The final authenticated version is available online at: https://doi.org/10.1097/CCM.0000000000004522

Effect of electrical muscle stimulation on upper and lower limb muscles in critically ill patients: A two-center randomized controlled trial

Short running title: Electrical muscle stimulation on upper and lower limb muscles in critically ill patients

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Reprints: None

Conflict of interest: None

Funding: Departmental fund

ABSTRACT

Objectives: Electrical muscle stimulation (EMS) is widely used to enhance lower limb mobilization. Although upper limb muscle atrophy is common in critically ill patients, EMS application for the upper limbs has been rarely reported. The purpose of this study was to investigate whether EMS prevents upper and lower limb muscle atrophy and improves physical function.

Design: Randomized controlled trial.

Setting: Two-center, mixed medical/surgical intensive care unit (ICU).

Patients: Adult patients who were expected to be mechanically ventilated for >48 h and stay in the ICU for >5 days.

Interventions: Forty-two patients were randomly assigned to the EMS (n = 17) or control group (n =

19).

Measurements and Main Results: Primary outcomes were change in muscle thickness and cross-sectional area of the biceps brachii and rectus femoris from day 1 to 5. Secondary outcomes included incidence of ICU-acquired weakness (ICU-AW), ICU mobility scale (IMS), length of hospitalization, and amino acid levels. The change in biceps brachii muscle thickness was −1.9% vs. −11.2% in the EMS and control (p = 0.007) groups, and the change in cross-sectional area was −2.7% vs. −10.0% (p = 0.03). The change in rectus femoris muscle thickness was −0.9% vs. −14.7% (p = 0.003) and cross-sectional area was −1.7% vs. −10.4% (p = 0.04). No significant difference was found in ICU-AW (13% vs. 40%; p = 0.20) and IMS (3 vs. 2; p = 0.42) between the groups. The length of hospitalization was shorter in the EMS group (23 [19–34] vs. 40 [26–64] days) (p = 0.04). On day 3, the change in the branched-chain amino acid level was lower in the EMS group (40.5% vs. 71.5%; p = 0.04).

Conclusion: In critically ill patients, EMS prevented upper and lower limb muscle atrophy and attenuated proteolysis and decreased the length of hospitalization.

Keywords: Electrical muscle stimulation, Muscle atrophy, Intensive care unit-acquired weakness, Proteolysis, Critically ill patients

INTRODUCTION

Skeletal muscle atrophy and physical disability are common in critically ill patients. Muscle atrophy rapidly occurs after admission to the intensive care unit (ICU), and muscle atrophy reaches 18.8%–20.7% in the lower limbs within 7 days (1). Although most studies have evaluated lower limb muscle atrophy, our previous study revealed that upper limb muscle mass decreased by 13.2%–16.9% within 7 days of ICU admission (2). Functional disability also occurs in both the upper and lower limbs in ICU-acquired weakness (ICU-AW), which is found in nearly 50% of critically ill patients (1).

Early mobilization in the ICU is proven to be effective in preventing muscle atrophy and physical disability. However, it is not necessarily applicable in all patients. Hence, electrical muscle stimulation (EMS) has been used as an additional physical therapy for critically ill patients (3). Previous studies showed that EMS prevented lower limb muscle atrophy in critically ill patients (4, 5) but did not improve physical function and mobility at discharge from the ICU (6). Lower limb EMS did not decrease the length of ICU and overall hospitalization (7, 8). EMS is usually used for lower limb training to enhance mobilization; we believed that its application to only the lower limbs was the cause of patients' unchanged physical condition and morbidity level.

Upper limbs play an important role for maneuvers on the bed, rib cage movements, and mobilization ability. However, investigations on the effects of upper limb rehabilitation on muscle atrophy and physical function in critically ill patients are limited. To our knowledge, no randomized controlled trial has investigated the effects of upper limb EMS on muscle atrophy in critically ill patients. We hypothesized that early application of EMS on the bilateral upper and lower limbs prevents

upper and lower limb muscle atrophy and improves physical function and mobility level.

MATERIALS AND METHODS

Study design

This single-blind, randomized controlled trial conducted in the mixed medical/surgical ICUs of Tokushima University Hospital and Tokushima Prefectural Central Hospital between July 2017 and January 2020 was approved by both clinical research ethics committees (approval numbers 2849 and 1740, respectively). This study was registered as a clinical trial (UMIN Clinical Trials Registry: 000027054). At the time of enrollment, written informed consent was obtained from patients or their authorized surrogate decision makers. Patients were randomly assigned to the EMS group (EMS application in addition to mobilization protocol) or control group (mobilization protocol alone) using sequentially numbered envelopes. Further details regarding the protocols are described in the supplemental file.

Study population

We enrolled consecutive adult patients who were expected to be mechanically ventilated for >48 h and to stay in the ICU for >5 days. Patients were prospectively recruited within 24 h following ICU admission on weekdays. Patients who met the following criteria were excluded: age under 18 years, trauma or amputation of upper and lower limbs, diagnosis of primary neuromuscular disease, systolic blood pressure <80 mmHg even with inotropic or vasopressor support, heart rate <40 or >140

beats/min, and peripheral oxygen saturation <88% with ventilatory support.

Mobilization program

Patients in both groups were mobilized using the same progressive mobilization protocol described by Morris et al. (9). The same protocol was used by the two centers in this study. Within 5 days of intervention, the median ICU mobility scale (IMS) level of all patients was 1 (1–3) in both groups.

Electrical muscle stimulation

EMS was applied in addition to the mobilization program in the EMS group. EMS was performed using a stimulator (Solius, Minato Medical Science, Co., Ltd., Osaka, Japan). A square wave on the positive side (650 μS) and an exponential decay wave on the negative side were applied. The frequency was 20 Hz (200 Hz only at the start of energization). EMS was continuously applied for 30 min with the cycle 400 mS “on” and 600 mS “off.” The 4-channel EMS stimulated four muscle groups simultaneously using eight electrodes. Rectangular electrodes (40 × 80 mm) were attached to the motor points of the biceps brachii and rectus femoris muscles. The motor points were identified by scanning the skin surface where current resulted in maximal muscle contraction. An image of the intervention was shown in a previous report (1). The EMS group received daily EMS sessions for 30 min from day 1 to day 5. The output current was adjusted to ensure visible contraction of muscles. The EMS intensities used were 30 mAp (23–37 mAp) for the biceps brachii and 41 mAp (33–50 mAp) for the rectus

femoris. Compliance of protocol was 80% (60%–100%) through stimulation sessions (EMS sessions/study days). We did not use a sham control group because muscle contraction was apparent in the EMS group.

Ultrasound measurement

Muscle mass of the biceps brachii and rectus femoris muscles was evaluated by measuring the muscle thickness and cross-sectional area using ultrasound on day 1 and day 5. Two investigators (N.N. and Y.U.) performed the measurements. Measurement was performed three times with the median value used for evaluation. Intra-observer and inter-observer correlation coefficients ranged from 0.98 to 0.99 (**Table S1**). The investigators conducting the measurements were not blinded to patients' assignment, but image analyses were blinded by concealing patients' status as previously reported (10, 11).

Physical assessment

In awake and attentive patients, the Medical Research Council (MRC) score and ICU-AW were evaluated at day 5 by physical therapists blinded to the randomization group (12). ICU-AW was defined as an MRC score of <48 on two separate occasions, and patients with preadmission MRC score of <48 were excluded (13). IMS is a scale of mobilization capabilities from 0 (lying in bed) to 10 (walking independently) (14). IMS was also assessed at the time of discharge from the ICU.

Amino acid level

Amino acid levels in whole blood were measured to evaluate proteolysis. We used metabolome analysis using capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS: Agilent Technologies, Santa Clara, CA, USA). One milliliter of blood was collected from an arterial line when available. Level of all 20 amino acids were measured. Because they are important components of muscles, we also measured the percentage increase or decrease in branched-chain amino acid (BCAA) levels, including valine, leucine, and isoleucine, from day 1 to day 3 or 5.

Outcome

The primary outcomes were change in muscle thickness and cross-sectional area of the biceps brachii and rectus femoris muscles from day 1 to 5. To assess changes in muscle mass, we calculated the muscle atrophy rate, defined as the percent variation in muscle mass compared with the values at admission. The secondary outcomes were MRC score and ICU-AW incidence at day 5, IMS at discharge from the ICU, ventilator- and ICU-free days, length of hospitalization, and percentage change in amino acid level.

Sample size and statistical analyses

Sample size was calculated using the method described by Gerovasili et al. (4), who reported that EMS prevented 5.9 ± 5.1% lower limb muscle atrophy within 7 days. We hypothesized a 4.2% difference in the upper limb muscle mass within 5 days, with 3.7% standard deviation (SD), and aimed to obtain a sample size in which such a difference could be observed with alpha 0.05 and power 90%,

which yielded 35 patients. Assuming that 15% of patients were discharged from the ICU within 5 days, a total of 42 patients were needed.

Changes in muscle mass were presented as mean and standard error of the mean. Other continuous data were presented as mean ± SD or median (IQR), whereas categorical data were expressed as number (%). Normally distributed continuous variables were compared between groups using the t-test, whereas non-normally distributed variables were compared using the Wilcoxon rank-sum test. Categorical data were compared using the chi-squared test. All statistical tests were two-tailed, and $p < 0.05$ was considered statistically significant. Statistical analyses were performed using JMP statistical software, version 13.1.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 42 patients were enrolled, and 6 were excluded (death until day 5, rejection due to pain, and insufficient muscle contraction due to edema or obesity). Finally, we assigned 17 patients to the EMS group and 19 to the control group (**Fig. 1**). Mean age was 73 ± 3 vs. 66 ± 3 years ($p = 0.09$) and median acute physiology and chronic health evaluation II (APACHE II) score was 25 (20–31) vs. 22 (19–30) ($p = 0.57$) in the EMS and control groups, respectively (**Table 1**).

Changes in upper and lower limb muscle mass

The changes in upper limb muscle thickness were −1.9 ± 2.4% and −11.2 ± 2.1% in the EMS and control groups, respectively ($p = 0.007$), and changes in cross-sectional area were −2.7 ± 2.6% and

−10.0 ± 1.5% in the EMS and control groups, respectively (p = 0.03) (**Fig. 2**). Changes in lower limb muscle thickness were −0.9 ± 3.1% and −14.7 ± 2.7% in the EMS and control groups, respectively (p = 0.003), and changes in cross-sectional area were −1.7 ± 2.9% and −10.4 ± 2.8% in the EMS and control groups, respectively (p = 0.04).

Secondary outcomes

Physical function was assessed in 8 and 10 patients in the EMS and control groups, respectively. No significant difference was found in MRC score, ICU-AW incidence, and IMS at discharge from the ICU (**Table 2**). The EMS group had a shorter length of hospitalization. BCAA levels on day 3 were significantly lower in the EMS group than in the control group. Moreover, glycine levels on days 3 and 5 and proline levels on day 3 were lower in the EMS than in the control group (**Table S2**).

DISCUSSION

We found that EMS of the upper and lower limb muscles prevented muscle atrophy. To our knowledge, this is the first study demonstrating that EMS prevented upper and lower limb muscle atrophy simultaneously. This study did not reveal any differences in physical function. However, we found that EMS attenuated proteolysis and reduced the length of hospitalization.

Most studies apply EMS to the lower limbs, and we found one study in which EMS was applied to the hemilateral side of the upper and lower limbs in 14 patients (15). They reported unclear

results that patients exhibited significant muscle atrophy in the limb without EMS, but EMS intervention did not show significant differences on the stimulated or unstimulated side. This muscle mass measurement is based on arm circumference and muscle thickness measured by ultrasound, which are easily affected by edema and the angle of the legs (16-18). In contrast, cross-sectional area is more precise and accurate or at least less susceptible to measurement bias (19). Hence, in our study, we assessed muscle atrophy using cross-sectional area and not muscle thickness.

A recent large-scale study by Fossat el al. demonstrated that rectus femoris muscle thickness did not change between patients with or without EMS during ICU stay (6). This study is also based on the measurement of muscle thickness, similar to most studies (4, 15, 17, 20). Moreover, the timing of intervention in the study by Fossat et al. is clearly different from that in our study. We intervened only in the early phase, when patients' mobilization could not be actively performed, achieving a maximum IMS level of 1 during the first 5 days. In contrast, Fossat et al. intervened from ICU admission to discharge and actively conducted mobilization in the intervention period. EMS is less useful once patients are actively mobilized (3). EMS was also effective for patients whose mobilization was limited due to impaired consciousness or mechanical ventilation (5, 21). EMS can be used as an additional therapy to maintain muscle mass in this population.

Although our study involved both the upper and lower limbs, our results were consistent with those of a previous large-scale study that showed that EMS did not improve physical function and mobility at discharge from the ICU (6). There are several possible reasons for this. First, the number of included patients was insufficient to analyze physical functions. Physical assessment could be

performed in only 50% of included patients because of impaired consciousness. Second, we did not include patients requiring prolonged ICU stay. In a previous study, Silva et al. reported that 14 days were needed to treat muscle weakness using EMS. Our study aimed at maintaining muscle mass during the early phase of critical illness, and we did not include patients requiring prolonged ICU stay (21). Therefore, our treatment duration may not have been long enough to improve clinical outcomes. Third, although we applied EMS on both upper and lower limbs, the effect of muscle stimulation was limited to the biceps brachii and rectus femoris muscles. It was better but not feasible to stimulate all muscles.

Our results indicate that maintaining muscle mass during the early phase of critical illness may reduce the length of hospitalization. In a previous study, enhanced rehabilitation of mechanically ventilated patients reduced the length of hospitalization (22). We believe that maintaining upper limb muscle mass smoothly led to mobilization because the upper limbs contribute to the ability to transfer from a bed to a wheelchair, to sit up, and to lie down. Furthermore, upper limbs are important for writing, drinking, and eating. Sufficient upper limb function may prevent aspiration or other problems in the ward and may reduce the length of hospitalization. Most studies applied EMS only to the lower limbs and did not report reduced length of hospitalization (7, 8). The simultaneous application of EMS to the upper limb may change the clinical outcomes of critically ill patients.

In critically ill patients, muscle atrophy occurs in the diaphragm and rib cage muscles as well as limbs. We previously reported intercostal muscle atrophy in mechanically ventilated patients, which was associated with weaning difficulties (10). In another study, the pectoralis major muscle was associated with a high 6-month survival rate (23). Upper limbs play some role in respiration in terms of

rib cage expansion. Therefore, our results indicate that EMS application may enhance respiratory muscle training. A recent study showed that EMS application to the diaphragm was feasible (24). A pilot study applied EMS to the abdominal muscles to assist in weaning from mechanical ventilation and found that the intervention decreased the duration of mechanical ventilation use (25). Moreover, upper limb training indirectly improves pulmonary function (26). In patients with chronic obstructive pulmonary disease, 8-week upper limb training improved lung function and functional capacity (27). Additional clinical studies will be needed to prove if EMS application to the upper limbs reduces mechanical ventilation time.

Interestingly, EMS not only prevented muscle atrophy but also attenuated proteolysis in critically ill patients. A previous study showed that EMS attenuated protein degradation, as evidenced by decreased 3-methylhistidine levels (28). In this study, we found lower BCAA levels in patients in the EMS group. BCAA is an important component of muscles (29). Lower BCAA levels may reflect decreased level of catabolism or increased consumption for protein synthesis in the muscles as previously reported (30). Moreover, glycine and proline levels were lower in EMS group. Because it is essential to prevent muscle atrophy (31), lower levels of glycine may indicate increased glycine consumption for protecting muscles. In contrast, lower levels of proline may also indicate decreased level of catabolism because muscle atrophy increases proline levels (32). Overall, in our study, the levels of most amino acids were lower in the EMS group, with or without statistical significance, suggesting an attenuated catabolic response. However, our experiments on amino acids are at a preliminary stage of research and are less precise with a wide range of values.

Limitations

Our study has several limitations. First, this study included a small sample size, especially in the analysis of physical function. Second, complete blinding could not be achieved. Investigators could not completely avoid seeing the intervention, and patients could understand the intervention or control. Thus, some bias may remain although the data analysis was blinded in a previously reported reliable method (10, 11). Third, as our study compared the effect of EMS added to the mobilization protocol with the mobilization protocol alone, the outcomes are associated with EMS application in combination with the mobilization protocol and not EMS alone.

CONCLUSIONS

In this trial, early application of EMS prevented upper and lower limb muscle atrophy in critical ill patients. EMS also attenuated proteolysis and decreased the length of hospitalization. Further research is required to determine if EMS improves physical function and long-term outcomes of critically ill patients.

ACKNOWLEDGEMENTS

The authors thank Yoshihiro Okayama (the Clinical Trial Center for Development Therapeutics, Tokushima University Hospital) for support in the statistical analysis. The authors thank the ICU staff of Tokushima University Hospital and Tokushima Prefectural Central Hospital, who made this study possible.

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FIGURE LEGENDS

Figure 1. Flowchart of patients included in this study

EMS = electrical muscle stimulation

Figure 2. Change in biceps brachii and rectus femoris muscle mass

a. EMS prevented reduction of biceps brachii muscle thickness ($p = 0.007$) and cross-sectional area ($p = 0.03$). b. EMS prevented reduction of rectus femoris muscle thickness ($p = 0.003$) and cross-sectional area ($p = 0.04$). Data are presented as means and standard error. P values were derived from t-test.

EMS = electrical muscle stimulation, CSA = cross-sectional area

Figure 1

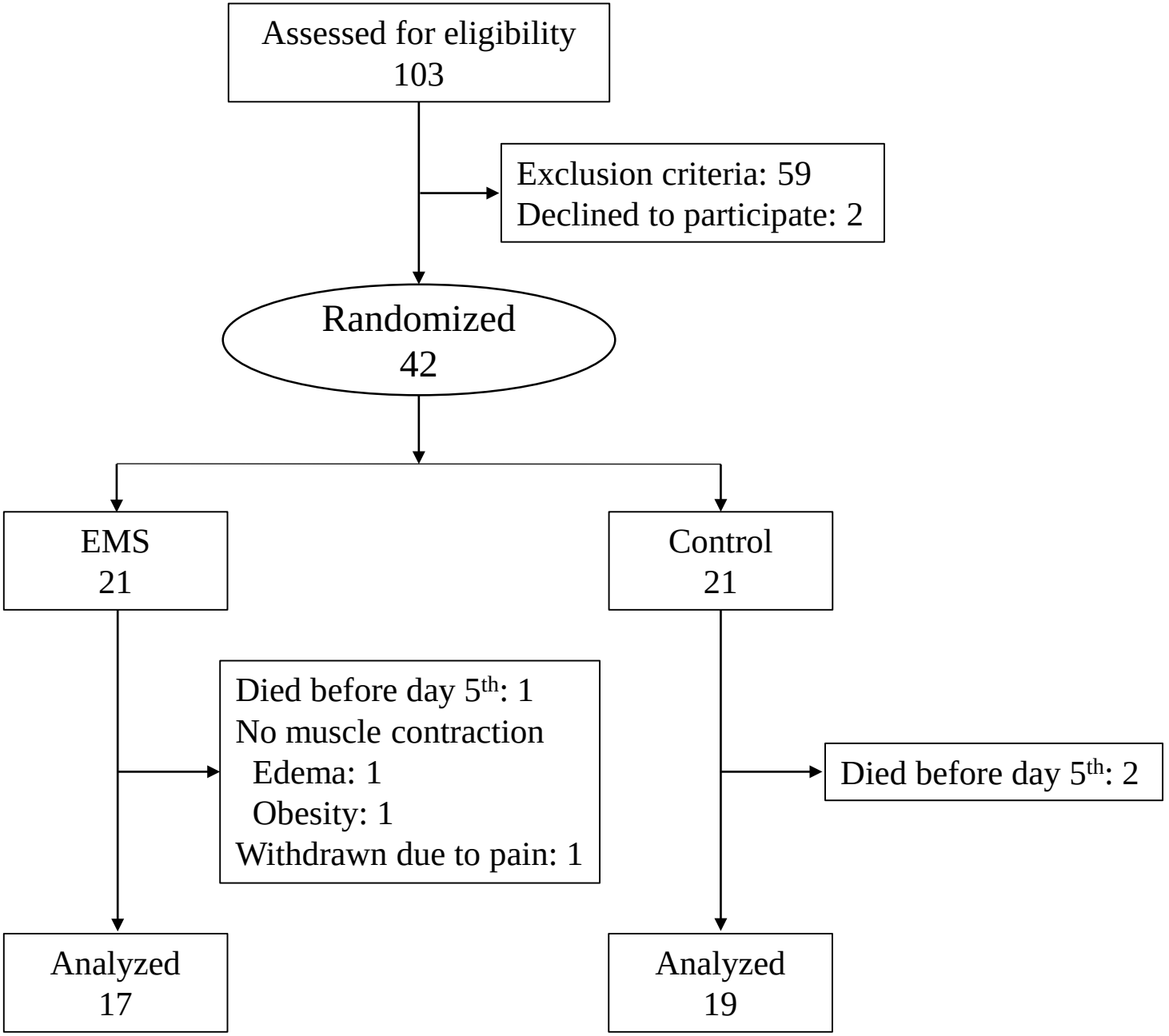
Assessed for eligibility
103
Exclusion criteria: 59
Declined to participate: 2
Randomized
42
EMS
21
Control
21
Died before day 5th: 1
No muscle contraction
Edema: 1
Obesity: 1
Withdrawn due to pain: 1
Died before day 5th: 2
Analyzed
17
Analyzed
19

Figure 2

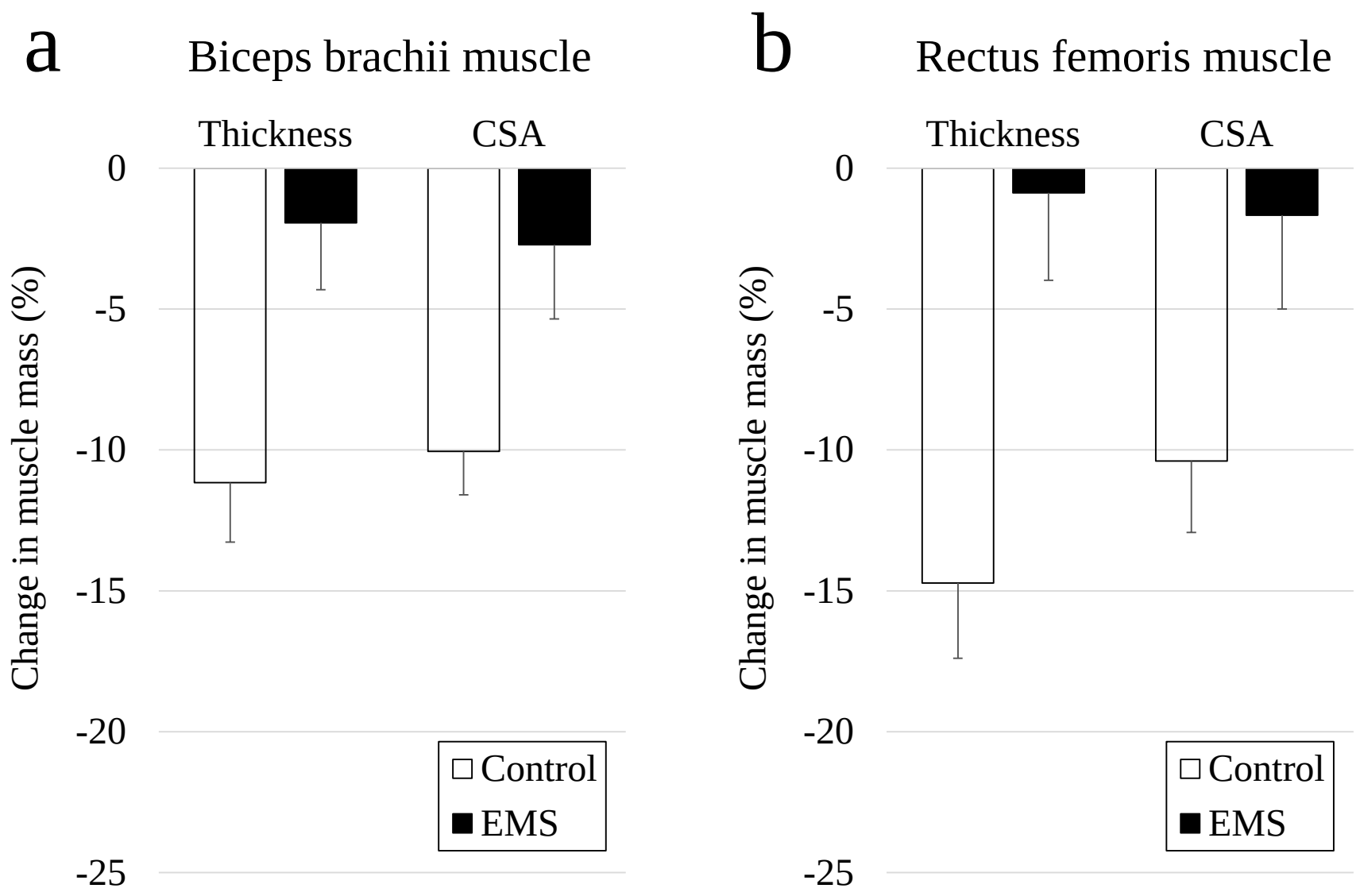
a
Biceps brachii muscle
Thickness
CSA
0
-5
-10
-15
-20
-25
Change in muscle mass (%)
Control
EMS
b
Rectus femoris muscle
Thickness
CSA
0
-5
-10
-15
-20
-25
Change in muscle mass (%)
Control
EMS

TABLE 1. Patient Characteristics

Variables	EMS, n = 17	Control, n = 19	*p*
Age, mean ± SD, y	73 ± 3	66 ± 3	0.09
Gender (M/F)	12/5	12/7	0.64
Body mass index (kg/m^2)	23 ± 4	24 ± 4	0.33
APACHE II	25 (20–31)	22 (19–30)	0.57
SOFA, mean over the first 3 days	9 (6–12)	7 (5–10)	0.13
Sepsis-3 criteria on admission, n (%)	8 (46)	11 (58)	0.51
ICU admission reasons, n (%)			
Respiratory failure	6 (35)	6 (32)	0.40
Heart failure	2 (12)	1 (5)	
Neurologic	1 (6)	3 (16)	
Post-cardiac surgery	5 (29)	2 (11)	
Sepsis, nonrespiratory	2 (12)	4 (21)	
Others	1 (6)	3 (16)	
Comorbidities, n (%)			
Diabetes mellitus	4 (25)	6 (35)	0.52
Cancer	0 (0)	1 (5)	0.32
Medications, n (%)			
Catecholamine*	14 (82)	12 (63)	0.20
Neuromuscular blocking agents†	0 (0)	0 (0)	-
Steroids‡	6 (35)	6 (32)	0.81
Aminoglycoside	0 (0)	1 (5)	0.34
Opioids	14 (82)	14 (74)	0.53
Sedatives§	11 (65)	14 (74)	0.56
Nutrition			
Days to enteral nutrition	2 (1–2)	2 (2–2)	0.42
Calorie at day 5, kcal/kg	9.8 (8.8–15.8)	13.2 (8.8–21.9)	0.11
Protein at day 5, g/kg	0.6 (0.4–0.7)	0.6 (0.4–0.8)	0.41

EMS = electrical muscle stimulation, APACHE = Acute Physiology and Chronic Health Evaluation, SOFA = Sequential Organ Failure Assessment, IQR = interquartile range

*Catecholamine (dopamine, dobutamine, noradrenaline, or adrenaline), †Neuromuscular blockers

with continuous use, ‡Steroids with intravenous or peroral use, §Sedatives (midazolam, propofol) Data were presented as median (IQR) unless otherwise indicated.

TABLE 2. Secondary Outcomes

Variables	EMS, n = 17	Control, n = 19	*p*
Functional outcomes (n = 8, 10)			
MRC score at day 5	55 (50–58)	52 (35–59)	0.53
ICU-AW (%) at day 5	13%	40%	0.20
IMS at discharge from the ICU	3 (1–4)	2 (1–3)	0.42
Ventilator-free days, d	23 (19–25)	22 (10–24)	0.45
ICU-free days, d	21 (12–23)	20 (9–23)	0.97
length of hospitalization, d	23 (19–34)	40 (26–64)	0.04
length of hospitalization (survivor), d	24 (20–32)	40 (29–55)	0.03
After hospital discharge			
Home	3	4	
Transfer	11	11	0.42
Death	3	4	
Amino acid level			
Day 1 to day 3 (n = 14, 17)			
BCAA, %	40.5 (−7.4 to 75.3)	71.5 (38.8 to 116.9)	0.04
Glycine, %	−23.4 (−54.5 to −0.6)	12.3 (−1.8 to 39.7)	< 0.01
Proline, %	2.6 (−43.4 to 48.3)	54.3 (−11.4 to 147.9)	0.04
Day 1 to day 5 (n = 12, 15)			
BCAA, %	18.6 (−12.8 to 67.2)	21.6 (9.0 to 106.1)	0.59
Glycine, %	−37.3 (−40.8 to −16.1)	4.0 (−5.5 to 37.4)	< 0.01
Proline, %	−11.0 (−28.6 to 25.4)	37.9 (−27.6 to 95.1)	0.26

MRC = medical research council, ICU-AW = intensive care unit-acquired weakness, IMS = intensive care unit mobility scale, ICU = intensive care unit, BCAA = branched-chain amino acid, IQR = interquartile range

Data were presented as median (IQR) unless otherwise indicated.

Supplemental File

Electrical muscle stimulation on upper and lower limb muscles in critically ill patients

METHODS

Mobilization program

Mobilization level was decided according to patients' neurological function (conscious or unconscious) and muscle strength. Passive range of motion was carried out for unconscious patients, whereas in conscious patients, the intensity was gradually increased to active resistance, sitting on the edge of bed, and ambulation. Mobilization level was limited in patients with hemodynamic or respiratory instability.

Ultrasound measurement

All scanning was done with patients supine and elbows and knees in passive extension. Generous amounts of contact gel were applied to avoid compression of the muscles by the transducer, and the transducer was placed perpendicular relative to the long axis of the limbs. A B-mode ultrasound with a linear transducer was used. Biceps brachii muscle was measured at two-thirds of the way between the acromion and the antecubital crease. Rectus femoris muscle was measured at midway between the anterior superior iliac spine and the proximal end of the patella. Biceps brachii muscle thickness (including the underlying brachialis muscle) was defined as the depth between the superficial fascia of the biceps brachii muscle and the uppermost part of the humerus, and cross-sectional area of the biceps brachii muscle was measured by tracking the area in the transverse plane. Rectus femoris muscle thickness (including the underlying vastus intermedius muscle) was defined as the depth between the superficial fascia of the rectus femoris muscle and the uppermost part of the femur, and cross-sectional area of rectus femoris muscle was measured by tracking the area in the transverse plane.

Physical assessment

Consciousness was evaluated as adequate when patients respond to at least three of the five orders (“open/close your eyes,” “look at me,” “open your mouth and put out your tongue,” “nod your head,” and “raise your eyebrows”). In responsive patients, we evaluated MRC score including bilateral shoulder abductors, elbow flexors, wrist extensors, hip flexors, knee extensors, and ankle dorsiflexors.

Amino acid level

The collected blood was immediately mixed with methanol with internal standard solution. High performance liquid chromatography and Milli-Q water (Millipore, Bedford, MA, USA) were added to the sample. The tube was centrifuged at 4°C for 5 minutes at 3150 rotations per minute. After the water layer was transferred into centrifuge filter tubes, the filtered sample was centrifuged again at 4°C for 4 hours at 9100 rotations per minute. Next, it was dried with vacuum centrifugal dryer for 2 hours and stored at −80°C. Finally, it was added to 50 μL of Milli-Q water with internal standard solution and analyzed by CE-TOF-MS.

TABLE S1. Reproducibility of Measurements

	Correlation coefficient		Bland-Altman 95% CI	
Variables	**r**	***p***	**Bias**	**95% CI**
Intra-observer reproducibility				
Thickness				
Biceps brachii muscle	0.99	< 0.01	0.025 ± 0.081	−0.137 to 0.187
Rectus femoris muscle	0.99	< 0.01	−0.024 ± 0.100	−0.223 to 0.175
Cross-sectional area				
Biceps brachii muscle	0.99	< 0.01	−0.001 ± 0.046	−0.093 to 0.090
Rectus femoris muscle	0.98	< 0.01	−0.086 ± 0.045	−0.177 to 0.004
Inter-observer reproducibility				
Thickness				
Biceps brachii muscle	0.99	< 0.01	0.140 ± 0.105	−0.069 to 0.349
Rectus femoris muscle	0.99	< 0.01	−0.058 ± 0.125	−0.307 to 0.191
Cross-sectional area				
Biceps brachii muscle	0.99	< 0.01	−0.015 ± 0.055	−0.126 to 0.095
Rectus femoris muscle	0.98	< 0.01	−0.103 ± 0.054	−0.211 to 0.005

CI = confidence interval

Reproducibility was assessed for 36 patients at 72 measurements in rectus femoris muscle. The Pearson correlation coefficient and Bland-Altman plot were determined by using JMP statistical software version 13.1.0 (SAS Institute Inc., Cary, NC, USA).

TABLE S2. Amino Acid Level

		Percentage change from Day 1 to Day 3 or 5			
Variables	**Group**	**Day 3**	***p***	**Day 5**	***p***
Glycine	Control	12.3 (−1.8 to 39.7)	< 0.01	4.0 (−5.5 to 37.4)	< 0.01
	EMS	−23.4 (−54.5 to −0.6)		−37.3 (−40.8 to −16.1)	
Alanine	Control	6.5 (−26.7 to 71.3)	0.36	−3.6 (−37.1 to 70.3)	0.53
	EMS	−7.6 (−26.2 to 36.8)		0.8 (−38.1 to 22.2)	
Serine	Control	46.3 (14.8 to 85.0)	0.21	29.8 (−8.4 to 57.9)	0.86
	EMS	29.3 (−6.2 to 60.5)		22.3 (−6.8 to 57.4)	
Proline	Control	54.3 (−11.4 to 147.9)	0.04	37.9 (−27.6 to 95.1)	0.26
	EMS	2.6 (−43.4 to 48.3)		−11.0 (−28.6 to 25.4)	
Valine	Control	59.5 (30.9 to 98.0)	0.07	27.2 (14.7 to 68.0)	0.53
	EMS	31.5 (−21.5 to 65.7)		20.5 (−13.7 to 60.3)	
Threonine	Control	97.4 (2.4 to 206.7)	0.18	92.8 (−17.7 to 209.1)	0.31
	EMS	32.7 (−15.7 to 108.9)		26.4 (−9.8 to 95.2)	
Cysteine	Control	74.7 (7.1 to 143.8)	0.07	59.4 (−15.3 to 128.9)	0.70
	EMS	−12.7 (−26.4 to 29.9)		17.1 (−19.6 to 115.6)	
Isoleucine	Control	125.4 (55.6 to 235.1)	0.10	50.5 (5.0 to 340.2)	0.81
	EMS	65.4 (−16.3 to 147.9)		33.2 (1.9 to 174.8)	
Leucine	Control	61.8 (46.3 to 144.4)	0.14	16.9 (−3.5 to 152.6)	0.81
	EMS	59.8 (15.0 to 81.0)		8.1 (−12.1 to 66.9)	
Asparagine	Control	38.4 (7.8 to 62.5)	0.08	25.9 (−3.2 to 39.9)	0.77
	EMS	16.9 (−15.2 to 34.2)		11.8 (−13.4 to 44.0)	

Aspartic acid	Control	−0.1 (−18.6 to 27.4)	0.81	9.8 (−28.0 to 29.7)	0.31
	EMS	−5.2 (−24.7 to 47.8)		22.5 (−16.3 to 48.7)	
Glutamine	Control	19.3 (−7.6 to 29.2)	0.25	−4.7 (−28.2 to 48.7)	0.63
	EMS	4.1 (−34.5 to 35.1)		−3.1 (−32.8 to 37.1)	
Lysine	Control	129.6 (50.1 to 178.2)	0.10	108.4 (15.5 to 175.3)	0.20
	EMS	55.1 (−35.5 to 147.9)		39.9 (3.3 to 105.3)	
Glutamic acid	Control	−3.3 (−19.1 to 32.8)	0.58	7.4 (−16.9 to 27.2)	0.70
	EMS	−9.0 (−25.5 to 14.6)		−0.9 (−31.8 to 46.1)	
Methionine	Control	114.3 (33.0 to 176.3)	0.37	45.6 (−10.5 to 192.5)	0.92
	EMS	15.8 (−15.5 to 139.3)		49.7 (−24.7 to 127.6)	
Histidine	Control	12.4 (2.6 to 28.3)	0.87	6.2 (−33.6 to 14.3)	0.66
	EMS	14.0 (−18.1 to 56.0)		−5.0 (−28.4 to 21.9)	
Phenylalanine	Control	31.2 (5.0 to 44.2)	0.94	6.8 (−16.4 to 24.7)	0.63
	EMS	20.1 (1.2 to 84.1)		9.5 (−24.0 to 71.4)	
Arginine	Control	73.6 (29.8 to 242.8)	0.23	61.4 (10.4 to 191.4)	0.53
	EMS	63.8 (−45.7 to 147.8)		41.6 (6.8 to 144.6)	
Tyrosine	Control	48.6 (13.0 to 100.7)	0.18	31.7 (−8.9 to 62.6)	0.63
	EMS	6.5 (−14.0 to 61.5)		26.3 (−19.9 to 57.6)	
Tryptophan	Control	22.3 (−2.8 to 108.2)	0.38	16.9 (−18.8 to 82.0)	0.85
	EMS	14.4 (−18.9 to 59.8)		15.4 (−18.7 to 74.7)	
BCAA	Control	71.5 (38.8 to 116.9)	0.04	21.6 (9.0 to 106.1)	0.59
	EMS	40.5 (−7.4 to 75.3)		18.6 (−12.8 to 67.2)	

EMS = electrical muscle stimulation, BCAA = branched-chain amino acid

Amino acid level was assessed in EMS and control (14 and 17 on day 3, 12 and 15 on day 5). The Mann-Whitney test was used for the comparison.