ist. In einer weiteren Analyse dieser Arbeit haben wir evaluiert, ob eine Kontraktionsantwort zum Erfolg der Stimulation beitragen kann. Und auch hier gibt es ein Signal, dass eine höhere Antwortrate zu verbesserter Kraft bei Entlassung von der Intensivstation führt. Wir haben der Publikation ein Protokoll angefügt, welches bei zukünftigen Studien mit elektrischer Muskelstimulation Aufschluss über die verwendete Stromstärke und den Stimulationserfolg geben soll. Bisher werden diese Daten nicht in den Publikationen anderer Arbeitsgruppen aufgeführt und lassen so keine Rückschlüsse auf erfolgreiche oder frustrane Stimulationsversuche zu, die zur Bewertung des Erfolges jedoch essentiell sind. So erkläre ich mir auch das Negativergebnis der Studie von Fossat et al. die in 300 Patienten eine funktionelle elektrische Muskelstimulation in Kombination mit einem Bettfahrrad untersucht haben und keine Vorteil finden können (64). Ob und wie eine erfolgreiche Kontraktion hier überprüft wurde und somit überhaupt suffizient stimuliert wurde bleibt völlig offen. Es gilt für die Zukunft Patienten, die auf eine elektrische Muskelstimulation ansprechen von denen, die das nicht tun zu separieren und so individuell, patientenadaptiert eine Physiotherapie mit ergänzenden Verfahren anwenden zu können (4).

3.4.3 Frühe Prävention

Alle bisherigen Untersuchungen deuten darauf hin, dass gerade die Frühphase der Erkrankung entscheidend für den Präventionserfolg sein kann. So zeigen alle Physiotherapeutischen Studien an Intensivpatienten, die innerhalb von 72 Stunden mit der Intervention beginnen einen funktionellen Vorteil bei Entlassung oder im Follow-Up Verlauf, wohingegen alle Studie, die nach 72 Stunden die Intervention initiieren keinen Vorteil zeigen (113, 115, 116, 120). Darüber hinaus sehe ich perioperative Patienten grundsätzlich einem ähnlichen Risikoprofil ausgesetzt wie Intensivpatienten (5). Der chirurgische Reiz führt zu einer

inflammatorischen Reaktion des Immunsystems, die Patienten erhalten zu einem großen Anteil Muskelrelaxantien und sind für die Dauer der Operation immobilisiert. Ebenso fällt bei vielen Patienten intraoperativ ein gestörter Glukosestoffwechsel auf, der sowohl durch die inflammatorische Reaktion als auch durch die Immobilisation begünstigt sein kann, und mit Muskelschwäche assoziiert ist (127, 128) . Und tatsächlich konnten wir in unserer Untersuchung eine perioperative Muskelschwäche auch ohne intensivmedizinische Behandlung nachweisen. Selbst im 3-Monats Follow-Up zeigen einige Patienten nach elektiver Operation und gutem präoperativem Ausgangsstatus körperliche Funktionseinschränkungen, die nicht auf die Operation selbst zurückzuführen sind (5). Hier ist der perspektivische Ansatz Patienten schon vor der elektiven Operation physiotherapeutisch zu behandeln, um erstens eine bestmögliche Ausgangssituationen für die Pateinten zu erhalten und zweitens so unmittelbar postoperativ die gewohnten Übungen wieder aufnehmen zu können, um auch den Krankenhausaufenthalt ohne Intensivstation möglichst ohne Begleiterscheinungen, wie die perioperativ erworbene Muskelschwäche zu überstehen.

3.5 Aktuelle Empfehlungen zur Prävention des neuromuskulären Organversagens

Für die klinische Umsetzung möchte ich mich an die aktuelle S2e Leitlinie zur Lagerungstherapie und Frühmobilisation halten, die eine Physiotherapie empfiehlt, die innerhalb von 72 Stunden nach Aufnahme auf die Intensivstation initiiert wird, deren möglichen Maßnahmen und Intensität patientengerecht in einem Protokoll festgehalten sind und nach Möglichkeit zweimal täglich durchgeführt wird (63). Erweiterte Maßnahmen wie elektrische Muskelstimulation, Ganzkörpervibrationstherapie oder Bettfahrräder sind nicht Bestandteil der Leitlinie, können jedoch bei entsprechender Erfahrung von Physiotherapeuten angewendet werden. Es sollte allerdings im interprofessionellen Team aus Arzt, Pflege und Therapeut eine Einzelfallentscheidung zu diesen Maßnahmen geben, da es bisher keine Evidenz für erweiterte Maßnahmen gibt, die in eine mir bekannte Leitlinie oder Handlungsempfehlung aufgenommen wurde.

3.6 Ausblick

Hinter dem Begriff der ICUAW verstecken sich verschiedene Phänomene des neuromuskulären Organschadens. Muskelmassenverlust, Muskelfunktionsverlust, Maximalkraftverlust und Ausdauerkraftverlust sind nicht klar voneinander abgrenzbar. Ebenso wird die Beteiligung von Muskeln, Nerven oder beiden an der erworbenen Muskelschwäche noch nicht klar diagnostiziert, da bisher auch die klinischen Konsequenzen zu einer derartigen Diagnose fehlen. Zukünftige Untersuchung müssen weiter die pathologischen Prozesse des multifaktoriellen Geschehens erworbene Muskelschwäche aufklären, um dadurch möglicherweise auch neue Therapie- oder Präventionsansätze entwickeln zu können. Ein akuter Schritt wird meinerseits eine klinische Studie zur Identifizierung von den Patienten sein, die von der Anwendung einer elektrischen Muskelstimulation profitieren können sein, sowie die Beschreibung eines Kollektives welche Patienten überhaupt und von welchen Maßnahmen in welchem Umfang und zu welchem Zeitpunkt profitieren.

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Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
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- mir die geltende Habilitationsordnung bekannt ist.

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.

……. ……..
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