Neuromuscular electrical stimulation prevents muscle wasting in critically ill, comatose patients

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Abstract

Fully-sedated patients, being treated in the ICU, experience substantial skeletal muscle loss. Consequently, survival rate is reduced and full recovery after awakening is compromised. Neuromuscular electrical stimulation (NMES) represents an effective method to stimulate muscle protein synthesis and alleviate muscle disuse atrophy in healthy subjects. We investigated the efficacy of twice-daily NMES to alleviate muscle loss in six fully-sedated ICU patients admitted for acute critical illness (n=3 males, n=3 females; age 63±6 y; APACHE II disease severity-score: 29±2). One leg was subjected to twice-daily NMES of the quadriceps muscle for a period of 7±1 d while the other leg acted as non-stimulated control (CON). Directly before the first and on the morning after the final NMES session, quadriceps muscle biopsies were collected from both legs to assess muscle fiber-type specific cross-sectional area (CSA). Furthermore, phosphorylation status of key proteins involved in the regulation of muscle protein synthesis was assessed, and mRNA expression of selected genes was measured. In the CON leg, type I and type II muscle fiber CSA decreased by 16±9 and 24±7%, respectively ($P<0.05$). No muscle atrophy was observed in the stimulated leg. NMES increased mTOR phosphorylation by 19% when compared to baseline ($P<0.05$), with no changes in the CON leg. Furthermore, mRNA expression of key genes involved in muscle protein breakdown either declined (FOXO1; $P<0.05$) or remained unchanged (MAFBx and MuRF1), with no differences between legs. In conclusion, NMES represents an effective and feasible interventional strategy to prevent skeletal muscle atrophy in critically ill, comatose patients.

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Introduction

Critically ill patients suffer from extensive muscle wasting, which occurs rapidly at the onset of an ICU stay [1-3]. Aside from an increased risk of mortality [4, 5], consequences to this muscle loss include muscle weakness, prolonged mechanical ventilation, fatigue, decreases in muscle strength, impaired glucose homeostasis and delayed recovery and rehabilitation [6-9]. Muscle atrophy in ICU patients exceeds that seen in normal hospitalized or bedridden persons [10, 11]. Moreover, ICU patients who are mechanically ventilated and deeply sedated are thought to be even more susceptible to muscle wasting and subsequent negative health consequences due to a complete lack of muscle contraction. Despite this, no data are currently available concerning muscle fiber atrophy in this specific ICU patient subpopulation.

Early ambulation has been proven a successful rehabilitation strategy in non-sedated ICU patients in terms of improving functional outcomes and overall prognosis [12]. However, in fully-sedated patients, early ambulation is not feasible and, as such, alternative strategies should be defined to alleviate muscle wasting. Neuromuscular electrical stimulation (NMES) is an effective means to invoke involuntary muscle contractions. Previously, NMES has been shown to attenuate the loss of muscle mass and strength experienced by non-sedated ICU patients [13] and healthy individuals subjected to limb immobilization [14]. However, the potential for NMES to rescue muscle mass in fully-sedated, comatose ICU patients has not been investigated. In the present study, we investigated our hypothesis that daily NMES attenuates skeletal muscle fiber atrophy in fully-sedated, comatose ICU patients. Fully-sedated ICU patients, expected to be sedated for a minimum of three days, were included in the present study. NMES was performed twice-daily on the quadriceps of one leg, whereas the other leg served as a sham-treated control. Prior to and immediately after the intervention, plasma samples were taken to assess any systemic changes in amino acid availability during the experiment, and muscle biopsies were taken from both legs to assess muscle fiber atrophy and myocellular characteristics. Additionally, RT-PCR and Western blotting were performed on collected muscle tissue samples to assess the potential impact of NMES on basal mRNA and protein expression levels of key genes involved in the regulation of muscle mass maintenance.

Methods

Patients

All patients admitted to the Intensive Care Unit (ICU) of Jessa Hospital, Hasselt, Belgium between March 2012 and July 2013 were assessed for eligibility for the present study (see **eFigure1**). Patients admitted to the ICU were screened by the nursing staff, and were excluded if one or more of the following exclusion criteria were met: <18 or >80 y old, not expected to undergo complete sedation, suffering from spinal cord injury, recent arterial surgery on the legs, local wounds that prohibit the application of neuromuscular electrical stimulation (NMES), chronic use of corticosteroids, intake of certain antithrombotic drugs, or the presence of an implantable cardioverter-defibrillator (ICD) and/or pacemaker. Secondly, the expected sedation time was estimated by the responsible physician and patients were excluded if this was <3 days. All patients who were excluded based on an expected short sedation time were re-evaluated after 24 h, and included if the revised expected sedation time was >3 d. Participants were accepted into the study after written informed consent was obtained from their legal representatives. The study was approved by the Medical Ethical Committee of the Jessa Hospital in accordance with the Declaration of Helsinki.

Study design

An overview of the experimental protocol is depicted in **eFigure 2**. Patients were included in the study directly after informed consent was obtained from their legal representatives, which was generally given within 2.5 d after admission to the ICU (depicted in column 'Time to inclusion' in **Table 1**). After this, patient's legs were randomly assigned as either the control (CON) or stimulated (NMES) leg, counterbalanced for left and right legs. Randomization was performed by an independent investigator, and treatment allocation was performed by using sequentially labeled envelopes which were opened after inclusion of subjects. Baseline measurements were then taken, which consisted of assessment of leg circumference (measured at different locations on the upper leg), obtaining an arterial blood sample, and obtaining a muscle biopsy from both legs. After the pre-measurements, NMES was performed twice-daily on one leg (NMES) whereas the other leg served as a control (CON). Post-measurements were performed on the final day of sedation, with a minimum study duration of 3 days and a maximum of 10 days. The study duration for each patient is depicted in **Table 1**. Post-measurements were performed prior to subjects being awake. Standard medical care was not altered, and passive mobilization was performed on both legs according to standard care procedures.

Data collection

At baseline, data on demographic and clinical characteristics of the patients were obtained, including information necessary to determine the severity of illness. These data were scored according to the Acute Physiology and Chronic Health Evaluation II (APACHE II) system with higher values indicating more severe illness and more therapeutic interventions, respectively [15].

Arterial blood samples were collected from the catheter already placed in the *arteria radialis*. Blood (10 mL) was collected into EDTA-containing tubes and immediately centrifuged at 1,000*g* for 10 min at 4°C. Aliquots of plasma were directly snap-frozen in liquid nitrogen and stored at -80°C until further analysis. Processing and storage of the samples was done by UBiLim (Universitaire Biobank Limburg, Hasselt, Belgium). Plasma amino acid concentrations were measured using ultra-performance liquid chromatography tandem mass spectrometry as described previously [16], and results are displayed in **eTable2**.

In addition, during the pre- and post-measurements, a muscle biopsy sample was collected from each leg. After injection of local anesthesia, percutaneous needle biopsy samples were collected from *m. vastus lateralis*, approximately 15 cm above the patella using the Bergström technique [17].

Neuromuscular electrical stimulation

Neuromuscular electrical stimulation sessions were performed both in the morning (11:00 AM) and afternoon (4.30 PM). Four self-adhesive electrodes (2 mm thick, 50 x 50 mm) were placed on the distal part at the muscle belly of the *m. rectus femoris* and the *m. vastus lateralis*, and at the inguinal area of both muscles. The electrodes were connected to an Enraf-Nonius TensMed S84 stimulation device (Enraf-Nonius, Rotterdam, the Netherlands), discharging biphasic symmetric rectangular-wave pulses. The position of the electrodes was re-marked daily with a semi-permanent marker to maintain the same location of stimulation for each session. The NMES protocol was composed of a warm-up phase (5 min, 5 Hz, 250 μs), a stimulation period (30 min, 100 Hz, 400 μs, 5 s on (0.75 s rise, 3.5 s contraction, 0.75 s fall) and 10 s off), and a cooling-down phase (5 min, 5 Hz, 250 μs). The intensity of the stimulation was set to a level at which full contractions of *m. quadriceps femoris* were both visible and palpable. The intensity was raised approximately every 3 min when a full muscle contraction was no longer achieved with the current intensity. This protocol was based on our previous work showing increased rates of muscle protein synthesis after a single bout of NMES [18], and applied on the immobilized leg of healthy young adults [14]. During the NMES sessions, four electrodes and compatible cables were also applied to the control leg to standardize all procedures (representing a sham treatment).

Dietary intake

When patients were hemodynamically stable, enteral feeding was started according to routine guidelines of the ICU at Jessa Hospital as early as possible. Patients were fed Nutrison Multi Fibre (containing 420 kJ, 16 en% protein, 49 en% carbohydrates, and 35 en% fat per 100 mL). Generally, patients were fed maximally 80 mL per hour with short intervals during which nutritional supply was paused. Gastric emptying was determined by the nursing staff, and food administration was altered accordingly. Nutritional support was not modulated and was applied according to the standard medical care in this ICU.

Muscle analyses

Muscle samples were freed from any visible non-muscle tissue and separated into different sections; the first part (~30 mg) was imbedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, the Netherlands), frozen on liquid nitrogen cooled isopentane and used to determine muscle fiber-type specific cross-sectional area (CSA) and satellite cell content as done previously.[19] The second part (~15 mg) was snap frozen in liquid nitrogen and used for real time-PCR analysis to determine mRNA expression of selected genes as described before,[14, 20] and compared with mRNA expression of *n*=6 healthy, age- and gender-matched controls. The third part (~40 mg) was snap frozen in liquid nitrogen for Western Blot analysis to determine the total content and phosphorylation status of several key proteins of interest as described previously [18]. All muscle analyses were performed by an investigator blinded to treatment. A detailed overview of the muscle analyses is presented in the supplemental information.

Statistics

Based on data from previous studies in healthy subjects in our laboratory [14, 21], we calculated that 8 patients would be required to detect a 8% difference in muscle fiber CSA between CON and NMES over 7 days (using an α level of 0.05 and a β level of 0.10). All data presented are expressed as means±SEM. Baseline differences between legs were compared with a paired samples t-test. Pre- and post-intervention data were analyzed using repeated measures analysis of variance (ANOVA) with time (pre vs post) and treatment (CON vs NMES) as factors. Fiber type (type I vs type II) was added as a third within-subjects factor when analyzing all muscle fiber characteristics. In case of significant interaction (*time x treatment*), paired-samples t-tests were performed to determine time effects within the CON and NMES leg separately. Alternatively, when a *time x treatment* effect was observed for muscle fiber characteristics, a 2-way ANOVA was performed for the CON and NMES leg separately, with time and treatment as factors. For the mRNA analyses, differences between patients and healthy controls were tested by means of an independent samples t-test between the mean value of the CON and NMES leg in patients and the values observed in healthy controls. Statistical analyses were performed using the SPSS version 20.0 software package (SPSS Inc., Chicago, IL, USA), with $P<0.05$ as the value for statistical significance.

Results

Patients

Between March 2012 and July 2013, 9 patients were included in the present study. Two patients awoke after <3 study days and one patient died. Therefore, the presented results represent data collected from 6 patients. Clinical characteristics of the included patients are listed in **Table 1**. Energy intake per day averaged 5.31±0.56 MJ, with a mean protein intake of 0.56±0.06 g·kg body weight^{-1}·day^{-1}.

Neuromuscular electrical stimulation

Within 5 min of the start of the actual 30 min stimulation period, a full muscle contraction was achieved. The intensity of the NMES intervention for subjects averaged 29.9 mA during the first session and was progressively increased to 32.3 mA in the final session.

Muscle fiber characteristics

Figure 1 illustrates the delta change in muscle fiber cross-sectional area (CSA) in both the NMES and CON legs throughout the study. **Table 2** details skeletal muscle fiber type specific characteristics at baseline and following 7±1 d of full sedation in both legs. In the CON leg, a significant decline of 16±9% and 24±7% was observed in type I and II muscle fiber CSA, respectively (time effect; $P<0.05$). In contrast, the NMES leg showed no atrophy in either type I or II muscle fibers (*time x treatment* interaction effect; $P<0.05$). Muscle fiber type distribution showed an overall significant *time x treatment* interaction effect (see **Table 2**; $P<0.05$), with a shift from type I towards type II fibers in the CON leg, and a shift towards more type I fibers in the NMES leg. At baseline, satellite cell content was greater in type I vs type II muscle fibers (expressed per muscle fiber, per millimeter squared, and as a percentage of total myonuclei). No differences in muscle fiber type specific myonuclear content, myonuclear domain size or satellite cell content were observed between legs or over time.

mRNA expression

Figure 2 displays the relative muscle mRNA expression of key genes involved in the regulation of muscle protein synthesis and breakdown in the CON and NMES leg before and after the intervention, as well as for a group of healthy, age- and gender-matched controls. At baseline, mRNA expression did not differ between NMES and CON legs. However, MAFBx, MuRF1, FOXO1, mTOR and P70S6K were all more highly expressed in the patients compared with healthy controls ($P<0.01$). There was a significant time effect ($P<0.05$) such that FOXO1 and P70S6K expression decreased during the period of sedation, with no differences between legs. Expression levels for all other genes did not reveal any interaction or time effects. The mRNA expression of additional genes involved in the regulation of myogenesis, oxidative metabolism, mechano-sensing and cellular amino acid transport are presented in **eFigure 3** (supplemental material).

Signaling proteins

The skeletal muscle content and phosphorylation status of key proteins involved in the regulation of muscle protein synthesis are displayed in **Figure 3**. Neither total protein content, nor phosphorylation status of Akt was affected by time or the intervention (both $P>0.05$). Whereas muscle mTOR content was unaffected by time or treatment, a significant *time x treatment* interaction effect ($P<0.05$) was found for the phosphorylation status of mTOR. mTOR phosphorylation increased by as much as 19±5% in the NMES leg ($P<0.05$), with no changes in the CON leg ($P>0.05$). Muscle P70S6K total protein content decreased following the intervention in both legs (time effect, $P<0.05$), without changes in phosphorylation status ($P>0.05$).

Discussion

In the present study, we demonstrate for the first time that fully-sedated patients experience substantial type I and type II muscle fiber atrophy during a ~7 d stay in the ICU. Daily application of neuromuscular electrical stimulation (NMES) effectively prevents skeletal muscle fiber atrophy, offering an effective and feasible interventional strategy to alleviate muscle wasting in comatose ICU patients.

General admission to the ICU has been shown to cause substantial muscle wasting [22] with a decline in type I and type II muscle fiber cross-sectional area of 3% and 4% per day, respectively [2]. In keeping with this, we show a 2.8% and 4.4% decline in muscle fiber size in type I and II muscle fibers, respectively, in fully-sedated patients (i.e. no possibility of voluntary muscle contraction) during on average 7 days in the ICU (**Figure 1**). By way of comparison, muscle atrophy brought about by disuse only in healthy humans (i.e. limb immobilization) leads to a 0.5% and 0.9% per day decline in type I and II muscle fiber cross-sectional area (CSA), respectively [21]. This implies that the mechanisms responsible for muscle wasting in the ICU are not simply attributed to disuse. One possible contributing factor could be inadequate nutritional status. Sufficient dietary protein is considered a key factor in the maintenance of muscle mass [23-25], and previous research has shown that sufficient protein intake is associated with reduced mortality rates in critically ill patients [26, 27]. In the current study, patients received 0.56±0.06 g protein·kg body weight^{-1}·day^{-1}, which is below the current guidelines of 1.3-2.0 g protein·kg body weight^{-1}·day^{-1} recommended during critical illness [28, 29], and has likely contributed to the extensive level of muscle wasting. In support, plasma amino acid concentrations in our patients declined throughout the sedated state (**eTable 2**). In agreement, previous work has reported declines in circulating amino acid concentrations during critical illness [30]. Such a decline in circulating amino acid concentrations likely reduces amino acid uptake in muscle [31] and, as such, could modulate the efficacy of NMES as a means to stimulate muscle protein synthesis rates.

From a mechanistic viewpoint, disuse atrophy has been primarily attributed to declines in muscle protein synthesis rates [20, 32-34]. However, it has been suggested that in various conditions associated with rapid muscle wasting a multitude of other factors (e.g. increased inflammation, higher metabolic stress responses etc.) may stimulate muscle proteolysis, driving much of the muscle loss [35]. In line with this, we see evidence of the severely metabolically compromised condition of our patients as demonstrated by numerous clinical chemistry indictors obtained throughout the study (e.g. high white blood cell counts and C-reactive protein (CRP) concentrations; **eTable 1**). In keeping with this, molecular markers that have been used as a proxy for changes in muscle protein breakdown rate were elevated upon admission to the ICU, when compared with a group of healthy subjects (i.e. MAFBx, MuRF1 and FOXO1; **Figure 2**). The subsequent decline in the expression levels of these genes suggest a decline in muscle protein turnover during hospital stay but expression levels remained elevated when compared to healthy controls. This is not unexpected given the metabolic stress response upon ICU admission [36]. In contrast to previous work investigating the impact of NMES on an immobilized leg [14], we observed no significant differences in the expression levels of various genes between the stimulated and unstimulated leg in this comatose ICU setting. The absence of such differences may be attributed to various factors, but underline our understanding that changes in the expression and phosphorylation levels of various genes being used as a proxy for changes in muscle protein breakdown and synthesis do not necessarily represent changes in muscle protein breakdown and synthesis rates and do not necessarily translate to a net increase or decrease in muscle mass [37]. Taken together, the present data highlight the need for immediate and effective intervention at the onset of ICU admission to stimulate muscle protein synthesis and inhibit proteolysis, thereby preventing or attenuating extensive muscle wasting. An interesting observation in the stimulated leg was that NMES reversed the decline in phosphorylation status of mTOR (**Figure 3D**), which seems to be in line with previous work showing that NMES increases muscle protein synthesis rates [18].

Daily application of NMES has been shown to prevent muscle atrophy in healthy subjects during a week of leg immobilization [14]. Moreover, clinical trials have demonstrated beneficial effects of NMES on muscle function in various bed-rested populations, including patients suffering from COPD [38, 39] and sepsis [40, 41]. The current study demonstrates, for the first time, that NMES is capable of preventing muscle wasting in fully-sedated patients during 7 days in the ICU (with a +7±12% change in mixed muscle fiber CSA in the stimulated leg compared with a -21±8% decline in mixed muscle fiber CSA in the control leg; **Figure 1)**. The prevention of muscle atrophy in these individuals can have profound clinical implications. For instance, maintaining muscle mass during critical illness has been shown to reduce mortality rates [4, 5]. Additionally, since muscle mass is vital for functional capacity [42], metabolic homeostasis [9], and immune function [43], maintaining muscle mass during an ICU stay is essential to allow proper recovery during rehabilitation. As such, preventing muscle wasting is imperative for promoting quality of life after hospital discharge and reducing the likelihood of re-hospitalization. NMES in fully-sedated patients can be easily applied by nursing staff, is relatively cheap and does not seem to cause any adverse effects on vital parameters during or after the sessions [44]. Some difficulties applying NMES in ICU patients have been reported previously and are likely due to increased skin/soft tissue impedance and/or edema [13]. Despite experiencing similar problems in the present study, all NMES sessions could be successfully performed without any adverse effects. Taken together, our data demonstrate that NMES is practical and feasible as a countermeasure for muscle wasting in clinically compromised ICU patients. Future studies should address whether these findings would translate into longer-term benefits such as increased survival rates, reduced hospitalization length of stay and/or improved rehabilitation outcomes.

Conclusion

NMES represents an effective and feasible interventional strategy to prevent skeletal muscle wasting in critically ill, comatose patients. ~~NMES may be applied effectively to offset negative consequences of muscle wasting and, as such, may increase survival and improve subsequent rehabilitation in these patients.~~

Clinical Perspectives

Fully-sedated patients experience substantial skeletal muscle loss that reduces survival rate and compromises full recovery. We investigated the efficacy of twice-daily neuromuscular electrical stimulation (NMES) to attenuate skeletal muscle loss in fully-sedated ICU patients admitted for acute critical illness. The non-stimulated leg showed substantial type I and type II muscle fiber atrophy (a 16±9 and 24±7% decline in muscle fiber cross sectional area, respectively; *P*<0.05). In contrast, no atrophy was observed in the muscle fibers collected from the stimulated leg. Both mRNA and protein expression of key proteins involved in muscle protein metabolism were assessed to understand the molecular mechanisms involved. In conclusion, NMES represents an effective and feasible interventional strategy to prevent skeletal muscle atrophy in critically ill, comatose patients.

Author contributions

M.L. Dirks had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data. None of the authors disclose any conflicts of interest. *Study concept and design:* M.L. Dirks, D. Hansen, A.van Assche, P. Dendale, and L.J.C. van Loon. *Acquisition of data:* M.L. Dirks and D. Hansen. *Analysis and interpretation of the data:* M.L. Dirks, D. Hansen, P. Dendale and L.J.C. van Loon. *Drafting of the manuscript:* M.L. Dirks. *Critical revision of the manuscript for important intellectual content:* D. Hansen, A. van Assche, P. Dendale and L.J.C. van Loon. *Study supervision:* A. van Assche, P. Dendale and L.J.C. van Loon.

Additional contributions

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Figure legends

Figure 1: Changes in muscle fiber cross sectional area (CSA) in the control (CON) and stimulated (NMES) leg of sedated patients, after 7±1 days of twice-daily NMES. A significant interaction effect (*P*<0.05) was observed, and a time effect in the CON leg (*P*<0.05). * Significantly ~~change~~ different from zero (*P*<0.05).

Figure 2: Skeletal muscle mRNA expression of genes of interest. Abbreviations: FOXO1, Forkhead box protein O1; MAFbx, Muscle Atrophy F-box; MuRF1, Muscle RING-finger protein-1; mTOR, mammalian target of rapamycin; P70S6K, P70S6 kinase. * Significantly different from patients at baseline (*P*<0.05). # Significantly different from pre-value (*P*<0.05).

Figure 3: Skeletal muscle protein expression of Akt, mTOR and P70S6K in the control (CON) and stimulated (NMES) leg, before (white bars) and after (black bars) 7±1 days of twice-daily NMES. Left graphs: total protein expression, right graphs: phosphorylated/total expression. Abbreviations: mTOR, mammalian target of rapamycin; P70S6K, P70S6 kinase. * Significantly different from pre-intervention values (*P*<0.05).