

The effect of transcutaneous electrical diaphragmatic stimulation on respiratory parameters of Chronic Obstructive Pulmonary Disease patients

Efeito da estimulação diafragmática elétrica transcutânea em parâmetros respiratórios de pacientes com Doença Pulmonar Obstrutiva Crônica

Efecto de la estimulación diafragmática eléctrica transcutánea en parámetros respiratorios de pacientes con Enfermedad Pulmonar Obstrutiva Crónica

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ABSTRACT | The objective of this study was to evaluate the effect of transcutaneous electrical diaphragmatic stimulation (TEDS) on respiratory muscle strength and endurance, thoracic-abdominal expansibility and spirometric variables of subjects with chronic obstructive pulmonary disease (COPD). Eight COPD patients submitted to respiratory physiotherapy received treatment with TEDS twice a week for 06 weeks, totaling 12 sessions. Before and after TEDS they were evaluated by the following parameters: maximal inspiratory pressure (MIP); maximal expiratory pressure (MEP); axillary, xiphoid and abdominal cyrtometry; and spirometry. After the Shapiro-Wilk test, the paired Student's-t test and the Mann-Whitney test were applied for comparison of the two stages (before and after TEDS). For comparison of the before, after (post-1st session), 1st, 2nd, 3rd, 4th week stages, the ANOVA followed by Tukey test were applied ($p < 0.05$). In accordance with the results obtained it was observed that TEDS promoted significant increase in: MIP (47.3%); MEP (21.7%); axillary (55.5%); xiphoid (59.2%) and abdominal (74.2%) cyrtometry, but not in the spirometric variables. In longitudinal analysis (in the 4 following weeks) the increase found in MIP and in thoracic-abdominal expansibility was maintained. Thus, we conclude that TEDS promoted improvement in respiratory muscle strength and thoracic-abdominal expansibility in COPD patients without alterations in spirometric variables, and some parameters were maintained in the following 4 weeks.

Keywords | Electric Stimulation; Pulmonary Disease, Chronic Obstructive.

RESUMO | O objetivo do estudo foi avaliar o efeito da estimulação diafragmática elétrica transcutânea (EDET) sobre a força e endurance muscular respiratória, expansibilidade toracoabdominal e variáveis espirométricas de indivíduos com doença pulmonar obstrutiva crônica (DPOC). Oito pacientes com DPOC submetidos à fisioterapia respiratória receberam tratamento com EDET duas vezes por semana durante 06 semanas, totalizando 12 sessões. Antes e depois do tratamento eles foram avaliados pelos seguintes parâmetros: pressão inspiratória máxima (Plmáx); pressão expiratória máxima (PEmáx); cirtometria axilar, xifoideana e abdominal; e espirometria. Após o teste Shapiro-Wilk, o teste t de Student pareado e o teste Mann-Whitney foram aplicados para a comparação dos dois estágios (antes e após a EDET). Para a comparação dos estágios antes, após (pós-1ª sessão), 1ª, 2ª, 3ª e 4ª semana, a ANOVA seguida do teste de Tukey foram aplicados ($p < 0,05$). De acordo com os resultados obtidos, foi observado que a EDET promoveu aumento significativo em: Plmáx (47,3%); PEmáx (21,7%); cirtometria axilar (55,5%); xifoideana (59,2%) e abdominal (74,2%), mas não nas variáveis espirométricas. Na análise longitudinal (nas 4 semanas seguintes) o aumento encontrado na Plmáx e na expansibilidade toracoabdominal foi mantido. Assim conclui-se que a EDET promoveu melhora na força muscular respiratória e na expansibilidade toracoabdominal em pacientes com DPOC sem alteração nas variáveis espirométricas; e alguns parâmetros foram mantidos nas quatro semanas seguintes.

Descritores | Estimulação Elétrica; Doença Pulmonar Obstrutiva Crônica.

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RESUMEN | El objetivo del estudio fue evaluar el efecto de la estimulación diafragmática eléctrica transcutánea (EDET) sobre la fuerza y endurance muscular respiratoria, expansibilidad toracoabdominal y variables espirométricas de individuos con enfermedad pulmonar obstructiva crónica (EPOC). Ocho pacientes con EPOC sometidos a fisioterapia respiratoria recibieron tratamiento con EDET dos veces por semana durante 6 semanas, totalizando 12 sesiones. Antes y después del tratamiento fueron evaluados por los siguientes parámetros: presión inspiratoria máxima (Plmáx); presión expiratoria máxima (PEmáx); cirtometría axilar, xifoidea y abdominal; y espirometría. Después del test Shapiro-Wilk, el test t de Student pareado y el test Mann-Whitney fueron aplicados para la comparación de los dos niveles (antes y después de la EDET). Para la comparación de los niveles

antes, después (post-1a sesión), 1ª, 2ª, 3ª y 4ª semana, la ANOVA seguida del test de Tukey fueron aplicados ($p < 0.05$). De acuerdo con los resultados obtenidos, fue observado que la EDET promovió aumento significativo en: Plmáx (47,3%); PEmáx (21,7%); cirtometría axilar (55,5%); xifoidea (59,2%) y abdominal (74,2%), pero no en las variables espirométricas. En el análisis longitudinal (en las 4 semanas siguientes) el aumento encontrado en la Plmáx y en la expansibilidad toracoabdominal fue mantenido. Así se concluye que la EDET promovió mejora en la fuerza muscular respiratoria y en la expansibilidad toracoabdominal en pacientes con EPOC sin alteración en las variables espirométricas; y algunos parámetros fueron mantenidos en las cuatro semanas siguientes

Palabras claves | Estimulación Eléctrica; Enfermedad Pulmonar Obstructiva Crónica.

INTRODUCTION

Among different aspects of skeletal muscle dysfunction, muscle mass regulation has attracted the attention of many researchers¹ due to their clinical relevance, especially in patients with Chronic Obstructive Pulmonary Disease (COPD) and other chronic affections.

In COPD, specifically, the inspiratory muscle dysfunction is associated with unfavorable clinical consequences such as dyspnea, respiratory failure with hypercapnia and even early mortality^{2,3}, which justify the exhaustion of techniques that may prevent it.

According to Testelmans *et al.*⁴, inspiratory muscle weakening in COPD patients is clinically relevant, because the maximal inspiratory pressure (MIP) is correlated to their survival. Some studies have reported that an adaption occurs in the diaphragm in this condition, being characterized by an increase in type I fibers and decrease in type II fibers^{4,8}, becoming more resistant to fatigue due to the increase in activity this clinical condition requires^{9,10}.

However, this change also leads to a reduction in the muscle strength of the diaphragm, with decrease in myosin content and sensibility to calcium, which favors muscle weakness at submaximal activation¹¹.

Respiratory Physical Therapy may contribute with the improvement of inspiratory muscle strength with transcutaneous electric diaphragmatic stimulation (TEDS). Aiming at better understanding this dynamics that acts directly on the diaphragm, studies have been conducted with animals^{12,13} and humans, for instance, in the postoperative period of gastropasty for obesity¹⁴.

However, we found no studies about TEDS in patients with COPD, and as this tool could be useful for patients with inspiratory muscle weakness this is a reason to conduct studies that can produce evidence of the technique benefits.

Therefore, this study was aimed at evaluating the effects of TEDS in respiratory muscle strength, thoracoabdominal expansibility and spirometric variables in patients with COPD at short term (after treatment) and four weeks later (residual effects). The hypothesis raised is that TEDS improves respiratory muscle strength, mainly inspiratory, because TEDS stimulates the contraction of the diaphragm.

METHODOLOGY

Sample

Eighteen patients diagnosed with COPD (according to criteria established by Rabe *et al.*¹⁵) were selected, out of which eight were enrolled in the study sample. The number of subjects was 8 with 80% sample power.

Amongst all patients, those aged above 80 years ($n=03$), in home oxygen therapy ($n=02$), using cardiac pacemaker ($n=02$), presenting cancer ($n=01$) and gastrointestinal disease ($n=02$) were excluded from the sample. Some other exclusion criteria were: recent history of COPD exacerbation or respiratory tract infections,

uncontrolled arterial hypertension, allergic rhinitis and tuberculosis. Patients with COPD had their disease reported in a form, including medication related to the respiratory tract; and all of them were smokers or former-smokers, none presenting clinical or physiological characteristics of bronchial asthma.

The COPD group was clinically stable and participating in a program for pulmonary rehabilitation (PR) and attending respiratory physical therapy sessions twice a week. This program consisted of strength and endurance exercises (upper and lower limbs) associated with respiratory reeducation, lengthening, aerobic exercises, orientations for daily life activities and techniques of energy conservation. Respiratory muscle training was not performed in the period of TEDS or post-TEDS. All patients had been participating in the program for at least one year.

The study was approved by the Ethics committee of the institution (Protocol 114/2009) and all patients signed the informed consent form before starting the research.

Intervention

To perform TEDS, we used the equipment Phrenics Dualpex 961 (Quark®; Brazil). The electrical current was pulsed, biphasic and symmetric, in the following patterns: 30Hz frequency (cycles per second), 0.4 ms band phase, rise time of 0.7 seconds and frequency of 14 respirations per minute. The intensity of the current was the minimum required to provide the diaphragm with a muscle contraction that was comfortable for patients¹⁶.

Four carbon-silicone electrodes (3x5 cm) were put on patients' skin — previously cleaned — with a conductor gel and micropore for fixation (3M®). Two electrodes were put on each side of the trunk, specifically on the third intercostals space near the middle part of the sternum and on the 7th intercostals space, on the middle axillary line. Sessions lasted 30 minutes and patients were oriented to coordinate their breath (inspiration) with the contraction generated by the electrical current.

Patients remained in semi-Fowler position (30°), with their lower limbs extended and upper limbs positioned at their sides.

The intervention was performed twice a week for six weeks, totaling 12 sessions.

Assessment

Before and after intervention, the following assessments were made:

- Respiratory muscle strength: assessed by MIP and MEP by an analogical manovacuometer (Ger-Ar®), gauged in cmH₂O, with variations of ± 300 cmH₂O, equipped with a bucal adaptor with a 2-mm diameter orifice, approximately, to avoid intraoral pressure that can be generated by unwelcome muscle contractions in the mouth cavity and, therefore, interference in results, in compliance with recommendations of the literature^{17,18}. Patients remained seated, with their trunk in a 90° position to the hips, using a nose clip during the maneuver. MIP was obtained from a maximal inspiration sustained for at least two seconds, after a maximal expiration, at the level of residual volume (VR). MEP was obtained from a maximal expiratory effort after a maximal inspiration at the level of total lung capacity (TLC), also sustained for at least two seconds¹⁸. Three technically satisfying measures were taken at each pressure, avoiding air escaping through the mouth or the nose, and with similar values (without exceeding 10% difference between them). The highest value obtained for each pressure was considered for analysis¹⁹.
- Thoracoabdoinal expansibility: assessed by axillary, xiphoid and abdominal cyrtometry using a tape measure. Three anatomical reference points were considered — axillary fold, xiphoid appendix, and umbilical line. The examiner asked the patients to perform maximal inspiration and expiration for each reference point three times. Data were registered in centimeters²⁰.
- Spirometry: performed using a portable spirometer (Easy One®) for the following variables: Slow Vital Capacity (SVC), Forced Vital Capacity (FVC), Maximum Voluntary Ventilation (MMV, muscle endurance), First Second Forced Ventilatory Volume (FVV₁) and index FVV₁/FVD. Measures were all obtained while using a nose clip. For each maneuver, three acceptable and reproducible measures were taken, according to the guidelines by the American Thoracic Society²¹.

Volunteers remained seated in the 1st and 3rd evaluations, and standing up in the 2nd evaluation. The Experimental protocol can be seen in Figure 1.

Statistical analysis

After the application of Shapiro-Wilk normality test, the following procedure was adopted: comparison of two phases (pre and post-intervention) using the paired Student's T (parametric) or Mann-Whitney (non-parametric) test, depending on the normality result; comparison between pre, post-intervention and 1, 2, 3 and 4 weeks after intervention using one-way ANOVA and Tukey tests. The significance level was set at 5% ($p < 0.05$) and we used the *software* Graph Pad Prism 5® for data processing.

RESULTS

The general and respiratory characteristics of patients with COPD are shown in Table 1.

After TEDS, there was an increase in respiratory muscle strength values (mean \pm SD; cmH₂O), represented by 47.3% at MIP (before: -59.4 ± 17.0 ; after: -87.5 ± 22.4 ; $p < 0.001$ compared to the pre-intervention phase) and by 21.7% in MEP (before: 71.9 ± 18.1 ; after: 87.50 ± 16.0 ; $p < 0.001$ compared to the pre-intervention phase).

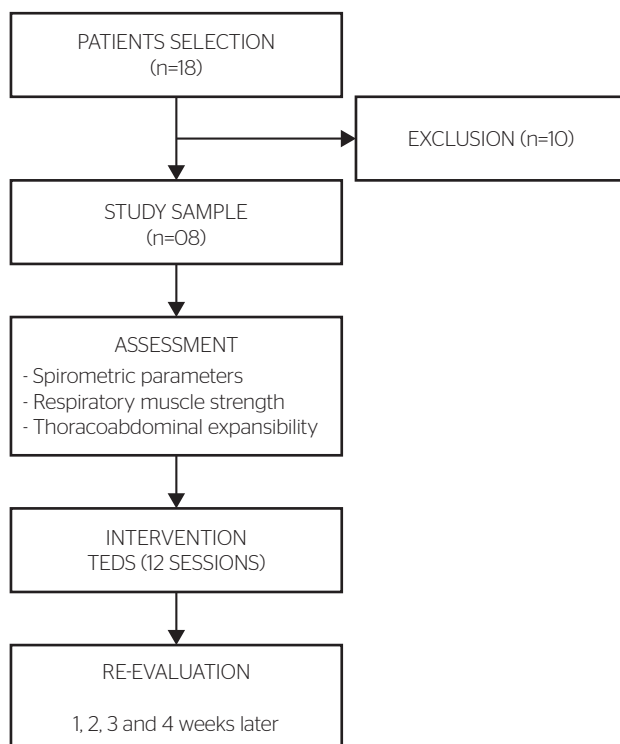


Figure 1. Experimental protocol

In the evaluation after intervention, MIP values (cmH₂O) (Figure 2A) still showed an increase, represented by: 28.4% (-76.3 ± 15.1 ; $p < 0.001$ compared to the pre-intervention phase) in the first week; 24.2% (-73.8 ± 16.0 ; $p < 0.01$ compared to the pre-intervention phase) in the second week; 29.5% (-76.9 ± 16.7 ; $p < 0.001$ compared to the pre-intervention phase) in the third week; and 28.4% (-76.3 ± 16.6 ; $p < 0.001$ compared to the pre-intervention phase) in the fourth week. These values are percentages, compared to those of the pre-intervention phase.

MEP values (cmH₂O) were not different in the following evaluations when compared to pre-intervention phase (71.9 ± 18.1), but they did differ from those of the post-intervention phase (87.50 ± 16.0), that is, values were lower in: first week (76.9 ± 17.1 ; $p < 0.05$ compared to the post-intervention phase); second week (77.5 ± 19.6 ; $p < 0.05$ compared to the post-intervention phase); and fourth week (76.9 ± 17.1 ; $p < 0.05$ compared to the post-intervention phase), as shown in Figure 2B.

In spirometry, there was no difference in the predicted value percentages for the studies variables, including VC (before: 74.2 ± 18.2 ; after: 72.9 ± 22.3 ; $p = 0.65$), FVC (before: 75.4 ± 18.8 ; after: 75.1 ± 20.9 ; $p = 0.95$) and MMV (before: 50.4 ± 22.1 ; after: 55.5 ± 30.5 ; $p = 0.25$).

Thoracoabdominal expansibility, assessed by cirtrometry (cm) presented difference, with increase by 55.5% at the axillary level (before: 3.6 ± 1.2 ; after: 5.6 ± 0.4 ; $p = 0.0004$), 59.2% at the xiphoid level (before: 2.7 ± 1.3 ; after: 4.3 ± 1.1 ; $p = 0.0022$) and 87.1% at the abdominal level (before: 3.1 ± 1.5 ; after:

Table 1. Anthropometric and spirometric variables of the Chronic Obstructive Pulmonary Disease Group (n=8)

Variable	COPD
Age (years)	68.5 \pm 6.2
Gender (M/F)	6/2
Height (cm)	163.1 \pm 14.2
Weight (kg)	67.7 \pm 9.0
BMI (kg/m ²)	25.6 \pm 3.3
HR (bpm)	84.8 \pm 9.9
SpO ₂ (%)	95.6 \pm 2.7
Spirometry	
CVF (% previsto)	75.4 \pm 18.8
VEF ₁ (% previsto)	51.2 \pm 25.7
VEF ₁ /CVF (% previsto)	64.2 \pm 16.8

Values expressed as mean \pm SD (standard deviation); COPD: Chronic Obstructive Pulmonary Disease; M: male; F: female; BMI: body mass index; HR: heart rate (bpm); SpO₂: peripheral oxygen saturation; FVC: forced vital capacity; FVV₁: first-second forced ventilatory volume

5.8 ± 1.5 ; $p=0.0011$). In the four following weeks, these increases were sustained at the three levels. No contralateral effects were observed in the studied patients.

DISCUSSION

Our results show that TEDS increase inspiratory and expiratory muscle strength, as well as thoracoabdominal expansibility in patients with COPD without interfering in spirometric variables. This increase in inspiratory muscle strength and thoracoabdominal expansibility was maintained in four weeks after the intervention. According to Sarlabous *et al.*²², the diaphragm plays a central role in the mechanical inspiratory activity. Patients with COPD have their inspiratory muscle and thoracic mechanical functions severely affected. Muscle efficiency (the relation between electrical activities and muscle mechanics) is significantly reduced due to changes in the thoracic-diaphragmatic space caused by the disease and which cause the muscle contractions to be ineffective, leading to energy loss.

This impaired contraction of the diaphragm in COPD may be explained by image studies such as magnetic resonance imaging, which shows a debasement of the diaphragm and limitations of its movements compared to healthy individuals^{23,24}. According to Iwasawa *et al.*²⁴, the exact mechanism of the paradoxal movement of the diaphragm has not

been completely understood yet, but this can be related to the low efficacy of its contraction in COPD.

In addition to the debasement seen at imaging evaluation, many studies have reported an adaption of the diaphragm in COPD, with consequent increase of type I fibers, which makes the organ more resistant to fatigue. Due to the continuous overload to the muscle in this disease, some authors^{4,8,11} have suggested that this adaption is considered beneficial to patients. In association with this changes in the type of muscle fibers, there is also a reduction in the cross-sectional area and in both fibers, type I and II, which characterizes atrophy of the muscle, even though it is submitted to continuous overload with the increase in this activity⁴.

Levine *et al.*²⁵ studied two patients with severe COPD and concluded that the fibers of the diaphragm are less stronger than the fibers of healthy patients. Ottenheim *et al.*²⁶ studied the fibers of this muscle as well, but in eight patients with COPD, and concluded that the strength of these muscles was reduced in COPD compared to healthy individuals. Stubbings *et al.*⁵, on their turn, reported that type I fibers of the diaphragm are increased in COPD.

In agreement with the results reported by these authors, our study showed reduced inspiratory muscle strength, represented by lower values of MIP. Compared to the two equations — by Neder *et al.*¹⁹ and Costa *et al.*²⁷ — for predicted values, patients with COPD presented reduced MIP and MEP values in our study.

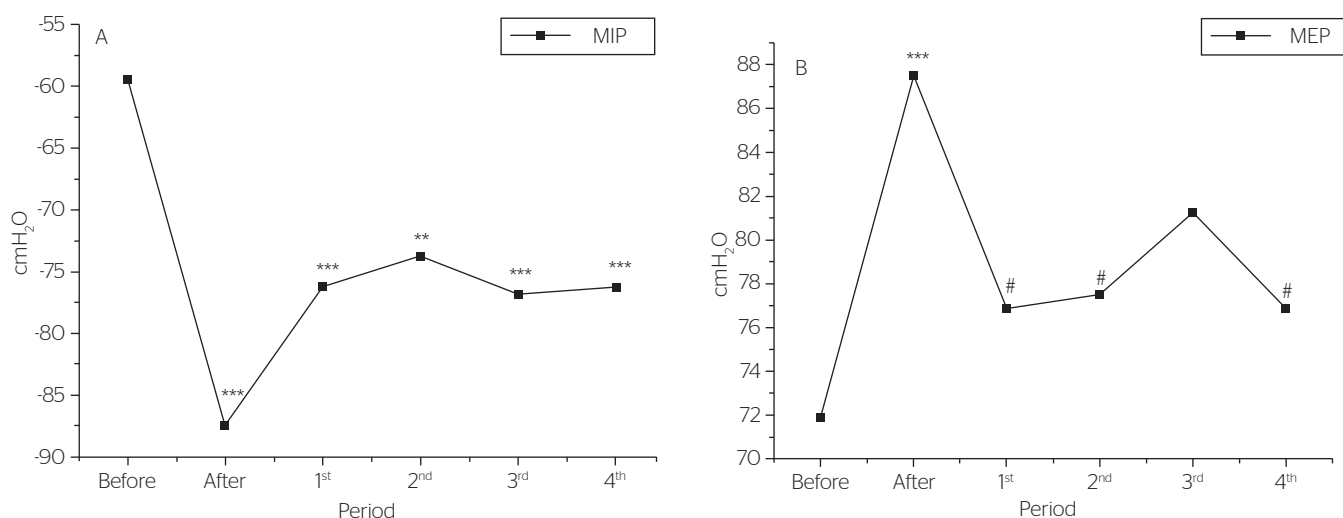


Figure 2. Maximal inspiratory pressure (MIP; -cmH₂O) (A) and maximal expiratory pressure (MEP; cmH₂O) (B) of the Chronic Obstructive Pulmonary Disease group treated by transcutaneous electric diaphragmatic stimulation (n=8) before and after intervention, and after 1, 2, 3 and 4 weeks of treatment.

Based on the equations by Neder *et al.*¹⁹, the studied sample had a MIP (cmH₂O) (-59.4 ± 17.0) below prediction (predicted MIP: -95.9 ± 6.1) and, compared to the equations by Costa *et al.*²⁷, the values obtained were slightly lower than prediction (predicted MIP: 62.9 ± 11.6). Expiratory muscle strength, represented by MEP (cmH₂O) (71.9 ± 18.1), was also lower compared to predictions by Neder *et al.*¹⁹ (100.5 ± 14.3) and Costa *et al.*²⁷ (89.9 ± 9.4).

In view of the muscle changes, TEDS becomes important for patients with COPD as an alternative treatment, especially because it not only increases inspiratory muscle strength, but also sustains it for a period (four weeks on average). This result may be related to the alterations in the type of fibers caused by electrical stimulations. This has been reported in a previous study with rats, where the authors observed an increase in Type IID fibers of the diaphragm, reduction of type I fibers and no changes in types IIA and IIB fibers¹².

Although the effects of TEDS is specifically on the inspiratory muscle, this technique also helps to increase MEP values for a short period (right after intervention), and the stimulation of the expiratory muscles by the electrical field generated may have occurred, as reported by Cancelliero *et al.*¹³ in animals and in other muscle groups with rats²⁹. Associated with the increase in respiratory muscle strength, an increase in thoracoabdominal expansibility was seen at cirtometry, a method considered to be reliable for the exploration of the dimensions and amplitudes of thoracoabdominal movements²⁰ and that, despite being hardly ever mentioned in literature, it has been widely used in clinical practice to assess thoracic mobility at respiration movements²⁹.

Our results agree with those by Costa *et al.*³⁰, who reported an improvement in thoracoabdominal mobility at cirtometry in women submitted to gastropasty and receiving TEDS, which played an important role in the mechanical recovery of thoracic and abdominal movements in the bariatric postoperative period.

According to the results of increase in thoracoabdominal mobility, one can infer that TEDS has effects similar to those obtained by physical exercises directed to thoracic mobility in cases of COPD, also assessed by cirtometry, according to the results reported by Paulin *et al.*³¹. Similarly, these authors have not found changes in the spirometric results of

patients with COPD after they had been through a program of physical exercises.

The abdominal mobility was the feature most likely to be altered at the three levels (axillary, xiphoid and abdominal) studied by Costa *et al.*³⁰, when a program of physical exercises was applied in obese patients, as well as in the study by Yamaguti *et al.*³², which showed an increase in the diaphragm mobility, evaluated by pletismography after a program of respiratory exercises (three series of ten exercises in different positions) at short term in patients with COPD. Their results are all in agreement with ours, once the increase observed was seen at these three levels.

Physiologically, inferior ribs are more oblique than the superior ones, and the more oblique, the greater the movement they are likely to do. This aspect is even more relevant when we consider that in patients with COPD these ribs usually arrange themselves in an oblique position, hence more horizontal, due to hyperinsufflation caused by the loss of pulmonary elasticity. Therefore, the increase in abdominal mobility can also be characterized as indicative of improvement in the mechanics of thoracoabdominal movements and consequent improvement in the pulmonary ventilation.

Although the authors were using different techniques in this study, the results were in agreement, emphasizing the increase in thoracoabdominal expansibility and respiratory strength, important features in the respiratory mechanics and dynamics.

Despite difficulties faced in maintaining patients' compliance to treatment until the completion of the study, thus characterizing a possible limitation, the results provided important support for therapeutic alternatives in the process of pulmonary rehabilitation in patients with COPD. Our hypothesis was confirmed because TEDS improved respiratory muscle strength, notably inspiratory.

CONCLUSION

Based on our results, TEDS was shown to be a therapeutic alternative for the recovery of clinical conditions of inspiratory muscle weakness and thoracoabdominal mobility limitations observed in patients with COPD, being therefore characterized as additional resource in pulmonary rehabilitation.

The maintenance of results for the period of four weeks may also be seen as a strong indication of success, but we suggest, however, that further studies be conducted with longer follow-ups.

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REFERENCES

- Doucet M, Dubé A, Joanisse DR, Debigaré R, Michaud A, Paré MÈ, *et al.* Atrophy and hypertrophy signalling of the quadriceps and diaphragm in COPD. *Thorax*. 2010;65(11):963-70.
- Bégin P, Grassino A. Inspiratory muscle dysfunction and chronic hypercapnia in chronic obstructive pulmonary disease. *Am Rev Respir Dis*. 1991; 143(5 Pt 1):905-12.
- Gray-Donald K, Gibbons L, Shapiro SH, Macklem PT, Martin JG. Nutritional status and mortality in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1996;153(3):961-66.
- Testelmans D, Crul T, Maes K, Agten A, Crombach M, Decramer M, *et al.* Atrophy and hypertrophy signalling in the diaphragm of patients with COPD. *Eur Respir J*. 2010; 35(3):549-56.
- Stubbings AK, Moore AJ, Dusmet M, Goldstraw P, West TG, Polkey MI, *et al.* Physiological properties of human diaphragm muscle fibres and the effect of chronic obstructive pulmonary disease. *J Physiol*. 2008;586(10):2637-50.
- Gosker HR, Wouters EF, van der Vusse GJ, Schols AM. Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: underlying mechanisms and therapy perspectives. *Am J Clin Nutr*. 2000;71(5):1033-47.
- Levine S, Gregory C, Nguyen T, Shrager J, Kaiser L, Rubinstein N, *et al.* Bioenergetic adaptation of individual human diaphragmatic myofibers to severe COPD. *J Appl Physiol*. 2002;92(3):1205-13.
- Levine S, Kaiser L, Leferovich J, Tikunov B. Cellular adaptations in the diaphragm in chronic obstructive pulmonary disease. *N Engl J Med*. 1997;337(25):1799-806.
- Nguyen T, Rubinstein NA, Vijayasathay C, Rome LC, Kaiser LR, Shrager JB, *et al.* Effect of chronic obstructive pulmonary disease on calcium pump ATPase expression in human diaphragm. *J Appl Physiol*. 2005;98(6):2004-10.
- Bellemare F, Grassino A. Force reserve of the diaphragm in patients with chronic obstructive pulmonary disease. *J Appl Physiol Respir Environ Exerc Physiol*. 1983;55(1 Pt 1):8-15.
- Ottenheijm CA, Heunks LM, Dekhuijzen RP. Diaphragm adaptations in patients with COPD. *Respir Res*. 2008;9:12.
- Costa D, Cancelliero KM, Campos GE, Salvini TF, Silva CA. Changes in types of muscle fibers induced by transcutaneous electrical stimulation of the diaphragm of rats. *Braz J Med Biol Res*. 2008;41(9):809-11.
- Cancelliero KM, Costa D, Silva CA. Transcutaneous electrical stimulation of the diaphragm improves the metabolic conditions of respiratory muscles in rats. *J Phys Ther*. 2006;10(1):59-65.
- Forti E, Ike D, Barbalho-Moulim M, Rasera I Jr, Costa D. Effects of chest physiotherapy on the respiratory function of postoperative gastroplasty patients. *Clinics*. 2009; 64(7):683-9.
- Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, *et al.* Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Resp Crit Care Med*. 2007;176(6):532-55.
- Geddes LA, Voorhees WD, Lagler R, Riscili C, Foster K, Bourland JD. Electrically produced artificial ventilation. *Med Instrum*. 1988;22(5):263-71.
- Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to age and sex. *Am Rev Respir Dis*. 1969;99(5):696-702.
- Sobush DC, Dunning M. Assessing maximal static ventilatory muscle pressures using the "bugle" dynamometer. suggestion from the field. *Phys Ther*. 1984;64(11):1689-90.
- Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests II: maximal respiratory pressures and voluntary ventilation. *Braz J Med Biol Res*. 1999;32(6):719-27.
- Caldeira VS, Starling CCD, Britto RR, Martins JA, Sampaio RF, Parreira VF. Reliability and accuracy of cirtometry in healthy adults. *J Bras Pneumol*. 2007;33(5):519-26.
- Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al.* ATS/ERS task force: standardisation of lung function testing. *Eur Respir J*. 2005;26:319-38.
- Sarlabous L, Torres A, Fiz JA, Gea J, Martinez-Llorens JM, Jane R. Evaluation of the respiratory muscular function by means of diaphragmatic mechanomyographic signals in COPD patients. *Conf Proc IEEE Eng Med Biol Soc*. 2009;2009:3925-28.
- Etlík O, Sakarya ME, Uzun K, Harman M, Temizoz O, Durmus A. Demonstrating the effect of theophylline treatment on diaphragmatic movement in chronic obstructive pulmonary disease patients by MR-fluoroscopy. *Eur J Radiol*. 2004;51(2):150-4.
- Iwasawa T, Kagei S, Gotoh T, Yoshiike Y, Matsushita K, Kurihara H, *et al.* Magnetic resonance analysis of abnormal diaphragmatic motion in patients with emphysema. *Eur Respir J*. 2002;19(2):225-31.
- Levine S, Nguyen T, Kaiser LR, Rubinstein NA, Maislin G, Gregory C, *et al.* Human diaphragm remodeling associated with chronic obstructive pulmonary disease: clinical implications. *Am J Respir Crit Care Med*. 2003;168(6):706-13.
- Ottenheijm CA, Heunks LM, Sieck GC, Zhan WZ, Jansen SM, Degens H, *et al.* Diaphragm dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2005;172(2):200-5.
- Costa D, Gonçalves HA, Lima LP, Ike D, Cancelliero KM, Montebelo MI. New reference values for maximal respiratory pressures in the Brazilian population. *J Bras Pneumol*. 2010;36(3):306-12.
- Durigan JLQ, Cancelliero KM, Bosi PL, Delfino GB, Montebelo MIL, Guirro RRJ, *et al.* Metabolic and morphometric alterations inherent to neuromuscular electric stimulation in the antagonist muscle submitted to ankle joint immobilization. *Braz Arch Biol Technol*. 2009;52(1):85-91.
- Costa D, Sampaio LMM, Pires de Lorenzo VA, Jamami M, Damaso AR. Evaluation of respiratory muscle strength and thoracic and abdominal amplitudes after a functional reeducation of breathing program for obese individuals. *Rev Latino-Am Enfermagem*. 2003;11(2):156-60.

30. Costa D, Forti EMP, Barbalho-Moulim MC, Rasera Jr. I. Study on pulmonary volumes and thoracoabdominal mobility in morbidly obese women undergoing bariatric surgery, treated with two different physical therapy methods. *Braz J Phys Ther.* 2009;13(4):294-300.
31. Paulin E, Brunetto AF, Carvalho CRF. Effects of a physical exercises program designed to increase thoracic mobility in patients with chronic obstructive pulmonary disease. *J Pneumol.* 2003;29(5):287-94.
32. Yamaguti WP, Claudino RC, Neto AP, Chammas MC, Gomes AC, Salge JM, *et al.* Diaphragmatic breathing training program improves abdominal motion during natural breathing in patients with chronic obstructive pulmonary disease: a randomized controlled trial. *Arch Phys Med Rehabil.* 2012; 93(4):571-7.