Effects Of Home-Based Inspiratory Muscle Training In Sickle Cell Disease (Scd) Patients

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Research

Keywords: Inspiratory muscle training, sickle cell disease, Volumetric capnography, exercise tolerance, PImax, PEmax

Posted Date: March 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17509/v1

Version of Record: A version of this preprint was published at Hematology, Transfusion and Cell Therapy on September 1st, 2020. See the published version at https://doi.org/10.1016/j.htct.2020.08.005.

Abstract

PURPOSE: to evaluate the effects of inspiratory muscle training (IMT) in adult sickle cell disease (SCD) patients in relation to respiratory muscle strength (RMS) variables; lung function; exercise tolerance; blood lactation concentration; limitation imposed by dyspnea in activities of daily living and; impact of fatigue on quality of life.

METHODS: a randomized, single-blind study, with true load (TG) and sham load group (SG) design of IMT. Initial assessment included spirometry, volumetric capnography (VCap), and measurement of RMS by maximal inspiratory and expiratory pressure (PImax and PEmax). The Medical Research Council (MRC) dyspnea scale and the modified fatigue impact scale (MFIS) were also applied, and blood lactate concentration (Lac) was measured before and after the 6-minute walk test (6MWT). After this initial assessment, the patient used the IMT device at home daily, returning every 6 weeks for RMS reassessment. Both groups used the same device and were unaware of which group they were in. After a total period of 18 weeks, the patients underwent the final evaluation as initially performed.

RESULTS: 25 patients in total participated up to the end of the study (median age 42 years). There were no significant differences between TG and SG groups based on age, sex, body mass index, severity of genotype. At the end of the training, in both groups there was a significant increase in PEmax and PImax, improvement in Vcap and in exercise tolerance and reduction of dyspnea in performing daily life activities. The same improvement was observed in patients grouped according to disease severity (SS and Sβ 0 vs SC and Sβ +), without differences between groups.

CONCLUSION: home-based inspiratory muscle training brings benefits to outpatients with SCD.

Backgroud

Sickle cell disease (SCD) is an autosomal recessive, clinically heterogeneous disorder affecting millions worldwide, and is characterized by hemolytic anemia, progressive vascular injury, vase-occlusive crises, end-organ injury and a reduced life expectancy (1,2). Pulmonary complications, including acute chest syndrome, pulmonary hypertension, venous thromboembolism, asthma/recurrent wheezing and sleep-disordered breathing are an important cause of morbidity and mortality in SCD(3,4).

These pulmonary disorders often lead to breathlessness and mild abnormalities in pulmonary function tests (PFT) (5). In addition, pulmonary complications, as well as other complications inherent to SCD, such as arterial oxyhemoglobin desaturation, anemia, damage of organs and tissues related to the adaptation to the exercise (such as heart or skeletal muscles) may cause exercise intolerance (6,7).

Several mechanisms are related to exercise intolerance observed in sickle cell disease patients such as reduced oxygen carrying capacity related to high hemoglobin S (HbS) levels, changes in cardiac function and structure due to chronic volume overload, leading to heart failure (7,8). Pulmonary complications, such as pulmonary hypertension, lead to less tissue oxygenation, decreasing exercise tolerance (9). In

addition, other factors such as anemia, pulmonary vascular disease with oxygen desaturation and musculoskeletal problems further influence the low tolerance to exercise, conferring to this complaint a multifactor character (10).

One way to assess exercise intolerance is to measure the moment when a rapid increase in the concentration of lactate in the blood ([Lac]) occurs and the lactate threshold is reached (11). However, patients with sickle cell disease may present a much higher value of [Lac] when compared to healthy individuals, given the lower availability of oxygen reaching the muscle cells, which increases the use of anaerobic metabolism (12).

Inspiratory muscle training (IMT) has been shown to be an efficient method of improving exercise tolerance and inspiratory and expiratory muscle strength in a number of diseases (13,14). Some studies have shown that IMT even enables increased oxygen uptake in the blood, decreases the perception of dyspnea and delays the onset of premature fatigue (15,16). However, the effects of IMT in patients with sickle cell anemia are relatively unknown. To our knowledge a single case study evaluated the application of this training, and showed, in that patient, improvement in exercise tolerance and respiratory muscle strength (17).

The aim of the current study was to investigate the effects of inspiratory muscle training in SCD patients. We hypothesized that eighteen weeks of IMT would enhance respiratory muscle strength and exercise tolerance, improve measurements in pulmonary function tests and would reduce the impact of fatigue on quality of life.

Methods

Participants: Patients with diagnosis of sickle cell disease (homozygote HbS, compound heterozygote HbS and HbC or β-thalassemia) were recruited from the Hemoglobinopathy outpatient clinic of the Hematology and Transfusion Medicine Center of the School of Medical Sciences, University of Campinas (UNICAMP).

Exclusion criteria comprised disorders that could prevent proper assessment or IMT during the study period, such as asthma or neurological disorders. For result analysis, patients who missed the reevaluation more than once or who remained for more than one week without training were also excluded. All patients were instructed to maintain their medical treatments throughout the research period.

Experimental design: This was a randomized, single-blind study, with true and sham load group (TG and SG respectively) design of inspiratory muscle training for SCD patients. All patients who agreed to participate signed a written informed consent form. The study was approved by the UNICAMP ethics committee and was performed according to the Declaration of Helsinki 2008. Patients were randomized by order of evaluation, being alternated between SG and TG sequentially. TG performed the IMT with true load, whereas SG performed the IMT with an equipment with no true load.

Both groups used the same equipment model for IMT and were unaware of which group they were in. The study began with an assessment that included spirometry, volumetric capnography (VCap), and assessment of respiratory muscle strength by maximal inspiratory and expiratory pressure, PImax and PEmax, respectively. Two scales, the Medical Research Council (MRC) dyspnea scale and the modified fatigue impact scale (MFIS) were then applied, and [Lac] was subsequently assessed before and after the 6-minute walk test (6MWT). After this initial evaluation, the patients were instructed on the use of the IMT device and trained in their home daily assignment, returning every 6 weeks for PImax and PEmax assessment, when the training load was adjusted. Each newly included patient received a training device with true or sham load according to an alternating list kept by the investigator. After a total period of 18 weeks, the patients underwent the final evaluation, which was composed of the same tests as the first evaluation (Fig. 1).

Entire study was carried out at Hospital de Clínicas of UNICAMP. All evaluations at baseline and at the end of the 18th week were performed by the same physiotherapists according to protocols and under the same conditions. The evaluations were carried out by three physiotherapists, one being responsible for volumetric capnography, the other for spirometry and the third, along with the second, for the other evaluations.

Lung Function

All patients underwent spirometry before and 20 minutes after inhalation of 400 µg of salbutamol using a spirometer (EasyOne-PC®, NDD Medizintechnik AG, Zurich, Switzerland). The test was performed according to American Society guidelines (18) and the analyzed values were forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC ratio and mean forced expiratory flow between 25–75% of expired vital capacity (FEF25-75%). PFT was always performed during the mornings at the Pulmonary Function Laboratory of the Pulmonary Diseases Service from Hospital das Clínicas of UNICAMP.

Volumetric Capnography

To perform the volumetric capnography VCap, a CO2SMOS Plus 8100 (Dixtal/Novametrix; Respironics, Murrisville, PA, USA) was used. The VCap examinations lasted approximately 10 minutes each and were always performed by the same technician in the mornings at the Pulmonary Function Laboratory of the Pulmonary Diseases Service at Hospital das Clínicas of UNICAMP.

The patient remained seated in a comfortable position during the entire time of the test. During the first five minutes, the patients would rest, while the instructions for the examination were provided. The carbon dioxide (CO_2) sensor, flow sensor and pulse oximeter were connected to a non-invasive monitor. A pulse oximetry sensor was installed on the index finger.

All patients were asked to breathe room air, regularly, through a mouthpiece, which was connected to the capnograph sensors; patients used a nasal clip and breathed with no visual feedback, for some seconds

in order to adapt to this situation.

The Analysis Plus software was used for online recording of respiratory cycles, and this continued for five minutes. An off-line sequence of patients' respiratory cycles was selected at the end of the data collection to accommodate a variation of less than 15% for the expiratory tidal volume and less than 5% for the end tidal partial pressure of CO_2 concentration ($PetCO_2$). In addition, sequences showing phases 2 and 3 slopes equal to zero were eliminated, along with discrepant values (19). The following parameters were analyzed from the data collected in the VCap: alveolar minute volume (MV alv), anatomical dead space (VD), production of CO_2 per minute (VCO_2), inspiratory volume (Vi), expiratory volume (Ve), respiratory rate (RR), oxygen saturation (SpO_2), inspiratory time(Ti), expiratory time (Te), peak inspiratory flow (PIF), peak expiratory flow (PEF), Tobin index (RSBI), elimination of CO_2 per breath (VCO_2/br), phase 2 slope (P2Slp), phase 3 slope (P3Slp), phase 3 slope normalized according to expired tidal volume (P3Slp/Ve).

Respiratory muscle strength

The maximum static expiratory and inspiratory pressures (PEmax and PImax, respectively) were determined using a digital manuvacuometer (MVD30-Globalmed). The patient used a nasal clip and remained seated during these maneuvers. The PImax and PEmax evaluation maneuvers were performed from residual volume (RV) and total lung capacity (TLC), respectively.

For data analysis, the highest value was recorded, and each maneuver was performed at least five times, providing that the difference between the highest and the lowest value among three values presented a difference less than 10% (20).

Medical Research Council dyspnea scale (MRC)

The MRC scale consists of only five items and the patient chooses the item that corresponds to how much dyspnea limits their daily living activities (DLA). The patient then reports the degree of dyspnea subjectively, choosing a value between 1 and 5, being: 1 (only suffers from shortness of breath during intense exercise), 2 (suffers from shortness of breath when walking hastily or climbing a ramp 3) (walking slower than people of the same age due to shortness of breath or having to stop to breathe even when walking slowly), 4 (stops to breathe after walking less than 100 meters or after a few minutes) and 5 (feels so short of breath that does not leave the house anymore, or feels short of breath when getting dressed). The higher the score, the greater limitation of DLA due to dyspnea. When the patient reported no dyspnea status the classification was 0 (5). In Brazil, the MRC scale was validated by Kovelis et al 2008 (21)

Modified Fatigue Impact Scale (MFIS)

MFIS consists of 21 items distributed in three domains: physical (9 items), cognitive (10 items) and psychosocial (2 items), so that for each item there are 4 possible answers, graded from 0 to 4, in which the highest scores reflect greater impact of fatigue.

For the physical domain the scores vary from 0 to 36, for the cognitive range from 0 to 40 and for the psychosocial range from 0 to 8. Therefore, the total score of the MFIS is given by the sum of the three domains and ranges from 0 to 84 points. Values below 38 are classified as absence of fatigue. For values above 38, the higher the score, the greater the degree of patient fatigue.

The MFIS underwent cross-cultural adaptation and was validated for the Portuguese language (in the Brazilian standard), by Pavan et al, in 2007 (22).

Blood lactate concentration

[Lac] was measured pre and immediately after the 6MWT, using a portable lactate meter (Accutrend plus, Roche, Switzerland), which has a procedure previously validated in the scientific literature (23). Measurements were performed in blood collected from fingertips after superficial perforation with a disposable lancet. Cleaning of the skin was carried out before and after the perforation with 70% alcohol-soaked cotton.

Six-minute walk test

All participants performed six-minute walk test (6MWT) according to standardized protocol (24) before and after the IMT period. A pulse oximeter (Nonin Medical, Inc; MN, USA) was used to evaluate pulse rate and oxygen saturation. The modified Borg scale (Borg) was used to evaluate dyspnea and perceived exertion rate scores before and after 6MWT, ranging from 0 to 10 (0 being "nothing" and 10 being "maximal") (25). Patients were gently encouraged periodically to walk on a level surface 30 m long during the 6-minute test.

Inspiratory muscle training procedure

A specific inspiratory training device (POWER®Breathe Wellness, IMT Technologies Ltd., Birmingham, UK) was used for IMT with load adjustment based on PImax. On the day of the first evaluation, the patients were instructed in relation to the handling of the device, since all IMT was performed in their homes. On the day of the first assessment, patients were instructed on how to handle the device, since all IMT was performed at home. The instructions consisted of doing diaphragmatic breathing and not using accessory respiratory muscles during training. The training group used an adjusted load of 30% of the PImax during the first two weeks of training, aiming to facilitate the adaptation to the use of the device, with the load being adjusted by 40% of the PImax during the third week, 50% of the PImax during the fifth week and 60% of the PImax during the sixth week of IMT by the end of the study. Every 6 weeks of IMT, respiratory muscle strength (PImax and PEmax) was reassessed; if PImax was altered the load was readjusted. The SG group performed the training with the same device, but without the flap valve, however, load adjustments were simulated so that the patients did not realize that they used a sham load. Patients from both groups were instructed to perform the IMT daily for 10 minutes per day with a 1-minute rest after 5 minutes, 7 days a week until completing 18 weeks of training. The number of repetitions was between 13 and 15 breaths per minute during the 10 minutes of daily training.

All participants received a paper notepad for notes on the date and time of the IMT. If they did not do IMT on a given day, they could also write down the reason that prevented them from doing so. In addition, for greater reliability when performing IMT at home, patients were also contacted by cell phone (voice or text message) to verify that they were performing the training correctly and daily or to clarify any problems they might have had with the use.

Statistical Analysis

Exploratory data analysis was performed through summary measures (median, minimum and maximum). Comparison between groups was performed using the Mann-Whitney, Fisher's exact or Chi-Square tests. The comparison between times and groups was performed through ANOVA for repeated measures with the transformed responses in ranks. Differences were considered significant in the face of a p-value < 0.05. All statistical analyses were performed using Statistical Analysis System (SAS) for Windows, version 9.4 (SAS Institute Inc, Cary, NC, USA).

Results

Thirty patients were initially recruited, however five were excluded. Three patients from the SG group dropped out for personal reasons and two other patients belonging to the TG group were excluded for remaining more than 7 days without training, one due to pneumonia and the other due to a sickle cell crisis. Therefore, 25 patients in total participated up to the end of the study. Descriptive characteristics of participants are shown in table I. There were no significant differences between TG and SG groups based on age, sex, body mass index (BMI), hemoglobin gene mutation (P > 0.05) (Table 1).

Table 1

Characteristics of the study groups.

Baseline characteristics	All patients	Sham Group (SG)	Training Group (TG)	Statistic comparation between SG and TG
Sex (M/F)	11/14	05/07	06/07	0.8213#
Age (years)	42.0 (28–64)	46.5 (30–64)	40.0 (28–56)	0.1343+
BMI (kg/m^2)	24.9 (15.8–39.7)	24.4 (15.8–39.7)	24.9 (21.1–32.2)	0.8490+
Hemoglobin diagnosis HbSS HbSC HbSβ (0/+)	11 08 03/03	06 05 00/01	05 03 03/02	0.2697§

Values are median (min-max). Statistical tests used: + Mann-Whitney; # Chi-Square; § Fisher's exact test. M: male; F: female; BMI: body mass index; HbSS: homozygous for hemoglobin S; HbSC: heterozygote composed of hemoglobin S and hemoglobin C; HbSβ^0: sickle cell/β-thalassemia phenotype with higher degree of hematological involvement in comparison to HbSβ$^+$ patients; HbSβ$^+$: sickle cell/β-thalassemia phenotype with lower degree of hematological involvement in comparison to HbSβ$^+$ patients.

Lung Function

Regarding lung function, there was no significant change in FVC comparing pre and post IMT for both the SG and TG groups, however FEV1, FEV1/FVC and FEF25-75% presented a significant increase comparing pre and post bronchodilator for both pre and post IMT groups ($p < 0.05$), with no changes in the comparison between the initial and final evaluations (Table 2).

Table 2

Pulmonary function test pre and post bronchodilator.

	Sham Group (N = 12)				True Load Group (N = 13)				
	Initial Evaluation		Final Evaluation		Initial Evaluation		Final Evaluation		P Value
Variable (%predicted)	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD	
FVC	72 (50–92)	69 (50–93)	70 (52–92)	68 (50–89)	78 (56–92)	78 (59–92)	79 (64–94)	80 (64–93)	0.8400
FEV1	72 (49–101)	75 (49–103)	70 (49–95)	72 (51–99)	76 (52–92)	79 (56–97)	76 (65–91)	82 (66–98)	0.0011*
FEV1/FVC	99 (90–111)	101 (92–115)	99 (91–116)	89 (101–114)	99 (93–105)	102 (96–105)	102 (96–105)	99 (91–107)	< 0.0001*
FEF25-75%	75 (38–156)	85 (50–149)	72 (41–140)	78 (39–163)	75 (41–95)	85 (50–115)	76 (58–120)	93 (73–143)	< 0.0001*

Values are median (min-max) * The statistical test used was ANOVA on ranks transformation, showing a time effect of the training for both groups ($p < 0.05$) in relation to the pre and post bronchodilator (BD) test for each evaluation (initial and final) in all analysis, except for FVC. Pre BD < Post BD. Statistical analysis showed no interaction or group effect. N: number of subjects per group; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; FEV1/FVC ratio: ratio of forced expiratory volume in 1 second to forced vital capacity; FEF25-75%: forced mean expiratory flow.

Volumetric Capnography

The results of volumetric capnography showed no statistically significant change ($p > 0.05$) in the variables evaluated in the comparison between the two groups (SG and TG), except for the inspiratory time (Ti) variable which was significantly higher in SG compared to TG ($p < 0.05$, group effect). However, the following variables: inspiratory tidal volume (Vi), peak expiratory flow (PEF), phase 3 slope of the capnography curve (P3Slp), phase 3 slope normalized according to expired tidal volume (P3Slp/Ve), peak inspiratory flow (PIF) and Tobin Index (RSBI) had a significant change regarding the time effect in both groups ($p < 0.05$) (Table 3).

Table 3

Volumetric capnography

	Sham Group (N = 12)		True Load Group (N = 13)		
Variable, Unit	Initial Evaluation	Final Evaluation	Initial Evaluation	Final Evaluation	P Value
RR (cpm)	14.3 (12.1–17.9)	15.3 (11.5–20.4)	18.3 (11.3–25.0)	18.1 (10.0-21.7)	0.2649
HR (bpm)	61.6 (49.7–91.2)	68.3 (51.6-90.5)	64.7 (56.6–80.2)	68.7 (55.1–89.3)	0.1019
SpO_2 (%)	97.1 (93.7–98.2)	96.6 (92.3–98.1)	97.1 (93.1–98.6)	96.8 (92.8–98.1)	0.0528
VD (aw) (ml)	113 (84–181)	116 (73–166)	116 (58–170)	119 (72–159)	0.0696
$PeCO_2$ (mmHg)	20.4 (17.0-25.9)	20.4 (16.8–25.9)	19.5 (13.8–25.3)	19.5 (16.0-26.3)	0.6182
Vi (ml)	399 (240–696)	471 (235–992)	353 (153–763)	404 (217–757)	0.0264*
Ti (sec)	1.7 (1.4–1.9)	1.6 (1.2–2.1)	1.4 (0.9-2.0)	1.4 (1.0-2.4)	0.0483#
Te (sec)	2.4 (1.8-3.0)	2.4 (1.7–3.4)	2.0 (1.9–3.5)	2.0 (1.5–3.7)	0.0621
PIF (l/min)	20.7 (13.8–41.1)	24.0 (16.2–57.8)	25.1 (11.3–48.2)	25.8 (14.2–44.5)	0.0285*
RSBI (VT/RR)	33.1 (20.5–71.2)	28.9 (15.6–87.7)	50.1 (20.8-128.7)	37.7 (16.8–95.4)	0.0207*
P2Slp (mmHg)	336 (215–572)	263 (172–614)	339 (134–738)	304 (189–581)	0.1434
P3Slp (mmHg)	23.5 (8.1–62.2)	19.1 (4.8–43.3)	29.1 (5.7-151.6)	24.7 (3.6–83.1)	0.0153*
NIP (cmH_2O)	0.0 (-0.5-0.0)	0.0 (-1.4-0.0)	0.0 (-0.9-0.0)	0.0 (-0.7-0.0)	0.8099
MV alv (ml)	3.8 (2.0–9.0)	4.2 (2.6–13.9)	4.7 (1.5–13.9)	4.5 (2.4–11.6)	0.2882
VCO_2 (ml/min)	122 (91–309)	135 (81–337)	151 (47–268)	150 (90–284)	0.5718
$PetCO_2$ (mmHg)	34.6 (25.9–42.2)	33.9 (25.0-41.5)	36.7 (23.2–40.7)	33.7 (23.0-42.2)	0.7927
Ve (ml)	393 (237–700)	456 (205–1018)	366 (142–743)	400 (226–755)	0.1301
PEF (l/min)	17.0 (11.2–28.0)	19.4 (12.7–44.6)	18.8 (7.6–58.3)	19.0 (8.1–54.3)	0.0382*

	Sham Group (N = 12)		True Load Group (N = 13)		
VCO_2/br (ml/breath)	8.5 (5.2–19.5)	9.6 (3.8–20.4)	9.6 (2.7–14.2)	8.6 (5.1–16.0)	0.2774
P3Slp/Ve	0.06 (0.01–0.22)	0.04 (0.00-0.19)	0.08 (0.00-1.07)	0.05 (0.00-0.37)	0.0254*

Values are median (min-max). * The statistical test used was ANOVA on ranks transformation, showing a time effect of the training for both groups ($p < 0.05$) in all analysis, except for Ti (#), where significant difference between groups were independent of the training (group effect). Vi: initial evaluation < final evaluation; Ti: sham group > true load group; PIF: initial evaluation < final evaluation; RSBI: initial evaluation > final evaluation; P3Slp: initial evaluation > final evaluation; PEF: initial evaluation < final evaluation; P3Slp/Ve: initial evaluation > final evaluation There was no significant result in the statistical analysis of interaction in any of the variables. RR: respiratory rate; HR: heart rate; SpO_2: oxygen saturation; VD (aw): anatomical dead space; $PeCO_2$: mean end-tidal partial pressure of CO_2; Vi: inspiratory tidal volume; Ti: inspiratory time; Te: expiratory time; PIF: peak inspiratory flow; RSBI: Tobin index; VT: tidal volume; P2Slp: phase 2 slope; P3Slp: phase 3 slope; NIP: negative inspiratory pressure; MV alv: alveolar minute volume; VCO_2: CO_2 production; $PetCO_2$: end-tidal partial pressure of CO_2; Ve: expiratory tidal volume; PEF: peak expiratory flow; VCO_2/br: CO_2 production per breath; P3Slp/Ve: phase 3 slope normalized according to expired tidal volume.

Respiratory muscle strength

In both groups, PImax and PEmax significantly increased after IMT ($p < 0.05$, time effect). For PEmax there was a significant increase in the second evaluation in relation to the first and in the fourth evaluation in relation to the third evaluation. Regarding PImax, there was a significant increase in the second evaluation compared to the first and in the third in relation to the second (Figs. 2A and 2B).

Medical Research Council (MRC) dyspnea scale and Modified Fatigue Impact Scale (MFIS)

The MRC scale showed a significant reduction in the final evaluation of the values measured ($p < 0.05$, time effect) pre-IMT, for both SG and TG (Fig. 2C), with the medians of both groups varying from identical way. No significant changes were detected in MFIS.

Six-minute walk test

In both groups there was a significant increase in the distance walked before the IMT compared to the post-period evaluation ($p < 0.05$) (Fig. 2D and Table 4).

Table 4

six-minute walk test and related tests.

	Sham Group (N = 12)		True Load Group (N = 13)		
Variable, Unit	Initial Evaluation	Final Evaluation	Initial Evaluation	Final Evaluation	P value
6MWT – distance (m)	493.1 (365.0-565.0)	500.4 (385.0-639.4)	493.5 (420.0-600.0)	540.0 (432.8–647.0)	0.0019*
Borg (0–10)					
Pre 6MWT	0.7 (0–3)	0.0 (0–5)	1.0 (0–6)	2.0 (0–3)	< 0.0001*
Post 6MWT	4.0 (2–7)	2.5 (1–8)	3.0 (0–8)	4.0 (0.5-9)	
[Lac] (mmol.L^{-1})					
Pre 6MWT	2.0 (1.0-5.2)	1.9 (0.8–6.1)	2.5 (0.8–6.1)	1.8 (0.8–8.1)	0.0144*
Post 6MWT	2.7 (1.6–4.7)	2.2 (1.5-6.0)	2.5 (0.8–6.4)	3.1 (1.2–8.6)	
SpO_2 (%)					
Pre 6MWT	96.5 (92–100)	96.5 (86–100)	97.0 (91–99)	96.0 (88–99)	0.0773
Post 6MWT	94.5 (89–100)	95.5 (83–99)	96.0 (80–99)	92 (82–99)	

Values are median (min-max) * The statistical test used was ANOVA on ranks transformation, showing a time effect of the training for both groups ($p < 0.05$) in all analysis. 6MWT - distance: initial evaluation < final evaluation; Borg: Pre 6MWT < Post 6MWT; [Lac]: Pre 6MWT < Post 6MWT. Statistical analysis showed no interaction or group effect. N: number of subjects per group; 6MWT: 6-minute walk test; Borg: Modified Borg scale; SpO_2: oxygen saturation.

Blood Lactate Concentration

[Lac] was significantly increased ($p < 0.05$) comparing pre and post 6MWT, before and after IMT in both groups, here were no changes however in the comparison between the initial and final evaluations (Table 4).

Modified Borg Scale

The values reported by the patients on the Borg, used before and after the 6MWT application, showed a significant increase between the pre and post 6MWT evaluation in both groups ($p < 0.05$), but there were no changes in the comparison between the initial and final evaluations (Table 4).

All data were also analyzed in patients grouped according sickle cell disease severity, ie HbSS plus $HbS\beta^o$ vs HbSC plus $HbS\beta^+$ and revealed no significant difference.

Discussion

This study aimed to evaluate the effects of long term IMT on sickle cell disease patients. In both groups: control or true load, there was a significant increase in inspiratory and expiratory muscle strength at the end of the training protocol, improvement in volumetric capnography variables, exercise tolerance and reduction of dyspnea in performing daily life activities. Regarding respiratory muscle strength, although the effects of IMT in patients with sickle cell disease have been poorly studied (17), studies conducted in individuals with chronic obstructive pulmonary disease (COPD) (13), older women (26) or with ankylosing spondylitis (14) showed an increase in PImax and PEmax after the termination of the training protocol. Ciesla includes IMT as one of the types of breathing exercises and points out several benefits these exercises, as it improves thoracic-cage mobility, increased tidal volume and increased inspiratory capacity (27). Our study however also showed a significant increase in respiratory muscle strength in the control group. We hypothesized that, as the two groups performed 10 min of deep inspiration daily, that is, they actually did breathing exercises, despite the real resistance to the inspiratory flow of home exercises for TG, the IMT was possibly able to modify the respiratory mechanics in both groups, leading to an increase of PImax and PEmax, not only for the true load group, but also for the control group.

Spirometry results here obtained showed no significant differences regarding initial and final evaluation, in neither group. No were there any changes in these variables reported for older women, COPD, and heart failure (26,28,29). On the other hand, in our study, the volumetric capnography showed significant changes in some variables after IMT, in both groups. Inspired volume (Vi), peak expiratory flow (PEF) and peak inspiratory flow (PIF) increased significantly, while the Tobin index (RSBI) decreased significantly. These changes suggest that daily deeper inspiratory exercises influenced an increase in inspired and exhaled air volume, even in the control group.

Another variable, the phase 3 slope (P3Slp), decreased significantly in both groups in the final assessment compared to the initial assessment. As phase 3 of the capnogram represents the elimination of pure alveolar gas, the greater the plateau of the curve in this phase the more homogenous is the distribution of ventilation in distal airspaces (30). Alveolar recruitment is related to reduced P3Slp (31). Therefore, the reduction in P3Slp observed in this study suggests that there was alveolar recruitment after IMT. Furthermore, considering that the higher the expired volume (Ve), the lower the P3Slp, this variable was normalized according to the expired tidal volume (P3Slp/Ve) (32), which also decreased after IMT, confirming the result presented by P3Slp.

In both groups after IMT, a significant reduction in dyspnea assessed by MRC was observed. This scale has been used for many years to classify the limitation imposed by dyspnea on DLA (21,33). The scale is a simple application tool, which allows the patient to indicate to what extent their dyspnea affects their mobility. This is a widely used scale for the assessment of dyspnea in patients with COPD, however, this scale has also been used as an instrument for the indirect measurement of dyspnea in a study of patients with sickle cell disease (5). Other studies that evaluated the effects of IMT in patients with pulmonary arterial hypertension and in COPD showed a reduction in dyspnea after the end of the training

protocol in the true load group (34,35). In our study, improvement in dyspnea may have occurred due to increased respiratory muscle strength and increased post-IMT alveolar recruitment in both groups.

The MFIS, which allows indirect assess of the impact of fatigue on quality of life, did not change significantly at the end of IMT in both groups. This scale, originally developed by Fisk et al in 1994 (36), though widely used to assess the impact of fatigue on quality of life in patients with multiple sclerosis, has also been used to assess indirect fatigue, in a study of patients with sickle cell disease (5). Both groups however, had a significant increase in the distance covered during the 6MWT, which can be considered a form of direct assessment of fatigue (5) and exercise functional capacity. Some studies in patients with COPD and asthma have also reported reduced muscle fatigue and increased exercise functional capacity after IMT (34,37). There is an increase in the level of metabolites in the circulation, such as lactate and hydrogen ions because of the increase in anaerobic metabolism induced by exercise in hypoxia (16). Especially in the respiratory muscles, IMT improves clearance ability and tolerance to the levels of lactate and hydrogen ions (38,39). Therefore, strengthening of inspiratory muscles probably attenuated the metaboreflex of inspiratory muscles, which increased blood flow and oxygenation of limb muscles, reducing premature fatigue during exercise in hypoxia (16,40,41). Nevertheless, these the results must be viewed with caution, since direct metaboreflex measurements were not performed in the present study.

Regarding [Lac], normal values in healthy individuals should be between 0.5 and 2.0 mmol/l (42). However, there was a tendency for [Lac] to be higher in patients evaluated before the 6MWT, therefore at rest, when compared to normal values in healthy individuals. Some of the evaluated patients had pre-TC6 maximum values between 5.2 mmol / l and 8.1 mmol / l, i.e. well above the maximum normal value found in healthy individuals. Some authors attribute the increase of [Lac] in patients with sickle cell disease to the adaptation to lower O_2 availability to body cells, caused by the change from basal to anaerobic metabolic pathway, due to the sickle cell process. Thus, anaerobic glycolysis increases the participation in cellular energy metabolism, favoring the production and increase of [Lac] (12,43).

[Lac] showed no significant change in the comparison of initial and final evaluation results. As expected, there was an increase in [Lac] after the 6MWT, probably related to the greater participation of anaerobic metabolism (11). [Lac] tends to increase significantly from levels between 50% and 60% of the maximum oxygen uptake rate (VO_2max) (44). Patients with sickle cell disease may have a reduction in VO_2max, due to the pathophysiological changes of the disease itself that reduce the half-life of red blood cells, leading to reduced oxygen transport. In addition, intrinsic lung disease also worsens peripheral oxygenation, contributing to the lower oxygen uptake peak in these patients (12,45). Thus, these patients tend to have a greater increase in [Lac] during exertion. Thus, IMT does not appear to have influenced an improvement in these conditions and, therefore, did not contribute to a lower elevation of [Lac].

Finally, comparison of patients grouped according to disease severity revealed no differences, suggesting homogeneity of the patients, probably due to the medical intervention in the natural history of the disease.

However, despite the important results found, we believe that other studies with a larger sample size and over a longer period should be made to test our hypothesis.

Conclusion

In this study in SCD, inspiratory muscle training at home is a strategy that can be easily applied in outpatients, such as increasing respiratory muscle strength, improvement in Vcap and exercise tolerance and reducing dyspnea in activities of daily living. Further studies in a larger sample and over a longer period should be performed to obtain more details on the effects of IMT in patients with SCD.

Abbreviations

6MWT: 6-minute walk test; CO_2: carbono dioxide; COPD: chronic obstructive pulmonary disease; DLA: daily living activities; FEF25-75%: mean forced expiratory flow between 25-75% of expired vital capacity; FEV1: forced expiratory volume in the first second; FEV1/FVC ratio: ratio of forced expiratory volume in 1 second to forced vital capacity; FVC: forced vital capacity; HbC: hemoglobin C; HbS: hemoglobin S; HR: heart rate; IMT: inspiratory muscle training; [Lac]: blood lactate concentration; MFIS: modified fatigue impact scale; MRC: Medical Research Council dyspnea scale; MV alv: alveolar minute volume; NIP: negative inspiratory pressure; PEF: peak expiratory flow; PEmax: maximal expiratory pressure; $PeCO_2$: mean end-tidal partial pressure of CO_2; $PetCO_2$: end-tidal partial pressure of CO_2; P2Slp: phase 2 slope; P3Slp: phase 3 slope; P3Slp/Ve: phase 3 slope normalized according to expired tidal volume; PIF: peak inspiratory flow; PFT: pulmonary function tests; PImax: maximal inspiratory pressure; RMS: respiratory muscle strength; RR: respiratory rate; RSBI: Tobin index; RV: residual volume; SCD: sickle cell disease; HbSC: heterozygote composed of hemoglobin S and hemoglobin C; SpO2: oxygen saturation; HbSS: homozygous for hemoglobin S; $HbS\beta^0$: sickle cell/β-thalassemia phenotype with higher degree of hematological involvement in comparison to $HbS\beta^+$ patients; $HbS\beta^+$: sickle cell/β-thalassemia phenotype with lower degree of hematological involvement in comparison to $HbS\beta^+$ patients; SG: sham load group; Te: expiratory time; TG: true load group; Ti: inspiratory time; TLC: total lung capacity; UNICAMP: University of Campinas; VCap: volumetric capnography; VCO_2: CO_2 production; VCO_2/br: CO_2 production per breath; VD (aw): anatomical dead space; Ve: expiratory tidal volume; Vi: inspiratory tidal volume; VO_2: maximum oxygen uptake; VT: tidal volume.

Declarations

ACKNOWLEDGMENTS

The authors would like to thank Prof.Alberto Cliquet Jr. for providing a space at the Laboratory of Biomechanics and Locomotive Apparatus Rehabilitation at UNICAMP, Roberto Zulli for the statistical analysis and Raquel S. Foglio for the writing assistance and English revision.

FUNDING

This study was funded *in part by* grant #2011/51959-0 and 2017/21801-2d, São Paulo Research Foundation (FAPESP).and Ministry of Science, Technology, Innovations and Communications (CNPq) grant # 301676/2013-5

AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analyzed during the current study are available upon request to the corresponding author.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study conformed to the declaration of Helsinki and was approved by the Research Ethics Committee of the University of Campinas (CAAE 44635415.2.0000.5404).

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests. There is no professional relationship with companies or manufacturers who will benefit from the results of the present study.

AUTHORS' CONTRIBUTIONS

Study design: FG, IAP and STOS; Data collection: FG, CTZ and MMM; Analysis of the data: FG, CTZ, IAP and STOS; Manuscript preparation: all authors. All authors read and approved the final manuscript.

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Tables

Table 1: Characteristics of the study groups.

Baseline characteristics	All patients	Sham Group (SG)	Training Group (TG)	Statistic comparation between SG and TG
Sex (M/F)	11/14	05/07	06/07	0.8213[#]
Age (years)	42.0 (28-64)	46.5 (30-64)	40.0 (28-56)	0.1343[+]
BMI (kg/m^2)	24.9 (15.8-39.7)	24.4 (15.8-39.7)	24.9 (21.1-32.2)	0.8490[+]
Hemoglobin diagnosis				
HbSS	11	06	05	0.2697[§]
HbSC	08	05	03	
HbSβ (0/+)	03/03	00/01	03/02	

Values are median (min-max). Statistical tests used: [+] Mann-Whitney; [#] Chi-Square; [§] Fisher's exact test. M: male; F: female; BMI: body mass index; HbSS: homozygous for hemoglobin S; HbSC: heterozygote composed of hemoglobin S and hemoglobin C; HbSβ^0: sickle cell/β-thalassemia phenotype with higher degree of hematological involvement in comparison to HbSβ$^+$ patients; HbSβ$^+$: sickle cell/β-thalassemia phenotype with lower degree of hematological involvement in comparison to HbSβ$^+$ patients.

Table 2: Pulmonary function test pre and post bronchodilator.

	Sham Group (N=12)				True Load Group (N=13)				
	Initial Evaluation		Final Evaluation		Initial Evaluation		Final Evaluation		P Value
Variable (%predicted)	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD	Pre BD	Post BD	
FVC	72 (50-92)	69 (50-93)	70 (52-92)	68 (50-89)	78 (56-92)	78 (59-92)	79 (64-94)	80 (64-93)	0.8400
FEV1	72 (49-101)	75 (49-103)	70 (49-95)	72 (51-99)	76 (52-92)	79 (56-97)	76 (65-91)	82 (66-98)	0.0011*
FEV1/FVC	99 (90-111)	101 (92-115)	99 (91-116)	89 (101-114)	99 (93-105)	102 (96-105)	102 (96-105)	99 (91-107)	< 0.0001*
FEF25-75%	75 (38-156)	85 (50-149)	72 (41-140)	78 (39-163)	75 (41-95)	85 (50-115)	76 (58-120)	93 (73-143)	< 0.0001*

Values are median (min-max) * The statistical test used was ANOVA on ranks transformation, showing a time effect of the training for both groups ($p < 0.05$) in relation to the pre and post bronchodilator (BD) test for each evaluation (initial and final) in all analysis, except for FVC. Pre BD < Post BD. Statistical analysis showed no interaction or group effect. N: number of subjects per group; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; FEV1/FVC ratio: ratio of forced expiratory volume in 1 second to forced vital capacity; FEF25-75%: forced mean expiratory flow.

Table 3: Volumetric capnography

Variable, Unit	Sham Group (N=12)		True Load Group (N=13)		P Value
	Initial Evaluation	Final Evaluation	Initial Evaluation	Final Evaluation	
RR (cpm)	14.3 (12.1-17.9)	15.3 (11.5-20.4)	18.3 (11.3-25.0)	18.1 (10.0-21.7)	0.2649
HR (bpm)	61.6 (49.7-91.2)	68.3 (51.6-9 0.5)	64.7 (56.6-80.2)	68.7 (55.1-89.3)	0.1019
SpO_2 (%)	97.1 (93.7-98.2)	96.6 (92.3-98.1)	97.1 (93.1-98.6)	96.8 (92.8–98.1)	0.0528
VD (aw) (ml)	113 (84-181)	116 (73-166)	116 (58-170)	119 (72-159)	0.0696
$PeCO_2$ (mmHg)	20.4 (17.0-25.9)	20.4 (16.8-25.9)	19.5 (13.8-25.3)	19.5 (16.0-26.3)	0.6182
Vi (ml)	399 (240-696)	471 (235-992)	353 (153-763)	404 (217-757)	0.0264*
Ti (sec)	1.7 (1.4-1.9)	1.6 (1.2-2.1)	1.4 (0.9-2.0)	1.4 (1.0-2.4)	0.0483#
Te (sec)	2.4 (1.8-3.0)	2.4 (1.7-3.4)	2.0 (1.9-3.5)	2.0 (1.5-3.7)	0.0621
PIF (l/min)	20.7 (13.8-41.1)	24.0 (16.2-57.8)	25.1 (11.3-48.2)	25.8 (14.2-44.5)	0.0285*
RSBI (VT/RR)	33.1 (20.5-71.2)	28.9 (15.6-87.7)	50.1 (20.8-128.7)	37.7 (16.8-95.4)	0.0207*
P2Slp (mmHg)	336 (215-572)	263 (172-614)	339 (134-738)	304 (189-581)	0.1434
P3Slp (mmHg)	23.5 (8.1-62.2)	19.1 (4.8-43.3)	29.1 (5.7-151.6)	24.7 (3.6-83.1)	0.0153*
NIP (cmH_2O)	0.0 (-0.5-0.0)	0.0 (-1.4-0.0)	0.0 (-0.9-0.0)	0.0 (-0.7-0.0)	0.8099
MV alv (ml)	3.8 (2.0-9.0)	4.2 (2.6-13.9)	4.7 (1.5-13.9)	4.5 (2.4-11.6)	0.2882
VCO_2 (ml/min)	122 (91-309)	135 (81-337)	151 (47-268)	150 (90-284)	0.5718
$PetCO_2$ (mmHg)	34.6 (25.9-42.2)	33.9 (25.0-41.5)	36.7 (23.2-40.7)	33.7 (23.0-42.2)	0.7927
Ve (ml)	393 (237 – 700)	456 (205-1018)	366 (142-743)	400 (226-755)	0.1301
PEF (l/min)	17.0 (11.2-28.0)	19.4 (12.7-44.6)	18.8 (7.6-58.3)	19.0 (8.1-54.3)	0.0382*
VCO_2/br (ml/breath)	8.5 (5.2-19.5)	9.6 (3.8-20.4)	9.6 (2.7-14.2)	8.6 (5.1-16.0)	0.2774
P3Slp/Ve	0.06 (0.01-0.22)	0.04 (0.00-0.19)	0.08 (0.00-1.07)	0.05 (0.00-0.37)	0.0254*

Values are median (min-max). * The statistical test used was ANOVA on ranks transformation, showing a time effect of the training for both groups ($p < 0.05$) in all analysis, except for Ti (#), where significant difference between groups were independent of the training (group effect). Vi: initial evaluation < final evaluation; Ti: sham group > true load group; PIF: initial evaluation < final evaluation; RSBI: initial evaluation > final evaluation; P3Slp: initial evaluation > final evaluation; PEF: initial evaluation < final evaluation; P3Slp/Ve: initial evaluation > final evaluation There was no significant result in the statistical analysis of interaction in any of the variables. RR: respiratory rate; HR: heart rate; SpO_2: oxygen saturation; VD (aw): anatomical dead space; $PeCO_2$: mean end-tidal partial pressure of CO_2; Vi: inspiratory tidal volume; Ti: inspiratory time; Te: expiratory time; PIF: peak inspiratory flow; RSBI: Tobin index; VT: tidal volume; P2Slp: phase 2 slope; P3Slp: phase 3 slope; NIP: negative inspiratory pressure; MV alv: alveolar minute volume; VCO_2: CO_2 production; $PetCO_2$: end-tidal partial pressure of CO_2; Ve: expiratory tidal volume; PEF: peak expiratory flow; VCO_2/br: CO_2 production per breath; P3Slp/Ve: phase 3 slope normalized according to expired tidal volume.

Table 4: six-minute walk test and related tests.

Variable, Unit	Sham Group (N=12)		True Load Group (N=13)		P value
	Initial Evaluation	Final Evaluation	Initial Evaluation	Final Evaluation	
6MWT – distance (m)	493.1 (365.0-565.0)	500.4 (385.0-639.4)	493.5 (420.0-600.0)	540.0 (432.8-647.0)	0.0019*
Borg (0-10)					
Pre 6MWT	0.7 (0-3)	0.0 (0-5)	1.0 (0-6)	2.0 (0-3)	< 0.0001*
Post 6MWT	4.0 (2-7)	2.5 (1-8)	3.0 (0-8)	4.0 (0.5-9)	
[Lac] (mmol.L $^{-1}$)					
Pre 6MWT	2.0 (1.0-5.2)	1.9 (0.8-6.1)	2.5 (0.8-6.1)	1.8 (0.8-8.1)	0.0144*
Post 6MWT	2.7 (1.6-4.7)	2.2 (1.5-6.0)	2.5 (0.8-6.4)	3.1 (1.2-8.6)	
SpO_2 (%)					
Pre 6MWT	96.5 (92-100)	96.5 (86-100)	97.0 (91-99)	96.0 (88-99)	0.0773
Post 6MWT	94.5 (89-100)	95.5 (83-99)	96.0 (80-99)	92 (82-99)	

Values are median (min-max) * The statistical test used was ANOVA on ranks transformation, showing a time effect of the training for both groups ($p < 0.05$) in all analysis. 6MWT - distance: initial evaluation < final evaluation; Borg: Pre 6MWT< Post 6MWT; [Lac]: Pre 6MWT < Post 6MWT. Statistical analysis showed no interaction or group effect. N: number of subjects per group; 6MWT: 6-minute walk test; Borg: Modified Borg scale; SpO_2: oxygen saturation.

Figures

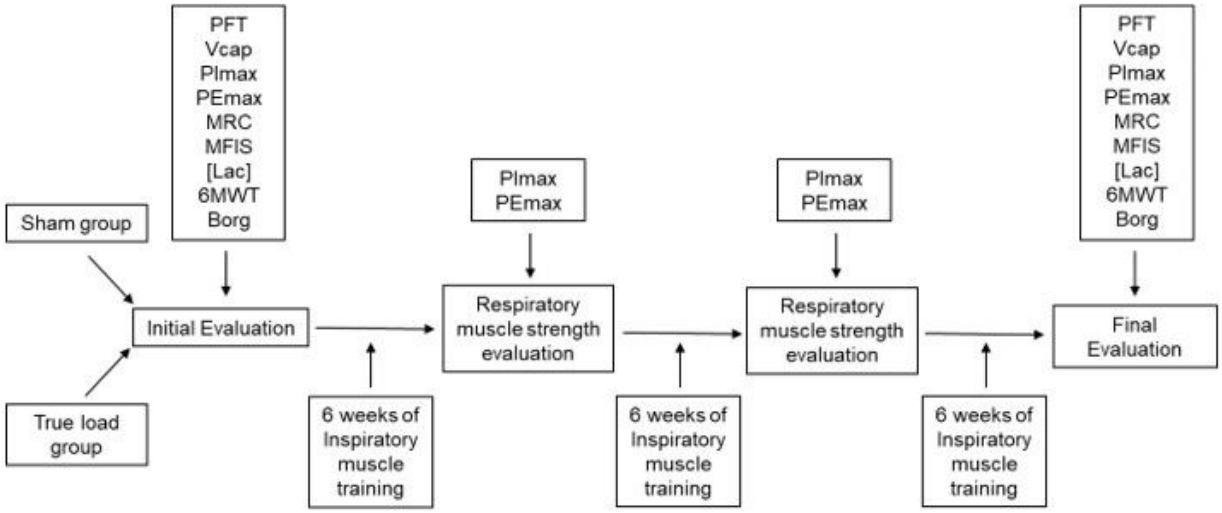

Figure 1

Patients flow diagram. PFT: Pulmonary function test; Vcap: Volumetric capnography; PImax: maximum static inspiratory pressure; PEmax: maximum static inspiratory pressure; MRC: Medical Research Council dyspnea scale; MFIS: Modified Fatigue Impact Scale; [Lac]: Blood lactate concentration; 6MWT: 6-minute walk test; Borg: modified Borg scale.

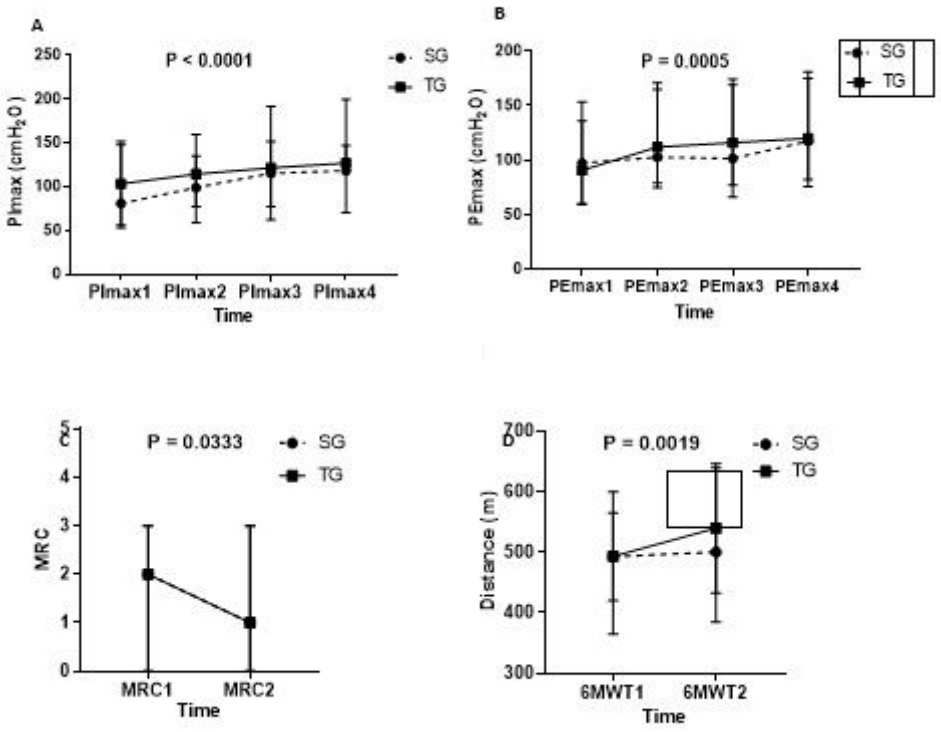

Figure 2

: A: Maximal static inspiratory pressure (PImax) in the first, second, third, and last evaluation, PImax1 < PImax2 < PImax3, PImax4. B: Maximal static expiratory pressure (PEmax) in the first, second, third, and last evaluation, PEmax1 < PEmax2, PEmax3 < PEmax4. Figure C: Medical Research Council (MRC) scale of dyspnea in the first and last evaluation, MRC1 > MRC2. Figure D: Distance measurements of the 6-minute walking test (6MWT) in the first and last evaluation, 6MWT1 < 6MWT2. The statistical test used was ANOVA on ranks transformation, showing a time effect of the training for both groups ($p < 0.05$). Statistical analysis showed no interaction or group effect.