






Comparison of different frequencies of transcutaneous electrical diaphragmatic stimulation in healthy subjects: a randomized crossover clinical trial

Comparação de diferentes frequências da estimulação diafragmática elétrica transcutânea em indivíduos saudáveis: ensaio clínico randomizado cruzado

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ABSTRACT

Most transcutaneous electric diaphragmatic stimulation (TEDS) studies use a stimulation frequency (SF) of 30 Hz, although the reason for this SF value is not completely understood. **Objective:** The purpose of this study was to compare the acute effect of two TEDS frequencies on the respiratory muscle strength and endurance, muscle activation, muscle thickness, diaphragmatic mobility, cardiovascular variables and safety in healthy subjects. **Methods:** Randomized crossover clinical trial with 20 healthy subjects subjected to two interventions: SF=30 Hz group and SF=80 Hz group. TEDS was applied at the diaphragm motor points with a symmetric biphasic pulsed current (pulse duration= 500 μ s) for 30 minutes. The evaluated outcomes were systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), respiratory muscle strength by maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), inspiratory peak flux (PIF), diaphragm thickness during inspiration (DTI) and expiration (DTE), diaphragm mobility and activation, and endurance (S-Index). **Results:** SF-30 Hz showed a reduction with respect to baseline values for SBP ($p= 0.04$), HR ($p<0.001$), DTE ($p= 0.02$), IPF ($p= 0.01$), and S-Index ($p= 0.03$). SF-80 Hz showed a reduction with respect to baseline values for HR ($p<0.001$) and an increase in MEP ($p<0.001$). However, these changes were not clinically important and there were no between-groups differences for any of the evaluated outcomes. No complications were observed. **Conclusion:** TEDS with SF-80 Hz produces similar effects to SF-30 Hz in healthy subjects and both frequencies proved to be safe (NCT03844711).

Keywords: Electric Stimulation, Diaphragm, Muscle Strength, Healthy Volunteers

RESUMO

A maioria dos estudos utilizam a estimulação diafragmática elétrica transcutânea (EDET) com frequência (F) de estímulo de 30Hz e testar diferentes frequências torna-se necessário para uma aplicação otimizada. **Objetivo:** Foi comparar o efeito agudo de duas frequências diferentes da EDET sobre a força muscular respiratória e endurance, ativação diafragmática, espessura muscular e mobilidade diafragmática, variáveis cardiovasculares e segurança em indivíduos saudáveis. **Métodos:** Estudo randomizado cruzado com 20 indivíduos saudáveis submetidos a duas intervenções: Grupo I com F= 30Hz e Grupo II com F= 80Hz. A aplicação foi nos pontos motores do diafragma, com duração do pulso de 500 μ s, durante 30 minutos. Foram avaliados a pressão arterial sistólica (PAS) e diastólica (PAD), frequência cardíaca (FC), força muscular respiratória pela pressão inspiratória máxima (PI_{max}), pressão expiratória máxima (PE_{max}), endurance e espessura muscular em inspiração (EDI) e expiração (EDE), mobilidade e ativação diafragmática. **Resultados:** O GI apresentou redução significativa em comparação às condições basais para os desfechos de PAS ($p= 0.04$), FC ($p<0.001$), EDI ($p= 0,02$), PIF ($p= 0.01$), e S-Index ($p= 0.03$). O GII apresentou redução significativa em comparação às condições basais para FC ($p<0.001$) e aumento da PE_{max} ($p<0.001$). Porém, estas alterações não foram clinicamente importantes e não houve diferença entre os grupos para nenhum desfecho avaliado. Nenhuma intercorrência foi observada. **Conclusão:** A EDET com F-80Hz produz efeitos semelhantes a F-30Hz em indivíduos saudáveis e ambas as frequências provaram ser seguras (NCT03844711).

Palavras-chaves: Estimulação Elétrica, Diafragma, Força Muscular, Voluntários Saudáveis

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INTRODUCTION

In physiological conditions, the good function of the respiratory system depends on the respiratory muscles' adequate force and resistance. Any dysfunction affecting the diaphragm excursion will reduce the pulmonary volume, thereby to changes in the ventilation/perfusion relation, which is crucial for the adequate gas exchange.¹ The physiological aging process promotes a reduction in the number of motor units and muscle fibers' type I and II volume, resulting in muscle force reduction,² which also affects the respiratory musculature.

However, these aging effects can be postponed with training of the respiratory musculature.³ Transcutaneous diaphragmatic electrical stimulation (TEDS) is a technique that can be used for training,⁴ as it promotes an improvement in the resistance and force of the respiratory muscles,^{5,6} in addition to maintaining the pulmonary function.⁷ According to the literature, TEDS parameters generally used are either a pulsed current^{4,8} or an alternated current (AC-2500 Hz)⁹ with 30Hz of stimulation frequency.

Stimulation frequency is an important parameter to evoke muscle force that affects the way a muscle contracts (fused or non-fused tetanic contractions) and the level of evoked force when motor nerves are stimulated.^{10,11} During neuromuscular electrical stimulation, all muscle fibers are activated synchronically. However, to obtain stable (fused tetanic) evoked contractions, stimulation frequencies need to be greater or equal to the muscle fibers fusion, with the most used frequencies varying between 20 and 80 Hz.¹²

The stimulation frequency versus evoked force relation has been determined, and it shows that force increases in a sigmoidal fashion with increasing stimulation frequency, with a plateau being reached between 60 and 100 Hz.¹³ However, as electrical stimulation usually generates submaximal force levels compared to the muscles' maximal force production capacity, higher stimulation frequencies, at the plateau of the force-frequency relation, are preferable as they generate a higher force summation, thereby increasing the level of evoked torque. We have used 80 Hz of stimulation frequency for the knee extensors' strengthening in the elderly with good results,¹⁴ and 80 Hz showed a slightly higher evoked torque compared to 60 Hz.¹⁵

TEDS' effects have been studied in mechanically ventilated patients,⁶ elderly,¹⁶ patients with chronic obstructive pulmonary disease,¹⁷ and post-bariatric surgery.⁷ However, as none of the existent studies evaluated higher stimulation frequencies than 30 Hz clinically, and we were unable to find studies evaluating the effects of higher frequencies even in healthy subjects, little is known about higher stimulation frequencies on cardiovascular and respiratory parameters.

OBJECTIVE

The purpose of this study was to compare the acute effects of two TEDS frequencies (30 Hz and 80 Hz) on respiratory muscle strength and endurance, muscle activation, muscle thickness, diaphragmatic mobility, cardiovascular variables and safety in healthy subjects.

METHODS

A crossover randomized clinical trial approved by the institution Research Ethics Committee (Nr. 80271517.2.0000.5327), was developed according to the CONSORT (Consolidated Standards of Reporting Trials) recommendations and registered at ClinicalTrials.gov (NCT03844711). All participants signed an informed consent form agreeing to participate in the study.

Participants

Male and female adult participants (between 18 and 35 years of age) were evaluated according to the following exclusion criteria: unstable angina, non-controlled systemic arterial hypertension, epilepsy, fever and/or infectious disease, neoplasia, diabetes mellitus, chronic kidney disease, musculoskeletal pathology, having implanted cardiac pace maker and/or decline to participate in the study. The study was conducted at the Exercise Research Laboratory of the School of Physical Education, Physiotherapy and Dance of the Universidade Federal do Rio Grande do Sul. Participants were enrolled through oral invitation in the period of January to March 2019.

Intervention

TEDS was applied with a symmetrical, bipolar, biphasic pulsed current stimulator (Ibramed, model Neurodyn II, Porto Alegre, RS, Brazil) with the following parameters: Group I with a 30 Hz stimulation frequency (SF-30 Hz), and Group II with 80 Hz of stimulation frequency (SF-80 Hz). For both groups, pulse duration was set at 500µs, ramp up time of 1 sec, contraction time of 1 sec, and ramp down time of 2 sec, with 15 rpm of respiratory frequency and lasting for 30 min or until muscle fatigue.

Current intensity was gradually increased up to the maximal tolerated intensity. Four self-adhesive surface electrodes (5x9 cm; Carci, São Paulo, Brazil) were positioned bilaterally besides the xiphoid process, between the 7th and 8th intercostal space, at the mean axillar line.^{9,17} The diaphragm muscle was identified with an ultrasound system (VIVID i®, GE Healthcare, USA) and to obtain an intercostal view, a higher frequency linear array transducer (7 to 18 MHz) is placed at the anterior axillary line, with the transducer positioned to obtain a sagittal image at the intercostal space between the 7th and 8th, or 8th and 9th ribs.¹⁸

Outcomes and evaluations

Evaluations were executed before (T0) and post-intervention (T1), by the same trained rater, in the following order: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) (primary outcomes), diaphragm thickness during expiration (DTE), diaphragm thickness during inspiration (DTI), diaphragm mobility (MOB), diaphragm activation (ACT) and respiratory endurance (END). The rater was trained by experienced researchers. Assessments were performed before and immediately after the interventions.

Cardiovascular variables and safety

The safety of the techniques was evaluated by measuring the systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR), obtained with using the automatic blood pressure measuring device model HEM-7200 (Omron Healthcare Co., Ltd. Kyoto, Japan). The occurrence of complications such as burns caused by the use of electrical stimulation was also evaluated.

Respiratory muscle strength

MIP and MEP were evaluated with an analogic manovacuometer (Comercial Médica®, São Paulo, Brazil), with a scale of ± 120 cmH₂O MIP was measured based on the residual volume, while MEP based on the Total Pulmonary Capacity (TPC). The maneuvers were performed with maximum respiratory effort, at 1-minute intervals, and maintained for at least 1 sec. At least three acceptable (without air leak) and reproducible (range < 10% between the main maneuvers) measurements were obtained, with the highest recorded value being adopted.¹⁹ All measurements were performed following the guidelines for pulmonary functional tests.²⁰ MIP and MEP predicted values were obtained individually based on sex and age.²¹

Diaphragmatic mobility and muscle thickness

An ultrasound system (VIVID i®, GE Healthcare, USA) with a linear transducer (60 mm, 7.5 MHz -VIVID i®, GE Healthcare, USA) was used for mobility assessment. The transducer was embedded in a water-soluble transmission gel promoting acoustic contact without depressing the skin surface, and was positioned perpendicular to the diaphragm, in the right hemithorax, between the nipple and axillary line at the height of the xiphoid process, to locate the hemi-diaphragm.^{18,22}

Ultrasound was used in B-mode to visualize the diaphragm and, after the best view of the hemi-diaphragm, the ultrasound M-mode was activated, frozen and the images of three respiratory movements were analyzed. The difference between the lower and upper mapped displacements were evaluated and compared, adopting the mean of the three values for analysis.^{18,22}

With the same ultrasound devices, diaphragmatic muscle thickness was evaluated, in B-mode, with the probe positioned over the diaphragm apposition zone, close to the costophrenic angle, between the right anterior axillary line and the middle axillary line.^{18,22} The diaphragm thickness was measured from the most superficial hyperechoic line (pleural line) to the deepest hyperechoic line (peritoneal line), with the volunteer in the supine position and being measured in Functional Residual Capacity (FRC) and then in TPC. The images of the diaphragm muscle were frozen^{18,22} and analyzed with the ImageJ software (National Institute of Health, Bethesda, USA), adopting the mean of three values for analysis.^{18,22}

Ventilatory muscles' endurance

An electronic and computerized device (Powerbreathe, K5 model, England, UK) was used with the Breathelink feedback software, with which the strength index (S-index) and an endurance test of the ventilatory muscles were evaluated.²³

The endurance test (resistance) consisted of 30 inspiratory

maneuvers, starting from the residual volume and with a load corresponding to 50% of the MIP. The load was gradually increased, reaching the pre-adjusted load (50% of the MIP) after the 4th inspiratory maneuver. With each resistive effort maneuver, the device measured the sustained average pressure, peak flow and inspired volume reached. From these parameters, the ventilatory power and efficiency were also measured.²³

Diaphragm activation

An electromyograph (EMG System do Brasil Ltda, São Jose do Campos, SP, Brazil), the AqDados software (Lynx Tecnologia Eletrônica Ltda, São Jose do Campos, SP, Brazil) and an HP Notebook computer, equipped with a A/D converter (EMG System do Brasil Ltda, São Jose do Campos, SP, Brazil) were used for diaphragmatic activation evaluation. Pairs of surface electrodes (Ag/AgCl; 1 cm in diameter; with self-adhesive) were used in the bipolar configuration to record the diaphragm muscle electromyographic (EMG) activity. The electrodes were positioned bilaterally at the diaphragm motor points, namely: parasternal region, next to the xiphoid process and between the 7th and 8th intercostal spaces, at the anterior axillary lines²⁴ located through ultrasound. The reference electrode was positioned at the clavicle, a neutral location with no muscle activity. Before placing the electrodes, the skin was shaved and the area cleaned with alcohol.

The EMG signals were stored on the computer and later quantified by calculating the root mean square (RMS) values in the AqDados software (Lynx Tecnologia Eletrônica Ltda, São Jose do Campos, SP, Brazil). Initially, the signal was filtered with a 20-500 Hz band-pass filter and smoothed with a 4th order Butterworth filter. The average of a two-second window from the diaphragmatic contractions was used for data analysis.

Randomization and blinding

The order of intervention was randomized with data generated by a computer program (www.random.org) by a researcher blinded to the groups. Subsequently, the data were kept in a hidden place. The subjects were also blinded as to the order of intervention.

Sample size calculation

The sample size was calculated using the G*POWER 3.1.9.4 software (Fraunhofer Universität Kiel, Germany), estimated based on results of a pilot study previously carried out. After analyzing the MIP between the intervention groups, measured before and after TEDS 30 Hz (-102.5 ± 7.5 cmH₂O) versus TEDS 80 Hz (-109.5 ± 6.8 cmH₂O), and accepting a type I error rate (α) of 0.05, an effect size of $d=0.97$, and power ($1-\alpha$) of 95%, a sample size of 18 individuals was estimated. Assuming a 10% loss, we recruited a total of 20 patients to avoid possible loss or exclusion of patients.

Statistical analysis

The data were presented as mean \pm standard deviation (SD). The distribution normality test was performed using the Shapiro-Wilk test. Significant differences between groups and between assessment-periods were assessed using Generalized Estimation Equations (GEE) with a Bonferroni post hoc test.

In addition, the effect size (ES) was calculated using Cohen's Equation²⁵ for independent data, by calculating the between-groups mean difference (MD) after intervention, and dividing this result by the pooled SD. We also calculated the effect size between the pre and post value of each group, characterizing the within group effect size. Cohen-d to set ES (d) were categorized as trivial (<0.20), small (0.20-0.49), moderate (0.50-0.79), large (0.80-1.29), and very large (>1.30) effect.²⁶ In addition, the mean relative change between the pre- and post-training for each group was calculated.²⁶ A value of $p < 0.05$ was defined for statistical significance. All statistical analyzes were performed using commercial software (Statistical Package for the Social Sciences, version 20.0 SPSS Inc., Chicago, IL, USA).

RESULTS

Thirty individuals were selected,²⁰ of whom met the eligibility criteria and were included (Figure 1). Their mean aged 28.2 ± 7.2 years, weight 71.8 ± 16.6 kg and body mass index 24.8 ± 4.2 kg/m². There was no loss of patients or follow-up. ES will only be shown for statistically significant comparisons, because ES were trivial ($d < 0.2$) for all non-significant comparisons.

Regarding hemodynamic responses, there was no difference between groups in relation to SBP, DBP and HR. However, within the group, there was a reduction in SBP from T0 to T1 in SF-30 Hz ($MD = -3.5 \pm 1.7$ mmHg; $p = 0.04$) with small ES (Table 1). A reduction in HR was observed in both groups (MD -SF-30 Hz = -7.8 ± 1.5 bpm; $p < 0.0001$; SF-80 Hz = -5.05 ± 1.7 bpm; $p = 0.003$) and SF-30 Hz with large ES and SF-80 Hz with moderate ES however between groups with trivial ES (Table 1).

Table 1 . Outcome variables' results

Outcomes	SF-30 Hz			SF-80 Hz			Between Groups Difference	
	Pre (n= 20)	Post (n= 20)	d1	Pre (n= 20)	Post (n= 20)	d1	d2	
<i>Hemodynamics</i>								
SBP (mmHg)	117.5 (11.6)	114 (13.13)*	0.39	116.5 (9.3)	113.5 (10.4)	0.32	0.04	0.84
DBP (mmHg)	78 (5.23)	77 (5.71)	0.16	79 (6.4)	78.5 (4.89)	0.05	0.28	0.79
HR (bpm)	76.4 (10.5)	68.6 (7.59)*	0.95	73.4 (10.1)	68.3 (7.6)*	0.56	0.03	0.28
<i>Echography</i>								
DTE (cm)	0.14 (0.03)	0.15 (0.04)	0.52	0.14 (0.03)	0.15 (0.03)	0.37	0	0.67
DTI (cm)	0.29 (0.11)	0.26 (0.08)*	0.30	0.28 (0.1)	0.27 (0.07)	0.15	-0.13	0.25
MOB (cm)	1.82 (0.59)	1.84 (0.53)	0.05	1.74 (0.55)	1.85 (0.52)	0.34	-0.01	0.28
<i>Respiratory Pressure</i>								
MIP (cmH2O)	-107 (20.5)	-108 (19.3)	0.10	-111 (17.7)	-111 (18.8)	0	0.15	0.30
MEP (cmH2O)	98 (23.9)	100.5 (22.1)	0.13	97 (24.3)	103.5 (19.5)*	0.55	-0.14	0.18
<i>Endurance</i>								
Power (Watts)	11.3 (7.7)	12.02 (9.5)	0.20	10.4 (5.9)	11.7 (7.04)	0.34	0.03	0.54
Energy (J)	193.4 (132.2)	194.3 (135.5)	0.01	168.6 (82.2)	180.2 (105.3)	0.31	0.11	0.25
Pressure (cmH2O)	36.2 (17.1)	35.4 (15.5)	0.10	32.8 (10.2)	36.1 (14.2)	0.30	0.04	0.05*
Flow (L/s)	4.6 (1.5)	4.8 (1.7)	0.37	4.7 (1.5)	4.7 (1.4)	0	0.06	0.37
Volume (L)	3.6 (1.03)	3.6 (1.01)	0	3.5 (1.05)	3.6 (1.1)	0.21	0	0.59
PIF (L/s)	4.7 (1.4)	4.4 (1.7)*	0.47	4.3 (1.5)	4.3 (1.4)	0	0.06	0.31
S-Index (cmH2O)	84.1 (28.3)	78.7 (32.61)*	0.42	77.2 (28)	71.3 (29.5)	0.17	0.23	0.95
<i>Diaphragm Activation</i>								
6° RIS (RMS μ V)	19.12 (9.9)	17.9 (10.5)	0.31	18.1 (9.4)	16.3 (7.1)	0.30	0.17	0.54
6° LIS (RMS μ V)	23.4 (8.7)	19.9 (7.4)*	0.51	20.4 (9.7)	19.2 (8.2)	0.27	0.08	0.13
8° RIS (RMS μ V)	17.8 (5.5)	18.6 (6.8)	0.18	15.7 (4.6)	15.6 (4.1)	0.02	0.53	0.39
8° LIS (RMS μ V)	24.2 (17.1)	19.5 (7.05)	0.30	20.03 (8.5)	19.4 (9.2)	0.09	0.01	0.29

Values= mean (standard deviation); Analysis of Variance followed by Bonferroni test. * $p < 0.05$; Values Cohen's d, d1 effect size for within group difference, d2 effect size for difference between groups; vs: versus; SF: Stimulation Frequency; SF-30 Hz vs SF-80 Hz; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HR: Heart Rate; DTE: Diaphragmatic Thickness during Expiration; DTI: Diaphragmatic Thickness during Inspiration; MOB: Mobility; MIP: Maximal Inspiratory Pressure; MEP: Maximal Expiratory Pressure; PIF: Peak Inspiratory Flux; S-INDEX: Inspiratory Muscles Intensity Index; RIS: Right Intercostal Space; LIS: Left Intercostal Space; RMS: root of mean square

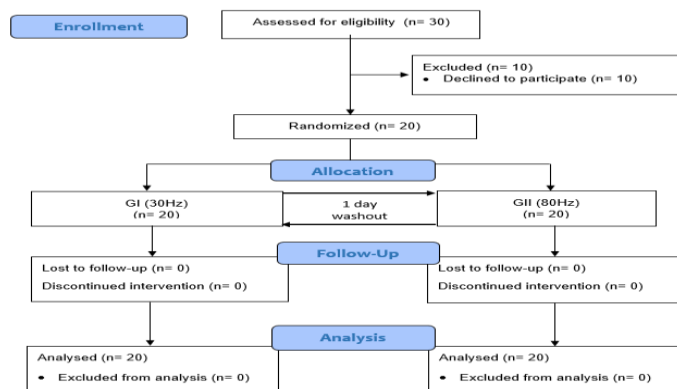


Figure 1. Flowchart of the participants' selection

There was no between-groups difference in MIP and MEP at the two evaluated moments. However, there was a significant increase in MEP compared to baseline ($MD = 6.5 \pm 1.7$ cmH₂O; $p < 0.0001$) with SF-80 Hz (Table 1) with moderate ES (Table 1).

No between-groups difference was found for the ultrasound outcomes. However, DTI decreased from T0 to T1 in the SF-30 Hz group ($MD = -0.032 \pm 0.14$ cm; $p < 0.02$) with small ES (Table 1). As for endurance, there were no between-groups differences for the incremental test (power, energy, flow and volume). On the other hand, SF-30 Hz showed a reduction from T0 to T1 in the PIF ($MD = -0.36 \pm 0.14$ L/s; $p = 0.01$) with moderate ES and in the S-Index ($MD = -5.46 \pm 2.6$ cmH₂O; $p = 0.03$) with moderate ES. Diaphragmatic activation also did not show significant differences between groups. No complications were observed during this study.

DISCUSSION

The results of this clinical trial demonstrated no difference between the TEDS frequencies (SF-30 Hz vs SF-80 Hz) in healthy individuals in relation to respiratory muscle strength and endurance, mobility, diaphragm thickness, muscle activation and cardiovascular variables after the intervention. So far, this is the first known study that evaluated the acute effect of TEDS by comparing different frequencies and evaluating the outcomes here presented. Although there is no information available to compare our results to, some studies have evaluated the effect of TEDS separately, with different protocols and in different populations.

Despite the reduction in HR in both groups (SF-30 Hz = -7.8, SF-80 Hz = -5.05 bpm) and SBP in SF-30 Hz (-3.5 mmHg), there was no minimal clinically important difference.²⁷ The hemodynamic change identified in our results may be the result of central mechanisms related to the metaboreflex²⁸ or associated with a reduction in sympathetic activation, similar to the use of transcutaneous electrical nerve stimulation (TENS) that can interfere with baroreflex sensitivity.²⁹

According to Schoser et al.³⁰ MIP can be used to predict survival in patients with chronic diseases³¹⁻³³ and mechanically ventilated.³⁴ In healthy women, Cancelliero et al.⁴ identified an increase in MIP with 12 sessions of TEDS (SF-30 Hz), and our results are not in agreement with their results. This can be explained by the different number of sessions that were carried out, since in our study a single session was performed per group, as our goal was to analyze the acute effects of the two TEDS frequencies on our variables of interest.

Although the effect of TEDS is directed to the inspiratory muscles, we found an increase in MEP in SF-80 Hz. The same was not found in a previous study with the use of TEDS with greater expiratory muscle strength, represented by MEP, in patients with COPD.⁹ It appears that the increase in MEP is due to the muscular overlap of the stimulated region and that the applied electric current generates a wide electric field, which would be sufficient to stimulate other muscle groups,^{4,9,35} include those of the abdominal wall and of the rib cage ones,²⁴ as was also found previously in studies carried out with rats, observing the stimulation of other muscle groups.^{36,37}

The TEDS with SF-50 Hz in rats improved the energetic conditions of the respiratory muscles³⁷ and promoted changes in the diaphragmatic muscle fibers profile, reducing type I fiber and increasing fiber IId.³⁶ Although there was no difference in TEDS with an SF-80 Hz in relation to SF-30 Hz in this study, the main goal of electrical stimulation is to gain muscle strength, and both frequencies activate slow and fast motor units, even at low levels of evoked diaphragm force.

Nevertheless, SF-80 Hz may produce higher evoked forces due to the force-frequency relation where a greater force summation is observed with SFs between 60 and 100 Hz (i.e., the plateau of the force-frequency relation).¹³ Histochemical studies reveal that the diaphragm is composed of 55% type I fibers, approximately 20% of type IIa fibers and 25% type IIb fibers which are more susceptible to fatigue however which are of fast contracting with high glycolytic and oxidative capacity.³⁸

Thus, type II fibers generate a higher level of strength and have a higher excitability threshold, which may justify a greater gain in MEP in SF-80 Hz, probably due to a larger activation of

these fast muscle fibers.

As previously mentioned, the effect of electrical stimulation for muscle strength production is proportional to SF, according to the evoked force-frequency relation. However, the higher the frequency, the greater the effects on metabolic demand and muscle fatigue.³⁹ With SF around 20 Hz, it is possible to promote tetanic contractions in type I fibers, as they have smaller caliber nerve fibers; however, fast fibers (type II) have larger caliber motor axons and, consequently, lower excitability threshold, requiring the use of higher SF, around 50 Hz.³⁹ This may be one of the explanations for why SF-80 Hz produced greater pressure in the manovacuometry and endurance test in relation to SF-30 Hz. As SF-80 Hz generates higher evoked forces, it may be better suited, for example, to stimulate the fast contraction fibers that are diminished in the elderly² and in COPD patients,⁴⁰ SF-80 Hz would be preferable, as the greater sum of force generated with 80 Hz, as described in the force-frequency ratio and would allow greater mechanical overload to strengthen the respiratory muscles. Therefore, SF-80 Hz may be preferable for strength training and SF-30 Hz may be preferable for endurance training, so the choice of SF should be in accordance with the objective of the patient treatment.

Regarding muscle thickness and diaphragmatic mobility, there are no studies evaluating the effects of TEDS on these outcomes in healthy individuals. We found a reduction in DTI with SF-30 Hz, and this reduction in diaphragm thickness may be associated with a reduction in strength. Since the diaphragm is the most important muscle in the respiratory system, any reduction in diaphragm muscle strength is of clinical importance, because the threshold of diaphragm fatigue can be approached during periods of increased respiratory load⁴¹ and is a proven failure predictor of wean ventilation mechanics.⁴²

However, the diaphragmatic thicknesses observed in our results corroborate reported a normal diaphragm thickness on expiration being 0.15 cm, and an increase of at least 20% of the diaphragm thickness up to the total lung capacity^{22,43,44} and range of motion was reported in the range of 1.9 to 9cm.^{18,22,45}

Situations involving fatigue have been correlated with increased muscle activation⁴⁶ therefore the assessment of muscle activation and its relation with fatigue is clinically important. Our results demonstrated that there were no changes in muscle activation in both hemi-diaphragm, demonstrating that TEDS was safe and did not interfere with respiratory muscle work, corroborating with Lin, Guan, Wu and Chen's²⁴ findings, who investigated the activation of respiratory muscles in healthy individuals and COPD patients. The authors identified an increase in muscle activation values in COPD patients, which was not identified in healthy individuals, suggesting that COPD patients had greater respiratory work, obviously due to the disease. In real clinical conditions, muscle activation has high sensitivity and suffers easy interference by neighboring muscles, due to cross talk.²⁴

Although this study does not show differences regarding the acute effects of TEDS on different SFs, our results demonstrate that the use of the higher frequency increased the MEP in the SF-80 Hz, while the lower frequency in SF-30 Hz reduced the PIF and the S-index, being related to the dynamic strength of the inspiratory musculature and to the respiratory musculature performance. It is necessary to take into account that during the study there was no adverse events such as skin

irritation, burns, allergy, pain and no significant changes in heart rate and bradypnea. The intervention is safe, feasible, and promising for the rehabilitation of the respiratory muscles.

Clinical Applicability

The study showed that both SF are similar, however, the choice of frequency should be in accordance with the purpose of training being strength or resistance. Therefore, it is important to take into account, the higher the SF greater the motor recruitment, which will produce greater muscle strength. In addition, this study allows towards rehabilitation protocols with greater mechanical overload, specificity and reversibility.

Study Limitations

The scarcity of studies on TEDS evaluating endurance, diaphragmatic structure with ultrasound and diaphragm muscle surface EMG, makes it difficult to compare the results of this study with the findings of other authors. As disadvantages of surface EMG, there is the possibility of interference from neighboring abdominal muscles due to the diaphragm depth and heart beats interference in the left hemi-diaphragm. However, the choice of electrodes, their proper positioning and the diaphragm location by ultrasound examination, defined after extensive literary investigation, probably minimized this interference.

Another limitation of the study was the use of the linear transducer for the assessment of diaphragmatic mobility, since the recommended would be the low frequency (2 to 6 MHz) curvilinear transducer.¹⁸ The evaluation with the linear transducer did not preclude the analysis for this outcome, since the results showed normal values.

CONCLUSION

This study demonstrated that there were no differences for the acute effect of TEDS at the 30 and 80 Hz of stimulation frequencies for the evaluated outcomes and both frequencies proved to be safe in healthy individuals.

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