Skeletal muscle effects of electrostimulation after COPD exacerbation: a pilot study

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ABSTRACT

Muscle dysfunction is a major problem in chronic obstructive pulmonary disease (COPD), particularly after exacerbations. We thus asked whether neuromuscular electrostimulation (NMES) might be directly useful following an acute exacerbation and if such a therapy decreases muscular oxidative stress and/or alters muscle fibre distribution.

A pilot randomized controlled study of NMES during 6 weeks was carried out in 15 inpatients (n=9 NMES; n=6 Sham) following a COPD exacerbation. Stimulation was delivered to the quadriceps and hamstring muscles (35Hz). Primary outcomes were quadriceps force and muscle oxidative stress.

At the end of the study, quadriceps force improvement was statistically different between groups (p=0.02), with a significant increase only in the NMES group (median 10 (4.7-11.5) kg, p=0.01). Changes in the 6-minute walking distance were statistically different between groups (p=0.008), with a significant increase in the NMES group (165 (125-203) m, p=0.003). NMES did not lead to higher muscle oxidative stress as indicated by the decrease in total protein carbonylation (p=0.02) and Myosin Heavy Chain carbonylation (p=0.01) levels. Finally we observed a significant increase in type I fibres proportion in the NMES group.

Our study shows that following COPD exacerbation, NMES is effective in counteracting

muscle dysfunction and decreases muscle oxidative stress.

Key words: chronic obstructive pulmonary disease exacerbation, neuromuscular

electrostimulation, oxidative stress, fibre type distribution.

INTRODUCTION

Significant skeletal muscle dysfunction has been observed during exacerbations of chronic obstructive pulmonary disease (COPD) [1-3]. Several mechanisms may contribute to this effect, such as nutritional imbalance [4], medications [5], physical inactivity [2], systemic inflammation [3] and oxidative stress (OS) [6, 7]. It has been suggested that OS is increased in COPD patients during an acute exacerbation because these patients show increased plasma [6] and skeletal muscle antioxidant capacities [7]. Indeed, OS is already considered to play a major role in peripheral muscle dysfunction of COPD patients, both at rest and after exercise [8-10]. OS affects myofibrillar proteins and especially myosin heavy chain (MHC), which elicits functional and structural changes in muscle type fibres [11, 12].

After COPD exacerbation, the development of muscle dysfunction is rapid, whereas recovery is slow and partial [3]. Early respiratory rehabilitation has been shown to be effective in counteracting this negative progress [1, 13-18]. Direct stimulation of muscle nerves through application of electrical currents using NMES has beneficial effects on muscle strength and performance in stable COPD patients [19-22], but has never been used in unstable COPD patients. Since this technique does not induce a ventilatory response and dyspnoea [22], it may be considered as an alternative strategy to increase the muscle work performed in unstable COPD patients. Nevertheless, it remains to be elucidated whether NMES might be useful following an acute exacerbation and if it could stabilize or even decrease muscular OS in unstable patients.

We thus carried out a pilot study in severe COPD patients during recovery from acute exacerbation to test whether NMES could counteract the deleterious effects of COPD exacerbations. All patients were randomly distributed in two groups. Our primary objective was to explore whether a 6-week NMES program could improve muscle strength during recovery of acute COPD exacerbation. Moreover, skeletal muscle biopsies were performed before and after the trial to investigate the effects of NMES on muscle OS, particularly MHC oxidation. To achieve this objective, we measured protein oxidation (carbonyl formation) and lipid peroxidation (4-hyrdroxynonenal protein adduct formation and thiobarbituric acid reactive substances). Lastly, the proportion and composition of fibre type were determined.

MATERIALS AND METHODS

A detailed description of the methods is available in Supplementary data.

Study subjects

Consecutive patients with COPD (forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) <70%) admitted to the Intensive Care Unit (ICU) of Gui Chauliac hospital for an exacerbation were included in this study. The following criteria were used for patients' selection: (1) diagnosis of acute exacerbation of COPD; (2) age < 75 years; (3) BMI<30 kg/m²; (4) no locomotor or neurological condition or disability that could limit the ability to perform exercise; (5) no pacemaker implantation. All patients gave their written informed consent. The ethics committee Montpellier Sud-Méditerranée-IV approved this study.

Study design and randomization procedure

Details on the randomization procedure are provided in the on-line supplement. Patients were randomly assigned to Sham or NMES training. The Sham group had weekly therapeutic education sessions, daily active-passive mobilization and sham electrostimulation. The NMES group followed the same program, but received effective electrostimulation. The program started in the ICU, after baseline evaluations, and continued in a inpatient rehabilitation unit (Clinique Souffle Vallonie, France).

NMES protocol

The quadriceps and hamstring muscles of both legs were electrostimulated (Phenix-S8-VIVALTIS, France) using biphasic symmetric, constant current impulses with a pulse width of 400 μs and a frequency of 35 Hz for 1 hr/day, 5 days/week for 6 weeks. Intensity was set as the maximal tolerable intensity for each patient in the NMES group. The Sham group was exposed to the same regimen, except that the stimulation did not cause visible or palpable contractions.

Clinical and functional evaluations

We assessed the MVC of the quadriceps of each leg and we reported the greatest value. Six-minute walking test: Patients were asked to walk at their own maximal rate without running for 6 minutes. Patients could stop and restart the test. Symptoms of dyspnoea were assessed using the Medical Research Council (MRC) scale [23].

Muscle biopsy analysis

Muscle oxidation was assessed by measuring total protein and MHC carbonylation [8, 24] and the level of 4-hydroxy-2-nonenal (4-HNE) protein-adducts [24] by immunoblotting; lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARs) [25].

Fibre typology was evaluated by immunohistochemistry on frozen sections from the muscle biopsies using a panel of anti-MHCI (A4.951-c), anti-MHCIIa (2F7-c) and anti-MHCIIx (6H1-c) monoclonal antibodies (University of Iowa) [26, 27]. Fibre number and size were identified with a micro-vision image analysis system (Histolab 6.1.0, Microvision-Instruments).

Statistical analysis

Data are presented as median and interquartile range in the tables and as box whisker plots in the figures. The Mann-Whitney non-parametric test was used for comparisons between groups. The Wilcoxon non-parametric paired test was employed to compare different variables at baseline and at the end. Multivariate analysis of variance was employed to compare fibre type distribution in both groups. Spearman's coefficient was used to assess correlations between variables. Bonferroni-type adjustments were performed for multiple comparisons. A p-value of 0.05 or less was considered significant. In addition, the effect size (ES) was calculated for results that approached significance ($0.05 < p < 0.16$), but not for variables that were significant ($p < 0.05$). Cohen's conventions for effect size were used for interpretation, where ES < 0.2, 0.5, and 0.8 are considered as small, medium, and large,

respectively. Statistical analysis was performed using the statistical package Sygmastat 1.0 (Jandel Scientific, San-Rafael, CA-USA).

RESULTS

Patients

Of the 47 patients with COPD exacerbation who were initially screened, only 17 were enrolled in the study (Figure S1 in supplementary-data). 19 were not included due to the presence of one or more exclusion criteria: unstable ischemic heart disease (n=2), BMI>30 (n=5), lung cancer (n=4), associated orthopaedic problems (n=3), scheduled surgery (n=5) or inclusion in another protocol (n=1). Eleven patients declined to participate, citing "fear of biopsy" (n=3), "returning home" (n=6), and family refusal (n=1). Among the 17 included patients, one was later excluded due to readmission to ICU following a new exacerbation and another withdraw consent, 48h after inclusion, because family refusal. Finally, 9 patients in the NMES group and 6 in the Sham group completed the study. All patients received standard treatment, including nebulised bronchodilators, oral or intravenous antibiotics and oral corticosteroids (30-40 mg daily) for one to two week. At admission to the ICU, patients were placed under oxygen (3 NMES and 2 Sham patients), non-invasive ventilation (3 NMES and 2 Sham patients) during 17 days and 20 days, respectively NMES and Sham patients, or oro-tracheal intubation with mechanical ventilation (3 NMES and 2 Sham patients) during 12 days and 11 days, respectively NMES and Sham patients. The patients' baseline characteristics (Table 1) were similar in the two groups. The NMES program began on average at 12±8 days of hospitalization in the Respiratory ICU.

Table 1. Patients' baseline characteristics

Variables	Sham (n = 6)	NMES (n = 9)	p value
Sex, No (F/M)	0 / 6	2 / 7	
Age (yrs)	67 (59-72)	59 (57-69)	0.74
Weight (kg)	56 (52-66)	64 (52-71)	0.57
BMI (kg/m²)	19 (17-25)	23 (17-24)	0.62
FEV_1 (% pred)	15 (10-27)	25 (17-41)	0.16
FVC (% pred)	34 (31-54)	53 (42-64)	0.28
FEV_1/FVC (% pred)	35 (31-39)	42 (34-52)	0.43
Walking distance (m)	52 (0-90)	0 (0-135)	0.82
Dyspnea (MRC)	5 (5-5)	5 (5-5)	0.49
MVC (kg)	7 (3-14)	4 (2-9)	0.40

Table 1. Values are medians (25th and 75th percentiles). BMI, body mass index; FEV_1, forced expiratory volume in 1s; FVC, forced vital capacity; MVC, maximal voluntary contraction.

Tolerance of the NMES sessions

No adverse effect was recorded during the study. All patients could complete 29±1 sessions on average. In the NMES group the intensity of the stimulation was of 22±11 mA and of 15±5 at inclusion and of 47±8 mA and 32±1 mA at the end for quadriceps and hamstring muscles, respectively.

Functional effects of the NMES

MVC changes were statistically different between groups (p=0.02, Figure 1A), with a clinically significant increase in the NMES group (median 10 [4.7-11.5] Kg, p=0.01) between inclusion and week 6 (Figure S2A in supplementary data), whereas only a slight and not significant increase was observed in the Sham group (median 3 [1-5] Kg, p=0.12). Changes of the 6-minute walking distance were significant between groups (p=0.008, Figure 1B), with a clinically significant increase in the distance covered by the NMES group (median 165 [125-203] m, p=0.03) and only a smaller and not significant improvement in the Sham group (median 58 [43-115] m, p=0.24) (Figure S2B in supplementary data). Finally, the dyspnoea score decreased of two points in the NMES group and of only one point in the Sham group

Muscle oxidative stress

As shown in Figure 2A, the anti-DNP antibody detected several protein bands in the quadriceps muscles from patients in the Sham and NMES groups. At baseline, the carbonylation levels of total proteins and MHC were comparable in the two groups (Figures S3A and S3B in supplementary data). At the end of the program, the changes in carbonyl group formation in total proteins and MHC were significantly different between groups (p=0.02 and p=0.04, respectively) (Figure 2B and 2C). Indeed carbonylation was significantly decreased in the NMES group when compared to baseline values (p=0.02 and p=0.01, respectively), whereas it remained unchanged in the Sham group (p=0.21 and p=0.62, respectively) (Figures S3A and S3B in supplementary data). The carbonylated MHC/total MHC content ratio was also significantly reduced in the NMES group (p=0.03) at the end of the trial, but did not change in the Sham group (p=0.40). Although the difference in carbonylated MHC/total MHC content ratio were not significantly different between groups, the effect size tended to be large (ES =1.09, p=0.08).

The level of 4-HNE protein adducts and lipid peroxidation (data not shown) did not differ between groups at baseline and remained unmodified after the program (Figure S4A and Figure S4B in supplementary data). However, the effect size for 4-HNE protein levels tended to be medium (ES=0.4, p=0.1), that could be considered as a moderate effect.

The levels of Cu/Zn-SOD, GR and CAT anti-oxidant enzymes did not significantly change in both groups (Figures 3 A, 3B, 3C and 3D). The effect size for the anti-oxidant enzymes levels was not calculated because all p > 0.16.

Muscle structure

No significant differences were found at baseline between groups (Table 2). Changes in the proportion of type I and IIa/IIx fibres were significantly different between groups (p=0.03 and

p=0.04, respectively) at the end of the study. The proportion of type I and IIa/IIx fibres significantly increased (from 13±4% to 25±3%, p=0.002 and 9±2% to 23±4%, p=0.003, respectively) in the NMES group, whereas they remained unchanged in the Sham group (Table 2). A significant negative correlation was observed between changes in the proportion of type I fibres and variation of carbonylated MHC (r= - 0.60; p=0.03) (Figure 4A). Although the changes in the proportion of IIx fibres were not significantly different between groups, a significant decrease in the proportion of type IIx fibres in both groups was observed (Table 2).

Table 2. Distribution of fibre types (in percentage) at baseline and at the end of the study.

	Sham		NMES	
	Before	After	Before	After
Type I (%)	12±3	13±5	13±4	25±3*
Type I-IIa (%)	2±1	9±2*	6±2	11±3
Type IIa (%)	32±5	32±8	36±5	27±3
Type IIa-IIx (%)	11±2	15±5	9±2	23±4*
Type IIx (%)	43±5	30±8*	35±4	15±3*

Table 2. Values before and after the trial are expressed as means (SEM).

* Significantly different between before and after the program.

The size of type I but not of type IIx fibres was significantly increased in the NMES group at the end of the program (p=0.009 and p=0.16, respectively), whereas it remained unchanged in the control group (data not shown).

Finally, the changes in total MHC content were statistically different between groups (p=0.03,). Moreover, a significant positive correlation was found between changes in MHC content and changes in quadriceps strength (r=0.79, p<0.001) (Figure 4B).

DISCUSSION

The present study shows that during recovery from acute exacerbation, a 6-week training protocol using neuromuscular electrostimulation induced: (1) improvement of the muscle force and distance covered during the walking test; (2) reduction of total protein carbonylation, and particularly of carbonylated MHC; (3) increase in MHC content; (4) significant changes in muscle fibre typology in the NMES group in comparison to control patients.

We chose to study patients after COPD exacerbation because, it has been shown this represent the best model of extreme muscle dysfunction. Indeed, Spruit and *al*. have clearly demonstrated lower peripheral muscle force in patients after hospitalization for acute COPD exacerbation than in patients with stable COPD [3]. Moreover, the average quadriceps strength in all our patients at inclusion was extremely weak (MVC <10kg) and was much lower than the value usually observed in stable patients [28]. In addition, skeletal muscle

protein oxidation was more important in our patients than in patients with stable COPD *(personal data)*. Furthermore, according to the meta-analysis by Gosker and *al.*, a proportion of type I fibres <27%, as described in our study (13%), is abnormally low [29]. Likewise, it has been proposed that a proportion of type IIx fibres >29%, also observed in our patients at inclusion (39%), should be considered as abnormally high [29].

Functional effects of NMES program

The NMES group presented a more important and significant increase of quadriceps strength that the Sham group at the end of the 6-week program. Although the results were not significant, a slight spontaneous recovery of quadriceps strength occurred in the Sham group, which was probably due to the reversibility of the systemic effects of acute COPD exacerbation and the daily mobilization by a physiotherapist. This result is consistent with the study by Spruit *et al* [3]. These authors reported that, 90 day after hospitalisation, the quadriceps peak torque was only increased by a mean of 8% in comparison to the value at day 8 after exacerbation.

The distance covered during the walking test significantly increased in the NMES group, whereas in the Sham group the increase was small at the end of the 6-week program. This result is consistent with the study by Man *et al.* who have shown that, 3 months after exacerbation, patients in the group of usual care had decreased exesice capacity [17]. The meta-analysis by Puhan et al. has highlighted a significantly improved exesice capacity after early rehabilitation [13]. A recent study interesting showed that outpatient pulmonary rehabilitation immediately following an acute COPD exacerbation improves exercise capacity partly through increase of quadriceps strength [18].

Moreover, the score of the MRC dyspnoea scale started to decrease already at the beginning of the program in the NMES group, concurrently with the improvement in the walking test. Finally, in our study, we did not report the influence of NMES on the respiratory system, but other authors, have observed decreased resting respiratory rate in bed-ridden patients, after NMES training [21] and, a small but significant reduction in the ventilation dead space was found at peak exercise in treated individuals in comparison with the sham group [19]. These preliminary findings about the possible impact of NMES on the ventilatory response to exercise require confirmation.

In summary, the NMES program seems to be an effective strategy to facilitate muscle function recovery in COPD patients following ICU hospitalization for exacerbation. Nevertheless, the underlying molecular mechanisms of such clinical and physiological improvements remain to be elucidated.

Muscle redox balance

Our program decreased significantly the level of carbonylated proteins in muscle of the NMES group in comparison to the Sham group and did not increase the level of 4-HNE protein adducts and lipid peroxidation. This suggests that localized electrostimulation produces muscle contractions of sufficient intensity to induce functional improvement without enhancing OS within the muscle. This is an important finding since patients with COPD exhibit increased muscle OS not only after high intensity training [10] but also at rest [8]. The 6-week NMES induced especially a decrease of MHC carbonylation. Marin-Corral and *al.* reported that, in the diaphragm of severe COPD patients, highly carbonylated MHC is likely

to be degraded by the ubiquitin–proteasome pathway [30]. Consequently, although the decrease of carbonylated MHC and increase in MHC content in our patients could not be causally related, it is tempting to suggest that the reduction carbonylated MHC could permit, at least partly, the increase of MHC content. On the other hand, it has been proposed that MHC oxidation by reactive oxygen species may perturb myosin structure and impair myosin function [12]. Accordingly, we observed a correlation between changes in total MHC content and changes in quadriceps strength (r=0.79, p<0.001). Altogether, our results suggest that NMES increases muscle strength probably due to a reduction of the level of MHC oxidation and therefore by increasing the quantity of functional MHC in skeletal muscle. However, future work should explore the exact contribution of OS to the increase in muscle protein breakdown and myosin loss observed in the quadriceps of COPD patients.

Muscle structure

Our study is the first to report changes of muscle fibre types in patients with severe COPD after NMES. Consistent with the results obtained in healthy men [31], paraplegic men [32] and chronic heart failure patients [33] after an electrostimulation program, we observed an increase in type I fibre proportion and a decrease in type IIx fibre proportion in COPD patients after exacerbation. These changes are usually interpreted as transition from fast-to-slow fibre type. Interestingly, we observed a significant correlation between the changes of type I fibre proportion and of carbonylated MHC level (p=0.03, r = -0.60). It is thus tempting to suggest that the gradual shift in the content of MHC isoforms, in COPD patients, might be due to a decrease of MHC-I carbonylation and consequently of its degradation.

However, the signalling pathways involved in the control of fibre type transition are still unknown. Recently, Peroxisome Proliferator-Activated Receptor-Coactivator-1 (PGC-1α) was implicated in the formation of oxidative muscle fibres in mice [34]. Moreover, it has been reported that in COPD muscles, the proportion of oxidative fibres is reduced [29] and the level of PGC-1α mRNA is lower [35]. Therefore, it would be interesting to test whether the higher amount of type I fibres observed in the NMES group could be induced by up-regulation of PGC-1α.

Study limitation

One possible critique of our study is the small sample size. However, the retrospectively calculated statistical power of the primary outcome (quadriceps force) was of 96% and significant results were found despite the relatively small population. Furthermore, finding patients with COPD exacerbation who accepted to be included in a study that included two muscle biopsies was challenging, but offered the opportunity to study biochemical and morphological changes of muscle over time in the same patients. However, it is necessary to conduct a clinical study with more patients to confirm our results. In the literature, there is only one example of a similar project; but only one biopsy was carried out at the end of the program [15]. We believe that our study, as the previous study [15], provides new information for the management of unstable COPD patients.

Given our results and all the existing studies [1, 14, 15, 17, 18] on the effects of early rehabilitation on muscle function and exercise capacity in unstable patients; neuromuscular electrostimulation, not requiring any patient cooperation, may be considered as an alternative to the standard training method used during the first days of hospitalization following

exacerbation. However, when the patient becomes more autonomous, the standard training modality will be able to enhance and potentiate the effects of the neuromuscular electrostimulation. Therefore, further studies are needed to define if the combination of neuromuscular electrostimulation and standard training methods in COPD patients after exacerbation may lead to a greater improvement of overall patient clinical functions than standard training alone.

In conclusion, the application of an NMES program following exacerbation of COPD is an effective strategy to counterbalance the loss of skeletal muscle function. In our patients it effectively restored muscle function by decreasing OS, and particularly MHC oxidation, and by improving MHC content and distribution of type I muscle fibres. We believe that the decrease in muscle OS, together with the significant changes in muscle fibre typology, may serve as a basis for designing future studies, where electrical stimulation may be used as a muscle training modality in a larger population of unstable COPD patients.

Acknowledgements

Funding

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Figure legends

Fig.1 **Clinical effects of the 6 week-long neuromuscular electrostimulation program**.

Box plots express changes in MVC (A) and 6-minute walking distance (B), between inclusion and the end of the program. Standard box plots with medians (25th and 75th percentiles) are depicted. These changes were statistically different between groups.
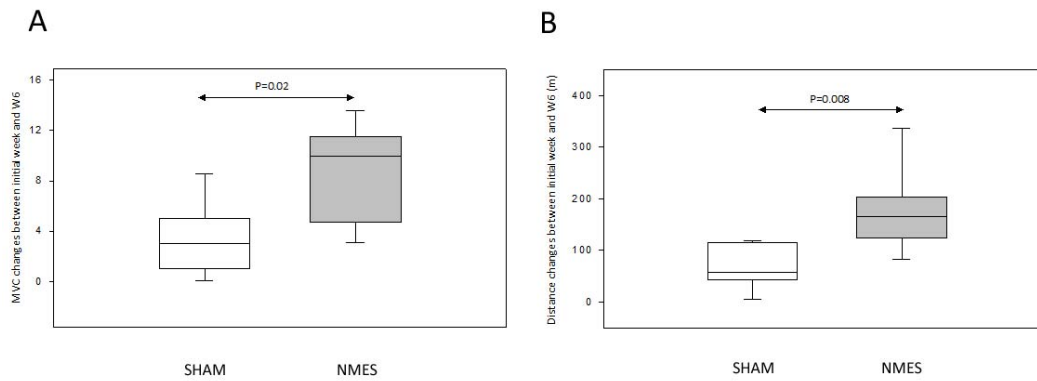

Figure 1

Fig.2 **Protein carbonylation**.

(A) Representative examples of carbonylated proteins in the vastus lateralis muscle from Sham and NMES patients; B, before and A, after the program. Carbonylated proteins of different molecular weights were detected. Quantification of the changes in carbonylation (detected as variations in optical density) of total proteins (B) and of MHC (C) in the Sham and NMES groups. Data are described using standard box plots with medians (25th and 75th percentiles). Total protein and MHC carbonylation changes were significantly different in the two groups, at the end of program.
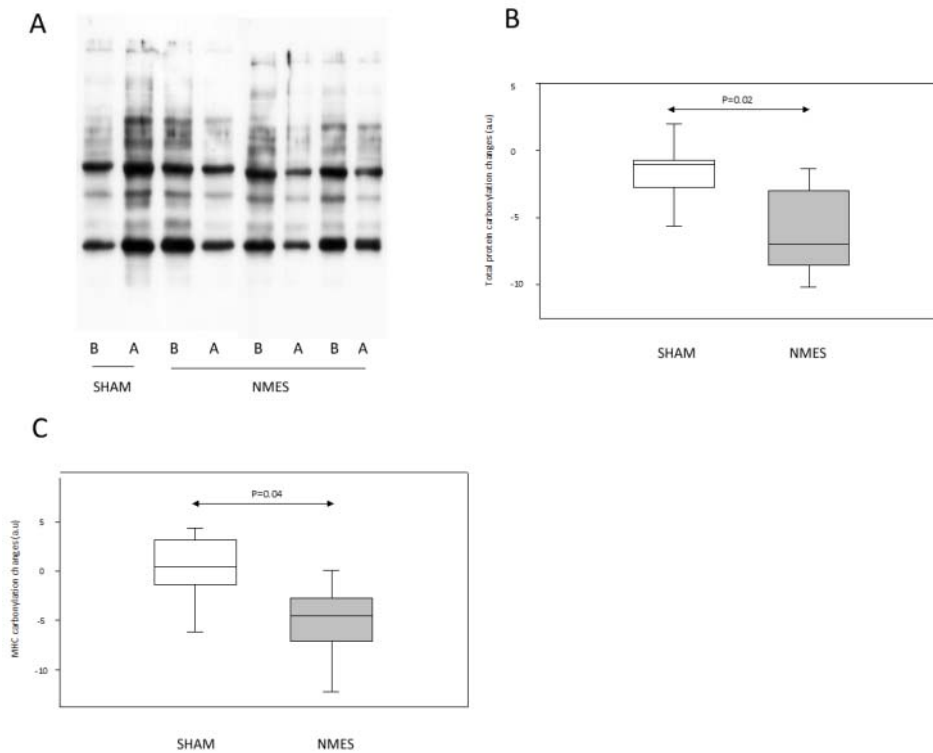
Figure 2

Fig. 3 **Anti-oxidant enzymes**.

(A) Representative examples of glutathione reductase (GR), catalase (CAT), Cu/Zn superoxide dismutase (Cu/Zn-SOD) and GAPDH expression in vastus lateralis from Sham and NMES patients. B, before and A, after the program. GAPDH was used as a loading control. Quantification of the change in Cu/Zn-SOD (B), CAT (C) and GR (D) expression. Data are described using standard box plots with medians (25th and 75th percentiles). Changes in the expression of anti-oxidant enzymes did not differ in the two groups.
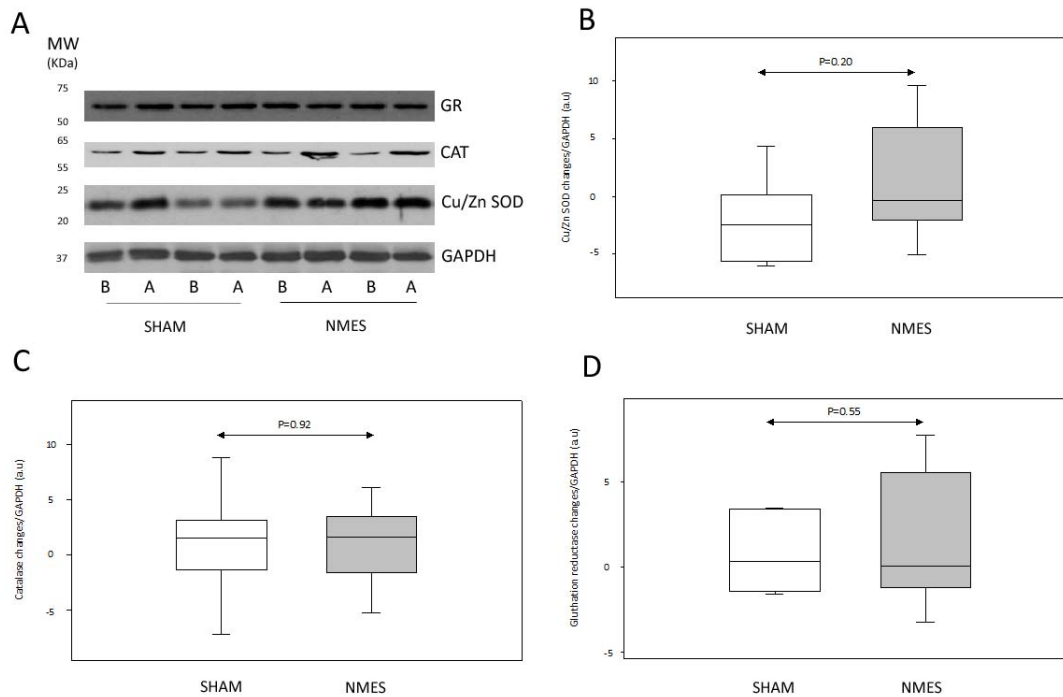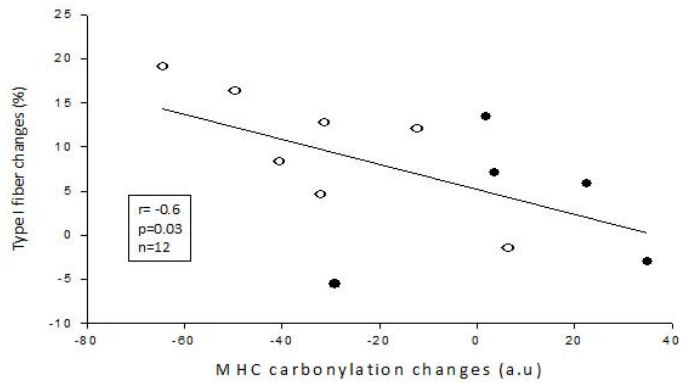
Figure 3

Fig. 4 **Muscle structure**.

(A) The changes in carbonylated MHC levels, expressed as optical density (OD) in arbitrary units (a.u), were inversely correlated with the changes in the proportion of type I fibres. Sham patients are represented by black circles and NMES patients by white circles (B) The increase in total MHC content, expressed as optical densities (OD) in arbitrary units (a.u), was positively correlated with improvement in the quadriceps MVC. Sham patients are represented by black circles and NMES patients by white circles.
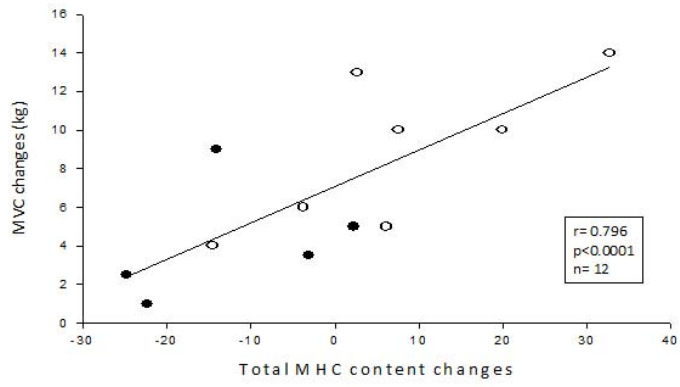
A

B

Figure 4