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PII: S0883-9441(20)30779-6

DOI: https://doi.org/10.1016/j.jcrc.2020.11.018

Reference: YJCRC 53719

To appear in: *Journal of Critical Care*

Please cite this article as: J. Segers, I. Vanhorebeek, D. Langer, et al., Early neuromuscular electrical stimulation reduces the loss of muscle mass in critically ill patients – A within subject randomized controlled trial, *Journal of Critical Care* (2019), https://doi.org/10.1016/j.jcrc.2020.11.018

Early neuromuscular electrical stimulation reduces the loss of muscle mass in critically ill patients – A within subject randomized controlled trial

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Abstract (word count: 200)

Purpose: To investigate the effect of Neuromuscular Electrical Stimulation (NMES) on muscle thickness, strength and morphological and molecular markers of the quadriceps.

Materials and Methods: Adult critically ill patients with an expected prolonged stay received unilateral quadriceps NMES sessions for 7 consecutive days. Before and after the

intervention period, quadriceps thickness was measured with ultrasound. After the intervention period, strength was assessed in cooperative patients and muscle biopsies were taken. Multivariable regression was performed to identify factors affecting muscle thickness loss.

Results: Muscle thickness decreased less in the stimulated leg (-6±16% versus -12±15%, p=0.014, n=47). Strength was comparable. Opioid administration, minimal muscle contraction and more muscle thickness loss in the non-stimulated muscle were independently associated with better muscle thickness preservation. Stimulated muscles showed a shift towards larger myofibers and higher MyHC-I gene expression. NMES did not affect gene expression of other myofibrillary proteins, MuRF-1 or atrogin-1. Signs of myofiber necrosis and inflammation were comparable for both muscles.

Conclusions: NMES attenuated the loss of muscle mass, but not of strength, in critically ill patients. Preservation of muscle mass was more likely in patients receiving opioids, patients with a minimal muscle contraction during NMES and patients more prone to lose muscle mass.

Trial Registration: clinicaltrials.gov: NCT02133300.

Key words: critical illness, physical therapy, muscle weakness, early mobilization, electric stimulation

COI: None of the authors have declared any conflict of interest related to the subject of this study. The authors acknowledge DJO Global (Herentals, Belgium) for providing the neuromuscular stimulation equipment used in this study.

INTRODUCTION

Critically ill patients admitted to the intensive care unit (ICU) often suffer from a dramatic loss of muscle mass and strength, known as intensive care unit-acquired weakness (ICUAW) [1]. On average, patients lose about 11% of their muscle mass in the first week after ICU admission and this loss is more pronounced in patients with multiple organ failure [2, 3]. It appears attractive to counteract this muscle mass loss before the patient is even able to cooperate [4]. Therefore, the effects of neuromuscular electrical stimulation (NMES) have been investigated in acutely critically ill patients [5-13]. Both Gerovasili [5] and Meesen [10] concluded that muscle mass was better preserved in groups receiving NMES as compared to control groups. However, other studies [7-9, 11] could not confirm these results. These studies are difficult to compare, mostly due to methodological heterogeneity in the assessment of muscle mass. Various outcome measures were used, including circumference measures, CT-scan and ultrasound. Other studies focused on the effect of NMES on muscle strength [6, 9, 11, 13, 14]. Routsi et al [6] and Rodriguez et al [9] showed that NMES resulted in a significantly better preserved muscle strength. Fischer et al [11] showed that muscle strength was higher in the NMES group at ICU discharge, but not at hospital discharge. Kho et al [13] and Grunow et al [14] did not find any difference in strength between the NMES and sham treated group. A few studies investigated the effect of NMES in muscle biopsies. Dirks et al showed that muscle fibre cross-sectional area (CSA) did not decrease in the stimulated muscles, compared to ~20% decrease in fibre CSA in the unstimulated muscles [15]. Wollersheim et al confirmed this preservation of fibre CSA and showed a higher gene expression for myosin heavy chains in the NMES treated muscles [16]. Weber-Carstens et al showed attenuation of atrophy in type II fibres only [17]. In addition, Strasser et al observed signs of more synthesis and less degradation of proteins in the muscles treated with NMES

[18]. None of the aforementioned studies investigated the effects of NMES on muscle thickness, muscle strength and morphological and molecular markers of muscle atrophy simultaneously.

The primary aim of this study was to investigate the effect of 7 days of unilateral NMES on quadriceps muscle thickness. The combined thickness of the rectus femoris and intermedius were used for this analysis. Secondary outcomes were muscle strength and morphological and molecular markers of muscle atrophy. In addition, factors associated with changes in muscle thickness were explored.

METHOD

Study design

The effect of unilateral NMES in critically ill patients was assessed in a randomized controlled design. Patients were included between day 2 and day 4 after their admission to the ICU. The attending intensivist judged whether the patient was expected to stay at least 7 more days in the ICU before the patient was considered for inclusion in the study.

Randomisation

Opaque sealed envelopes were used to randomize which quadriceps muscle was selected for stimulation. Five envelopes contained a paper with 'dominant' written on it, another 5 had a paper with 'non-dominant' written on it. Before the start of inclusion, these evelopes were shuffled by a secretary, unrelated to the study. After shuffling, the envelopes were placed on top of each other. After inclusion of a patient, the top envelope was opened to identify which leg was selected for stimulation. After inclusion of 10 patients, the process was

repeated with 10 new envelopes. This procedure was repeated until the required number of patients was included.

Patient population

All patients were adults (≥18 years) admitted to the medical or surgical ICU of the University Hospitals Leuven. Written informed consent was obtained by J.S. from the patients or next of kin for uncooperative patients. Exclusion criteria were: transfer from another ICU or other hospital, re-admission to the ICU, prognosticated lethal outcome, presence of a pace-maker, pregnancy, pre-existing neurological or neuromuscular disease, intracranial pressure >20cmH_2O, abnormal musculoskeletal and skin conditions that could interfere with the stimulation (e.g. femur fracture, burn injury on the thigh, skin disease). During a period of administration of neuromuscular blocking agents (NMBAs), the NMES session did not take place. Patients receiving NMBAs during the first 4 days of ICU admission were excluded from the study. The study was approved by the Medical Ethical Committee of the University Hospitals Leuven, Belgium (ML10058), and registered on clinicaltrials.gov (NCT02133300).

Intervention

All patients received physiotherapy and early mobilization in the accordance with a standardized local 'Start-to-Move as soon as possible' protocol [4]. Additionally, the patients received a 1-hour NMES session (including 5 minutes warming up and 5 minutes cooling down) daily for 7 consecutive days, even is the patient was discharged early, stimulation continued on the ward (applied by J.S., not blinded for intervention). During the intervention, the patient was in a supine position with the head end of the bed elevated to 30° and the leg extended with a solid support roll under the knee. To ensure the best possible contraction, 3 motor points were localized using a pen electrode (vastus medialis,

rectus femoris and vastus lateralis) as described by Gobbo et al [19]. A 5x5cm electrode was placed on each of the 3 motor points. Two 10x5cm electrodes were attached proximally on the upper thigh. During the warming-up, the frequency was 4Hz and the intensity was increased until a clear muscle response was visible. The settings on the NMES device (Chattanooga Physio, DJO Global, Herentals, Belgium) during the 8.5s contraction phase were 45Hz, 350µs, 1.5s ramp up and 1s ramp down. The rest phase consisted of 4Hz contractions during 12s. In both the stimulation and rest phase, the highest tolerable intensity was applied in order to obtain the best possible muscle response without discomfort. Conscious patients informed the investigator when the stimulation became painful. In uncooperative patients, facial expression was used to estimate pain responses in order to keep the intensity below the pain threshold. In the cooling down phase, the settings were equal to the warming-up. The maximal output of the stimulator was 120mA. The quality of obtained muscle contractions was evaluated using a 5-point scale [20]. A 'good' contraction was defined as a clearly visible and palpable contraction (scores of 4-5 out of 5). Just visible and/or palpable contractions (score of 3) or less were rated as 'minimal' contractions. The non-stimulated quadriceps muscle did not receive a sham treatment.

Outcome measures

Primary outcome: The combined muscle thickness of the rectus femoris and intermedius was assessed both before the start of the first NMES session and after the intervention period (i.e. either on the day of the last stimulation session or on the day after) using ultrasonography (Vivid 7, GE Healthcare, Herentals, Belgium) [2, 3, 21]. A blinded investigator (N.C.) measured muscle thickness of both quadriceps muscles. At least three

images per side were recorded to obtain three measurements of thickness that differed by less than 10%. The average of the three measurements was used for analysis.

Secondary outcomes:

Quadriceps muscle strength was assessed only in patients who were able to respond adequately to simple commands (assessed by their ability to respond to 5 standardized orders as described previously by De Jonghe et al [22]), namely: 1. open and close your eyes; 2. look at me; 3. open your mouth and put out your tongue; 4. nod your head; 5. raise your eyebrows when I have counted up to 5. Two blinded investigators (B.C. or I.D.) performed assessments by determining the Medical Research Council (MRC) score [23], either on the day of the last stimulation session or on the day after. For the MRC-score, the strength of the muscle is graded with a number of 0-5. The strength for value 0= no contraction; 1= visible and/or palpable contraction without limb movement; 2= contraction and movement in the horizontal plane; 3= movement against gravity; 4= submaximal strength; 5= maximal strength. When the MRC score was 3 or higher, quadriceps strength was also assessed more objectively with handheld dynamometry (HHD) [24]. At least 3 measurements that differed by less than 10% were performed on both quadriceps muscles [24]. The highest value of these 3 measurements was used for analysis.

Percutaneous muscle biopsies were taken bilaterally from the vastus lateralis of the quadriceps muscle at the level of the mid-thigh after the intervention period and after all measurements of ultrasonography and strength were performed, either on the day of the last stimulation session or on the day after. Biopsy collection and procedures for histological (hematoxylin-eosin and myofiber type staining) and molecular analyses (gene expression for myofibrillary proteins (MyHC-I, MyHC-II, actin), E3 ligases (MuRF1 and atrogin-1) and p62,

and protein expression of p62 and LC3 as markers of autophagy) evaluating muscle atrophy and quality were performed by blinded investigators (I.D., S.D., L.P., W.W.) as previously described [25] and are also outlined in the online supplement.

Data analysis

Statistical analyses were performed with the statistical package for the social sciences (SPSS 24) and JMP14. To obtain a power of 80% with an alpha error of 0.05, 45 patients were needed to be included. To anticipate a 10% drop-out, inclusion of 50 patients was set as the target sample size.

For the initial power calculation we referred to data of Gerovasili et al [5] and estimated that 90 patients had to be included to obtain a power of 80% with an alpha error of 0.05. The data from the study of Gerovasili, however, were derived from a between group analysis, whereas our study was a within subject comparison of the stimulated and non-stimulated muscle. After including 23 patients, a recalculation was performed based on the initial data to have a more accurate estimate of the number of patients that were needed to be included. This was computed for t-test analysis of the difference between two dependent means for matched pairs. As patients served as their own controls, paired analyses were used for continuous data (paired samples t-test or related-samples Wilcoxon signed rank test for normally or not-normally distributed data, respectively). To control for potentially confounding effects of baseline differences in thickness between muscles, the comparisons were also performed taking into account differences between baseline muscle thickness. This was performed using an analysis of covariance (ANCOVA) with baseline thickness as

covariate. Comparison of qualitative scores of the biopsies was performed with Chi square (Fisher exact) test.

An additional analysis was performed to identify factors that were potentially related to the effect of the stimulation on muscle thickness. This was performed using a multivariable linear regression analysis (enter method). Sepsis, edema and administration of vasopressors were *a priori* included in the model as previous research indicated that these variables are associated with the quality of muscle contractions obtained in response to NMES [20]. Additionally, a correlation analysis was performed based on our own data to identify other variables related to the percentage of change from baseline in muscle thickness of the stimulated muscle versus the change in the non-stimulated muscle. All variables that were correlated with the change in muscle thickness with $p<0.15$ were added to the model. A variance inflation factor (VIF) of 5 or higher was used to exclude variables that showed collinearity.

RESULTS

Patient characteristics and quality of NMES

Between May 2014 and September 2016, 1710 critically ill patients were screened. The consolidated standards of reporting trials (CONSORT) flow chart is depicted in figure 1. Fifty patients were included in the study. Three patients did not complete the study period as consent was withdrawn. Patient characteristics are shown in table 1. The online supplement shows the patient characteristics of the subgroups (table S1 shows the data for the patients that performed a strength test and the data for the patients that also had a biopsy analysis. Eight patient were both in the strength subgroups as well as in the biopsy subgroup).

The total number of delivered and analyzed sessions was 307 (93% of the planned sessions). All patients received at least five stimulation sessions. Eight patients (17%) left the ICU before the end of the study and received the remaining one or two sessions on the ward. Twenty-four patients received NMES of the quadriceps on the dominant side and 23 patients on the non-dominant side. Baseline quadriceps thicknesses of the dominant and non-dominant side were not different (dominant: 2.09 ± 0.70cm; non-dominant: 2.10 ± 0.74cm; p=0.840). In 32 patients (68%) a good muscle contraction (median type of contraction: 4, 95% confidence interval (CI): 4-5) was elicited. In 15 patients a minimal muscle contraction (median type of contraction: 3, 95%CI: 2-4) was observed.

Primary outcome: Muscle thickness

No difference in quadriceps thickness was present at the start of the study between the intervention and control muscle (2.07 ± 0.70 vs 2.12 ± 0.74cm, respectively; p=0.366). Muscle thickness was significantly decreased in both muscles by the end of the intervention period (intervention group: -0.14±0.31cm [-6±16%], p=0.003; control group: -0.27±0.37cm [-12±15%], p<0.001, effect size 0.33 (based on difference of 0,13 cm and group SD 0,34 cm). Average decline in the intervention group was significantly smaller than in the control group by 0.13cm (95%CI: 0.04 to 0.22cm, p=0.007) or 6% (95%CI: 1 to 11%, p=0.014) (Figure 2).

Secondary outcome: Muscle strength

Post-intervention assessments of muscle strength were performed in a subsample of 18 cooperative patients (38%). A median score of 4 (interquartile range (IQR): 4-5) on the MRC scale was observed in both muscles (p=0.317). Handheld dynamometry was performed in 15 patients (3 patients had a score lower than 3 on the MRC scale and did therefore not perform the HHD test). No differences were observed between the 2 muscles (stimulated

muscle: 106 ± 72 N (32 ± 22% predicted); control muscle: 101 ± 62 N (31 ± 19% predicted); p=0.48, effect size 0.06). In the subgroup of patients who were unable to perform a strength test, a difference in decline in muscle thickness between stimulated and non-stimulated muscle was observed (0.15 ± 0.36cm (95%CI: 0.01 to 0.38) (p= 0.034) (6 ± 16%)). In the subgroup of patients who performed a strength test, no difference in decline between stimulated and non-stimulated muscle was observed (0.10 ± 0.25cm (95%CI: -0.02 to 0.23) (p= 0.100) (5 ± 17%)).

Secondary outcome: Morphological and molecular markers of atrophy and muscle quality

Sixteen of the 47 patients were included for biopsy analysis. The other patients refused the collection of a biopsy or had coagulation disorders which did not allow to perform a biopsy. In table S4 in the online supplement, the baseline characteristics of this subgroup are presented. The results for preservation of muscle thickness in the biopsy subgroup are not different from the results of the total patient population (data not presented). The muscle biopsies of the stimulated muscle showed a slight shift towards larger myofibers as compared with the non-stimulated muscle, both for type 1 and type 2 myofibers (figure 3A). Muscle biopsies of non-stimulated and stimulated muscles showed no significant differences in signs of necrosis, inflammation and infiltration of myocytes with inflammatory cells or fibrosis, in the presence of muscle cells with centralized nuclei or vacuolation, or in the presence of adipocytes between the muscle cells (Table 2). As compared with biopsies taken from the non-stimulated muscles (2,07 ± 0.59), biopsies taken from the stimulated muscles showed a 37% higher gene expression for MyHC-I (2.83 ± 0.75, p= 0.03), but not for the other myofibrillary proteins MyHC-II or atrogin-1 (figure 3B). The E3 ligases MuRF-1 and atrogin-1 as markers of atrophy showed similar gene expression in stimulated and non-

stimulated muscles. No significant difference was observed between the non-stimulated and stimulated muscles for the protein content of p62, which is known to accumulate when autophagy is impaired, or LC3-II or the LC3-II/LC3-I ratio as marker of mature autophagosome formation (figure 3C).

Secondary outcome: Factors associated with change in muscle mass

The administration of opioids, type of contraction and percentage muscle loss in the non-stimulated muscle were significantly correlated with the difference in the decline in muscle thickness between stimulated and non-stimulated muscle and were added to the multivariable linear regression model together with the *a priori* included variables sepsis, edema and administration of vasopressors. No collinearity was observed between these variables of which the administration of opioids, poorer muscle contraction and more loss of muscle mass in the non-stimulated muscle were independently and significantly associated with better preservation of muscle mass (r^2= 0.382, table 3).

DISCUSSION

This study showed that NMES can attenuate muscle mass loss in critically ill patients. No differences in strength were observed. A shift towards larger myofibers and significantly higher gene expression for MyHC-I in the stimulated muscle was observed, while no differences in signs of muscle fiber necrosis or inflammation were observed in both muscles. Preservation of muscle mass was more likely in patients who received opioids, who had a poorer muscle contraction during NMES, or who were more prone to loss of muscle mass.

Our results are in accordance with Gerovasili et al [5] who showed that NMES could not completely prevent muscle wasting, but better preserved muscle thickness than

standard care alone (-8% vs -14% respectively, p=0.009). In contrast to our findings, Poulsen et al [8] observed in a small sample (n=8) no differences in septic patients between the stimulated

(-20%) and non-stimulated (-16%) quadriceps muscle CSA with MRI-scans (p=0.12). The stimulation intensities reported by Poulsen et al were much lower than in our study. They used mean intensities of 31mA (23-48mA) for vastus medialis and 42mA (37-54mA) for vastus lateralis. In our study, the mean intensity was 75mA. Grunow et al [14] also did not find a beneficial effect of NMES, but intensities were lowered to 40mA if no sufficient response was seen at 70mA. As reported in our study, patients with an insufficient response to NMES showed better preservation of muscle mass, but our intensities remained high. It could be speculated that the intensity in their study was not sufficient to elicit a favorable muscle response. A higher intensity may be needed, as Strasser et al [18] showed a positive correlation between applied current and higher expression of mechano-growth factor mRNA levels. Fischer et al [11] reported an intensity of 40mA and also did not find a difference in muscle thickness between stimulated and non-stimulated muscles in cardiothoracic surgery patients.

The observed differences in muscle thickness between the stimulated and non-stimulated muscle theoretically might reflect intramuscular edema due to muscle damage. Fouré observed muscle damage with MRI after NMES of the quadriceps muscle in healthy subjects [26]. In our biopsy results, however, inflammation and infiltration of myocytes with inflammatory cells was not different between the stimulated and non-stimulated muscle. In accordance with our results, Wollersheim et al [16] found no effect of NMES on necrosis or inflammation in the muscles of critically ill patients.

Our results showed no differences in muscle strength measurements, whereas other studies reported beneficial effects of NMES on muscle strength [6, 11, 12, 27, 28]. However, only 18 awake and cooperative patients in our study could perform strength measurements. This led to a selection of patients in whom less neuromuscular impairment was present as shown in the results. The patients who performed a strength test showed no significant difference in decline between the stimulated and non-stimulated muscle, whereas the patients who were not cooperative and able to undergo strength testing showed significantly more decline of muscle thickness in the non-stimulated muscle. The combination of low number of subjects and selection of patients with better preserved muscle thickness might have contributed to the absence of differences in strength in our study.

Our study showed a shift towards larger myofibers and a significantly higher MyHC-I gene expression in favour of the stimulated muscles, without differences between stimulated and non-stimulated muscles for atrogin-1 and MuRF-1. The shift towards larger myofibers is in agreement with the larger cross-sectional area or diameter with NMES observed by Dirks et al [15], Wollersheim et al [16] and Weber-Carstens et al [17]. The latter study suggested that the effect was only present for type II myofibres, whereas the other studies also observed an increase in size of type I myofibres. Strasser et al [18] found that total RNA content and total sarcoplasmic reticulum protein content increased, which may suggest muscle synthesis as contributor to the thicker muscle mass. Wollersheim also found an up-regulation of gene expression for several myosin heavy chain subtypes. Our study only confirmed the up-regulation of MyHC-I. With regard to muscle protein degradation, our study showed no differences in the expression of the E3 ligases atrogin-1 and MuRF-1 between stimulated and non-stimulated muscles. This is in accordance with the findings of

Dirks et al [15], who did not observe any difference between the stimulated and non-stimulated muscles in the expression of forkhead box protein O1 (FOXO1), atrogin-1 and MuRF1 as markers of atrophy.

Factors predicting better preservation of muscle were administration of opioids, eliciting poorer contractions and more muscle wasting in the non-stimulated leg. The explanation for the positive effect of opioids might be found in its analgesic properties and our approach to increase stimulation intensity within the pain threshold of the patient. The higher stimulation current in patients receiving opioids may have excited more motor units which might have resulted in better preserved muscle thickness. The mean intensity in our study was 75mA. This is much higher than the intensity used in other studies (20-42mA) [5, 8, 11, 12]. The reason why a lower amount of good contractions and more muscle wasting in the non-stimulated muscle led to better preservation of muscle mass may be attributed to the clinical status of the patients. Indeed, severely critically ill patients lose more muscle mass [3] and might be more likely to benefit from an intervention preserving muscle mass. Grunow et al [14] showed that the contractile response to NMES was lower in more severely critically ill patients. Our previous research showed that lower amount of good contractions was seen in patients with sepsis [20]. Thus, the poorer clinical status of the patient may explain why there is more muscle wasting and why it was more difficult to obtain a good contraction. However, trying to achieve even a minimal muscle contraction with a high stimulation intensity might have been sufficient to attenuate the loss of muscle mass in these subjects.

Limitations of the study

Our study has some limitations to address. First, our patients were not blinded for the intervention as the control muscle did not receive any sham stimulation. However, most patients were not cooperative during the intervention period. In addition, as our primary outcome is an effort-independent measure of muscle thickness performed by a blinded investigator, this is not considered to induce bias. Second, no long-term consequences or functional outcomes were investigated. Our focus was on the immediate effect of NMES on muscle mass and strength after a 7-day intervention period. Future studies should also focus on functional outcomes at ICU and hospital discharge. Third, for our secondary outcomes of strength and biopsy, less patients could be included. The outcomes in the subgroup of patients with muscle biopsy was, however, not different from patients without muscle biopsy and seems representative for our study population (effect size= 0.48). The trends in the outcomes of the biopsies were in accordance with our primary outcome, but should be confirmed in a larger study population. The strength results did not confirm our primary outcome. However, it is possible that the timeframe of intervention was too short to show an effect on strength. The positive results on muscle thickness might also lead to positive strength results if the intervention period extends beyond 7 days. However, this should be further explored. Furthermore, assessing strength with evoked peak torque in this setting might have been more appropriate to evaluate strength in uncooperative or less cooperative patients [29]. Fourth, the protocol did not allow adjustment, as it was preset and only intensity could be increased to elicit a better muscle contraction. This was chosen to have some consistency and reproducibility in the stimulation protocol. The optimal parameters for stimulation are not fully explored, but the characteristics used in this study did not differ substantially from those reported in the literature at the time this study started [5-9]. Also, in a more recent study by Silva et al [30] the chronaxie value was used to set the pulse width

for stimulation. They found that 300µs was ideal in the first stimulation session and tends to increase every day. This supports our choice of pulse width of 350µs over the 7 day intervention period.

Conclusions

Muscle mass was better preserved in the muscle treated with NMES as compared to the control muscle, coinciding with a shift towards larger type 1 and type 2 myofibers in the stimulated muscle as compared with the non-stimulated muscle. Muscle strength was not different between the stimulated and control muscle. Patients receiving opioids and those who exhibited more pronounced loss of muscle mass in the non-stimulated muscle benefitted most of NMES, while a good muscle contraction in all sessions was not compulsory.

Acknowledgements

AUTHOR CONTRIBUTIONS

Study concept and design: Gosselink, Hermans, Vanhorebeek

Acquisition of data: Segers, Charususin, Clerckx, Frickx, Demeyere, Casaer, Wei, Derde, Derese, Pauwels

Analysis and interpretation of data: Segers, Gosselink, Hermans, Vanhorebeek, Langer

Drafting of the manuscript: Segers, Gosselink, Hermans, Vanhorebeek

Critical revision of the manuscript for important intellectual content: Gosselink, Segers, Langer, Vanhorebeek, Hermans.

Statistical analysis: Segers, Langer, Hermans, Vanhorebeek

Obtained funding: Gosselink, Vanhorebeek, Van den Berghe.

Study supervision: Gosselink, Hermans, Vanhorebeek

Responsibility for the integrity of the whole work from incepton to published article: Gosselink

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Figure 1: CONSORT flow chart

ICU: Intensive Care Unit; NMBA: Neuromuscular blocking agents

Figure 2: Quadriceps muscle thickness assessed with ultrasonography before and after the

intervention period. The bar represents the mean, the whisker the standard error of the mean (n= 47).

Figure 3: Morphological and molecular markers of atrophy and muscle quality.

A. Size distribution of all myofibers and of type 1 and type 2 myofibers separately for the stimulated (light grey) and non-stimulated muscle (dark grey). **B**. Gene expression for myofibrillary proteins and markers of atrophy, for the stimulated and non-stimulated muscle per patient (thin lines) and for all patients as a group (thick line, with circles and whiskers representing mean and SEM). **C**. Gene or protein expression for markers of autophagy, for the stimulated and non-stimulated muscle per patient (thin lines) and for all patients as a group (thick line, with circles and whiskers representing mean and SEM).

Table 1: Patient characteristics (n = 47)

Patient characteristics at inclusion	N (%) or mean ± SD / median (IQR)
Gender, male, n (%)	25 (53)
Age, years, mean ± SD	60 ± 15
BMI, kg/m², mean ± SD	25 ± 6
APACHE II, mean ± SD	26 ± 8
ICU LoS at inclusion, days, median (IQR)	3 (2-4)
Patient characteristics during intervention period	
Sepsis, n (%)	20 (43)
Edema, n (%)	33 (70)
Administration of corticosteroids, n (%)	21 (45)
Administration of vasopressors, n (%)	37 (79)
Administration of inotropes, n (%)	11 (23)
Administration of opioids, n (%)	33 (70)
Administration of aminoglycosides, n (%)	6 (13)
Administration of neuromuscular blockers, n (%)	15 (32)
Admitted to medical intensive care unit, n (%)	32 (68)
Admission category	
Abdominal/pelvic surgery, n (%)	3 (6)
Cardiac surgery, n (%)	5 (11)
Gastrointestinal/hepatic disorder, n (%)	12 (26)

Respiratory failure, n (%)	14 (30)
Organ transplantation, n (%)	3 (6)
Thoracic surgery, n (%)	1 (2)
Hematology/oncology, n (%)	1 (2)
Other diagnoses*, n (%)	8 (17)

BMI: Body Mass Index; APACHE II: Acute Physiology And Chronic Health Evaluation II; ICU LoS: Intensive Care Unit Length of Stay

Other diagnoses include: sepsis, septic shock, meningitis, epidural haematoma, hyperglycemic coma

Table 2: Morphological abnormalities in muscle biopsies

Type of abnormality	**Non-stimulated** muscle **N=16**	**Stimulated** muscle **N=16**	**p**
Necrosis	2 (12.5)	4 (25.0)	0.361
Inflammation			0.513
Very mild	7 (43.8)	7 (43.8)	
Mild	8 (50.0)	6 (37.5)	
Moderate	1 (6.3)	3 (18.8)	
Myocyte infiltration with inflammatory cells	3 (18.8)	3 (18.8)	>0.999
Connective tissue			0.444
Mild presence	4 (25.0)	6 (37.5)	
Clear presence	12 (75.0)	10 (62.5)	
Central nuclei	10 (62.5)	12 (75.0)	0.444
Vacuolation			0.192
Absent	3 (18.8	7 (43.8)	
Mild presence	6 (37.5)	6 (37.5)	
Clear presence	7 (43.8)	3 (18.8	
Presence of adipocytes	6 (37.5)	6 (37.5)	>0.999

Data are shown as number (percentage).

Table 3: Multivariable analysis identifying factors associated with the difference in muscle loss between stimulated and non-stimulated muscle

Factors included in the multivariable analysis	**Unstandardized β**	**p**
Edema*	-4.068	0.398
Administration of vasopressors*	-5.907	0.250
Sepsis*	2.062	0.666

Administration of opioids*	10.931	0.018
Type of contraction, good*	-11.933	0.027
Muscle thickness change in non-stimulated muscle**	-16.383	0.006

*Data analyzed as dichotomous data (For edema, administration of vasopressors, sepsis and administration of opioids: yes versus no; for type of contraction: good contraction versus poor contraction).

**Data analyzed as continuous data (thickness post stimulation period – thickness pre stimulation period; expressed in centimeter).

Highlights

- Neuromuscular electrical stimulation diminishes the loss of muscle mass but not of muscle strength in critically ill patients
- Less reduction of muscle mass with NMES was observed in patients who are prone to lose muscle mass
- Obtaining a good muscle contraction was not compulsory for attenuating the loss of muscle mass

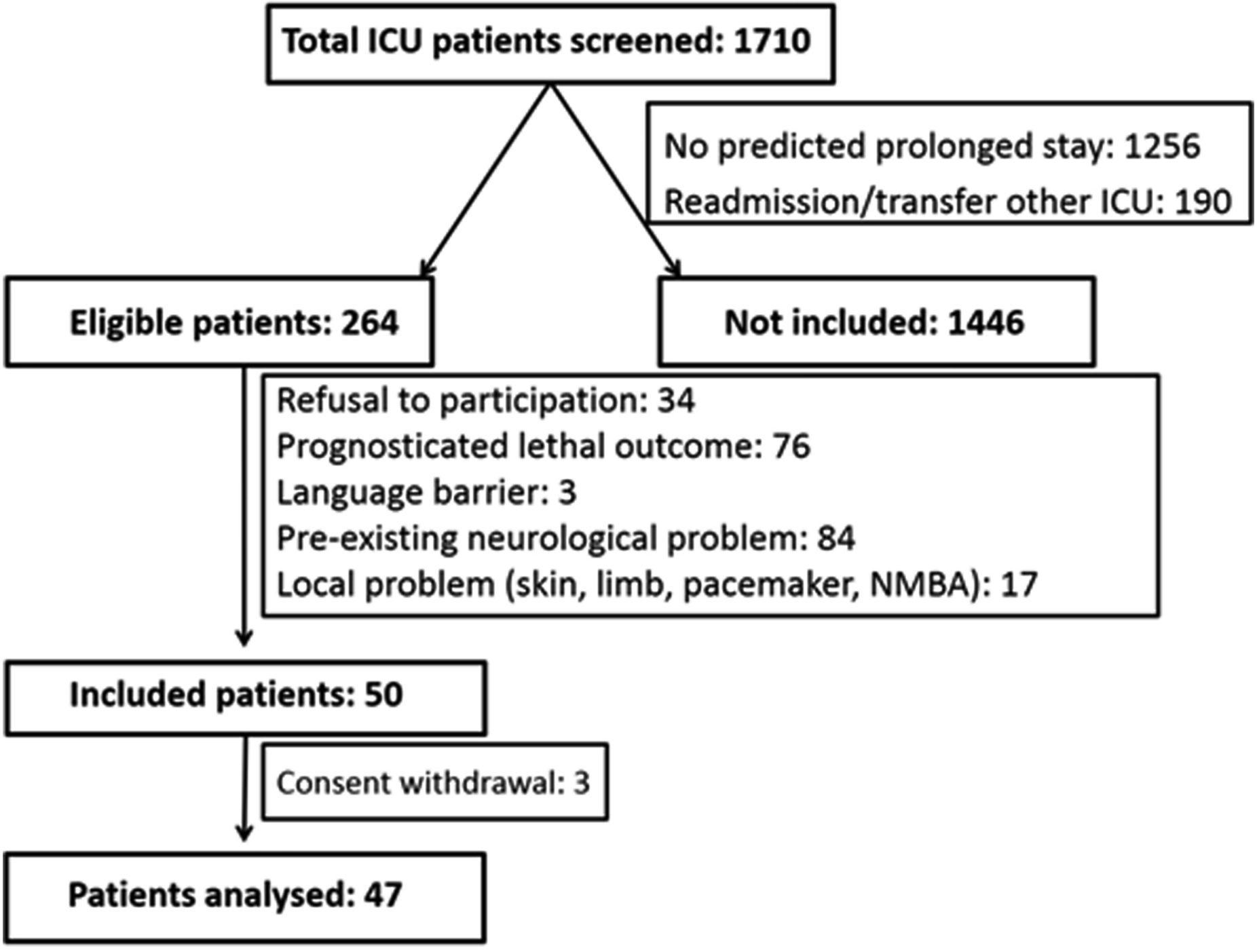
Total ICU patients screened: 1710
No predicted prolonged stay: 1256
Readmission/transfer other ICU: 190
Eligible patients: 264
Not included: 1446
Refusal to participation: 34
Prognosticated lethal outcome: 76
Language barrier: 3
Pre-existing neurological problem: 84
Local problem (skin, limb, pacemaker, NMBA): 17
Included patients: 50
Consent withdrawal: 3
Patients analysed: 47

Figure 1

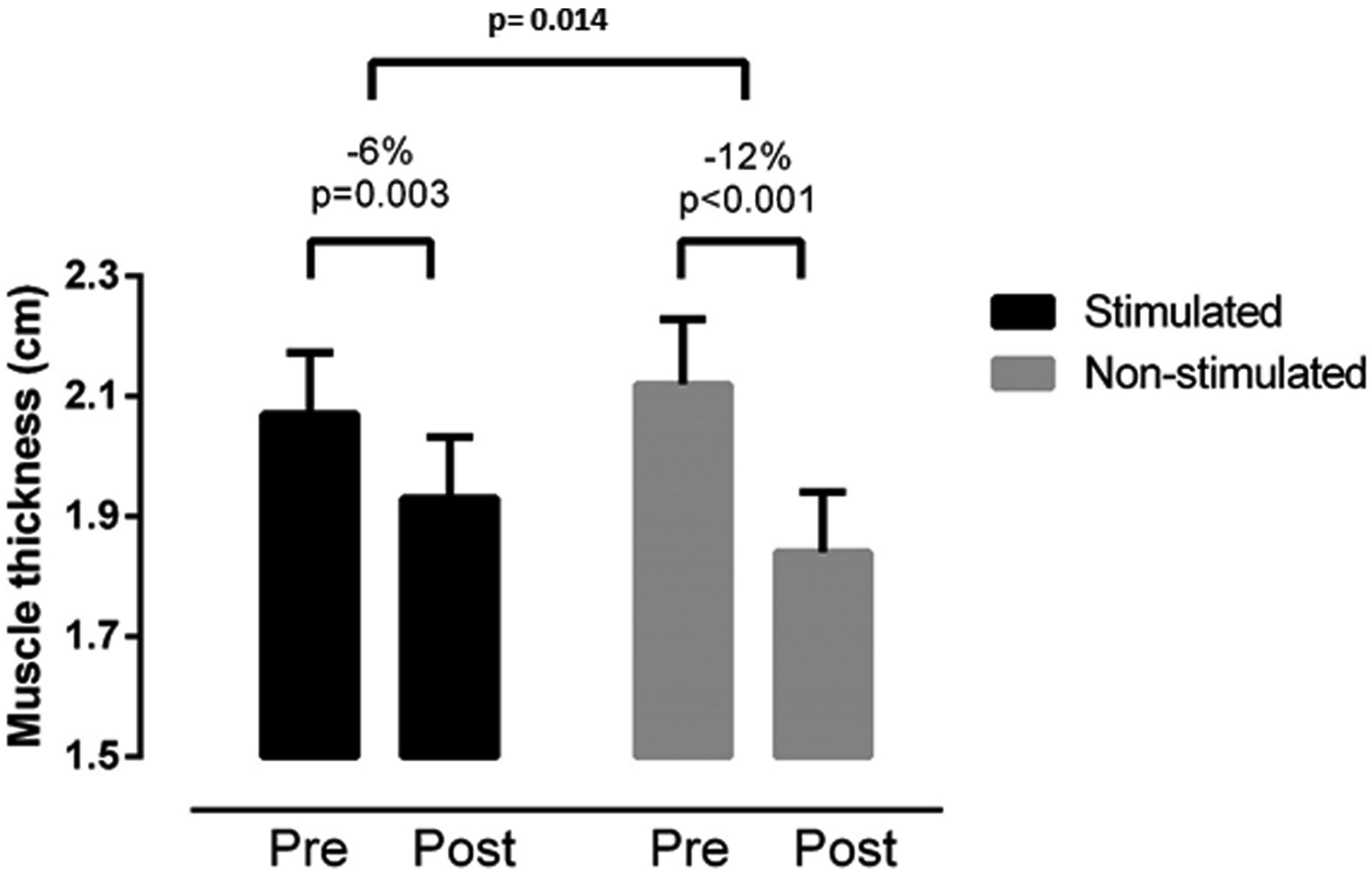
p= 0.014
-6%
p=0.003
-12%
p<0.001
Muscle thickness (cm)
2.3
2.1
1.9
1.7
1.5
Pre
Post
Pre
Post
Stimulated
Non-stimulated

Figure 2

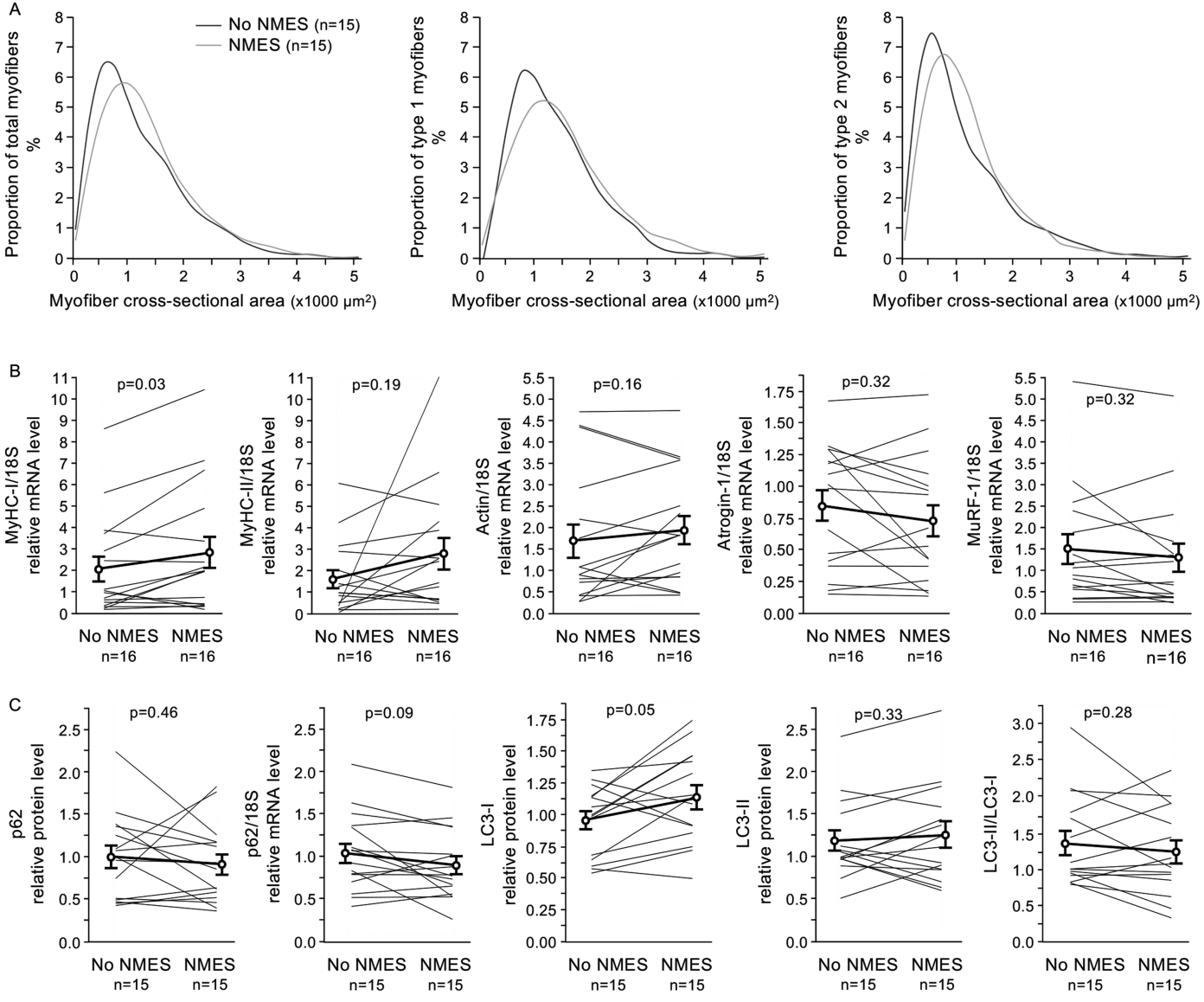
A
No NMES (n=15)
NMES (n=15)
Proportion of total myofibers %
Proportion of type 1 myofibers %
Proportion of type 2 myofibers %
Myofiber cross-sectional area (x1000 µm²)
B
MyHC-I/18S relative mRNA level
MyHC-II/18S relative mRNA level
Actin/18S relative mRNA level
Atrogin-1/18S relative mRNA level
MuRF-1/18S relative mRNA level
p=0.03
p=0.19
p=0.16
p=0.32
p=0.32
No NMES n=16
NMES n=16
C
p62 relative protein level
p62/18S relative mRNA level
LC3-I relative protein level
LC3-II relative protein level
LC3-II/LC3-I
p=0.46
p=0.09
p=0.05
p=0.33
p=0.28
No NMES n=15
NMES n=15

Figure 3