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

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Abstract

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

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

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

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

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

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[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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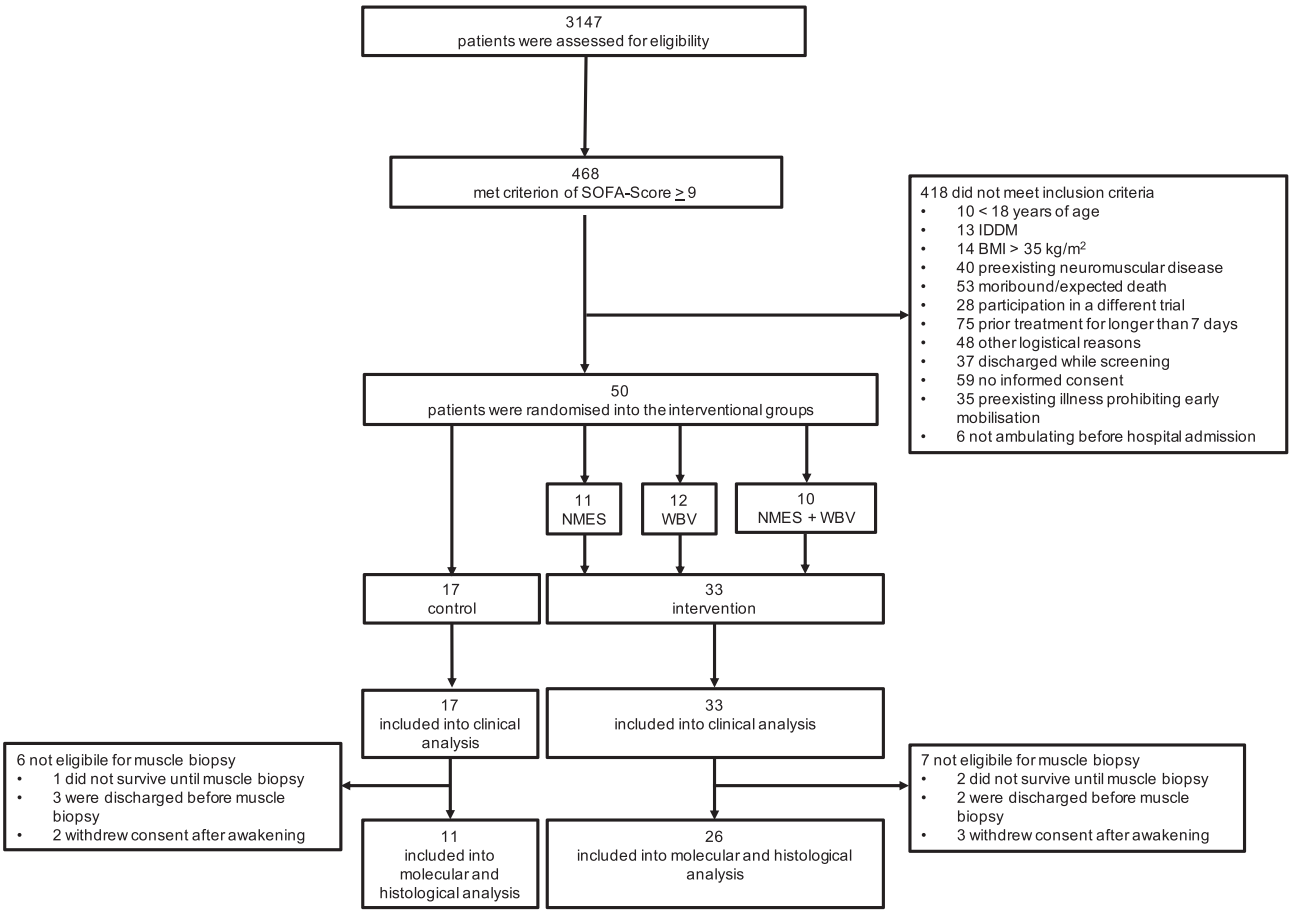
Figure 1 Trial enrolment scheme. 'Other logistical reasons' indicates cases where a legal proxy could not be appointed within the screening timeframe or study personal was not available for logistical reasons. IDDM, insulin-dependent diabetes mellitus; NMES, neuromuscular electrical stimulation; WBV, 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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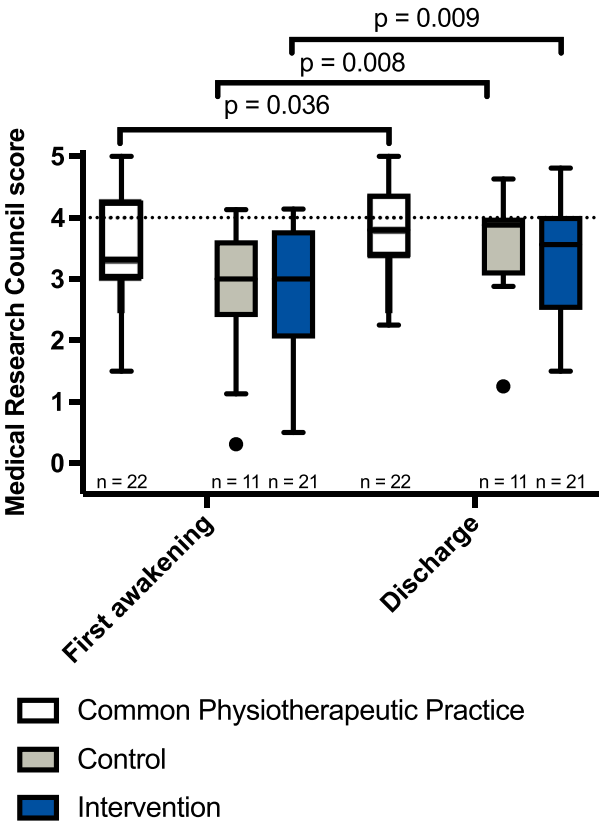
Figure 2 Muscle strength measured by Medial Research Council sum score. MRC score showed a significant increase for the control, intervention, and common physiotherapeutic practice group from first awakening until discharge, while no difference between the groups at either time point could be observed. 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.

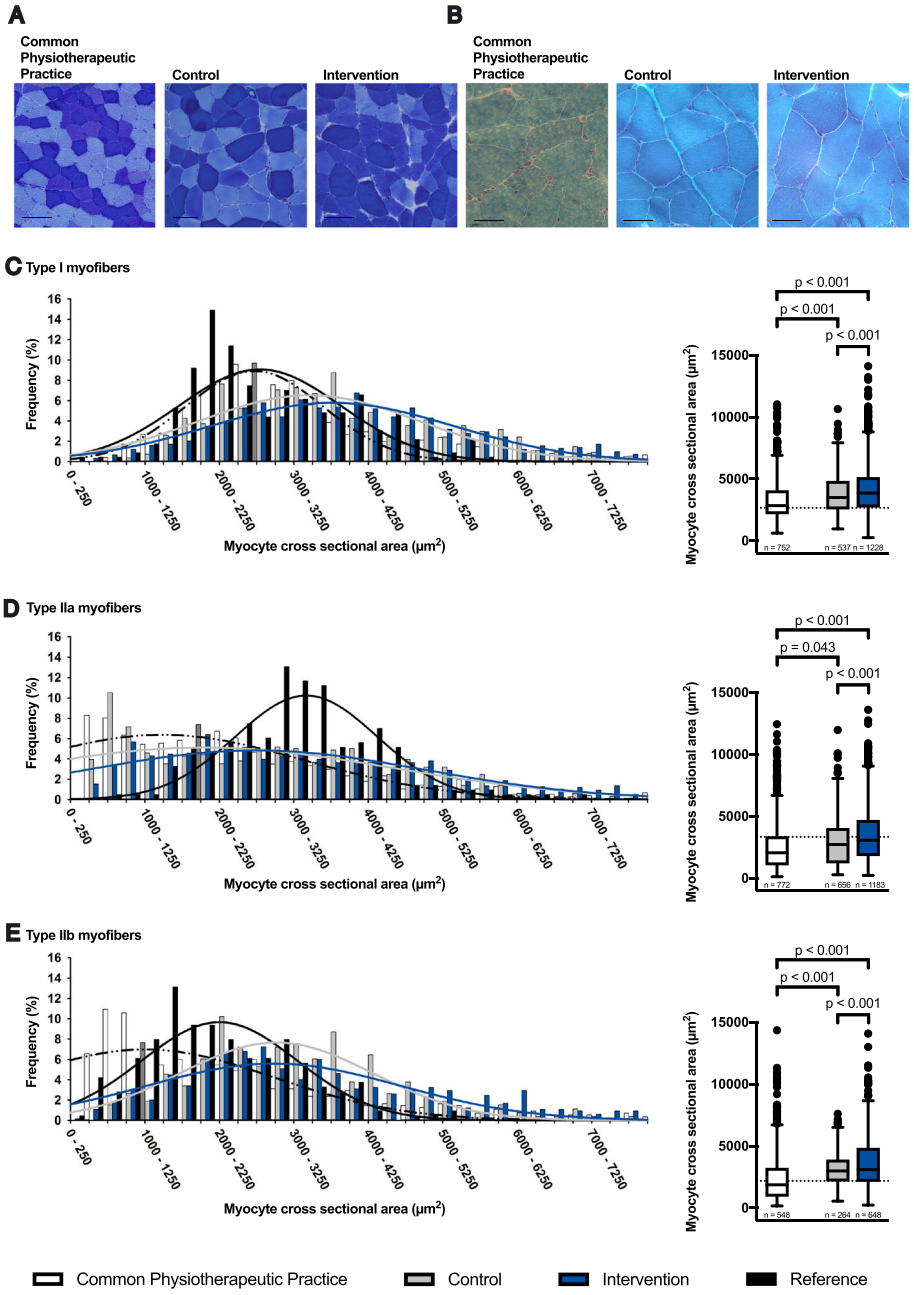


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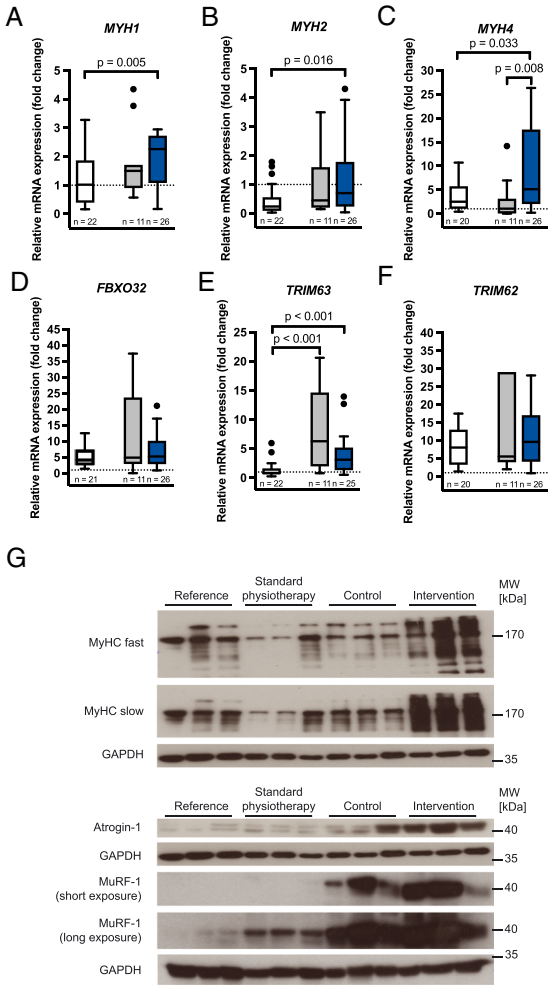
Figure 4 Gene expression for myosin heavy chains and atrogenes as well as protein content for myosin and key proteins of the ubiquitin proteasome system. (A) *MYH1* gene expression was significantly increased in the intervention group in comparison with the common physiotherapeutic practice group and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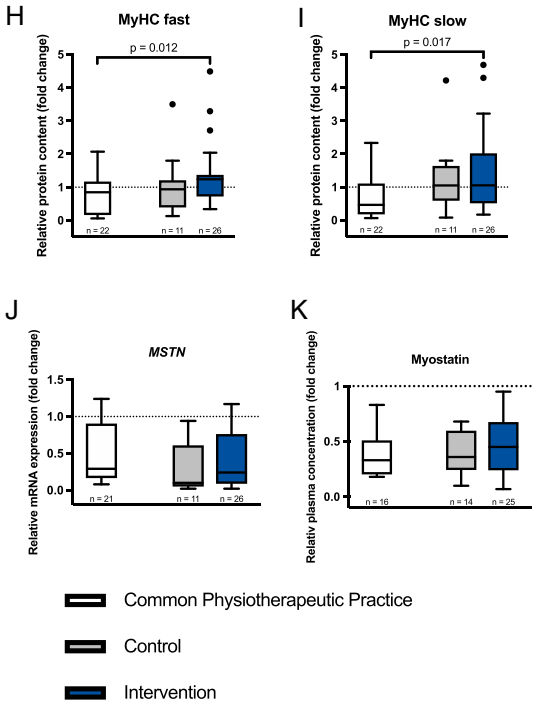
Figure 4 Continued



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

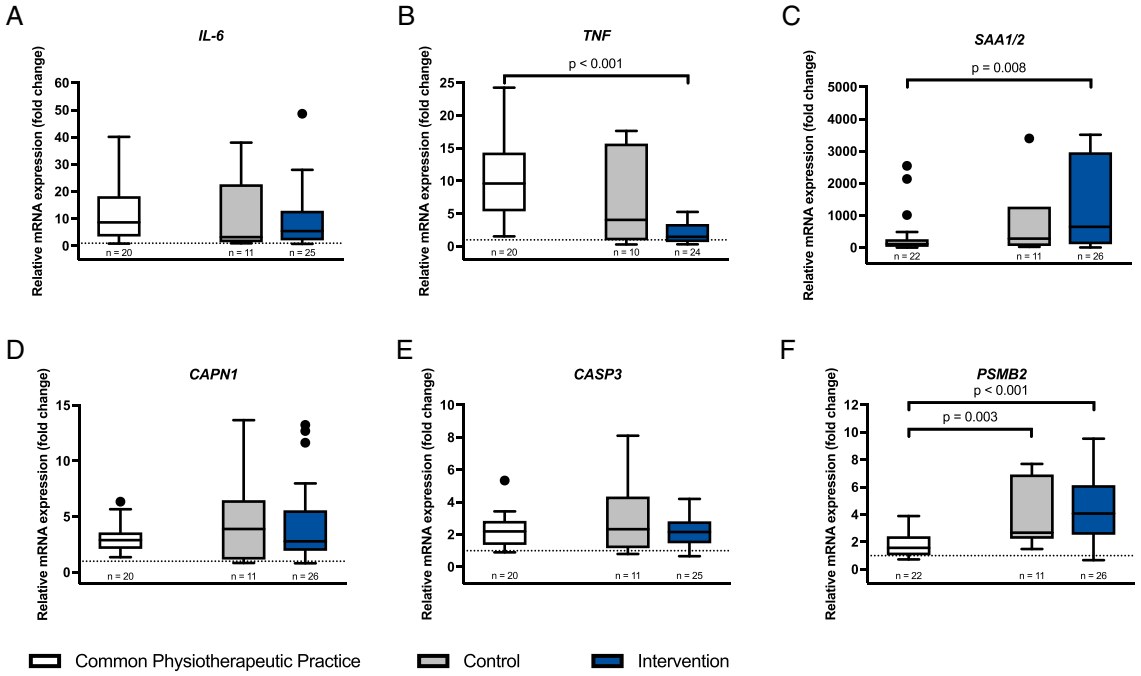
The inflammatory cytokines *IL-6* (encoding for interleukin 6) and *SAA1/2* (encoding for serum amyloid a1/2) were both significantly increased above values for healthy references while *TNF* (encoding for tumor necrosis factor alpha) presented values similar to healthy references for the intervention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

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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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