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Respiratory training in older women: Unravelling central and peripheral hemodynamic slow oscillatory patterns

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ABSTRACT

We hypothesized that inspiratory muscle training (IMT) increases the respiratory-induced low-frequency oscillations of mean blood pressure (MBP) and middle cerebral artery blood velocity (MCAv), upregulating cerebrovascular function in older women. Firstly, participants were recorded with free-breathing (FB) and then breathed at a slow-paced frequency (0.1 Hz; DB test) supported by sonorous metronome feedback. Blood pressure was recorded using finger photoplethysmography method, ECG, and respiration using a thoracic belt. To obtain the MCAv a transcranial ultrasound Doppler device was used. Spectral analysis of MBP, R-R intervals, and mean MCAv time series was obtained by an autoregressive model. The transfer function analysis (TFA) was employed to calculate the coherence, gain, and phase. After that, older women were enrolled in a randomized controlled protocol, the IMT-group ($n = 8$; 64 ± 3 years-old) performed IMT at 50 % of maximal inspiratory pressure (MIP), and Sham-group, a placebo training at 5 % MIP (Sham-group; $n = 6$; 66 ± 3 years-old). Participants breathed against an inspiratory resistance twice a day for 4-weeks. DB test is repeated post IMT and Sham interventions. IMT-group, compared to Sham-group, augmented tidal volume responses to DB (Sham-group 1.03 ± 0.41 vs. IMT-group 1.61 ± 0.56 L; $p = 0.04$), increased respiratory-induced MBP (Sham-group 26.37 ± 4.46 vs. IMT-group 48.21 ± 3.15 $mmHg^2$; $p = 0.04$) and MCAv (Sham-group 14.16 ± 31.26 vs. IMT-group 79.90 ± 21.76 $cm^2 s^{-2}$; $p = 0.03$) slow oscillations, and reduced TFA gain (Sham-group 2.46 ± 1.32 vs. IMT-group 1.78 ± 1.30 $cm{\cdot}s^{-1}{\cdot}mmHg^{-1}$; $p = 0.01$). Our findings suggest that IMT increases the respiratory-induced oscillations in MBP and MCAv signals and reduces TFA gain. It seems compatible with an improved dynamic cerebrovascular regulation following IMT in older women.

1. Introduction

The reciprocal nature of autonomic control between peripheral and central hemodynamics is an essential mechanism to maintain cerebral blood flow (CBF) (Koep et al., 2022). The baroreflex buffering in arterial pressure oscillations and the cerebral autoregulation play a fundamental role in the CBF control (Levine et al., 1994; Ogoh et al., 2010; Koep et al., 2022).

The central generated sympathetic rhythms and the peripheral reflex mechanisms within the closed-loop model seem to explain the low-frequency (0.1 Hz) oscillations from blood pressure and R-R intervals (Montano et al., 2001; Malliani et al., 1991), while in the high-frequency band respiration modulates the vagal activity to the heart (Montano et al., 1994). The CBF is under tonic autonomic neural control and autoregulated in the very-low-frequency (Zhang et al., 2002a).

Since breathing pattern influences cardiovascular control, respiratory training (e.g., slow breathing exercise and Yoga) have been proposed as non-pharmacological approaches to modulate heart rate and blood pressure in several populations (Bernardi et al., 2001; Santaella et al., 2011). Recently, the inspiratory muscle training (IMT) was proposed to offset deteriorative effects from sedentary aging on respiratory muscle function and cardiovascular autonomic control (Rodrigues et al., 2018, 2021a, 2021b). During IMT sessions, the participant breathes against an inspiratory resistance through an inspiratory threshold

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device. The training load is progressive across the weeks. (Rodrigues et al., 2018, 2020a, 2021a, 2021b). IMT increased vagal modulation to the heart in young (Abreu et al., 2019; Abreu et al., 2020) and older (Rodrigues et al., 2018, 2021a, 2021b) individuals. In older adults, a modified spontaneous breathing pattern by IMT (ie., shorter inspiratory time) explains, in part, the observed cardiac autonomic control improvement (Rodrigues et al., 2021b). Moreover, IMT changed heart rate responses to slow deep breathing (DB) test in older women, suggesting that the combination of increased tidal volume and reduced breathing frequency (e.g., respiratory sinus arrhythmia) is a putative mechanism underlying cardiac autonomic adaptations post-IMT (Rodrigues et al., 2018).

Regarding cerebrovascular control, IMT reduced cardiac output and middle cerebral artery blood velocity (MCAv) drops during initial orthostasis in older adults. It suggests that inspiratory muscles strength post-IMT may influence respiratory pump contributions to cardiac output and cerebral blood flow regulation (Rodrigues et al., 2020b). The respiratory pump contribution to venous return is optimized since intrathoracic pressure is reduced, augmenting the pressure gradient between the peripheral circulation and the central venous pressure. The intrathoracic pressure could be reduced by an acute inspiratory resistive loading (IRL), which avoids the CBF drop under hypovolemic conditions (Rickards et al., 2007; Rickards, 2019; Convertino et al., 2011). In older women, IRL improves the rapid recovery of MCAv during the initial orthostasis (Rodrigues et al., 2020a). Similarly, DB increases orthostatic tolerance due to beneficial changes in cerebrovascular function during tilt test plus low body negative pressure stimulus in healthy youngers (Lucas et al., 2013). Thus, increased respiratory muscles strength post-IMT may improve the mechanical effect from the respiratory pump in the rapid MCAv recovery during the initial orthostasis (Rodrigues et al., 2020a, 2020b). Nevertheless, the autonomic control mechanisms underlying peripheral and central hemodynamics oscillations post-IMT are still unclear.

From this background, we speculated that adaptations on respiratory muscles pump from IMT (i.e., augmented inspiratory muscle strength and tidal volume) may optimize the respiratory-induced oscillations from peripheral towards cerebral hemodynamics. We hypothesized that IMT increases the respiratory-induced low-frequency oscillations (0.1 Hz) of mean blood pressure (MBP) and MCAv, upregulating cerebrovascular responsiveness to deep breathing test. Thus, the purpose of the current study was to investigate the IMT effects on MCAv and MBP oscillations, through the cross-spectral analysis, during the DB test in older adults.

2. Methods

2.1. Study's design

Participants of the current study were recruited from a healthy aging program waiting list. They were not enrolled in any physical exercise program when participating in the current protocols.

Thirty older adults (both sexes over 60 years) were recruited by telephone, but three did not attend the first meeting. Further, seven individuals (six men) were excluded during the first meeting. Both older males and women were contacted to participate. However, males were excluded because they matched exclusion criteria as cardiovascular pathologies and medications in use. The exclusion criteria were the use of any medications with cardiovascular, respiratory or metabolic actions, presence of cardiovascular, respiratory or metabolic diseases, and lung function parameters below 80 % of the age-predicted reference value.

Twenty healthy older women, all in post-menopause (self-reported), were enrolled in protocol 1 (deep breathing vs. free breathing). After that, older women were enrolled in protocol 2 a double-blind study, randomized in inspiratory muscle training (IMT-group) or Sham-group. The participants were randomized by a coin toss for every pair of age-matched subjects. The first author, who analyzed the data, and participants were blinded to the groups' labels. The flowchart of the study's design is present in Fig. 1. All procedures were approved by the Fluminense Federal University institutional ethics committee (851.371). All volunteers provided informed consent for participation before being enrolled in the study.

2.2. Experimental procedures

In the first visit, after providing informed consent, body mass (BM) and height were obtained (Wellmy, W200, Brazil), and participants were familiarized with all experimental procedures. On a second day, blood pressure (BP) was recorded using a finger photoplethysmography device (FinometerPro; Finapres Medical Systems, Arnhem, The Netherlands), R-R interval was recorded by an Electrocardiogram (PhysioFlow, PF-05; Manatec Biomedical, Macheren, France) and respiration using a thoracic belt record (ADInstruments, Bella Vista, NSW, Australia). To obtain the middle cerebral artery velocity (MCAv), the middle cerebral artery was insonated using a 2-MHz pulsed transcranial ultrasound Doppler device (500 V; Multigon Industries, Mt. Vernon, NY, USA). The transducer was positioned over the right temporal window, and the MCAv signal features (depth and gain) were adjusted to the better signal-to-noise ratio. Mean BP (MBP) and MCAv were derived through the waveform integration (area-under-the-curve), using the time of the DBP value as the initial and endpoints of each cardiac cycle (Claassen et al., 2016).

Breath-by-breath metabolic and ventilatory data were recorded using a metabolic gas analyzer (Ultima CPX; Medgraphics, St Paul, MN, USA) with a medium-flow pneumotachograph. The breathing rate (BR), minute ventilation (VE), tidal volume (Vt), expired carbon dioxide pressure ($PetCO_2$) were variables considered for analysis.

All data were recorded continuously for 5 min in each breathing condition. All recorded data were input and sampled by a data acquisition device (PowerLab 16SP, ADInstruments, AUS) at the same frequency (1 kHz) and stored for offline analysis (LabChart, ADInstruments, AUS).

Twenty participants enrolled in Protocol 1, which consisted of free-breathing (FB) records at the first step, and slow deep breathing (DB), lately, on the same day. In the sitting position, participants were instructed to breathe with a rate of 6 cycles/min with sonorous metronome feedback. The breathing frequency is evaluated by the respiratory belt providing real-time biofeedback for the participant. The DB test is a simple maneuver commonly used to generate sinusoidal hemodynamic oscillations by slow breathing pattern at six breaths per minute (0.1 Hz), which was introduced by Diehl et al. (1995) as a clinical tool to investigate cerebrovascular control. Since DB may increase the squared coherence function (k^2) between MBP and MCAv at low-frequency compared to FB (Lucas et al., 2013), it was proposed as an autonomic maneuver to evaluate the cerebrovascular control at baseline and after IMT.

From protocol 2, the pulmonary function test (PFT) (Pulmowin2 MRS/DATALINK; Montpellier, France) was used to verify whether the candidates fulfilled the exclusion criteria. Three PFT evaluations were carried out, and the highest values of forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were retained (Miller et al., 2005).

The manovacuometry test (MVD300; GlobalMed, Porto Alegre, Brazil) was used to evaluate the maximal inspiratory pressure (MIP) (Gibson et al., 2002). MIP was determined as the highest value of pressure noted for three inspirations. The participants were encouraged to make an expiration (to eliminate the reserve expiratory volume) before the MIP test, and the MIP was evaluated at residual volume.

In the first week, the IMT and sham groups performed familiarization sessions without load. After the MIP test, the inspiratory threshold device (POWERbreathe Wellness; HaB International Ltd., Southam, United Kingdom) was adjusted to a moderate load (50 % MIP) for the IMT group and an inefficient load (5 % MIP) for the sham group. The IMT and sham

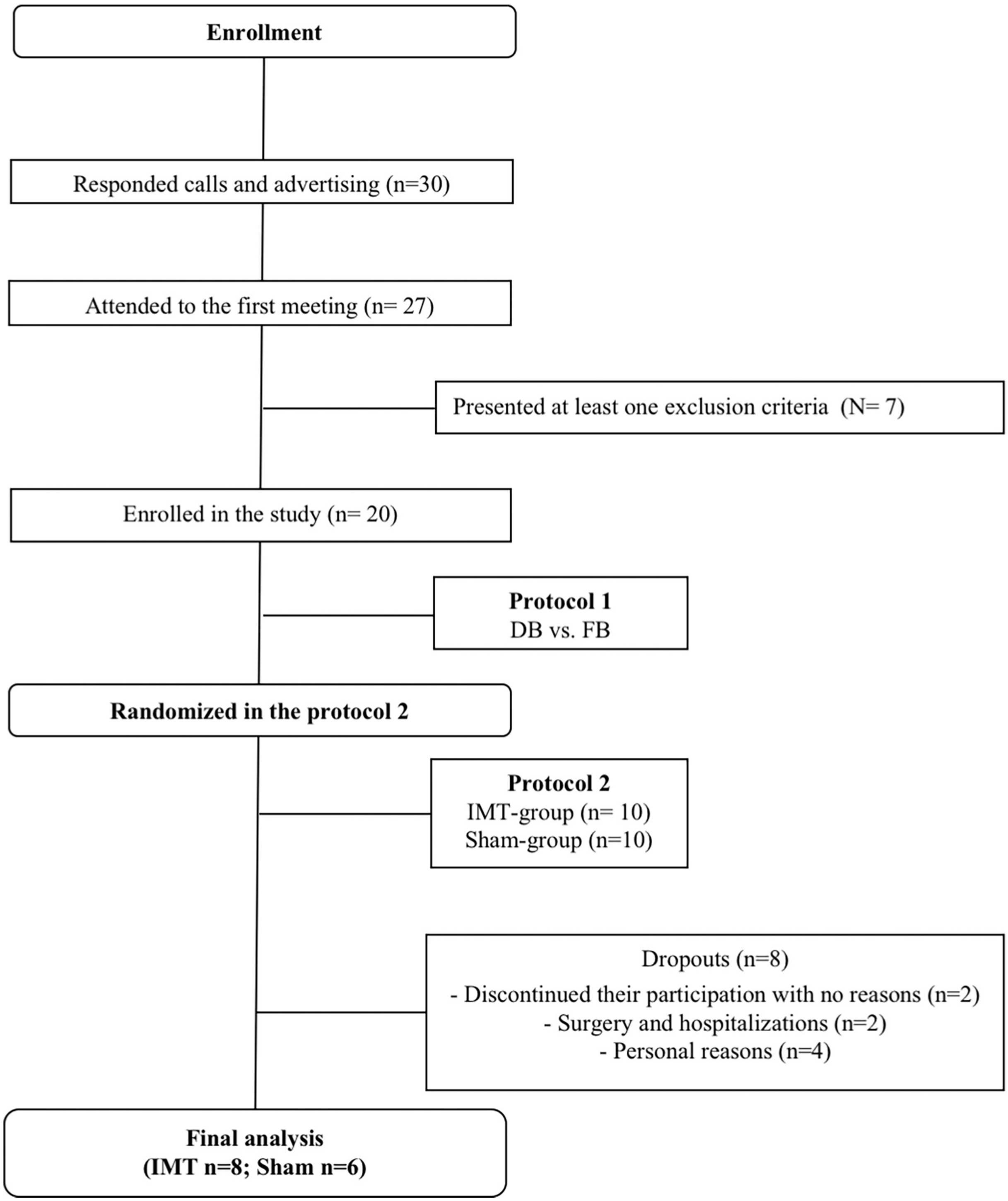

Fig. 1. Study's design. FB: free breathing; DB: deep breathing; IMT-group: experimental group performed IMT (50 % MIP), Sham-group: sham group performed placebo load (5 % MIP).

protocols were performed at home seven days per week, twice a day, for 4 weeks. During the study, all participants visited the laboratory once a week for MIP testing and load adjustment. Since MIP values increased during the follow-up Lab visits, inspiratory resistive loading is adjusted weekly for the IMT-group (50 % MIP) and Sham-group (5 % MIP) (Rodrigues et al., 2021a, 2021b). All tests were repeated after each intervention.

2.3. Data processing

The R-R, mean MCAv, and MBP time series were extracted from raw data, and data sets corresponding to 300 beats were subjected to autoregressive (AR) modeling based on the Akaike information criterion, with a Hanning window and 50 % overlap to obtain the spectral power. The model order was estimated for each segment by Akaike's criterion in the present data bounded between 5 and 14 (Badilini et al., 2005).

For heart rate (HRV) and blood pressure (BPV) variabilities analysis, times series were analyzed in the frequency domain, and the absolute and normalized powers in the very low frequency (VLF: 0.01 to 0.04 Hz), low frequency (LF: 0.04 to 0.15 Hz), and high frequency (HF: 0.15 to 0.40 Hz) bands were obtained. The normalized LF and HF spectral powers of R-R were also obtained in normalized units by dividing the LF and HF values expressed in absolute units by the total power minus the VLF component power, and the sympatho-vagal balance (LF/HF) was calculated.

For MCAv-MBP frequency domain analysis, times series were the very low frequency (VLF: 0.02 to 0.07 Hz), low frequency (LF: 0.07 to 0.20 Hz), and high frequency (HF: 0.20 to 0.50 Hz) bands were obtained (Claassen et al., 2016; Panerai et al., 2022). Cross-spectral analysis was performed to calculate the k^2 between the MBP and MCAv signals of each participant. The transfer function analysis (TFA) gain and the phase relationships between mean blood pressure and MCAv oscillations were calculated (Claassen et al., 2016; Panerai et al., 2022). For the DB condition, since we set the breathing frequency at 0.1 Hz, we report the spectral powers and TFA measures in this frequency. All parameters were analyzed through ad hoc software (HeartScope II; AMPS-LLC, New York, NY, USA).

2.4. Statistical analysis

The Shapiro-Wilk test was used to evaluate the normality of the data distribution for each variable. For DB and FB comparisons at baseline, paired *t*-test was used.

The analysis of covariance (ANCOVA) was employed to account for differences after Sham and IMT interventions. In randomized studies, ANCOVA has more power than the analysis of variance (ANOVA), especially when groups differences preexisting at baseline. Since ANCOVA assumes an absence of a baseline difference (i.e., there is no group effect at pre-test) it is recommended for randomized controlled studies with small sample sizes (Van Breukelen, 2006). Sidak's post-hoc was employed to confirm differences accounted by the ANCOVA. The primary variable outcome (TFA gain) has a moderate to large effect size (1.36) (Hopkins et al., 2009). The significance level was set at $\alpha < 0.05$.

The software used was GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego, CA, USA), SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA), and G-Power version 3.1.9.2 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).

3. Results

From protocol 1, a higher squared coherence function (k^2) at LF was found during DB (0.79 ± 0.31) compared to FB (0.28 ± 0.14; $p = 0.001$). No differences in TFA gain and phase were observed between DB and FB ($p > 0.05$). As expected, Table 2 shows that DB increases the Vt, VE, and reduces $PETCO_2$ compared to FB. Fig. 2 shows an example of spectral analysis of R-R, MBP, and MCAv series using an autoregressive model during free and deep breathing conditions.

From protocol 2, all participants presented lung function parameters above 80 % of the age-predicted reference value. All participant's characteristics were not statistically different at baseline (Table 1). MIP increased substantially from baseline to post-intervention in the IMT (88 ± 7 vs. 111 ± 10 cmH_2O) compared to Sham group (93 ± 14 vs. 95 ± 14 cmH_2O; $p < 0.001$), confirming IMT effects on inspiratory muscle strength.

The IMT increased respiratory-induced oscillations of RR-LF ($p = 0.01$), MBP-LF ($p = 0.04$), and MCAv-LF ($p = 0.03$) compared to Sham-group (Fig. 3). Also, IMT reduces the transfer function gain (p = 0.01) between MBP and MCAv during DB compared to Sham intervention, while coherence ($p = 0.10$) and phase ($p = 0.33$) did not change (Fig. 4). Otherwise, no differences were found for LF in absolute values, and all spectral indexes of R-R and MCAv under free-breathing excepted for LF indexes of MBP (Fig. 3).

Table 1
Participant's characteristics.

	Sham-group	IMT-group	*p*-Value
N	6	8	–
Age (years)	66 ± 3	64 ± 3	0.14
Sex (male/female)	0/6	0/8	–
Height (cm)	150 ± 5	157 ± 5	0.07
Body mass (kg)	63 ± 8	63 ± 13	0.90
BMI (kg/m^2)	28 ± 4	26 ± 5	0.56

IMT-group: experimental group performed IMT (50 % MIP); Sham-group: sham group performed placebo load (5 % MIP); MIP: maximal inspiratory pressure. Unpaired *t*-test mean ± SD; $p \leq 0.05$.

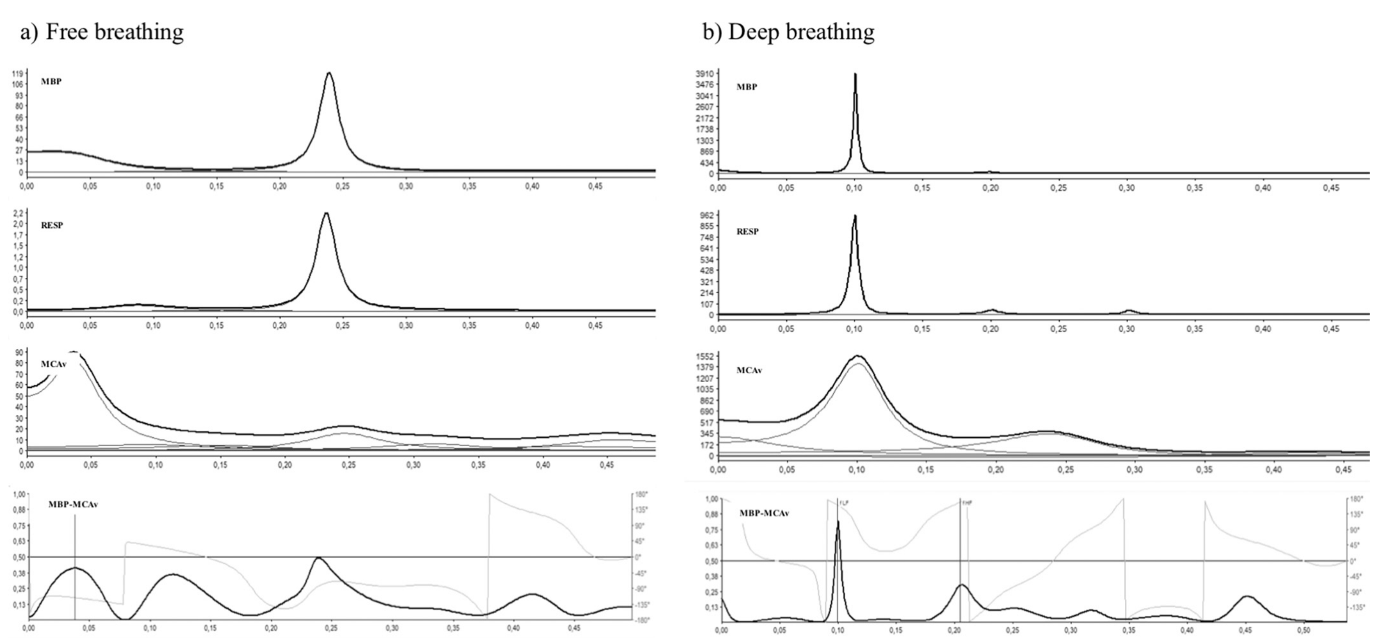

Fig. 2. The representative spectral and cross-spectral analysis during breathing (panel a) and deep breathing (panel b). In the cross-spectral analysis coherence and phase are represented by the left and right y axes, respectively.

Table 2
Comparison of ventilatory indexes between FB and DB at baseline.

	FB	DB	p-Value
$PetCO_2$ (mmHg)	41 ± 2	35 ± 5[a]	0.001
BR (breaths/min)	15 ± 2	6 ± 0[a]	0.001
Vt (L)	0.36 ± 0.01	1.13 ± 0.40[a]	0.001
VE (L/min)	4.98 ± 1.36	6.83 ± 2.40[a]	0.03

FB: free breathing; DB: deep breathing; PetCO2: end tidal CO2 partial pressure; BR: breathing rate; Vt: tidal volume; VE: minute ventilation; paired *t*-test; mean ± SD; $p \leq 0.05$.
[a] Differences from FB.

Table 3
Comparison of ventilatory indexes during DB before and after inspiratory muscle training and sham interventions.

	Sham-group		IMT-group		p-Value
	Baseline	Post-intervention	Baseline	Post-intervention	
$PetCO_2$ (mmHg)	37 ± 5	37 ± 6	35 ± 4	33 ± 7	0.66
BR (breaths/min)	6 ± 0	6 ± 0	6 ± 0	6 ± 0	0.79
Vt (L)	1.02 ± 0.35	1.03 ± 0.41	1.08 ± 0.18	1.61 ± 0.56[a,b]	0.04
VE (L/min)	6.09 ± 2.12	6.18 ± 2.45	6.55 ± 1.30	9.79 ± 3.57	0.06

DB: deep breathing; $PetCO_2$: end tidal CO_2 partial pressure; BR: breathing rate; Vt: tidal volume; VE: minute ventilation. Baseline pre-tests were corrected by ANCOVA.
[a] Difference from Sham-group.
[b] Differences from baseline.

As expected, IMT-group increased vagal modulation to the heart during free-breathing, as shown by HF in absolute (Baseline Sham-group 377.93 ± 279.63 and IMT-group 149.03 ± 204.61 ms^2; Post-intervention Sham-group 162.64 ± 85.70 vs. IMT-group 221.50 ± 234.00 ms^2; $p = 0.005$) and normalized (Baseline Sham-group 53 ± 21 and IMT-group 40 ± 15 n.u; Post-intervention Sham-group 50 ± 15 vs. IMT-group 60 ± 13 n.u; $p = 0.0001$) units. Because of that, IMT shifted the sympatho-vagal balance to a vagal predominance (Baseline Sham-group 1.2 ± 1.3 and IMT-group 1.6 ± 0.7; Post-intervention Sham-group 1.2 ± 0.8 vs. IMT-group 0.7 ± 0.3; $p = 0.02$).

Table 3 shows that IMT increases Vt during DB t in IMT- compared to Sham-group. No differences were found regarding $PetCO_2$, BR, and VE (Table 3).

4. Discussion

The main finding of the current study is that IMT: 1) augments tidal volume responses to deep breathing without changes on $PetCO_2$; 2) increases respiratory-induced cerebral and peripheral hemodynamic slow oscillations in older women; 3) reduces MBP-MCAv transfer function gain, which is compatible with an improved dynamic cerebrovascular regulation. Taken together, these findings suggest that improved respiratory pump response to deep breathing is an underlying mechanism of cerebrovascular and hemodynamic control following IMT in older women.

The IMT seems to improve cardiac autonomic control in young (Abreu et al., 2019; Abreu et al., 2020), and older (Rodrigues et al., 2018, 2021a, 2021b) healthy individuals, cardiovascular (Mello et al., 2012; Ferreira et al., 2013) and pulmonary (Cutrim et al., 2019) diseases. In older adults (Rodrigues et al., 2018, 2020a, 2021a, 2021b) and clinical populations (Mello et al., 2012; Ferreira et al., 2013; Cutrim et al., 2019), a moderate IMT intensity (30–50 % MIP) seems to provide

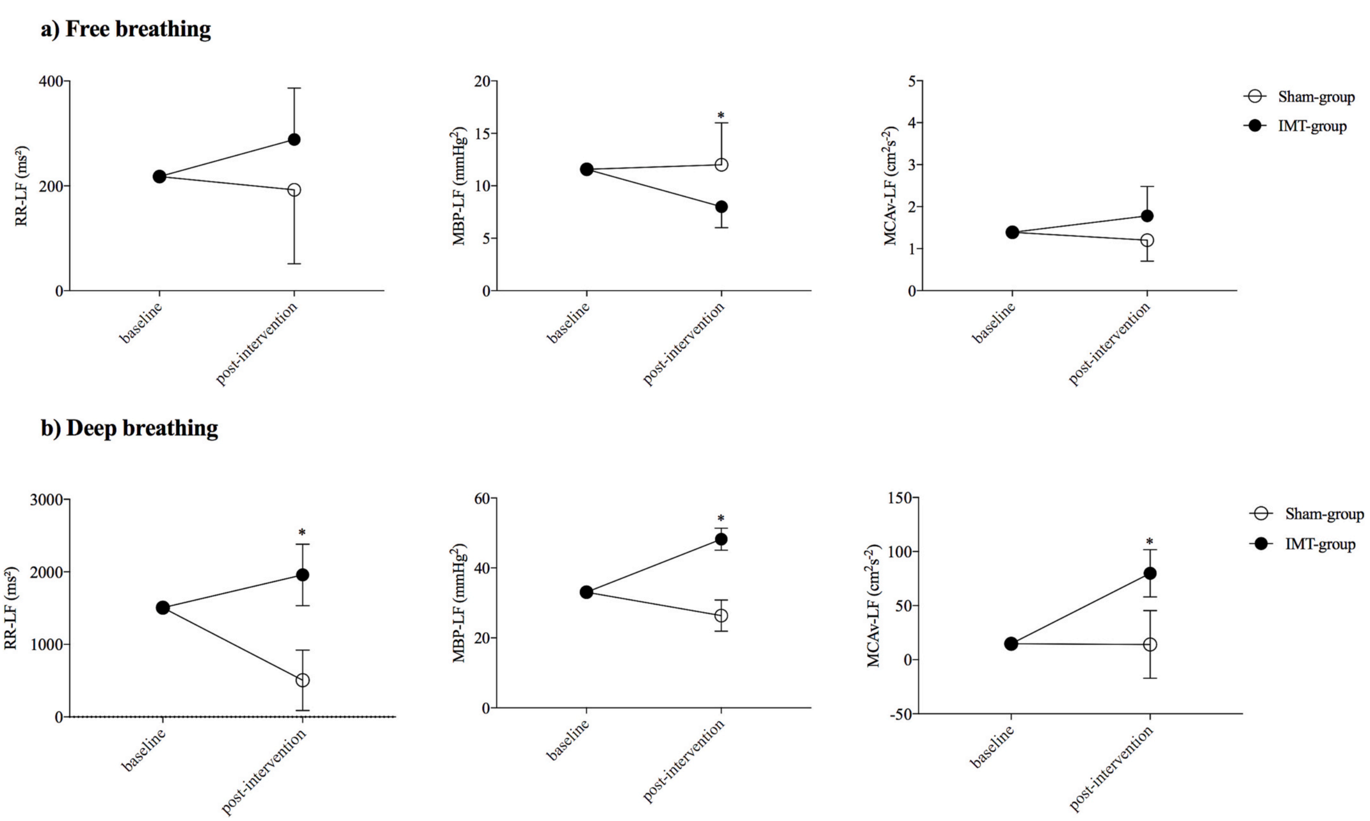

Fig. 3. Comparisons of R-R, MBP and MCAv low-frequency oscillations (0.1 Hz) during breathing (panel a) and deep breathing (panel b) between IMT and Sham group post-interventions. IMT-group: experimental group performed IMT (50 % MIP), Sham-group: sham group performed placebo load (5 % MIP). MIP: maximal inspiratory pressure; *differences between groups. Baseline pre-tests were corrected by ANCOVA. Mean ± SD. $p \leq 0.05$.

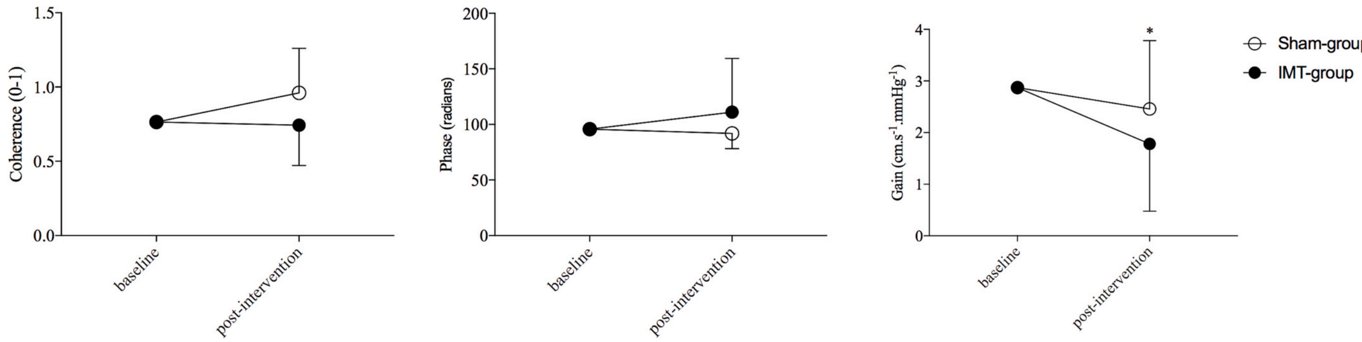

Fig. 4. Transfer function analysis of MBP and MCAv at low-frequency (0.1 Hz) between IMT- and Sham-group post-interventions. IMT-group: experimental group performed IMT (50 % MIP), Sham-group: sham group performed placebo load (5 % MIP). MIP: maximal inspiratory pressure; *differences between groups. Baseline pre-tests were corrected by ANCOVA. Mean ± SD. $p \leq 0.05$.

benefits in the cardiovascular autonomic control. From the current study, the IMT intensity of 50 % MIP provides benefits on cardiovascular and cerebrovascular compared to a placebo load (5 % MIP) in older women. However, experimental settings with different IMT intensities should be further investigated.

IMT may reduce ambulatory blood pressure, which is attributed to a reduced systemic vascular resistance (DeLucia et al., 2018). However, the spontaneous baroreflex sensitivity seems to be unchanged by IMT in healthy young (DeLucia et al., 2018) and older (Rodrigues et al., 2021b) subjects. Regarding physiological mechanisms, the shifted breathing pattern may influence the cardiac control during spontaneous breathing that may explain the vagal-mediated improvement in heart rate post-IMT (Rodrigues et al., 2018, 2021b). On the other hand, the putative mechanisms underlying blood pressure and cerebrovascular control following IMT remain unclear.

The voluntary increments in tidal volume (i.e., without respiratory fatigue) reduce sympathetic nerve activity discharge and increase limb vascular conductance in healthy adults, highlighting that peripheral reflexes elicited by increased tidal volume play a role in the respiratory modulation of vasoconstrictor tone (St. Croix et al., 2000). From the current study, IMT increases the tidal volume responses to slow deep breathing compared to the Sham-group (Table 3). We may speculate that increased tidal volume during slow deep breathing post-IMT plays a role in central inhibitory rhythms (Bernardi et al., 2001; Elstad et al., 2018), upregulating peripheral and central hemodynamics oscillations.

Recently, a low acute inspiratory resistive load (IRL; ~9 cmH_2O) was employed to optimize the role of respiratory pump on hemodynamic responses in humans. The IRL induces greater negative intrathoracic pressure variations that are transferred through the thoracic spine to the cerebrospinal fluid and the non-valvular veins around the spinal cord, causing immediate and proportional reductions in intracranial pressure (Convertino et al., 2011). It was demonstrated that MCAv and MBP high frequency oscillations (0.15–0.40 Hz) were higher during IRL use in the orthostatic position in older adults (Rodrigues et al., 2020b) and under lower body negative pressure stimulus in healthy young subjects (Rickards et al., 2007).

From a clinical point of view, IRL and DB seem to be acute maneuvers to prevent falls. In healthy youngers, DB increases orthostatic tolerance (improved time to pre-syncope) during tilt test plus low body negative pressure stimulus compared to spontaneous breathing. It could be explained by beneficial changes in cerebrovascular function (i.e., elevations in LF-MCAv and LF-MBP, increased LF phase, and reduced LF gain) during DB (Lucas et al., 2013). Likewise, we showed that IMT improves cerebrovascular function since increases LF-MCAv and LF-MBP oscillations and reduces TFA gain during deep breathing (Figs. 3 and 4).

In older women, the improvements in cerebrovascular and cardiovascular control induced by acute IRL or chronic IMT reduce the postural instability, suggesting a potential application of acute IRL to prevent falls in the older population (Rodrigues et al., 2020a, 2020b). The enhanced cerebrovascular function following IMT has applications in daily situations when cerebral blood flow is challenged, such as orthostatic stress (Rodrigues et al., 2020a). Taken together, these findings highlight a key mechanism of respiratory pump contributions in central and peripheral hemodynamic control in older women. It seems to be relevant for this population since both post-menopause and aging may impair cerebral blood flow, carotid artery reactivity, and peripheral vascular function (Brislane et al., 2020; Laitinen et al., 2004; Mehagnoul-schipper et al., 2000).

In some pathological conditions, such as aneurysmal subarachnoid hemorrhage (Giller, 1990), occlusive cerebrovascular diseases (Diehl et al., 1995), and type 2 diabetes mellitus (Brown et al., 2008), TFA reveals a phase-shifted, high coherence and gain that are compatible with an impaired cerebral autoregulation. Moreover, it was observed that a cognitive task shifts the TFA phase (in LF and VLF bands) and increases TFA gain in LF during cognitive activity, implying impaired dynamic cerebral autoregulation (Ogoh et al., 2018). Curiously, cerebral blood flow decrements seem to be an early pathophysiological mechanism in neurodegenerative disease (Korte et al., 2020). Thus, it could be speculated that an upregulated cerebrovascular control following IMT may promote clinical benefits in the older population regarding cerebral and cognitive protection, warrant further investigations.

Spontaneous respiratory-related blood pressure oscillations are typically found on high frequencies (>0.15 Hz), which is, to date, attributed to mechanical effects of fluctuations in stroke volume (Parati et al., 2003). Under a ganglion blockade, blood pressure variability (BPV) at high frequencies remained unchanged, while BPV at the low frequency was reduced. It suggests that the high frequency oscillations of blood pressure are determined, mainly, by non-neural effects of respiration on intrathoracic pressure and/or cardiac filling (Zhang et al., 2002b). Kawase et al. (2002) showed that blood pressure high frequency oscillations depended on absolute changes in blood volume in a model of hemorrhage in isoflurane-anesthetized dogs, highlighting the mechanical component of high frequency index of blood pressure in spectral analysis. Otherwise, since isoflurane suppresses autonomic nervous activity, the role of the neural modulation could not be excluded in the aforementioned study (Kawase et al., 2002).

Despite blood pressure variations, the cerebral autoregulation is an intrinsic cerebral mechanism that regulates CBF. The frequency-dependent properties of dynamic cerebral autoregulation are estimated by the TFA between MBP and MCAv variables (Zhang et al., 2002a; Claassen et al., 2016). It was demonstrated that dynamic cerebral autoregulation is under tonic autonomic neural control in humans. A ganglion blockade reduced the VLF-MBP oscillations and increased the TFA gain, while VLF-MCAV did not change (Zhang et al., 2002a).

Considering the central generated sympathetic rhythms and baroreflex activity at low frequency (0.1 Hz) (Montano et al., 2001; Malliani et al., 1991), the slow deep breathing could be a relevant tool in the autonomic control assessment of peripheral and central circulation (Bernardi et al., 2001; Diehl et al., 1995). During spontaneous breathing, the cerebral autoregulation mechanism is a paragon of a high-pass filter, shifting CBFV oscillations to the left (i.e., VLF band) (Panerai et al., 2022). In this way, our data showed that the DB test is a feasible method to generate periodic oscillations in blood pressure transmitted towards MCAv at the same frequency, augmenting the coherence between MBP and MCAv signals (Fig. 2). These results corroborate previous studies (Diehl et al., 1995; Lucas et al., 2013). Also, the DB test reveals an increased MBP and MCAv low oscillations and reduced TFA gain in IMT-compared to Sham-group. Although the origins of oscillatory phenomena in MCAv and MBP remain a controversial matter, it is reasonable that respiratory-induced blood pressure oscillations contribute largely to fluctuations seen in cerebral vasculature (Rickards, 2019; Lucas et al., 2013). From a mechanistic point of view, IMT seems to play a role in the sympathetic regulation over the cerebral vasculature, highlighted by increased MBP and MCAv oscillations and a reduced TFA gain at the frequency of central generated sympathetic rhythms (0.1 Hz). From a clinical perspective, it seems to be compatible with an enchased dynamic cerebrovascular responsiveness in healthy (Lucas et al., 2013), opposing to pathological conditions in which cerebrovascular function is impaired (Giller, 1990; Diehl et al., 1995; Brown et al., 2008).

5. Limitations

The current study has some limitations. The major limitation is the small sample size. However, our primary variable outcome (TFA gain) presents a large effect size (1.39). Indeed, even with a small sample, IMT produces a large effect regarding the cardiovascular and cerebrovascular variables as previously described (Rodrigues et al., 2020a, 2021a). Also, the study has a unisex sample (female only). Despite both older males and women being contacted to participate, males were excluded by cardiovascular pathologies and medications in use.

In conclusion, the inspiratory muscle training increases tidal volume, cerebral and peripheral hemodynamic oscillatory responses to deep breathing in older women. The reduced gain of the transfer function between blood pressure and cerebral blood flow seems to be a marker of improved dynamic cerebrovascular regulation following IMT. Otherwise, future studies should investigate the link between IMT effects on cerebrovascular control to clinically relevant outcomes.

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CRediT authorship contribution statement

Gabriel Dias Rodrigues: Conceptualization, Data collection and analysis, Writing – original draft, Writing – review & editing. **Antonio Claudio Lucas da Nobrega**: Supervision, Conceptualization, Writing – review & editing. **Pedro Paulo da Silva Soares**: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

The authors do not have permission to share data.

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