INSPIRATORY MUSCLE STRENGTH TRAINING: ACUTE EFFECTS, DYNAMIC IMPROVEMENTS AND CLINICAL SIGNIFICANCE

by

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ABSTRACT

Inspiratory muscle strength training (IMST) is a form of resistive breathing training traditionally used to strengthen respiratory muscles. In our hands, we have reported significant reductions in blood pressure following six weeks of daily IMST that are driven in part by reductions in systemic vascular resistance. The work set forth in this dissertation addresses outstanding questions resulting from our previous work. Study 1 characterizes the *acute* cardiovascular and sympathetic response(s) to a single bout of IMST in healthy young adults. We show that IMST *acutely* increases heart rate with concomitant reductions in sympathetic nervous system outflow. Study 2 assesses the potential for six weeks IMST to alter the cardiovascular response to respiratory muscle fatigue in college-aged adults. The results of this study suggest IMST improves respiratory muscle endurance and as a result, blunts the blood pressure and heart rate responses to respiratory muscle fatigue. Finally, Study 3 documents the effects of six weeks IMST on overnight blood pressure and mediators of systemic vascular resistance in older adults diagnosed with obstructive sleep apnea. In a population that exhibits elevated blood pressure and sympathetic nervous system activity, we show that IMST significantly lowers daytime blood pressure, nighttime systolic blood pressure and resting sympathetic nervous system activity. These studies are the first to characterize the acute cardiovascular effects of IMST and to explore the effects of IMST on mediators of systemic vascular resistance in both healthy and patient populations.

OVERVIEW

In the studies detailed below I explore the acute and dynamic effects of inspiratory muscle strength training (IMST) on cardiovascular function. The work builds thematically on work by Dr. Jennifer Vranish that established the IMST intervention framework and provided initial evidence of IMST-related reductions in blood pressure in healthy adults and adults with obstructive sleep apnea (OSA). However, in this most recent work the emphasis shifts toward mechanistic insights and how acute exposure to IMST works to bring about longer-term improvements in cardiovascular health.

The Foreword provides a brief review of the literature in which I outline the principles of IMST and make the point that not all IMST training protocols are created equal and that IMST protocols implemented in different laboratories differ in regard to training intensity and/or training volume. Given a diversity of training protocols, I focus in on studies that use moderate or high intensity IMST and the effects of this training on cardiovascular indices and aerobic exercise performance in healthy young adults. The concluding paragraphs focus on cardiovascular outcomes in older adults with obstructive sleep apnea (OSA).

The body of the dissertation is made up of three chapters. Chapters 1 and 3 are published manuscripts that appear in the *Journal of Applied Physiology* (Study 1 DOI: 10.1152/japplphysiol.01015.2020; Study 3 DOI: 10.1152/japplphysiol.00024.2020). The results of a second study are presented in Chapter 2 and currently is in preparation for submission. Each of the chapters is presaged by a brief commentary.

FOREWORD

Hypertension is a major health issue in the United States and the development of evidence-based nonpharmacological strategies for the prevention and treatment of hypertension is a high priority. The cardiovascular benefits of regular exercise are well documented and include reductions in BP and the prevention of cardiovascular disease. Indeed, new guidelines issued by the American College of Cardiology (ACC) and American Heart Association (AHA) advocate 150 minutes/week of aerobic exercise among the first-line treatments for all stages of hypertension (71).

Although the importance of daily exercise for maintaining a healthy lifestyle is widely recognized, an estimated 25% of US adults (ages 18-64 years) are considered sedentary and fail to meet the national guidelines with regard to weekly activity (40, 74). Regular aerobic exercise confers important physiologic benefits including lowered blood pressure (72) and reduced risk of cardiovascular disease (60) however, for sedentary individuals and patient populations, exercise-related dyspnea and limb fatigue (12, 35, 41, 76) can preclude sustained exertion (52). And, because the benefits of exercise on cardiovascular health typically are delayed until ~3-4 months after initiation of regular exercise (10, 72), many adults will discontinue exercise before making any health gains (6, 8). The need for novel forms of exercise that are time-efficient, well tolerated, and effective for men and women of all ages has never been greater. Unfortunately, rising rates of obesity, exercise intolerance, limited access to gym facilities and lack of time continue to deter many adults from initiating and/or maintaining a regular exercise program.

IMST: a novel intervention. Beginning in 2015, we implemented a novel *abbreviated* physical training regimen known as inspiratory muscle strength training or IMST, originally

developed to improve respiratory muscle strength in select patient populations (2, 3, 37). There are several features of this type of training which set it apart from more traditional forms of aerobic exercise (i.e., running or cycling) and which merit elaboration. First, IMST is performed in stationary standing and subjects train for brief periods each day (i.e., 5 minutes). Second, breathing rate is constrained to 10-12 breaths/minute in contrast to traditional forms of aerobic exercise during which breathing rates may reach upwards of 45 breaths/minute (26). Last, when performed 5 days/week for 6 weeks, this remarkably abbreviated training technique lowers blood pressure and systemic vascular resistance in healthy young adults (14, 67).

IMST protocols vary widely (29, 66) but typically entail repeated inspiratory efforts against a resistance. Each of the studies set forth in this dissertation implements a high intensity, low volume training format implemented over the *intermediate* term (i.e., 6 weeks) (14, 47, 67, 68). In this format, subjects first generate a maximal inspiratory effort against a near infinite resistance – a so-called PI_{max} maneuver. The pressure generated in this maneuver establishes the individual's training pressure which is set to 75% of their PI_{max}. It is important to note that the magnitude of the inspiratory pressure generated during IMST distinguishes it from all other forms of aerobic exercise. Thus, the average inspiratory pressure generated by a healthy adult performing IMST is between (-50) to (-70) mmHg (14, 30, 50, 67) easily exceeding inspiratory pressures generated in tidal breathing (~2.5 mmHg) (69), deep breathing (~5 mmHg) (67) or high-intensity aerobic exercise (95% VO_{2max}, ~22 mmHg) (26, 32). Indeed, we have identified the repeated exposure to large inspiratory (negative) pressures as the primary stimulus underpinning IMST-related reductions in blood pressure (67).

A second aspect of IMST is the interval nature of the training format — that is, individuals perform training in 5 sets of 6 breaths with one to two minutes between each set. In this regard, IMST resembles another popular exercise training regimen known as High Intensity Interval Training or HIIT. Although HIIT entails whole body exercise, it is similar to IMST in comprising repeated short bouts of *vigorous yet submaximal exertion* (11). Like IMST, HIIT protocols also elicit larger reductions in SBP in middle-aged and older adults (8-10 mmHg) (36, 45, 61) than steady state aerobic exercise (2-8 mmHg) (71). Certainly, the intermittent nature of the training stimulus distinguishes HIIT and IMST from all other forms of traditional aerobic exercise and likely is key to achieving favorable cardiovascular outcomes in a comparatively short time frame.

***IMST and vascular resistance*.** In 2018, I reported significant declines in systemic vascular resistance (21.8±1.30 mmHg*L/min to 18.3±1.71 mmHg*L/min) among healthy young adults following 6 weeks high intensity, low volume IMST. No similar effects were noted in sham trained peers (14). Because sympathetic nerve activity is a potent regulator of peripheral vascular resistance (15), I posited that the decline in SVR may be a result of declines in sympathetic outflow. Thus, while sympathetic nerve activity in recreationally active adults is unaffected by 4-8 weeks running or cycling training (7), it may be that IMST exerts distinct effects SNS activities at the level of the vasculature.

***Sympathetic nervous system outflow and IMST*.** Muscle sympathetic activity is directed to vascular smooth muscle within skeletal muscle. Elevations in muscle sympathetic nerve activity (MSNA) increases vascular smooth muscle contraction, contributing to increased systemic vascular resistance and resultant reductions in limb

blood flow. Thus, MSNA plays a critical role in regulation of arterial blood pressure regulation and blood flow during times of rest (18) and physical activity (53). Importantly, MSNA recordings are the standard technique for assessment of human sympathetic nervous system activity. Thus, multiunit MSNA recorded directly from peripheral nerves via microelectrode inserted into the common peroneal nerve is reported in terms of burst frequency (burst/min) and burst incidence (bursts per 100 heart beats) and total activity (mean burst area /min) obtained from the integrated MSNA signal (73).

Regulation of MSNA include the arterial baroreflex (31, 34), arterial chemoreflex (44, 56), cardiopulmonary receptors (20, 28), pulmonary stretch receptors (54, 58) and muscle metaboreceptors (55, 57). At rest, the arterial baroreflex, cardiopulmonary receptors and lung stretch receptors play a role in the within-breath modulation of MSNA and blood pressure swing driven increases/decreases in MSNA (58, 64). During exercise, muscle metaboreceptor activation in exercising muscle in addition to central increases in sympathetic drive contribute to increases in MSNA and as a result, increased blood pressure. Hence, MSNA is an important *player* in the regulation of blood pressure.

To identify the mechanism/s underlying improvements in cardiovascular function in the intermediate term it is helpful to determine the *acute* effects of the intervention on heart rate, blood pressure and vascular resistance. In light of my previous work that demonstrated IMST-related reductions in vascular resistance (14), a reasonable next step is to assess the effects of IMST on regulators of systemic vascular resistance. Given that MSNA plays a critical role in blood pressure regulation, and second, that subjects can perform IMST while seated, we are provided the rare opportunity to evaluate the effects of the intervention on this potent regulator of vascular resistance. Accordingly, **Study 1**

of this dissertation characterizes the acute effects of the intervention on blood pressure, heart rate and MSNA in healthy young women and men to determine whether this *intermittent training stimulus* contributes to a *short-term* reduction in resting blood pressure.

***IMST and exercise endurance*.** As outlined above, IMST originally was devised for use in select clinical populations. However, many more since have adopted forms of respiratory muscle conditioning as a tool to enhance athletic performance. A review of this literature reveals IMST used in sports including running, cycling and rowing. Of particular interest and importance from the athletic standpoint is whether IMST might improve respiratory muscle strength or delay time to fatigue. Accordingly, numerous groups have documented IMST related improvements in respiratory muscle strength (25), time trial and endurance exercise performance (9, 27, 38, 43, 49, 65) and perceptions of breathlessness all achieved using IMST protocols of varying intensity, duration and volume (5, 49, 51, 62). The results show consistent improvements in respiratory muscle conditioning (4, 39, 46), exercise tolerance (5) and exercise capacity (16, 24, 39, 43, 59, 63, 75) which correlate with reductions in diaphragm fatigability and dyspnea in healthy adults (17, 29, 50, 65) and patient populations (12, 22). Whereas the aforementioned studies monitor dyspnea, an *indicator* of respiratory muscle work, none monitored inspiratory pressure and in turn, none assessed respiratory muscle work. This contrasts with traditional aerobic exercise studies in which participants routinely are required to perform at the same running or cycling workload pre-post intervention or are required to operate at a specified limb muscle workload throughout a trial (43, 48, 59, 62).

IMST and respiratory fatigue. An alternative approach to the assessment of respiratory muscle fatigue is the resistive breathing trial. Resistive breathing trials typically require subjects to prolong inspiratory duration (T_i) relative to total respiratory cycle time (T_{tot}) and to breathe against a moderate constant resistance (50-65% PI_{max}) to exhaustion. Unlike whole-body exercise, subjects are seated and still throughout permitting a unique opportunity to record respiratory muscle function and cardiovascular responses *independent* of limb muscle movement/work (55, 57, 77).

Respiratory fatigue trials to exhaustion have been used to characterize the cardiovascular consequences of respiratory muscle fatigue including increased sympathetic outflow to vascular smooth muscle (57), reductions in limb blood flow (55), and resultant fatigue related increases in blood pressure (21, 70). Thus, at high workloads blood may be redirected away from exercising limb musculature and toward fatiguing respiratory muscles (33, 52) with important implications for aerobic exercise performance.

Given evidence that IMST improves exercise performance and reduces perceptions of breathlessness, Witt and colleagues evaluated heart rate and blood pressure responses to respiratory muscle fatigue pre- and post- 5 weeks moderate intensity (i.e. 50% of PI_{max}), high volume (3 sets of 75 breaths, 6 days/week) IMST. They reported significant blunting of the blood pressure response to respiratory muscle fatigue following the intervention and concluded IMST increased the respiratory muscle threshold for fatigue (77). However, given no evidence of increased respiratory muscle performance pre- *versus* post-IMST and no evaluation of within or between subject assessments of cumulative respiratory muscle work these claims appear unsubstantiated. Accordingly, **Study 2** evaluates whether IMST can blunt the cardiovascular response to respiratory muscle

fatigue via increases in respiratory muscle work capacity. Importantly, Study 2 addresses the conceptual gaps in our understanding and technical shortfalls of previous studies in characterizing the cardiovascular response to respiratory muscle fatigue, respiratory muscle endurance and *respiratory muscle work capacity* pre-post high intensity (i.e.,75% of PI_{max}), low volume (30 breaths/day) IMST.

IMST in clinical settings. IMST initially was devised to strengthen the respiratory muscles of mechanically ventilated patients to help wean them from their machines (3). Since that time, IMST has been used to strengthen respiratory muscles and improve respiratory function in patient populations with respiratory muscle weakness including adults with chronic obstructive pulmonary disorder (13, 37), neuromuscular disorders (19) and heart failure (12). Importantly, the results from each of these studies has reinforced the promise that IMST holds as a therapeutic tool suitable for use in clinical populations.

Beginning in 2015, we explored the effects of IMST on the respiratory and cardiovascular function in adults with obstructive sleep apnea (OSA) (68). OSA is a type of sleep disordered breathing that disrupts sleep via obstruction of upper airway (apnea) or repeated airflow limitation (hypopnea). Apneas and hypopneas result in repeated arousal from sleep and surges in blood pressure which, over the long term, disrupt autonomic balance and result in chronic sympatho-excitation. Indeed, elevated basal sympathetic nervous system outflow is considered a key contributor to persistent hypertension in some 30-70% of OSA adults (1).

In the initial study by Vranish and colleagues in adults diagnosed with OSA (68), we examined the potential for high intensity, low volume IMST to improve respiratory function

and in turn, reduce the severity of their sleep apnea. However, the results showed that whereas IMST increased respiratory muscle strength it did not reduce OSA severity. Remarkably, the study also found significant and unanticipated reductions in systolic and diastolic blood pressure and concomitant reductions in circulating plasma catecholamines (68). This was the first evidence of the cardiovascular benefits of IMST in this population.

The mechanism(s) underlying the IMST related reductions in blood pressure and sympathetic activity remain to be determined. OSA is recognized as a cause of secondary hypertension and sympathetic nervous system activity is identified as a fundamental contributor to elevated blood pressure in this population (12). Whether IMST-related reductions in blood pressure occur secondary to reductions in sympathetic nervous outflow is unclear. By extension, whether IMST impacts 24-hour blood pressure — a better predictor of blood pressure related cardiovascular complications (23, 42) also is unclear. Accordingly, **Study 3** of this dissertation addresses these shortcomings by assessment of cardiac baroreflex sensitivity, daytime and 24-hour blood pressure and MSNA in older adults with OSA pre-post 6 weeks IMST.

STUDY 1: ACUTE CARDIOVASCULAR RESPONSES TO A SINGLE BOUT OF HIGH INTENSITY INSPIRATORY MUSCLE STRENTH TRAINING IN HEALTHY YOUNG ADULTS

Given the favorable effects on intermediate resting blood pressure and vascular resistance, IMST appears well-suited as an alternative/adjunct form of exercise. However, it is unclear in what ways IMST effects on cardiovascular function differ from more traditional forms of whole-body exercise. Accordingly, the first element of my dissertation details the results of experiments conducted in healthy young women and young men (22 ±2 years) in which subjects were asked to perform 5 sets of 6 inspiratory efforts against a resistance of 75% PI_{max} — that is, *a single bout of high intensity, low volume IMST*.

As such, we recorded heart rate and blood pressure throughout however, the study is distinct from any previous study of this type in the following respects: a) we obtained recordings of muscle sympathetic nerve activity and b) female and male subjects were matched in regard to their PI_{max}, training pressures and inspiratory muscle work per breath. This level of subject matching is a particular strength of the study and which allowed the direct comparisons of heart rate and blood pressure responses across the sexes for the first time. Importantly, Study 1 yielded *de novo* insights into IMST-related changes in HR, BP and MSNA which when repeated daily over six weeks, likely contribute to intermediate term reductions in blood pressure and systemic vascular resistance.

Acute cardiovascular responses to a single bout of high intensity inspiratory muscle strength training in healthy young adults.

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New & Noteworthy: Previous studies show 6 weeks of high intensity, low volume inspiratory muscle strength training (IMST) lowers blood pressure (BP) and systemic vascular resistance in young adults. However, the acute response to IMST is unknown. We characterized BP, heart rate and sympathetic nervous activity (SNA) in healthy young adults at Baseline, during IMST and in Recovery. There was no acute effect of IMST on BP however, there was significant IMST-related suppression of SNA that was of greater magnitude in women than men.

Abstract

High intensity, low volume inspiratory muscle strength training (IMST) has favorable effects on casual systolic blood pressure and systemic vascular resistance. However, the acute effects of IMST on heart rate (HR), blood pressure (BP) and sympathetic regulation of vascular resistance and the trajectory of post exercise recovery are not known. We recruited fourteen young adults (7 women/7 men, age: 22±2 years) to perform a single bout of high intensity IMST (inspiratory resistance set at 75% of maximal inspiratory pressure) importantly, female and male subjects were matched in regard to the target inspiratory pressure *and* target inspiratory muscle work per breath. We recorded HR, beat-to-beat changes in BP and postganglionic, muscle sympathetic nerve activities (MSNA) continuously throughout Baseline, a single bout of IMST (comprising five sets of 6 inspiratory efforts) and in Recovery. We show that one bout of IMST does not effect a change in BP however, it effects a significant increase in HR (68.4 ±11.7 BPM vs. 85.4 ±13.6 BPM; $p<0.001$) and a significant decline in MSNA (6.8 ±1.1 bursts/15s bin; $p<0.001$ vs. 3.6 ±0.6 bursts/15s bin) relative to Baseline. Remarkably, among men MSNA rebounded to Baseline levels within the first minute of Recovery however in women, MSNA suppression persisted for 5 minutes. We show that in healthy young adults, high intensity, low volume respiratory training results in the acute suppression of MSNA. Importantly, MSNA suppression is of greater magnitude and longer duration in women than in men.

Introduction

Despite abundant evidence that aerobic exercise lowers blood pressure and improves cardiovascular health, an estimated 60-80% of adults do not adhere to guidelines for minimum physical activity (150 min/week of moderate intensity aerobic exercise or 75 min/week of vigorous intensity aerobic exercise) (26, 36). The most often cited reason for not exercising is a “lack of time” (10).

Beginning in 2013, we modified for use a respiratory strength training protocol originally devised for use among ventilator dependent patients (1, 2). In its original format, this respiratory strength training protocol known as inspiratory muscle strength training (or IMST) followed a traditional exercise format requiring 20-30 minutes training/day or ~150 min /week (13, 16, 24, 32, 35, 43). In our hands, IMST comprises 30 (5 sets of 6 breaths) inspiratory efforts against a resistance (75% of the individual’s maximum inspiratory pressure; PI_{max}). Training is performed 5 minutes each day, 5 days/week for a total weekly training time of ~30 minutes. In this high intensity, low volume format, IMST is considered somewhat akin to high intensity interval training or HIIT (10, 19, 45).

We have shown that young healthy adults who complete 6 weeks of this respiratory training regimen exhibit significant improvements in blood pressure, autonomic balance and systemic vascular resistance (14, 40), with similar outcomes in select patient populations (34, 41).

Despite these encouraging outcomes and burgeoning interest in HIIT-type protocols (3, 42), the acute and post-exercise responses to IMST have yet to be characterized. Characterizing the cardiac and autonomic response/s to an acute bout of IMST is a necessary first step toward understanding the intermediate and longer term (4-6 weeks)

health-related physiologic adaptations. Accordingly, we obtained continuous recordings of heart rate, blood pressure, and sympathetic nervous system activity, (via microneurography) in women and men at baseline, during high-intensity IMST and immediately post-training, testing the hypothesis that a single bout of high-intensity IMST will effect a short-term suppression of muscle sympathetic nerve activity (MSNA) in young healthy adults.

Methods

Ethical approval and human subjects. We recruited 14 healthy adults (7 women, 7 men; ages 18-30 years) from the student population at The University of Arizona (see Table 1). All were *casual* exercisers (i.e., ~30 minutes of exercise 3-5 days/ week) (23), non-smokers, non-obese, normotensive and free from overt cardiovascular disease. After obtaining written consent, all subjects underwent screening assessments of pulmonary function. All studies were performed in The University of Arizona's Human Neurophysiology Laboratory following 4 hours of fasting and 12 hours free from caffeine and exercise. Experimental procedures were approved by the University of Arizona Human Subjects Protection Program, and in accordance with the *Declaration of Helsinki*. Complete datasets were obtained for all subjects with one exception. In a male subject, MSNA data was not obtained during minutes 4 and 5 of Recovery.

Maximum inspiratory pressure (PI_{max}). While seated in a dental chair, subjects were coached to generate a PI_{max} from residual lung volume via a bespoke resistance device comprising a mouthpiece attached to a non-rebreathing valve (2600 series; Hans Rudolph, Shawnee, KS, USA) fitted with a flow limitation end cap on the inhalation port providing a constant, near-maximal resistance. Inspiratory pressure was detected via a

tube attached to the non-rebreathing valve and coupled to a pressure transducer (Omegadyne Inc., Stamford, CT). PI_{max} was determined for each subject as the average of at least three maximum inspirations against resistance that were within 5% of each other. For all subjects, 75% PI_{max} defined the individual's target training pressure (see General procedures below).

Blood pressure and heart rate. Beat-to-beat changes in blood pressure were monitored via an automated finger cuff pressure transducer (500Hz sampling rate; ccNexfin; Bmeye, Amsterdam, The Netherlands) on the non-dominant hand. Continuous lead-II ECG (1000Hz sampling rate; band pass filters 0.3-1.0 KHz) was sampled using surface electrodes (Kendall 133 foam electrodes; Covidien, Mansfield, MA) and recorded online (LabChart 8.0, ADInstruments. Colorado Springs, CO).

Muscle sympathetic nerve activity (MSNA). Concurrent with ECG recordings, sympathetic nerve traffic was recorded from the common peroneal nerve via tungsten microelectrode (200μm: 25-40mm, impedance: 5MΩ) (FHC, Bowdoin, ME) inserted percutaneously immediately posterior to the fibular head. Subjects rested semi-upright with the right knee and foot supported by positioning pillows (VersaForm™, Performance Health, Warrenville, IL). Microelectrode placement was confirmed via electrical stimulation (0.02 Ma, 1 Hz) as described previously (29). A second microelectrode, inserted just below the skin surface ~1.0 cm from the first served as a reference electrode. Electrode position in muscle fascicles was confirmed by pulse synchronous bursts of activity, elicitation of afferent nerve activity by mild muscle stretch and absence of response to light stroking of the skin or with startle response to loud noises (29). The recorded signal was amplified (gain 2 X 10^4), bandpass filtered (500-2.0kHz) using a pre-

amplifier (NeuroAmp Ex; ADInstruments, Colorado Springs, CO) and signals were full wave rectified (0.1 s moving window), sampled at 10 kHz. The resulting signal was monitored using a computer-based data acquisition and analysis system (LabChart 8.0 software, ADInstruments, Colorado Springs, CO) and loudspeaker throughout the experiment.

General experimental protocol. With the resistance device coupled to a pneumotachometer (PNT series 4183; Hans Rudolph) to record expiratory airflow, subjects were coached to inspire to their PI_{max} and to sustain inspiratory effort against the resistance for 1-2s before exhaling to end-expiratory volume. Accordingly, the breathing rate for all subjects was set at 12 breath cycles per minute (14, 40). After a period of rest breathing, subjects completed a single bout of IMST comprising 5 sets of 6 inspiratory efforts with a 60-90 second rest between sets (see Figure 1). Subjects were provided audio and visual cues to guide them in attaining correct breath timing and their inspiratory target pressure (75% of PI_{max}). Following the fifth and final set, subjects were allowed to recover (see Recovery). End tidal CO_2 levels were monitored via CO_2 analyzer (model 17515, Vacumetrics, Inc, Ventura, CA) and supplemental CO_2 was titrated as needed to prevent hypocapnia.

Data analysis. Beat-to-beat measures of systolic blood pressure, diastolic blood pressure, mean arterial pressure, instantaneous heart rate, respiratory muscle work and muscle sympathetic nerve activity were averaged in each of the following experimental segments: Baseline, IMST (sets 1-5), and Recovery. For Baseline, data were averaged over the entire 5-minute segment. For IMST (sets 1-5), data were averaged separately for each *set* (corresponding to 6 breaths against the resistance) and for each of the

intervening rest periods (corresponding to the 1 minute of rest between each IMST set). In Recovery, data were averaged each minute for 5 minutes. Data in IMST and Recovery were expressed as a percentage of Baseline (%baseline).

Respiratory work (mmHg/s) was calculated as the area beneath the inspiratory pressure waveform and respiratory volume (L), determined by calculating the area under the expiratory portion of the flow waveform.

Negative deflecting cardiac related sympathetic bursts were identified using unprocessed and root mean squared MSNA signals. Bursts that exceeded a predetermined threshold were marked and counted. Baseline MSNA was averaged over the entire 5-minute segment. In view of the abbreviated training window (<1.0 minute), MSNA burst frequency (bursts/minute) was expressed as number of bursts per 15 second bin and weighted averages used to quantify MSNA when experimental segments exceeded 15 seconds (e.g., Baseline, IMST and Recovery). Likewise, MSNA burst incidence (bursts/100 heart beats) was calculated over successive IMST bouts as follows:

$$\frac{Total\ number\ of\ MSNA\ bursts}{Total\ training\ time\ (s)}\ x\ \frac{60\ seconds}{Average\ HR\ during\ IMST}\ x100$$

Estimates of MSNA burst frequency and incidence recorded during IMST (sets and intervening rests) and in each minute of Recovery subsequently were expressed as a percentage of Baseline.

Statistical analyses. Differences in anthropomorphic data between the sexes were assessed using independent sample *t* tests with significance set at $p<0.05$. A mixed model with fixed effects was used to assess the main effects of IMST on each parameter

(HR, SBP, DBP and MSNA) in each experimental segment (IMST sets, intervening rests and Recovery) and in which subjects served a random factor. A 95% confidence interval (CI) was used to delineate significant differences between each experimental segment and Baseline. Significant differences between IMST sets, intervening rests and Recovery were determined by pairwise comparisons. Post hoc mixed models with fixed effects were used to test the effects of IMST on select experimental time points (i.e., IMST Set 5 and Recovery Minute 5) and the effect of sex. Significant differences in effects (time or sex) were set at $p<0.05$ and significance between experimental time points (IMST Set 5 and Recovery Minute 5) and Baseline were determined using 95% Cis. Sex differences at specific time points in Recovery (Minutes 1-5) were evaluated via pairwise contrasts with significance corrected to account for multiple comparisons ($p<0.01$). All data were normalized to Baseline. Accordingly, experimental time points were significantly different from Baseline if the 95% CI *excluded* zero.

Results

Subject characteristics. Female and male subject participants were matched for age ($p=0.386$), weight ($p=0.125$), body mass index ($p=0.751$), resting heart rate ($p=0.987$), casual systolic blood pressure ($p=0.946$), and casual diastolic blood pressure ($p=0.792$). Female subjects were shorter than their male counterparts ($p=0.028$). Details of menstrual phase and birth control status are presented in Table 2. At the time of assessment, 3 women were in the luteal phase and 4 in the follicular phase. Three of seven women were taking prescription oral contraceptives (combination progestin/estradiol).

Respiratory parameters. Maximal inspiratory pressures (F: -79.9mmHg; M: -78.3; p=0.879) and target inspiratory pressures (75% PI_{max}) (F: -58.6mmHg; M: -58.7mmHg; p=0.991) were the same for women and men. Further, both sexes performed the same inspiratory work per breath (F: 84.1mmHg.s; M: 85.40mmHg.s; p=0.918) and generated comparable expiratory volumes (F: 0.649L; M: 0.771L; p=0.189) (See Table 3) during IMST.

Cardiovascular responses to IMST. A representative continuous recording obtained at Baseline, and throughout IMST and Recovery in one subject is presented in Figure 2. As shown, in each of the 5 IMST sets, the subject generated large, negative intrathoracic pressures (approximately -66.5mmHg) with resultant increases in heart rate and BP fluctuations. Note that the suppression of sympathetic activity coincides with the transition into each IMST set. For this subject in Recovery, MSNA, blood pressure and heart rate all approximated Baseline levels.

Figure 3 shows averaged outcomes (%baseline) for HR, SBP, DBP and MSNA during each of the experimental segments (IMST sets, intervening rests and Recovery). During each IMST set, there were significant increases in heart rate (26.3 ±1.48% [95% CI 23.2, 29.4]) and significant declines in MSNA (-41.2 ±5.42% [95% CI -52.5, 29.8]). In the intervening rest periods, heart rate declined but remained elevated relative to Baseline (11.3 ±1.5% [95% CI 8.1, 14.5]). Subjects also exhibited modest increases in SBP (5.8 ±1.3% [95% CI 3.1, 8.5]) and DBP (4.8 ±1.2% [95% CI 2.3, 7.2]) while MSNA trended towards Baseline values (-11.1 ±5.6% [95% CI -22.7, 0.53]). In Recovery, heart rate returned to Baseline values [95% CI -4.6, 1.7] while SBP and DBP remained slightly

elevated above Baseline [SBP 95% CI 0.5, 5.9; DBP 95% CI 0.01, 4.8]. Conversely, MSNA suppression persisted throughout Recovery (-18.8 ±5.4% [95% CI -30.8, -7.4]). Figure 4 depicts results for HR, SBP and DBP at the terminus of the IMST training bout. By IMST Set 5, average heart rate was ~25% higher (25.2 ±2.1% [95% CI 20.8, 29.7]) but quickly returned to Baseline frequency by Recovery minute 5 (-4.1 ±2.2% [95% CI -8.7, 0.5]). In contrast, there was no consistent effect of IMST on blood pressure, and SBP and DBP were not different at training end (i.e., IMST Set 5) and Recovery (Minute 5) (SBP: 3.5 ±1.5%, p=0.154; DBP: 2.4 ±1.3%, p=0.473).

Figure 5 shows results for MSNA burst frequency (bursts/15s bin) and estimated burst incidence (bursts/100 heart beats) during IMST. Burst frequency was ~45% lower during IMST than at Baseline (-43.8 ±7.5% [95% CI -59.3, -28.2]) and on average, suppression persisted throughout Recovery (-23.3 ±7.8% [95% CI -39.5, -7.2]). Burst incidence followed the same trend, with burst incidence rates ~60% lower during IMST (-60.2 ±15.9% [95% CI -73.4, -47.0]) and continued suppression throughout Recovery (-17.1 ±29.8% [95% CI -30.3, -3.9]).

A more fine-grained analysis of MSNA as a function of sex (Figure 6), revealed comparable burst frequencies for women and men at Baseline (F: 14.1 ±2.3 vs. M: 18.7 ±2.7 bursts/min) that progressively declined during each IMST set, attaining a nadir in Set 5 (F: -54.1 ±8.9%; M: -33.3 ±8.4%, p=0.328). Importantly, trajectories in Recovery differed between women versus men. In men, MSNA burst frequency rebounded to Baseline by Recovery minute 1, whereas among women suppression was of greater magnitude (Minutes 1-3; $p<0.009$) and persisted throughout Recovery. It should be noted

that at the same time, heart rates were lower in women than in men (p=0.0298) however, SBP and DBP were not different between the two groups (p=0.269 and p=0.393). Despite lower heart rates, MSNA burst incidence was lower among women than men (F: -35.5 ±7.8%; M:1.3 ±10.2%, p=0.0148) and also slower to return to Baseline.

Discussion

Our objective in the current study was to characterize the *acute* effects of a single bout of high intensity (75% of PI_{max}) low volume (30 breaths) IMST on key cardiovascular parameters in young healthy adults. The primary novel finding(s) of the study are that IMST results in acute increases in heart rate (~25%) and the simultaneous acute suppression of MSNA (~40%) that reaches a nadir in the fifth and final training Set. Second, in Recovery, IMST-related suppression of MSNA is of greater magnitude and longer duration in women. Among men, sympathetic neural activity quickly rebounds and returns to Baseline within the first minute of Recovery.

IMST and the regulation of heart rate. In response to IMST, we report a transient increase in average HR (68.4 ±11.7 BPM vs. 85.4 ±13.6 BPM) that returns to Baseline values after cessation of training. The present findings are similar to those reported in other *non-fatiguing* inspiratory resistive breathing protocols and show both low (≤10% PI_{max}) and high (≥70% PI_{max}) workloads elicit comparable increases in heart rate (7-9).

The magnitude of HR change during IMST was not significantly different between female and male subjects (F: 28.9 ±4.4% vs M: 22.9 ±12.6%; p=0.257, main effect for sex). To our knowledge, little has been reported on sex-specific differences in HR responses to *non-fatiguing* respiratory muscle work. However, our findings are in line with evidence

from aerobic exercise, where high intensity interval/circuit training (5) and incremental exercise protocols (21, 30) elicit similar HR increases between women and men, especially when workload is matched at the same percentage of subject VO2max.

IMST and the regulation of blood pressure*.* An extensive literature documents the *acute* effects of resistive breathing on blood pressure and reveals effects that are distinct for low vs. high resistance breathing. Inspiratory efforts against low resistance (≤10% PI_{max}) have been used to clinical advantage to effect acute increases in stroke volume, cardiac output and SBP (7-9), whereas moderate (i.e., 60% PI_{max}) or higher (i.e. 90% PI_{max}) resistances yield different outcomes (31, 38).

Importantly, the current study comprising equal numbers of women and men matched in regard to target inspiratory pressure *and* inspiratory muscle work provides valuable new insight into the effects of the stimulus (IMST) on regulation of blood pressure. Specifically, where previous studies have documented sex differences in arterial baroreflex function dynamic exercise (27, 28), we report BP responses to each training set that are similar for both groups (SBP, p=0.803; DBP, p=0.283). In view of the similarity of the BP response to IMST and the transient (~30s) nature of the respiratory stimulus (22), we think it unlikely that IMST-related suppression of MSNA has its origins in the arterial baroreflex regulation of sympathetic nervous system activity.

IMST and suppression of muscle sympathetic nerve activity. Others previously have documented within-breath inspiratory-related inhibition of sympathetic outflow in the context of loaded and unloaded respiratory tasks (38, 39). Importantly, a key distinction between this earlier work and the current study lies in the magnitude of the inspiratory

pressure generated. Specifically, the inspiratory pressures reported by St Croix et al., encompass -1.0 and -13.0 mmHg as compared to -60 and -100 mmHg generated by the subjects in the current study. Thus, it is the much larger (~4 fold greater) inspiratory pressures generated in the context of high intensity low volume IMST that distinguishes the current protocol from previous studies of this type and which is the unique stimulus that modulates MSNA.

In recent studies, we attributed IMST-related reductions in BP and systemic vascular resistance to cardiopulmonary receptor inhibition of (central) sympathetic outflow secondary to repeated large (negative) intrathoracic pressures driving *respiratory pump* mediated venous return (14, 40). The current finding of MSNA suppression coincident with the initiation of the inspiratory efforts and therefore, in time with the respiratory pump-induced augmentation of venous return, provides further strong support for this hypothesis.

IMST and vascular endothelial function. Inspiratory efforts against a significant resistance also contribute to transient increases in cardiac output (20-40%) (6) presumably with downstream effects on regional and/or systemic blood flow and increases in shear stress sensed by endothelial cells. Although we did not attempt an assessment of flow-mediated arterial dilatation (FMD) in the acute context, *de novo* findings obtained in older men and women (70 years) following 6 weeks high intensity, low volume IMST show significant improvements in FMD and NO production with reductions in superoxide production (11, 12). In light of the current findings, an assessment of vascular endothelial function in young women and young men during IMST is warranted.

Experimental considerations: strengths and limitations. Female and male participants were matched for inspiratory work. To our knowledge only one other study has attempted a similar level of matching (18). Thus, the majority have evaluated sex differences in the cardiovascular response to inspiratory resistance/work in women and men matched for age and $FEV_{1.0}$ (%) (25, 37, 44). In matching across sex for absolute inspiratory muscle work *and* anthropomorphic data (save height), the current study provides a unique opportunity to assess the effects of IMST on cardiovascular parameters independent of differences in size/muscle mass.

Available published data for the effects of menstrual phase (4, 15, 33), birth control (20) and stressors (17) on sympathetic nervous activity in young women remain equivocal. Although the current findings indicate a comparable blunting of MSNA for subjects in the follicular vs. luteal phases (Table 2), the dataset is too small to draw a definitive conclusion. Additional studies that entail a larger female cohort and which control for menstrual phase and/or hormonal birth control are indicated.

Conclusions. Traditional aerobic exercise has well-documented and favorable effects on blood pressure and cardiovascular health and yet 60-70% of adults fail to meet the minimum weekly requirement for physical activity. We show here that a novel training protocol, referred to as IMST, results in acute increases heart rate and acute suppression of sympathetic nervous outflow. Among young women and men, IMST related reductions in sympathetic nervous system activation are comparable in magnitude however, suppression is of greater magnitude and longer lasting in women than in men. Our results provide new insight into the acute effects of IMST which when repeated daily over the

intermediate and longer term can modify cardiovascular health via reductions in BP, systemic vascular resistance and sympathetic nervous outflow.

Acknowledgements. The authors thank Dr. Mark Borgstrom for assistance with statistical analyses and undergraduate researcher Mr. Wyatt Snell for assistance with experimental procedures.

Table 1. Group averages (±SD) for anthropomorphic measures for female (n=7) and male (n=7) subject participants. ***Significant difference p<0.05.**

	Female		**Male**	
Measure	*Mean*	*SD*	*Mean*	*SD*
Age (years)	21.3	2.4	22.1	0.9
Height (cm)	***171.7**	**6.3**	**183.1**	**10.4**
Weight (kg)	68.0	13.4	78.6	10.5
BMI	22.9	3.3	23.4	2.3
HR (BPM)	75.2	7.7	73.6	15.1
SBP (mmHg)	114.0	9.1	114.0	4.7
DBP (mmHg)	71.9	8.7	70.8	4.7
$FEV_{1.0}$ (L)	3.6	0.5	4.2	0.7

Table 2. Birth control and menstrual phase status for female participants.

Subject	Birth Control	Menstrual phase	MSNA response (%baseline)
1	No	Follicular	-67.2%
2	Yes	Follicular	-23.2%
3	Yes	Follicular	-46.5%
4	No	Follicular	-33.7%
5	No	Luteal	-52.0%
6	Yes	Luteal	-61.4%
7	No	Luteal	-94.9%

Table 3. Group averages for maximum inspiratory pressure (PI_{max}), target training pressure (75% PI_{max}), inspiratory work per breath, and expiratory flow obtained from 30 IMST breaths for female (n=7) and male (n=7) subjects.

	Female		Male	
Measure	*Mean*	*SD*	*Mean*	*SD*
Max inspiratory pressure (PI_{max}, mmHg)	-79.9	17.7	-78.3	21.7
Target pressure (75% PI_{max}, mmHg)	-58.6	13.0	-58.7	16.3
Inspiratory work (mmHg.s)	84.1	22.2	85.0	25.9
Expiratory volume (L)	0.649	0.1	0.771	0.2

Figure 1. Schematic of experimental protocol. Baseline: subjects sat quietly for 5 minutes. IMST Sets 1-5: subjects performed 5 sets of 6 breaths against an inspiratory resistance (75%PI_{max}) interspersed with ~60 seconds rest. Recovery: subjects sat quietly for 5 minutes.

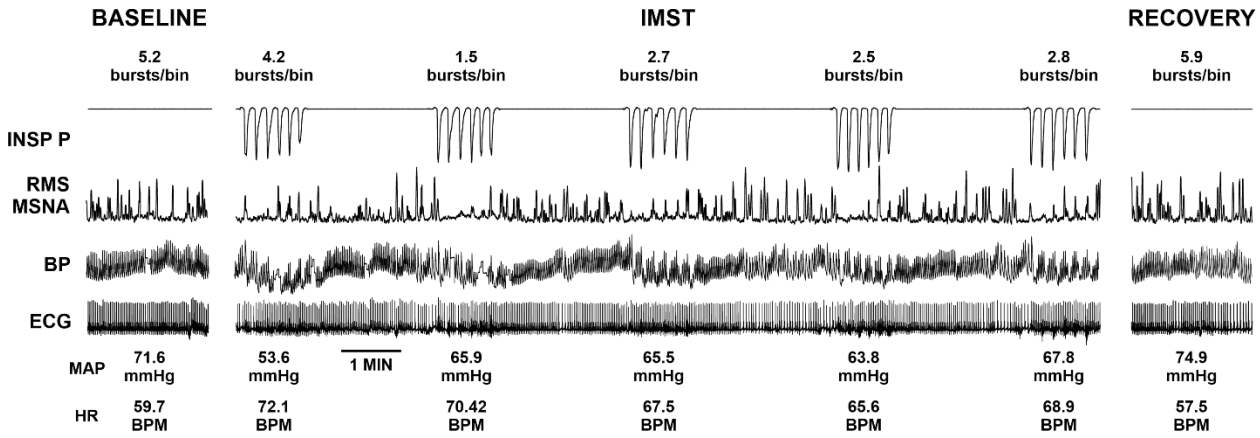

Figure 2. Representative recording of inspiratory pressure (INSP P), postganglionic, multiunit muscle sympathetic nerve activity (RMS MSNA), blood pressure (BP), and electrocardiogram (ECG) signals recorded from a subject at Baseline, during IMST, and in Recovery. Burst frequency counts (in bursts/15s bin), mean arterial pressure (MAP), and heart rate (HR) are provided for Baseline, each of the IMST sets and in Recovery.

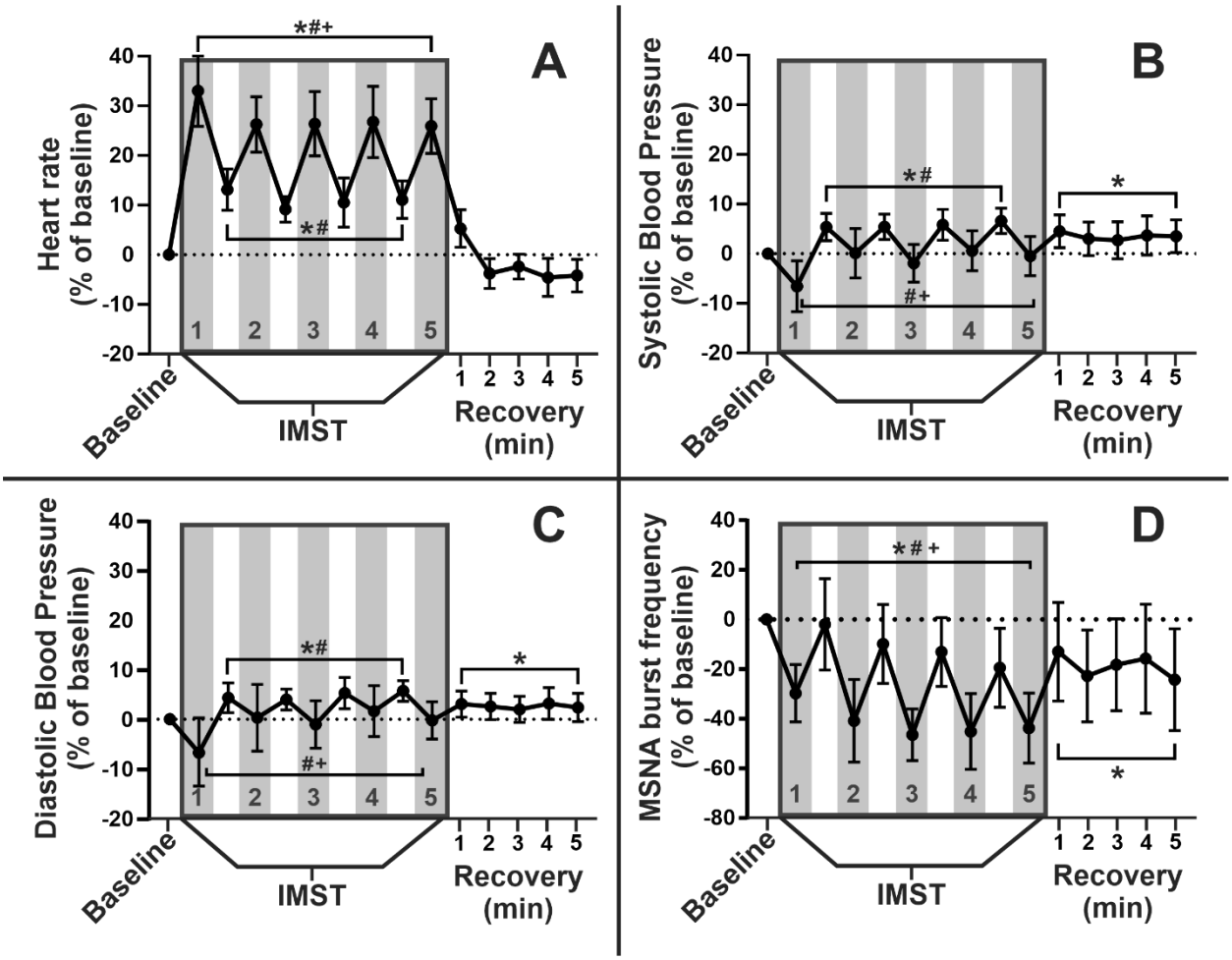

Figure 3. Group averages (±95%CI) for (A) heart rate; (B) systolic blood pressure (C) diastolic blood pressure; and (D) MSNA burst frequency expressed as percentage of Baseline (%baseline). For IMST, subjects performed five sets of six inspiratory efforts against a resistance (75%PI_{max}) (grey columns) interspersed with ~1 min of resistance-free, rest breathing (white columns). Following IMST, subjects sat quietly for 5 minutes of Recovery. ***Significant difference from Baseline (95% CI excludes zero); #Significant difference from Recovery (p<0.05); +Significant difference between IMST sets and intervening rests (p<0.05).**

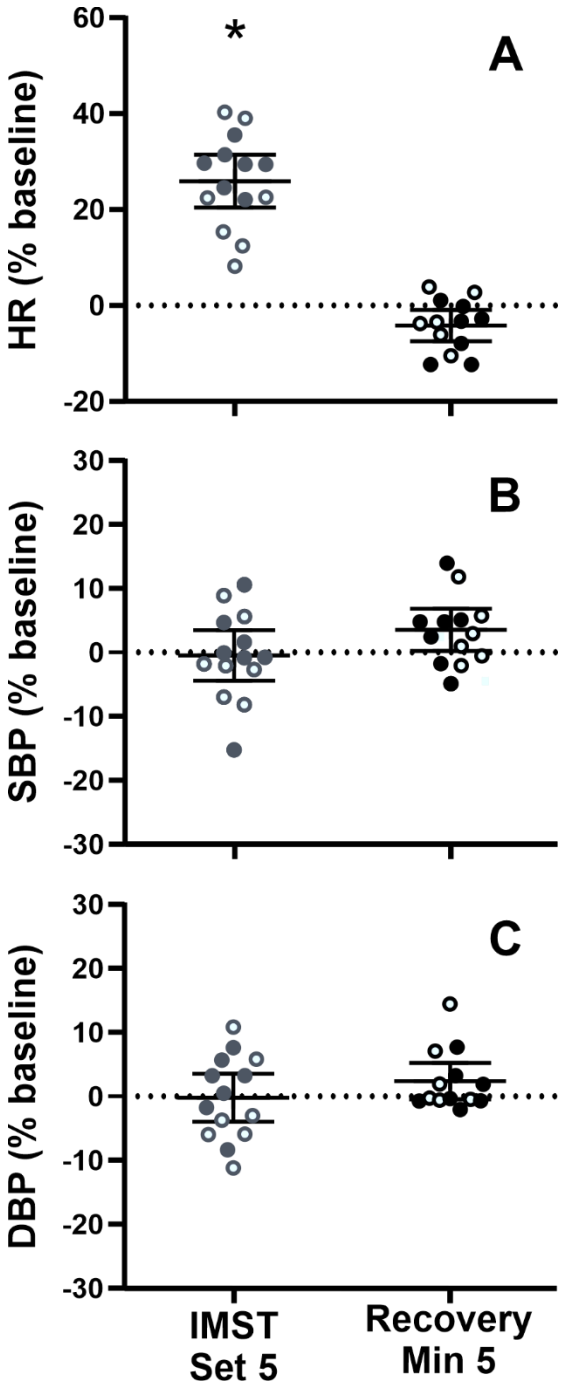

Figure 4. Group averages (±95%CI) for (A) heart rate (HR); (B) systolic blood pressure (SBP); and (C) diastolic blood pressure (DBP) (%baseline) during IMST Set 5 (grey symbols) and Recovery minute 5 (black symbols) for female (solid symbols) and male (open symbols) subjects. ***Significantly different from Baseline (95% CI excludes zero).**

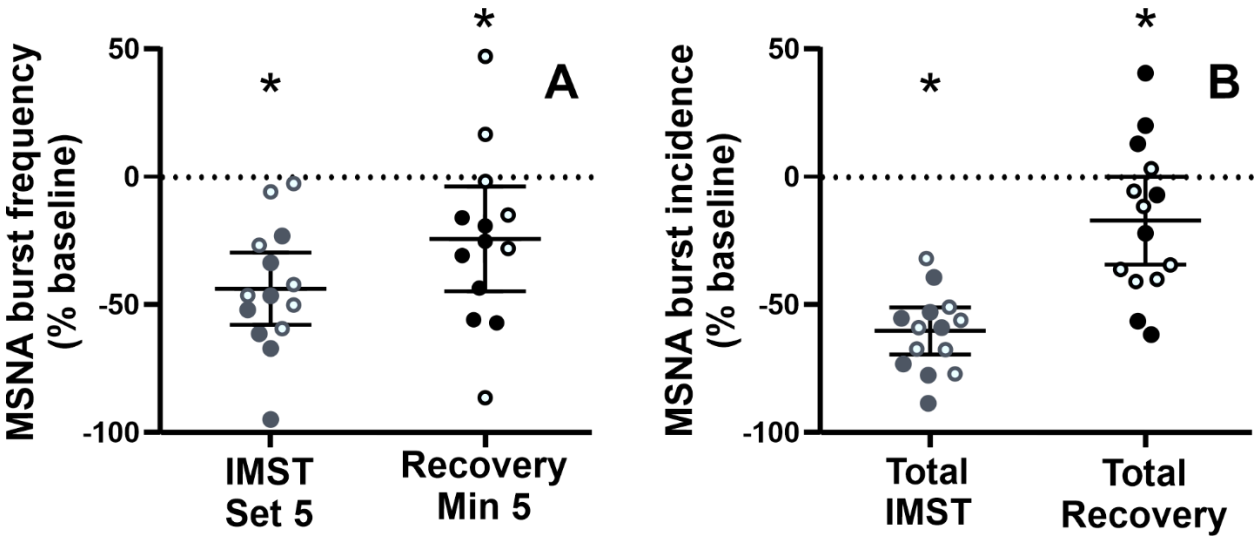

Figure 5. (A) Group averages (±95%CI) for MSNA burst frequency (%baseline) at IMST Set 5 (grey symbols) and at Recovery minute 5 (black symbols) for female (solid symbols) and male (open symbols) subjects. (B) Calculated MSNA burst incidence (%baseline) over the 5 discontinuous IMST sets and over the entire 5-minute Recovery period for female (solid symbols) and male (open symbols) subjects. ***Significantly different from Baseline (95% CI excludes zero).**

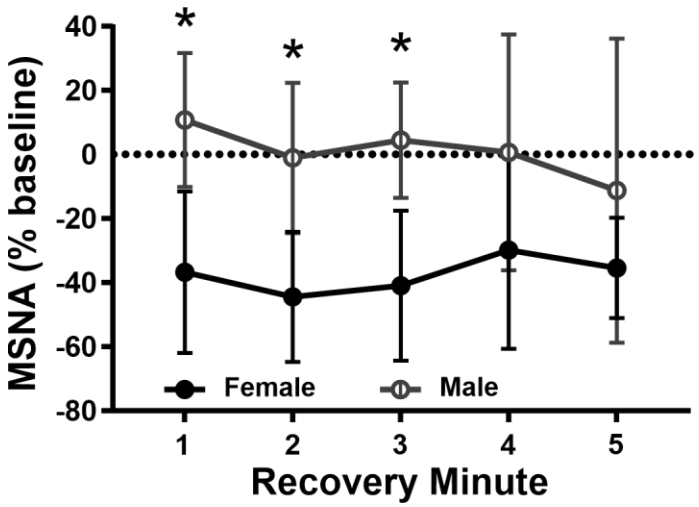

Figure 6. Group averages (±95%CI) for MSNA burst frequencies (%baseline) for men (open symbols) and women (filled symbols) in Recovery minutes 1-5. In men, MSNA rebounded to Baseline by minute 1. ***Significantly different from females (P<0.05).**

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STUDY 2: INSPIRATORY MUSCLE STRENGTH TRAINING INCREASES RESPIRATORY MUSCLE ENDURANCE AND ATTENUATES THE CARDIOVASCULAR RESPONSE TO FATIGUE

There is substantial interest in the potential for more abbreviated and/or high intensity interval type training formats like IMST to favorably impact *dynamic* cardiovascular function over the *intermediate* term. Despite several previous efforts to evaluate the potential for IMST to blunt the cardiovascular response to respiratory muscle fatigue none of the studies has considered/evaluated respiratory work. Accordingly, Study 2 of the dissertation details studies in healthy young adults (21.1 ±2.5 years) who perform respiratory muscle fatigue trials to exhaustion, before and after 6 weeks of IMST.

Throughout the fatigue protocol, we recorded heart rate, blood pressure and time to exhaustion however, unlike any previous studies of this type, it includes an assessment of *respiratory muscle work.* The latter is considered an essential element of the protocol which allows me to ask: Does respiratory muscle work plays a role in the cardiovascular response to fatigue? Importantly, results from Study 2 of this dissertation demonstrate IMST-related improvements in respiratory muscle endurance correlate significantly with improved cardiovascular responses to respiratory muscle fatigue[1].

[1] As a side note, the fatigue protocol also requires subjects to remain seated throughout thereby lending itself to MSNA recordings. Although we were successful in obtaining MSNA recordings in two subjects in the context of fatigue, pre-post 6 weeks IMST, these data are considered too preliminary and therefore, are not reported in Study 2 findings.

High intensity, low volume inspiratory muscle strength training extends respiratory endurance while attenuating the cardiovascular response to respiratory muscle fatigue in healthy adults.

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Author contributions
Conception and design of the experiments: CMD and EFB. Collection, analysis, and interpretation of data: CMD, DRD, SMS and EFB. Draft and revision of the article: CMD, DRD, SMS and EFB.

Abstract

Previous findings from our laboratory suggest six weeks of high intensity, low volume inspiratory muscle strength training (IMST) lowers resting blood pressure via reduced systemic vascular resistance. Although numerous reports underscore favorable outcomes for resting blood pressures, we know very little about the potential impact of IMST on the cardiovascular response to exercise. Pre- and post- intervention assessments comprised a respiratory muscle fatigue trial during which subject inspiratory muscle work, heart rate and blood pressure and breathing-related perceptions were recorded. Subjects exhibited reductions in resting systolic (-5.6mmHg, p=0.007), diastolic (-5.5mmHg, p=0.029) and mean arterial pressures (-5.6mmHg, p=0.004). Resting heart rates were unchanged (p=0.999). Remarkably, subjects exhibited a 58% increase in maximal inspiratory pressure (p<0.001) and an 82% increase in respiratory muscle endurance (p=0.001). When expressed as a function of cumulative work (unit), heart rate, systolic and diastolic blood pressure responses during the fatigue trial were significantly blunted post- relative to pre-intervention. Our results provide *de novo* observations on the potential for *high intensity* IMST to improve respiratory muscle endurance secondary to lowered resting blood pressure and attenuate the cardiovascular response to respiratory muscle fatigue in healthy young women and men.

Introduction

In healthy adults, respiratory muscle fatigue is evoked following sustained, high intensity aerobic exercise (5) or resistive breathing with prolonged duty cycles (1, 10). As respiratory muscles fatigue, mechanoreceptors increase firing rates and metabolic by-products (metabolites) accumulate within the working musculature. The result is a sudden decline in inspiratory force presaged by time-dependent increases in dyspnea (18), heart rate (HR) and sympathetic vasoconstrictor activity (23, 25) that diverts blood flow to the respiratory muscles — the respiratory muscle metaboreflex. Whether respiratory muscle conditioning can mitigate these cardiovascular responses and extend the time to fatigue (t_{lim}) is of interest and importance from athletic (3) and clinical (11) perspectives.

High intensity (75% PI_{max}), low volume (30 breaths/day) inspiratory muscle strength training (IMST) delivers significant improvements in inspiratory muscle strength (25-35mmHg gain in PI_{max}) and reductions in systolic blood pressure (4-6mmHg) (4, 21, 28, 29). Whether the intervention can reduce cardiovascular responsiveness to high levels of inspiratory muscle work and extend time to fatigue is unknown. Although previous studies assessed cardiovascular indices in the context of a respiratory muscle fatigue pre-post IMST (or similar intervention), the studies entailed minimal or moderate intensity IMST (i.e. 50-60% PI_{max}) and none evaluated respiratory work or time to fatigue (27, 32). In light of the above, we asked whether high intensity, low volume IMST could increase respiratory muscle endurance, attenuate respiratory-related dyspnea and in turn, dampen cardiovascular responsiveness to respiratory muscle fatigue. Specifically, we sought to test the hypothesis that in healthy young adults, 6 weeks IMST attenuates HR and BP

responses to continuous resistive breathing to exhaustion and extends the time necessary to reach respiratory muscle fatigue.

Methods

Ethical approval and human subjects. We recruited 10 healthy adults (3 women. 7 men; ages 18-30 years) from the student population of the University of Arizona (Table 1). All were *casual* exercisers (i.e., ~30 minutes of exercise 3-5 days/week), non-smokers, non-obese, normotensive and free from overt cardiovascular disease. All studies were performed in the University of Arizona's Human Neurophysiology Laboratory following 4 hours of fasting and 12 hours free from caffeine and exercise. Experimental procedures were approved by the University of Arizona Human Subjects Protection Program, and in accordance with the *Declaration of Helsinki*. All subjects underwent preliminary screening assessments to determine their comfort breathing against an inspiratory resistance. Only subjects able to breathe consistently via the resistance apparatus were considered eligible to participate and completed the preliminary assessments outlined below.

Spirometry. Assessments of lung function comprising assessments of forced expiratory volume in 1.0 sec ($FEV_{1.0}$), forced vital capacity (FVC), forced inspiratory volume in 1.0 sec ($FIV_{1.0}$), forced inspiratory capacity (FIVC), $FEV_{1.0}$/FVC, $FIV_{1.0}$/FVC, $FIV_{1.0}$/FIVC, peak expiratory flow (PEF) and peak inspiratory flow (PIF) (WinspiroPRO, Medical International Research, New Berlin, WI, USA) in accordance with the guidelines of the American Thoracic Society (17). Subjects with an $FEV_{1.0}$ >80% were eligible to participate.

Resting blood pressure. In-laboratory measures of resting (seated) blood pressure were obtained at intake, at study close, and once weekly throughout the 6-week intervention. Measures were taken in accordance with American Heart Association guidelines (19) with an automated oscillometric sphygmomanometer (SunTech CT40, SunTech Medical). Three measures, taken on alternate arms, were averaged to obtain systolic (SBP) and diastolic (DBP) blood pressures and to determine mean arterial pressure (MAP) using the equation: (MAP = DBP + 1/3[SBP – DBP]). Measures were obtained at the same time of day and on the same day each week for 6 weeks.

Maximal inspiratory pressure (PI_{max}). While seated in a dental chair, subjects were coached to perform a series of Mueller maneuvers (i.e., maximal inspiration against a closed glottis) from residual volume with ~2 minutes rest between each effort. For each maneuver, PI_{max} was defined as the peak pressure generated and sustained for at least 5 seconds. The average of three trials (within 5%) defined the individual's PI_{max} (2, 14).

Study design.

Following preliminary screening assessment, all subjects completed experimental protocols in sequence as follows: a) Pre-assessment; b) Intervention; c) Post-assessment.

Pre and Post assessments. All subjects completed pre- and post- intervention fatigue protocols comprising a baseline recording (5 minutes), the respiratory fatigue protocol and a period of recovery (10 minutes). In accordance with previously published literature (1, 10, 23), respiratory fatigue was induced by continuous breathing via a circuit at a predetermined duty cycle (T_i/T_{tot} = 0.7) and inspiratory resistance (target pressure = 65%

of PI_{max}). Subjects were provided audio and visual cues to ensure they maintained the correct inspiratory and expiratory durations and attained the target inspiratory pressure. The circuit comprising a mouthpiece attached to a non-rebreathing valve (2600 series; Hans Rudolph, Shawnee, KS, USA) fitted with a flow limitation end cap on the inhalation port, provided a constant, near-maximal inspiratory resistance. Inspiratory pressures were detected via a tube attached to a bespoke respiratory valve coupled to a pressure transducer (Omegadyne Inc., Stamford, CT), sampled at 500 Hz, digitized, stored using a Cambridge Electronic Design 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK). Online integration of inspiratory pressures yielded estimates of inspiratory muscle work (mmHg.s). Expiratory airflow was recorded using a pneumotachometer (PNT series 4183; Hans Rudolph) coupled to the expiratory port of the non-rebreathing respiratory valve. End tidal CO_2 levels were monitored via CO_2 analyzer (model 17515, Vacumetrics, Inc, Ventura, CA) and supplemental CO_2 was titrated as needed to prevent hypocapnia.

The target breathing frequency for the fatigue assessments was matched to each subject's rest breathing frequency. Subjects rated their perceived exertion via the Borg Scale at 30 second intervals throughout. The fatigue protocol was terminated when the subject; a) was unable to initiate a breath *or* b) performed *consecutive* breaths in which inspiratory muscle work declined by ≥10% relative to average inspiratory muscle work attained in minute 1. Termination was defined as t_{lim} and followed by Recovery (10 minutes).

Beat-to-beat changes in blood pressure and heart rate were sampled via an automated finger cuff pressure transducer (500Hz sampling rate; ccNexfin; Bmeye, Amsterdam, The Netherlands) on the non-dominant hand. Continuous lead-II ECG (1000Hz sampling rate; band pass filters 0.3-1.0 KHz) was sampled using surface electrodes (Kendall 133 foam electrodes; Covidien, Mansfield, MA), recorded online (LabChart 8.0, ADInstruments. Colorado Springs, CO) and stored on a computer for analysis.

Intervention. Following the Pre-assessment, subjects completed 6 weeks of high-intensity, low-volume inspiratory muscle strength training in accordance with previously published protocols (4, 21, 28, 29). Briefly, subjects reported to the laboratory each weekday (Monday-Friday) and completed 5 sets of 6 inspiratory efforts with a 1-2 minute rest between sets. Each subject's training pressure was displayed on a computer monitor and they were instructed to adjust their effort to achieve that target (75% of PI_{max}). In training, respiratory rate was set at ~12 breaths per minute. Each subject's PI_{max} was retested weekly and target training pressures adjusted accordingly.

Data analysis. Beat-to-beat measures of blood pressure (systolic and diastolic), instantaneous heart rate (and where available, MSNA) were averaged during baseline, for 10% intervals during the fatigue trial and during recovery. All values were expressed as percentage of baseline (%Baseline).

Estimates of respiratory muscle work per breath and expiratory volume per breath were determined by integration of the inspiratory pressure and expiratory flow signals, respectively during the Pre- and Post-assessments only. Given that time to fatigue varied between subjects and over time (Pre vs Post), comparisons were conducted on

normalized data and at the same time points (%total time). Thus, if a subject completed their Pre-assessment with a t_{lim} of 5 minutes and the Post-assessment with a t_{lim} of 10 minutes, the 50% time point would correspond to 2.5 minutes and 5 minutes, respectively. Linear regressions for fatigue related changes in heart rate, systolic blood pressure and diastolic blood pressure over time were determined at select time points corresponding to 20, 40, 60, 80 and 100% of total (fatigue protocol) time. Individual subject regression slopes were calculated for both Pre- and Post-assessments. Pearson correlations were used to examine the relationship between cumulative inspiratory muscle work and heart rate, systolic blood pressure and diastolic blood pressure pre- and post-intervention.

Statistical analyses. Differences in anthropomorphic data and resting maximal inspiratory pressure, systolic blood pressure, diastolic blood pressure and mean arterial blood pressure were assessed using paired samples *t* tests with significance adjusted according to the Bonferroni correction. Pre- *versus* Post-assessment differences (%change) in heart rate, systolic blood pressure and diastolic blood pressure were assessed via multiple *t* tests with the Bonferroni correction for multiple comparisons (include adjusted P value). Linear regression slopes were transformed into z-scores and used in paired samples *t* tests to depict the trajectories of heart rate, systolic and diastolic blood pressure throughout the fatigue protocol, pre- vs. post intervention. The relationship between cumulative inspiratory muscle work and %change in heart rate, systolic blood pressure and diastolic blood pressure was assessed by Pearson correlation.

Results

Subject characteristics. Anthropomorphic data are shown in Table 1. As there are only 2 women participants, data are collapsed across the sexes. As shown, there were no

significant changes in subject age, height, weight or BMI pre- vs. post-assessment. All subjects completed the intervention; thus, the study retention rate was 100%.

Respiratory muscle strength and endurance. All subjects made significant gains in respiratory muscle strength. The average increase in maximal inspiratory pressure (PI_{max}) was ~38mmHg, encompassing the range -64.6 ±16.0mmHg to -102.5 ±24.0mmHg, ($p<0.001$) (Table 2). Time to fatigue (t_{lim}) was significantly longer Post- relative to Pre-intervention. Compared to Pre-assessment, t_{lim} of 376.7±65.9s, t_{lim} Post-assessment rose to 686.2 ±320s, equivalent to a ~82% increase ($p=0.001$). Despite substantial gains in respiratory muscle strength, inspiratory work per breath was comparable Pre (126.8 ±58.8 mmHg.s) *vs.* Post intervention (139.2 ±65.9 mmHg.s) ($p=0.188$). With respect to perceived exertion during fatigue, subjects reported slightly reduced initial Borg ratings (Pre: 9.9 ±1.8 vs. Post: 8.7 ±1.6), similar final Borg ratings (Pre: 18.3 ±1.2 vs. Post: 18.6 ±1.3) and a similar change in Borg ratings from beginning to end of the fatigue trial (Pre: +8.4 ±2.1 vs. Post: +9.9 ±2.4) Pre- and Post-IMST (Figure 3).

Cardiovascular outcomes. Measures of resting systolic, diastolic, and mean arterial blood pressures at intake and study close are shown in Table 3. Group average systolic blood pressure declined ~5.6mmHg (Pre:119.0 ±9.1mmHg; Post: 113.4 ±10.4mmHg, $p=0.007$), diastolic pressure ~5.5mmHg (75.0 ±4.3mmHg vs. 69.5 ±6.2mmHg, $p=0.029$) and mean arterial pressure by ~5.6mmHg (89.7 ±5.1mmHg vs. 84.1 ±6.9mmHg, $p=0.004$). There was no change in resting heart rate ($p=0.999$).

Group outcomes for heart rate, systolic blood pressure and diastolic blood pressure time-based trajectories (%Baseline) throughout pre- and post-assessment fatigue protocols (%total time) are shown in Figure 1. As shown, heart rate (Pre: 0.2616, $r^2=0.4249$ *vs.*

Post: 0.2503, r^2=0.4706; p=0.999), systolic blood pressure (Pre:0.0421, r^2=0.6140 *vs*. Post:0.0224, r^2=0.1333; p=0.072) and diastolic blood pressure (Pre:0.0246, r^2=0.6608 *vs*. Post:0.0227, r^2=0.0501; p=0.245) outcomes were not different pre vs. post intervention.

Inspiratory work and cardiovascular function during the respiratory fatigue protocol. The relationship between cumulative inspiratory work, heart rate (Panels A and D), systolic blood pressure (Panels B and E) and diastolic blood pressure (Panels C and F) are shown in Figure 2. The strong *positive* correlation between heart rate and cumulative inspiratory muscle work and between systolic blood pressure and cumulative inspiratory muscle work evident in the pre-assessment (HR: r=0.5704, p<0.001; SBP: r=0.1675, p=0.0019) was eliminated post intervention (HR: r=0.0271, p=0.618; SBP: r=-0.0541, p=0.320). Likewise, evidence of a strong *positive* correlation between diastolic blood pressure and inspiratory work evident pre-intervention (r=0.3259, p<0.001) shifted *negative* post-intervention (r=-0.1472, p=0.007).

Discussion

Our objective was to evaluate the effects of a 6 weeks high intensity (75% of PI_{max}) low volume IMST on respiratory endurance, respiratory-related perceptions and the cardiovascular response to respiratory muscle fatigue.

We recruited recreationally active college-aged students and measured the time to fatigue (t_{lim}), respiratory muscle work and %change in blood pressure and heart rate during a respiratory muscle fatigue trial pre- and again post- IMST. Our principle finding(s) are that when performed according to this training format, IMST effects significant improvements in respiratory muscle strength and endurance while blunting blood pressure and heart

rate responses to increasing respiratory muscle work. In addition, we confirm significant IMST-related reductions in resting systolic, diastolic and mean arterial pressure absent any change in resting heart rates.

Effects of the intervention on PI_{max}. In these healthy young adults, PI_{max} was 58% greater i.e., more negative, post relative to pre intervention. The increase is within the range of improvements reported previously with high intensity IMST protocols (4, 21, 28, 29) but exceeds that reported following moderate intensity IMST protocol (i.e. 50% of PI_{max}) (32).

Effects on resting blood pressure. Consistent with previous work (4, 28), our subjects exhibited significant (~5mmHg) reductions in resting systolic, diastolic and mean arterial pressures pre- vs. post intervention. The effect of IMST on BP appears more potent than for traditional aerobic exercise which has not been shown to lower blood pressure within 6-8 weeks in recreationally active young adults (8, 16, 20). Consistent with prior intervention studies there was no effect of IMST on resting heart rate (4, 21, 28, 29).

Effects of the intervention on time to fatigue (t_{lim}). Remarkably, subjects in the current study exhibited an 82% increase in the average time to fatigue (t_{lim}) and a 69% increase in cumulative respiratory muscle work post- *versus* pre-intervention. Only two previous studies have evaluated respiratory muscle endurance within the context of a respiratory muscle fatigue trial pre-post IMST (27, 32). Unfortunately, as the first study did not report time to fatigue (32) and the second capped the post-intervention fatigue time to a preset value (27), comparison with the current outcomes for time to fatigue and respiratory muscle work are precluded.

Effects of the intervention on the cardiovascular response to fatigue. We report the no evidence of fatigue trial-related increases in blood pressure pre- or post-IMST (Figure 1) (23, 25). An absence of an effect on blood pressure is consistent with two previous *standalone* respiratory muscle fatigue trials (23, 25) but differs from others that reported significant increases in MAP (7, 31). In view of the apparent divergence in blood pressure outcomes, we focused on the cardiovascular response as a function of continuous *inspiratory muscle work* asking, do subjects' heart rate and blood pressure responses to cumulative inspiratory work differ pre- vs post- 6 weeks IMST? Our analysis reveals a strong positive correlation between heart rate, systolic and diastolic blood pressure and cumulative inspiratory muscle work pre-intervention that is effectively abolished following 6 weeks IMST (Figure 3).

Estimates of respiratory work are key in gauging the effects of IMST on fatigue-related blood pressure responses and in turn, respiratory endurance. Yet, to our knowledge, the present study is the first study to evaluate respiratory muscle work pre-post intervention and to assess the effect of work on heart rate and blood pressure responses in the context of fatigue (23, 25, 31). A previous study by Witt and colleagues also evaluated blood pressure responses to respiratory fatigue, pre- and post- IMST and noted fatigue-related increases in blood pressure were blunted post intervention (32). However, Witt et. al., adopted a moderate intensity (50%PI_{max}) high volume (75 breaths/day) IMST and participants' respiratory muscle work was not reported. Although the authors argued on the basis of their findings that IMST may shift the threshold for fatigue (12, 32), the present findings provide the first concrete evidence that at equivalent levels of cumulative

respiratory muscle work, 6 weeks high intensity, low volume IMST raises the threshold at which fatigue-related increases in blood pressure begin to emerge.

Effects of the intervention on fatigue-related perceptions. The Borg scale reliably and reproducibly allow individuals to report effort symptoms related to increased respiratory workloads (9, 15). Although the Borg scale has served as a proxy of total respiratory motor output (30), it is unclear whether or how well respiratory muscle training protocols like IMST may attenuate perceptions of respiratory effort given a linear relationship to increasing respiratory muscle work (26). Accordingly, we asked our subjects to rate perceptions of effort at 30 second intervals throughout the fatigue trial on the original (7.5-20 point) Borg scale. Consistent with previously published findings (27), despite subjects performing considerably more cumulative respiratory muscle work post- vs. pre-intervention, the trajectory of Borg ratings throughout each trial and at t_{lim} were not different pre- post-intervention (p=0.999 at t_{lim}).

Limitations and future directions. Our subject pool comprised unequal numbers of women and men. In view of differences in respiratory muscle fatigability between young women and men (7, 24, 31), an equal number of men and women would permit the *a priori* comparison of cardiovascular responses to fatigue between groups.

An important secondary cardiovascular consequence of respiratory muscle fatigue is a reduction in resting leg blood flow due to increased sympathoexcitation (13, 25). Although many previously have underscored the significant role the sympathetic nervous system plays in the respiratory muscle metaboreflex (6, 22), no study has reported on the potential for IMST to reduce fatigue-related sympathoexcitation. An evaluation of MSNA

in the context of fatigue pre-post IMST is a logical next step. Although we have obtained MSNA recordings in two subjects pre-post intervention, these data are insufficient for meaningful insights.

Conclusions. Our results highlight the respiratory and cardiovascular benefits of high intensity, low volume IMST for healthy adults. Six weeks IMST significantly lowers resting systolic, diastolic and mean arterial pressure independent of any change in resting heart rate and contributes to significant attenuation of fatigue-related increases in heart rate and blood pressure via increases in respiratory muscle strength and endurance.

Table 1. Mean (±SD) anthropomorphic data for 10 subjects (3F, 7M) at intake. There were no significant differences in age, height, weight or BMI by study close.

Measure	*Mean*	*SD*
Age (years)	21.1	2.5
Height (cm)	176.3	8.4
Weight (kg)	68.3	12.4
BMI (kg/m^2)	21.8	2.7

Table 2. Mean (±SD) respiratory measures both at rest and during the respiratory muscle fatigue trial pre- and post-IMST for 10 subjects. ***Significant difference from pre-IMST (adjusted p<0.05).**

	PRE		*POST*	
Respiratory Measure	*Mean*	*SD*	*Mean*	*SD*
PI_{max} (mmHg)	-64.6	16.0	**-102.5***	**24.0**
Avg inspiratory work (mmHg.s)	126.8	58.8	139.2	65.9
t_{lim} (s)	376.7	65.9	**686.2***	**320.0**

Table 3. Mean (±SD) resting cardiovascular measures at intake and at study close. ***Significant difference from pre-IMST (adjusted p<0.05).**

	PRE		*POST*	
Resting Measure	*Mean*	*SD*	*Mean*	*SD*
Systolic blood pressure (mmHg)	119.0	9.1	**113.4***	**10.4**
Diastolic blood pressure (mmHg)	75.0	4.3	**69.5***	**6.2**
Mean arterial pressure (mmHg)	89.7	5.1	**84.1***	**6.9**
Heart Rate (bpm)	69.7	11.8	68.4	11.0

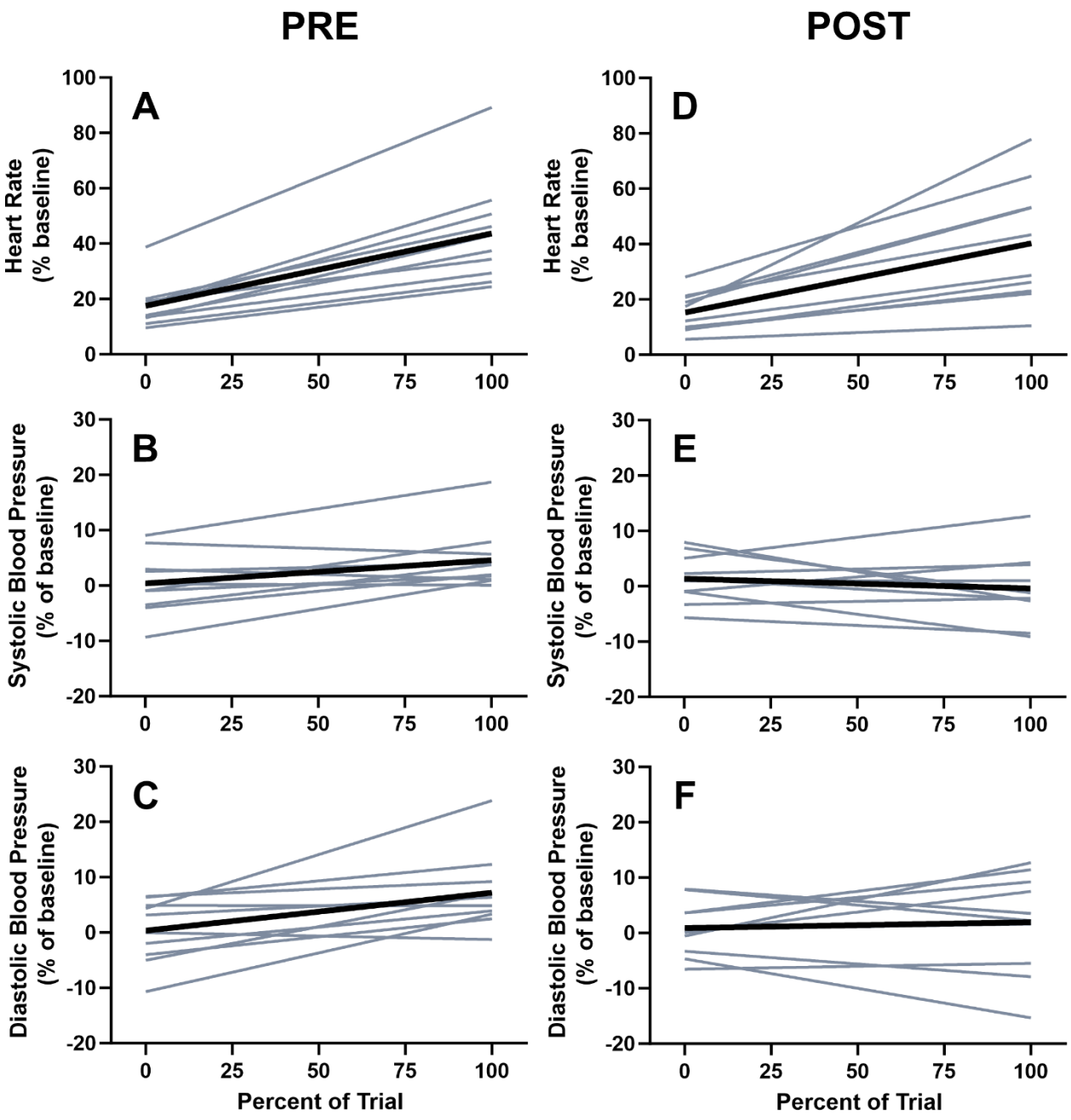

Figure 1. The heart rate (A & D), systolic blood pressure (B & E) and diastolic blood pressure (C & F) rates of change during the Pre-assessment (left) and Post-assessment (right) as a function of % trial completed. There were no significant differences in the rate of change in any cardiovascular variable between the Pre- and Post-assessments.

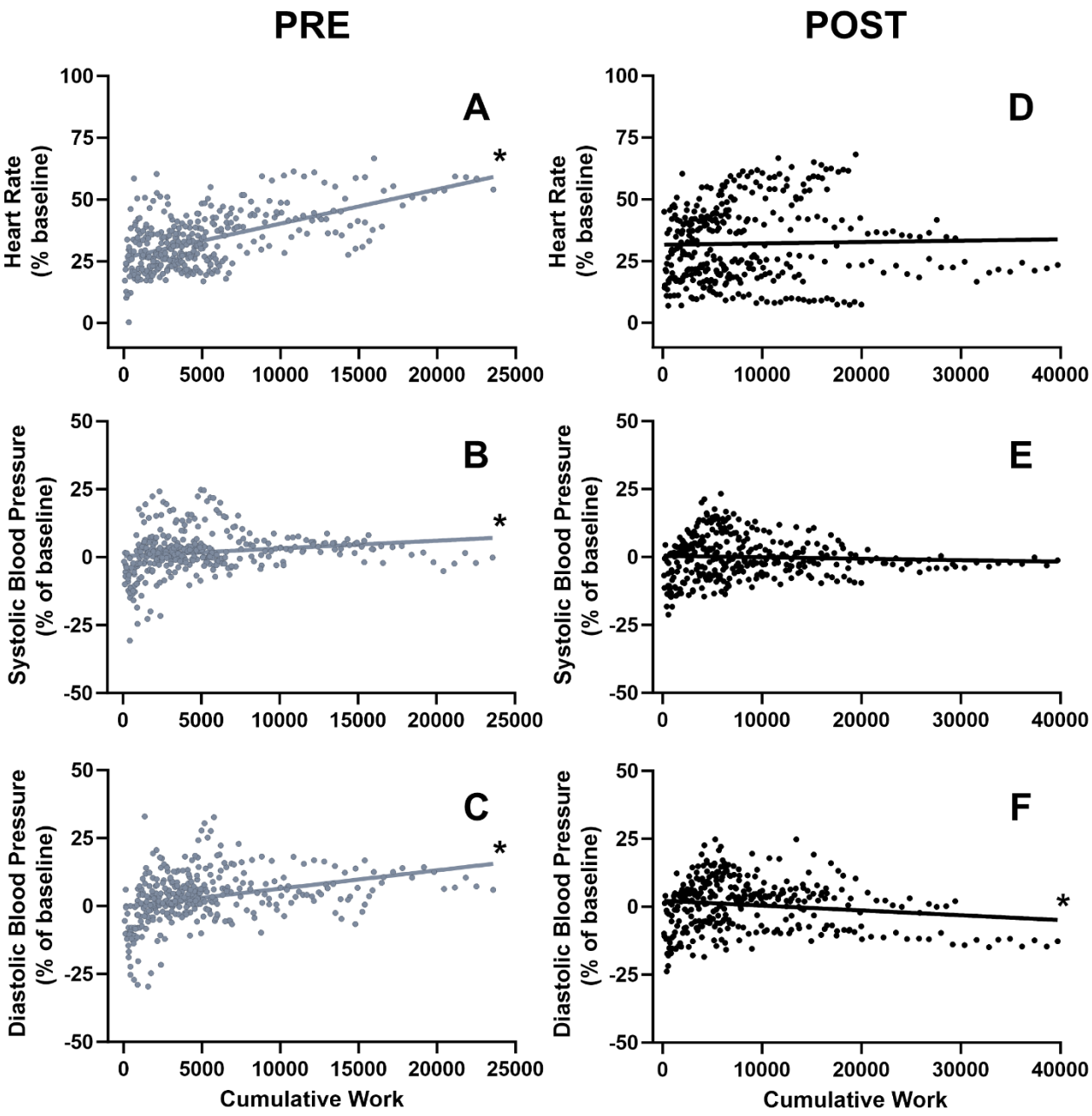

Figure 2. Correlations between cumulative inspiratory work and heart rate (A & D), systolic blood pressure (B & E) and diastolic blood pressure (C & F) during the Pre-assessment (grey) and Post-assessment (black). At study intake, there were significant correlations between cumulative inspiratory muscle work and heart rate, systolic blood pressure and diastolic blood pressure. By study close, these significant correlations were abolished (HR and SBP) or reversed (DBP). ***Significant correlation with cumulative inspiratory muscle work.**

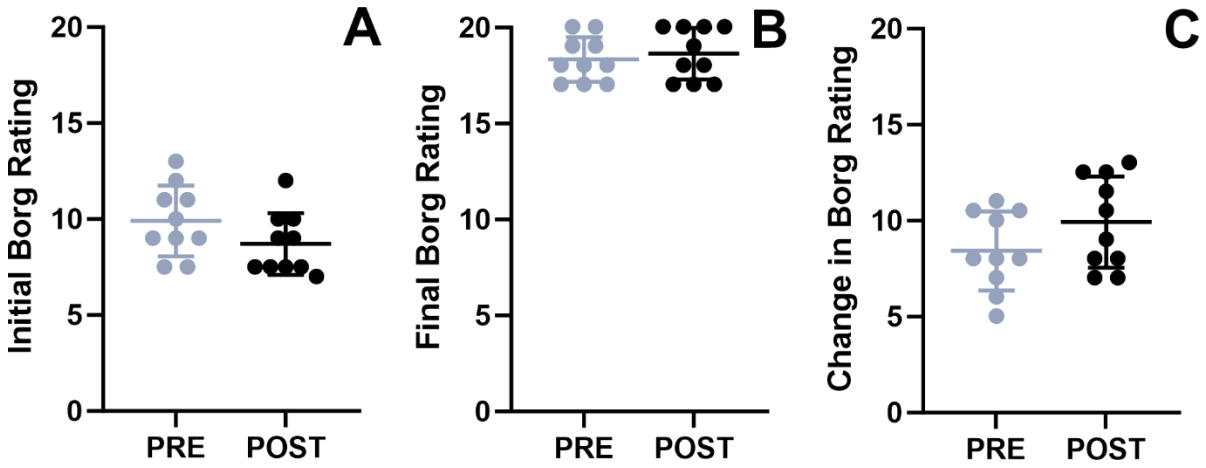

Figure 3. Individual Borg scale ratings (Lines Mean ±SD) Pre- (grey) *versus* Post-IMST (black) for 10 subjects. There were no significant differences in the initial (A), final (B) or change (C) in Borg scale perception of effort scores.

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STUDY 3: INSPIRATORY MUSCLE STREGTH TRAINING LOWERS BLOOD PRESSURE AND SYMPATHETIC ACTIVITY IN ADULTS WITH OSA: A RANDOMIZED CONTROLLED PILOT TRIAL

In view of their well-documented sympatho-excitation at rest, adults with OSA are an ideal population in which to implement IMST to assess the impact of training on systemic vascular resistance. Accordingly, Study 3 of the dissertation details studies in older adults (66 ±8 years) diagnosed with mild, moderate and severe OSA who participated in a randomized controlled pilot trial to evaluate the intermediate effects of IMST on daytime *and* 24-hour ambulatory blood pressure and muscle sympathetic nervous outflow. Subjects underwent either high intensity (75%PI_{max}), low volume (30 breaths/ day, 5 days a week) IMST or low intensity (15%PI_{max}), low volume (30 breaths/ day, 5 days a week) Sham training for 6 weeks.

The study is considered distinct from all previous exercise interventions in OSA because it is the first of its kind to; a) assess the intermediate (i.e. 6 week) effects of IMST on mediator(s) of systemic vascular resistance and, b) report on resting sympathetic nerve activity before and after high intensity, low volume IMST. Whereas previously we reported blood pressure improvements in adults with mild-moderate OSA, this small clinical trial recapitulates and expands on those findings by adding measures of overnight blood pressure. Our results provide a necessary first step in characterizing the sympathetic nervous outflow in older adults with OSA and in demonstrating the effects of 6 weeks IMST on this key mediator of vascular resistance.

Inspiratory muscle strength training lowers blood pressure and sympathetic activity in older adults with OSA: A randomized controlled pilot trial.

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ABSTRACT

Previous work has shown lowered casual blood pressure after just six weeks of inspiratory muscle strength training (IMST), suggesting IMST as a potential therapeutic in the prevention/treatment of hypertension. In this study, we assessed the effects of IMST on cardiovascular parameters in older, overweight adults diagnosed with moderate and severe obstructive sleep apnea (OSA). Subjects were randomly assigned to one of two interventions i) high intensity IMST (n=15, 75% maximal inspiratory pressure) or ii) a control intervention (n=10, 15% maximum inspiratory pressure). Subjects in both groups trained at home completing 30 training breaths/day, 5 days/week for 6 weeks. Pre- and post- training measures included maximal inspiratory pressure, casual and ambulatory blood pressures, spontaneous cardiac baroreflex sensitivity, and muscle sympathetic nerve activity. Men and women in the high intensity IMST group exhibited reductions in casual systolic, diastolic and mean arterial blood pressures (SBP: -8.82 ± 4.98 mmHg; DBP: -4.69 ± 2.81 mmHg; MAP: -6.06 ± 1.03 mmHg; $p<0.002$), and nighttime SBP (pre: -12.00 ± 8.20 mmHg; ($p<0.01$). Muscle sympathetic nerve activities also were lower (-6.97 ± 2.29 bursts/min^{-1}; $p=0.01$ and -9.55 ± 2.42 bursts per 100 heartbeats; $p=0.002$) by Week 6. Conversely, subjects allocated to the control group showed no change in casual blood pressure or muscle sympathetic nerve activity and a trend toward higher overnight blood pressures. A short course of high intensity IMST may offer significant respiratory and cardiovascular benefits for older, overweight adults with OSA. Clinical Trial Registration: URL: https://www.clinicaltrials.gov. Identifier: NCT02709941

INTRODUCTION

Obstructive sleep apnea (OSA) is characterized by repeated airflow obstruction (apnea) and airflow limitation (hypopnea) that result in sleep disruption and chronic intermittent hypoxemia (CIH). CIH has been linked to increases in reactive oxygen species and oxidative stress that contribute to sympathetic nervous system (SNS) hyperactivity (11, 37, 50, 67), and hypertension in an estimated 30-70% of OSA adults (1). The standard of care for OSA worldwide is continuous positive airway pressure (CPAP), which delivers a steady stream of pressurized air via a (nasal/oral) mask to stent the upper airway and stabilize breathing and blood oxygenation. Among adults with OSA and hypertension, nightly CPAP use improves spontaneous baroreflex sensitivity (BRS) (38, 69) and overall sympathetic nervous system activity (29, 41). However, these favorable outcomes are offset by uniformly low adherence i.e., <4.4 hours/night (20, 35, 40, 45) that continue to limit CPAP-related improvements in cardiovascular health (45).

Aerobic exercise is a first-line treatment for all stages of hypertension (76) and has well-documented benefits for blood pressure. Indeed, 2017 guidelines issued by the American Heart Association and American Cardiology Association advocate 150 minutes/week of aerobic exercise among the first-line treatments for all stages of hypertension (76) to lower blood pressure. Although traditional forms of aerobic exercise may improve BRS and lower blood pressure in OSA, the salient features of OSA including obesity (BMI >30) (21, 63, 77), lethargy (64, 68), and/or exercise intolerance (2, 7, 10, 28), often preclude sustained exertion (18, 43).

In recent years, a novel form of exercise known as inspiratory muscle strength training (IMST) has yielded surprising results including improvements in blood pressure and

autonomic balance in patients with hypertension (25, 41) or OSA (72), and reductions in systemic vascular resistance in healthy young adults (19, 71). These outcomes are of interest and importance because in each case they were attained within 6 weeks and with a training requirement of just 5 minutes a day/5 days/week or 25 min/week total training time (72).

Whereas there is evidence that IMST performed daily lowers casual (resting) blood pressure and plasma catecholamines in adults with OSA and elevated or Stage 1 hypertension (72), it is unclear what effect it may have on 24-hour blood pressure, a better predictor of blood pressure related end-organ damage (27, 46). Accordingly, in the current study we obtained measures of casual *and* continuous, non-invasive ambulatory blood pressure monitoring in a cohort of older (60-80 years), predominantly obese (i.e., BMI > 30) adults with moderate-severe OSA (apnea hypopnea index, AHI≥15) pre-post 6 weeks IMST. Because OSA is a recognized cause of secondary hypertension and sympathetic nervous system activity plays a fundamental role in raising blood pressure in this population (12), we also performed microneurography to quantitate sympathetic neural activity directed to vascular smooth muscle (i.e., muscle sympathetic nerve activity, MSNA). Last, whereas in a previous IMST study CPAP-users had been excluded, the current study permitted inclusion of participants identified as adherent to CPAP or mandibular advancement devices (>4 hours nightly use).

METHODS

This prospective, randomized double-blind controlled pilot clinical trial was conducted on adults with OSA who were recruited from the general population via advertisements placed in regional publications. Details about how the trial was conducted, reporting

enrollment, allocation, follow-up and analysis of subjects involved in the clinical trial are presented in a CONSORT (Consolidated Standards of Reporting Trials) flow chart (See Figure 1) (23, 60). Exclusion criteria included: asthma, history of respiratory disease, neurological impairment, head/neck or thoracic surgery, hypothyroidism, immune or nervous system impairments, recent history of infection, body mass index (BMI) >40 kg/m^2, apnea hypopnea index ≤15.0 events/hour sleep, majority mixed sleep apnea (i.e., obstructive and central apneas), majority central sleep apnea, anticoagulant medication, chronic heart failure, unstable angina, myocardial infarction, smoking, hypnotic or immunosuppressive medication, or cognitive disorders. Exclusion criteria for systolic blood pressure (SBP) was ≥150 and for diastolic blood pressure (DBP) ≥100. The upper limit for SBP is based on previous observations in OSA adults that showed a propensity for slight increases in BP in some subjects within the first training week. In view of this possibility, we adopted a somewhat conservative cutoff for purposes of a pilot trial.

In accordance with the training device manufacturer guidelines (http://www.powerbreathe-usa.com/), subjects with presence/history of dyspnea, ruptured eardrum or other middle ear condition, history of rib fracture, marked elevated left ventricular end-diastolic volume and/or pressure, also were excluded from participation. Note that individuals who were regular users of continuous positive airway pressure (CPAP) (or a related pressure therapy) or users of mandibular advancement dental devices *were eligible* to participate, as were subjects with elevated, stage 1 or stage 2 hypertension. The University of Arizona's Human Subjects Protection Program approved the study procedures and all subjects provided written informed consent prior to being enrolled. Some 200 adults responded to advertisements placed in a local

newsletter, 136/200 adults completed the on-line screening questionnaire and were deemed eligible to complete the pre-assessments outlined below.

Spirometry. Assessments of lung function comprising assessments of forced expiratory volume in 1.0 sec ($FEV_{1.0}$), forced vital capacity (FVC), forced inspiratory volume in 1.0 sec ($FIV_{1.0}$), forced inspiratory capacity (FIVC), $FEV_{1.0}$/FVC, $FIV_{1.0}$/FVC, $FIV_{1.0}$/FIVC, peak expiratory flow (PEF) and peak inspiratory flow (PIF) (WinspiroPRO, Medical International Research, New Berlin, WI, USA) in accordance with the guidelines of the American Thoracic Society (47). To determine maximal inspiratory pressure (PI_{max}), subjects generated a maximal inspiration from residual lung volume using the POWERbreathe™ training device in TEST mode. The average of the three trials defined the individual's PI_{max} (9, 33).

Home sleep apnea testing (HSAT). We used home sleep apnea testing (HSAT) to reliably identify those adults in our sample with moderate and severe OSA (52-54). The type 3 portable testing device (ApneaLink™, ResMed, Bella Vista Sydney, Australia) is validated for use in adults with moderate and severe OSA (22, 26, 48, 58) and captures blood oxygenation, nasal airflow and thoraco-abdominal movement and yields estimates of the severity of sleep-disordered breathing based on monitoring time. These results are referred to as the respiratory event index (REI). Home sleep apnea testing also permitted exclusion of other forms of sleep disordered breathing (e.g., obesity hypoventilation syndrome or Cheyne Stokes Respiration) on the basis of nasal airflow disturbance, awake resting and overnight oximetry measurement. Sleep quality, sleep duration, sleep efficacy, sleep latency, sleep disturbance and impact on daily function using the Pittsburgh Sleep Quality Index (PSQI) (55) also were recorded.

Ambulatory blood pressure monitoring. Eligible adults, who passed lung function assessments and had an AHI≥15, completed a period of 24-hour ambulatory BP monitoring (SOMNOmedics, Randersacker, Germany). Given the propensity for sleep disturbance and arousal reactions to contribute to perturbations in SBP, we obtained continuous measures of SBP and DBP using an FDA approved and European Society of Hypertension validated SOMNO-touch non-invasive ambulatory blood pressure monitor (8). The device includes a small control unit worn on the wrist to measure pulse transit time (PTT), three-channel electrocardiogram (ECG) leads placed on the chest and an oxygen monitor fitted to the finger that obviates the need for arm cuff inflations that may interfere with sleep quality (4).

After fitting each subject, the device was calibrated via a manual blood pressure measurement. Subjects were asked to refrain from any strenuous physical activity while wearing the device and to report back to the laboratory 24 hours later for data download. For ambulatory recordings exceeding 4.0 hours continuous recording overnight, beat to beat measures of blood pressure (BP) were averaged over the entire recording period and compared for consistency with repeated measures over shorter i.e., 10 minute, representative intervals (44).

In-laboratory procedures.

Subjects initially underwent an in-laboratory blood draw and on a separate day, in-laboratory assessments of resting blood pressure, resting muscle sympathetic nerve activity (MSNA) and cardiorespiratory measures (see details below). Subjects were asked to refrain from caffeine and alcohol for 12 hours and instructed not to eat for at

least 4 hours prior to their visit. Each of the measures was repeated at the 6 weeks timepoint after completion of training.

Plasma catecholamines. Subjects underwent a fasting blood draw and were instructed to refrain from eating or drinking (anything other than water) and from taking over the counter pain or allergy medications for the 12 hours leading up the draw. Venous blood samples were collected between the hours of 07:00 and 10:00 from the antecubital region following 30 minutes of supine rest in a quiet, temperature-controlled room. Samples were placed on ice in lithium-heparin coated tubes (BD Vacutainer, Franklin Lakes, NJ) immediately centrifuged (4°C, 1500 RPM, 15 ins) and the plasma frozen at -80°C. Plasma samples were analyzed via quantitative high-performance liquid chromatography (Associated Regional and University Pathologists – ARUP Laboratories, Salt Lake City, UT).

Resting blood pressure. In-laboratory measures of resting (seated) blood pressure were obtained at intake and study close, and once weekly throughout the 6-week intervention. Measures were taken in accordance with American Heart Association guidelines (57) with an automated oscillometric sphygmomanometer (SunTech CT40, SunTech Medical). Three measures, taken on alternate arms, were averaged to obtain systolic (SBP) and diastolic (DBP) blood pressures and to determine mean arterial pressure (MAP) using the equation: (MAP = DBP + 1/3[SBP – DBP]). Measures were obtained at the same time of day and on the same day each week for 6 weeks.

Resting spontaneous cardiac baroreflex sensitivity (BRS). While subjects were semi-recumbent and after 20 minutes rest, we recorded lead II-EKG continuously (0.3-1.0 kHz) via Ag-AgCl surface electrodes placed on the chest (2.0 kHz), and beat-to-beat changes

in systolic and diastolic blood pressures (SBP; DBP) at 1min intervals via automated finger cuff pressure transducer (400Hz) (ccNexfin; Bmeye, Amsterdam, The Netherlands). Data were recorded online using a PowerLab (ADInstruments, Colorado Springs, CO) interface and LabChart 8.0 software. Moment-to-moment changes in the R-R interval (RRI) coincident with fluctuations in SBP were used to obtain estimates of cardiac BRS (30, 31, 56) using proprietary software to identify "up" and "down" sequences (Cardioseries V2.4, Brazil). Sequences ≥3 cardiac cycles that showed in beat-to-beat increases in SBP (≥1 mmHg) and lengthening of the R-R interval (≥6ms) for each beat in the series *or* with beat-to-beat decreases in systolic blood pressure (≥1mmHg), and shortening of the R-R interval (≥6ms) for each beat in the series were included in this analysis (56, 65). Consecutive R-R intervals were plotted against SBP (mmHg) values in the preceding cycle to obtain regression lines and correlation values for each sequence. Correlation coefficients >0.85 were averaged to obtain individual subject estimates of spontaneous cardiac baroreflex sensitivity (ms/mmHg) (56). MSNA and beat-to-beat changes in (systolic and diastolic) blood pressure were recorded over 20 minutes of undisturbed rest. Data in the final 10 minutes of each recording were subject to analysis. Respiration-related motions of the chest wall (100Hz) were recorded using respiratory belt transducers (ADInstruments, Colorado Springs, CO) placed around the chest and abdomen. All data were recorded continuously throughout ~20 minutes of undisturbed rest.

Resting muscle sympathetic nerve activity (MSNA). Concurrent with ECG recordings, sympathetic nerve traffic was recorded from the common peroneal nerve using tungsten microelectrodes (200μm: 25-40mm, impedance: 5MΩ) (FHC, Bowdoin, ME) inserted

percutaneously immediately posterior to the fibular head. Subjects rested semi-recumbent in the dental chair with the right knee and foot supported by positioning pillows (VersaForm™, Performance Health, Warrenville, IL). Microelectrode placement was confirmed via electrical stimulation (0.02 Ma, 1 Hz) as described previously (42). A second microelectrode, inserted just below the skin surface ~1.0 cm from the first served as a reference electrode. Neural activity was amplified (gain 2 X 10^4) and filtered (500-2.0kHz) using a pre-amplifier (NeuroAmp Ex; ADInstruments, Colorado Springs, CO) and signals were full wave rectified (0.1 s moving window) and stored (10 kHz sampling) using a computer-based data acquisition and analysis system (LabChart 8.0 software, ADInstruments, Colorado Springs, CO). Electrode position in muscle fascicles was confirmed by pulse synchronous bursts of activity, elicitation of afferent nerve activity by mild muscle stretch and absence of response to startle (42).

Negative deflecting cardiac related sympathetic action potentials were identified using both unprocessed and root mean squared MSNA signals and quantitated as the number of bursts per 100 heart beats, number of bursts per minute and total activity (mean burst area /min) obtained from the RMS processed (root mean square, moving average time-constant 200 ms) signal (49, 51, 62, 70, 73, 74). The recording period was started no earlier than 15 min after insertion of the electrode and was continuous throughout ~20 minutes of undisturbed rest.

6-week intervention.

Twenty-five adults (17 men, 8 women) were prospectively assigned, via stratified block randomization to high intensity IMST (n=15) or to the control condition (n=10), outlined

below. All subjects were unfamiliar with IMST and all were blinded to their assigned training group.

Subjects in both groups trained independently at home completing 30 breaths i.e., 5 sets of 6 breaths with a ~1-2 min rest between each set, 5 days a week for 6 weeks on the POWERbreathe™ device (K3 Series, Warwickshire, UK). Training was performed at the same time each day, and data from each day's training were stored on the device and uploaded in the laboratory at the end of each training week. Subjects were instructed first to exhale to residual volume and then inhaled via the device mouthpiece to their target pressure. As previously, target pressures for the control group were set to 15% of the PI_{max} and those for the high intensity IMST group were set to 75% of the PI_{max} (19, 71, 72). Neither group encountered resistance to expiration. Because IMST improves inspiratory muscle strength and subjects in both groups typically show improvement in PI_{max} test performance, target pressures for both groups (i.e., 15% or 75% PI_{max}) were reassessed at the end of each training week.

Statistical Analysis

A per protocol, two-way repeated measures mixed model analysis of variance was used to test the main effects of treatment (IMST versus control) and time point (Week 1 *versus* Week 6). Statistical significance was set at $p \leq 0.05$. If the ANOVA revealed significance, planned post hoc within-group and between-group comparisons were performed using paired and independent sample t-tests respectively, with significance adjusted (p=0.025) according to the Bonferroni correction. Investigators were blinded to participant group assignment during data analysis.

RESULTS

Twenty-five adults were randomized to high intensity IMST or the control intervention. One subject was disqualified from continuing the study due to nonadherence to the training regimen. As a result, the study retention rate was 96%. There were no between-group differences in sex, age, neck circumference, BMI, systolic and diastolic BP, respiratory disturbance index or PSQI scores (p>0.1) at study intake. Details of subject number, anthropomorphic data and health status (i.e., sleep apnea severity, sleep apnea therapy, cardiovascular risk category, medications and physical activity levels) for high intensity IMST and control groups are presented in Table 1.

Overall, key sleep indices including awake and resting oxygen desaturations, and sleep duration, were unchanged pre-post intervention for both groups (p>0.1). Maximum inspiratory pressures (PI_{max}) were greater pre-post for IMST (82.6 ± 12.5 to 116.5 ± 13.6 cmH_2O) (p<0.001) and control groups (85.60 ± 4.5 to 101.2 ± 6.94 cmH_2O) (p<0.01) but there was no effect of either intervention on tests of pulmonary function: $FEV_{1.0}$; FVC; $FIV_{1.0}$; $FEV_{1.0}$/ FVC; $FIV_{1.0}$/FIVC; PEF; or PIF (p>0.05) (data not shown).

Individual results for casual in-laboratory measures of systolic, diastolic and mean arterial blood pressures are shown in Figure 2. For the high intensity IMST group, average (±SD) SBP, DBP and MAP all declined from Week 1 to Week 6 (SBP: -8.82 ± 4.98; DBP: -4.69 ± 2.81; MAP: -6.06 ± 1.03), (p<0.002). Heart rate and BRS were unchanged (Table 2). For the control group, measures of blood pressure (SBP: -2.23 ± 6.85; DBP: -1.10 ± 3.96; MAP: -1.48 ± 4.60), heart rate (2.2 ± 2.4 bpm) and BRS (-0.31 ± 1.9 ms/mmHg) were unchanged pre *vs*. post (p>0.1).

Ambulatory blood pressure and MSNA recordings were obtained in a subset of individuals, pre-post intervention (9 high intensity and 6 control). Group data for high intensity IMST are provided in Table 3 and results for individuals in both groups are shown in Figure 3. Despite overall declines in nighttime BP, only results for SBP (pre: 141.56 ± 18.93 mmHg; post: 129.55 ± 15.67 mmHg) attained significance (p<0.01). Nighttime DBP (pre: 76.56 ± 8.88 mmHg; post: 74.11 ± 9.22 mmHg) and MAP (pre: 98.67 ± 11.19; post: 92.78 ± 8.73 mmHg) (Figure 3) also declined but did not attain statistical significance (p<0.10). In the control group, average (± SD) nighttime SBP (pre:135.14 ± 13.13; post: 144.67 ± 19.10 mmHg), DBP (pre: 73.17 ± 13.63; post: 77.17 ± 13.72 mmHg) and MAP (pre:93.83 ± 13.67; post: 100.33 ± 15.67 mmHg) slightly increased post intervention (p>0.05) (Figure 3).

Results for in-laboratory measures of resting MSNA for high intensity IMST are provided in Table 3 and representative recordings shown in Figure 4. Average measures of MSNA bursts/min^{-1} (-6.97 ± 2.29; p=0.01) and bursts per 100hb (-9.55 ± 2.42; p=0.002), were lower at week 6 than in week 1 following high intensity IMST but did not change following the control intervention (4.87 ± 2.80 bursts/min^{-1}; p = 0.10 and 5.527 ± 2.96 bursts/100hb; p = 0.85) (Figure 5). Neither training protocol was associated with significant changes in plasma norepinephrine (high intensity IMST: pre-training 307.10 ± 69.15 vs. post training 293.82 ± 72.37; p = 0.64) and (control: pre-training 298.71 ± 89.43 vs. post training 313.29 ± 99.04; p = 0.67).

DISCUSSION

OSA is characterized by repeated airway obstructions that result in intermittent

hypoxemia and arousal from sleep which together drive increases in nighttime sympathetic nervous activity (66). Although previous studies confirm the benefits of CPAP and/or daily exercise on cardiovascular health (3, 5, 24, 38, 69), adherence rates for CPAP remain low (45). Furthermore, many adults with OSA are unwilling or unable to maintain a regular exercise program (2, 7).

Compared to traditional forms of aerobic exercise, retention rates for IMST are consistently high (92-95%) exceeding those of comparable duration lifestyle i.e., aerobic exercise and/or dietary interventions (32, 39). With no treatment-emergent adverse events and a 96% adherence rate (number of prescribed training sessions completed at the target pressure) IMST appears well-tolerated by the OSA population.

Strengths and Limitations. We acknowledge that we were unable to obtain complete data sets from all our subject participants and that the requirement for pre-post 24-hour blood pressure monitoring and MSNA recordings posed a particular challenge in this population. The loss of subjects limits the generalizability of findings. While not a concern for lower probability outcomes ($p<0.05$), the variability inherent in a smaller sample size may have given rise to a number of false negative outcomes for nighttime measures of BP.

Sleep and nighttime breathing including blood oxygen desaturation (34), nasal airflow and thoraco-abdominal movement were monitored using an in-home sleep apnea testing (HSAT) validated for use in adults with moderate and severe OSA (22, 48, 58). Importantly, the device assesses time spent with blood oxygen desaturation <90%, nasal airflow and thoraco-abdominal movement and the intraclass correlation between results obtained with this form of home sleep apnea testing and with overnight PSG is excellent (13).

Pre-post HSAT showed no evidence of intervention-related changes in apnea frequency (respiratory disturbance index), oxygen desaturation (<90%), total sleep time and modest improvements in subjects' subjective assessment of sleep quality (PSQI). The latter outcome differs from previously published findings in adults with mild-moderate OSA (72) who reported improved sleep quality (PSQI) under the same IMST protocol. Nevertheless, because weight, neck circumference, physical activity, medications, sleep quality and AHI each remained consistent throughout the study period, the observed reductions in casual and overnight SBP and SNS hyperactivity cannot be ascribed to change/s in the aforementioned variables.

Care was taken to exclude participants with prior knowledge of or experience with inspiratory muscle strength training. Whereas participants in both groups trained on the same hand-held pressure-threshold training device, followed the same training regimen (i.e., 30 breaths day/ 5 days/week for 6 weeks) and attended weekly laboratory visits and reassessments, all visits were coordinated to preclude participant overlap to ensure participant blinding to high intensity IMST vs. control intervention formats.

As described previously, training pressures for the control group were significantly lower than for the high intensity group (i.e., 15%PI_{max} *vs.* 75%PI_{max}). However, the pressure range for the control group encompassed -15.0 to -20.0 cmH_2O exceeding pressures typical of tidal (75) or deep breathing (36). We anticipated the control intervention may contribute some improvement in inspiratory muscle strength however, the magnitude of the increase in PI_{max} (~5cmH_2O) is consistent with previously published findings in healthy adults (19) and consistent with "learning" related improvements attributable to repeat testing over a short time span (59, 61).

Mechanistic insights into improvement in blood pressure. The assessment burden on the subject participants in this pilot trial was considerable and for this reason we excluded assessments of cardiac output (6, 14). However, the ccNexfin (Bmeye, Amsterdam, The Netherlands) estimates of cardiac output although not validated for measures obtained at different time points may provide some additional insight. Based on in-laboratory data obtained pre-post intervention, neither intervention affected CO (high intensity 98.43 ± 5.53%; control 97.69 ± 6.39% of pre-intervention values).

The mechanisms responsible for the beneficial effects of IMST require further elucidation. As described previously in healthy adults, large (positive or negative) intrathoracic pressures but *not* large lung volume excursions appear to be the primary (respiratory) stimulus underpinning IMST-related reductions in BP (71). While there is no evidence of IMST-related changes in baroreflex sensitivity, heart rate or cardiac output (19), there is evidence of IMST-related declines in plasma catecholamines (72), peripheral resistance (19), endothelial dependent dilation (16) and sympathetic nervous outflow (current findings) that point to changes in vascular function (15). Specifically, a focus on peripheral artery stiffness appears warranted given preliminary evidence of IMST-related improvements in peripheral artery distensibility and increased nitric oxide bioavailability in otherwise healthy older healthy adults (17). Whether a similar vascular benefit might occur in the context of OSA will require further study.

Summary. Our results confirm previously reported findings of IMST-related improvements in casual blood pressure. In addition, the current findings provide preliminary and novel support for the potential for high intensity IMST to reduce resting sympathetic neurogenic activity and nighttime systolic blood pressure among older, overweight adults with OSA

with 5 minutes training/day over 6 weeks. Given these findings we propose that high intensity IMST may be an effective preventative treatment for older adults with OSA. Whether IMST can confer similar benefits when implemented in younger adults with OSA and hypertension and whether the benefits aggregate over the long (6-12 months) term and diminish upon withdrawal awaits further attention.

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Table 1.

Intervention group	**Control (n=10)**	**High Intensity IMST (n=15)**
Subjects	6 men, 4 women	11 men, 4 women
Age	69.7 ± 6.7	65.9 ± 6.0
BMI (kg/m^2)	31.3 ± 6.5	30.7 ± 6.2
Neck Circumference (cm)	41.0 ± 3.9	41.6 ± 5.2
RDI	26.2 ± 13.5	22.9 ±11.0
PSQI	9.0 ± 5.0	8.6 ± 4.0
OSA therapies		
Continuous positive airway pressure	3	3
Mandibular advancement device	1	0
No device	6	12
Cardiovascular Risk Category		
Normal (systolic) BP (<120mmHg)	2	4
Elevated systolic BP (120-129 mmHg)	1	4
Stage 1 hypertension (130-139 mmHg)	2	1
Stage 2 hypertension (>140 mmHg)	5	6
BP Medications		
Beta-blocker	1	3
Angiotensin receptor blocker	1	3
Calcium channel blocker	0	3
ACE inhibitor	3	0
No BP medication	5	6
Physical activity levels		
Minimally active (0-2 hours exercise/week)	2	5
Moderately active (3-4 hours exercise/week	4	7
Vigorously active no. (>5 hours exercise/week)	4	3

Table 1. Average values (±SD) for age, body mass index (BMI), neck circumference, respiratory disturbance index (RDI), Pittsburgh Sleep Quality Index (PSQI), obstructive sleep apnea (OSA) therapy type, cardiovascular risk category, blood pressure (BP) medication/s and level of physical activity reported by participants in the control group (n=10) and high intensity IMST group (n=15) at study intake.

Table 2. High Intensity IMST	**Week 1**	**Week 6**
Home Sleep Apnea Assessment (n=15)	**Pre-Intervention**	**Post-Intervention**
Mild (RDI ≤ 15)	0	0
Moderate (RDI 15-29)	12	12
Severe (RDI ≥30)	3	2
Group average RDI	22.9 ± 11.0	21.2 ± 12.2
Cardiovascular measures (n=15)		
Systolic blood pressure (mmHg)	140.8 ± 17.9	**132.8 ± 14.2***
Diastolic blood pressure (mmHg)	74.9 ± 9.9	70.2 ± 8.6
Mean arterial pressure (mmHg)	95.0 ± 11.2	**89.0 ± 10.4***
Heart rate (bpm)	59.2 ± 5.4	59.7 ± 5.5
BRS (ms/mmHg)	10.4 ± 3.9	10.9 ± 5.8
Plasma norepinephrine (80-520 pg/Ml)	307.1 ± 69.1	293.8 ± 72.4
Plasma epinephrine (10-200 pg/Ml)	28.0 ± 13.8	26.5 ± 12.4

Table 2. Average values (±SD) for respiratory disturbance index (RDI), systolic, diastolic and mean arterial pressure (mmHg), heart rate (bpm) and cardiac baro-reflex sensitivity (ms/mmHg) (BRS) for subjects in the high intensity IMST group (n=14), pre (Week 1) and post (Week 6) intervention.

Table 3. High Intensity IMST	**Week 1**	**Week 6**
Cardiovascular measures (n=9)	**Pre-Intervention**	**Post-Intervention**
Ambulatory (Non-Invasive) Monitoring		
24hour systolic blood pressure (mmHg)	143.1 ± 18.5	136.4 ± 16.7
24hour diastolic blood pressure (mmHg)	77.7 ± 8.7	75.3 ± 7.9
24-hour heart rate (bpm)	66.1 ± 2.7	67.1 ± 6.8
Nighttime systolic blood pressure (mmHg)	141.6 ± 18.9	**129.6 ± 15.7***
Nighttime diastolic blood pressure (mmHg)	76.6 ± 8.5	74.1 ± 9.2
Nighttime heart rate (bpm)	57.8 ± 3.6	59.3 ± 4.7
Muscle Sympathetic Nerve Activity		
Burst incidence (burst /100 beats^{-1})	91.7 ± 6.7	**83.5 ± 9.3***
Burst frequency (burst min^{-1})	53.7 ± 7.0	**46.7 ± 8.0***

Table 3. Average values (±SD) obtained from 24-hour non-invasive blood pressure monitoring and in-laboratory measures of resting muscle sympathetic nerve activity (MSNA) for subjects in the high intensity IMST group (n=9), pre (Week 1) and post intervention (Week 6).

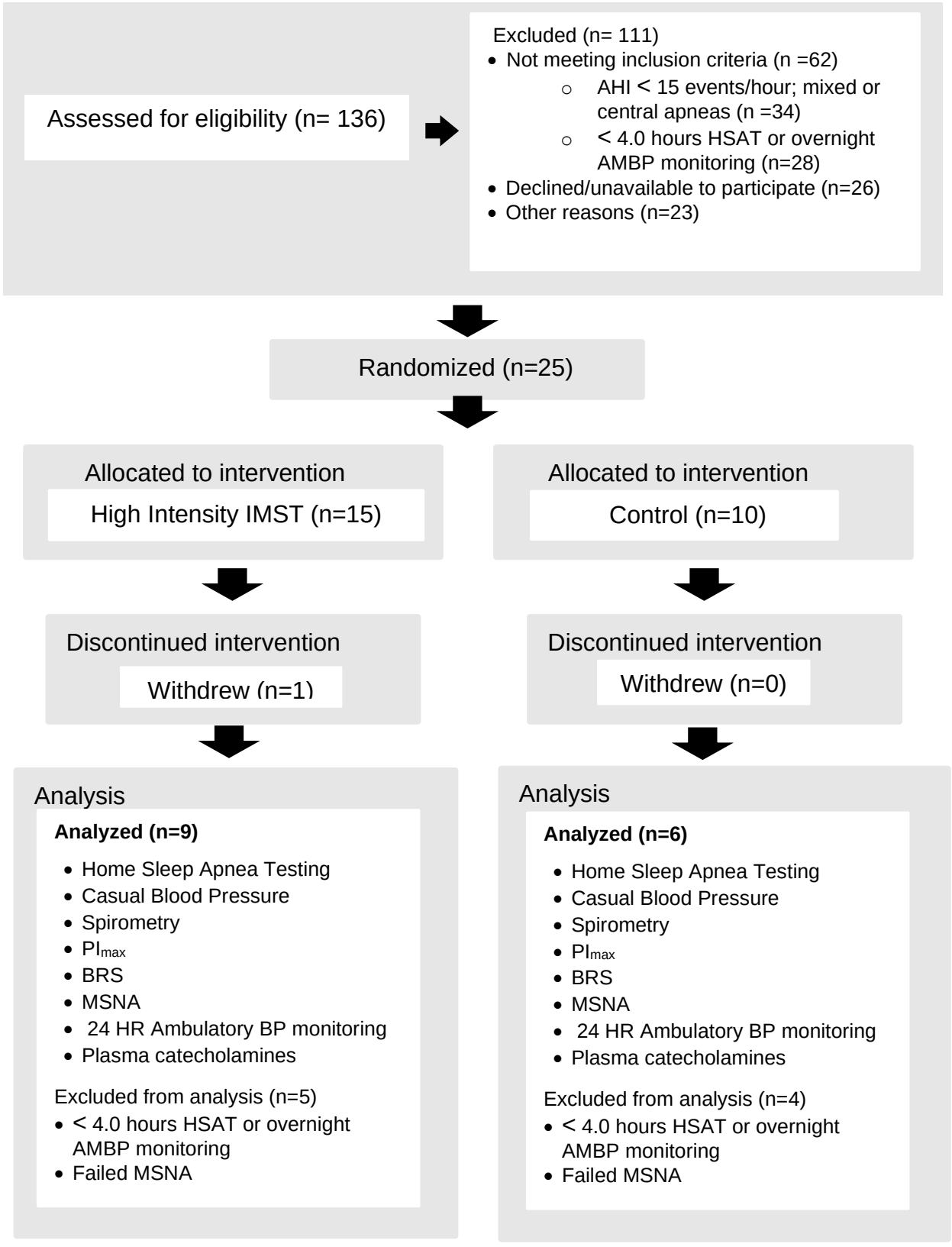

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow chart.

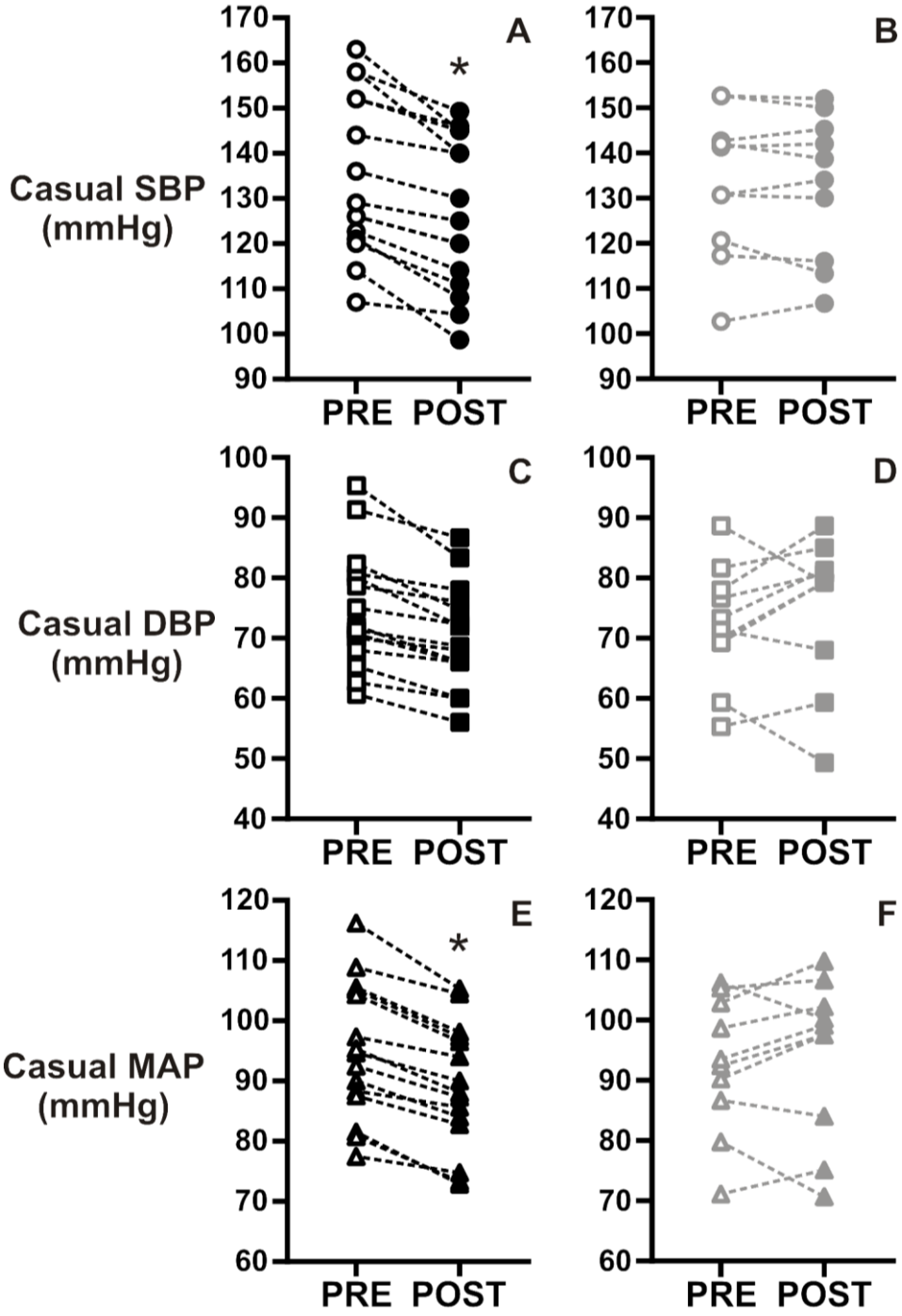

Figure 2. Panels A-F. Individual results for in-laboratory measures of resting blood pressure for subjects in the high intensity IMST (black symbols) (n=14) and control (gray symbols) (n=10) groups. Panels A-B: Individual results for casual systolic (SBP) (circles); C-D: Diastolic blood pressure (DBP); and E-F: Mean arterial blood pressure (MAP) measured pre (week 1) and post (week 6) intervention. Asterisks indicate significant differences in the main effect of time pre vs. post ($p<0.05$).

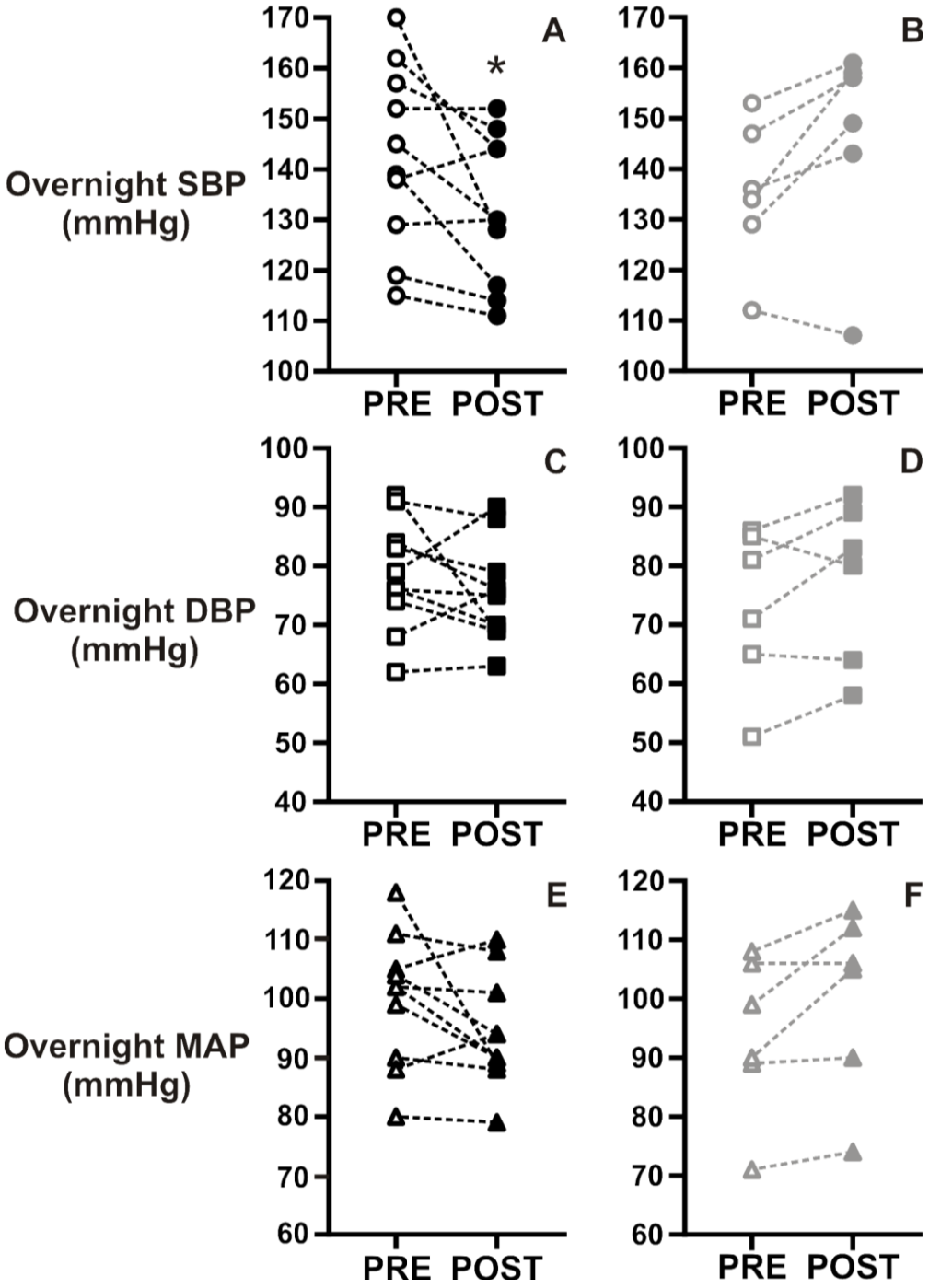

Figure 3. Panels A-F. Individual results from in home, overnight non-invasive monitoring of blood pressure for high intensity IMST (black symbols) (n=9) and control (gray symbols) (n=6) groups. Panels A-B: Individual results for systolic blood pressure (SBP); C-D: Diastolic blood pressure (DBP); and E-F: Mean arterial blood pressure (MAP) measured pre (week 1) and post (week 6) intervention. Asterisks indicate significant differences in the main effect of time pre vs. post ($p<0.05$).

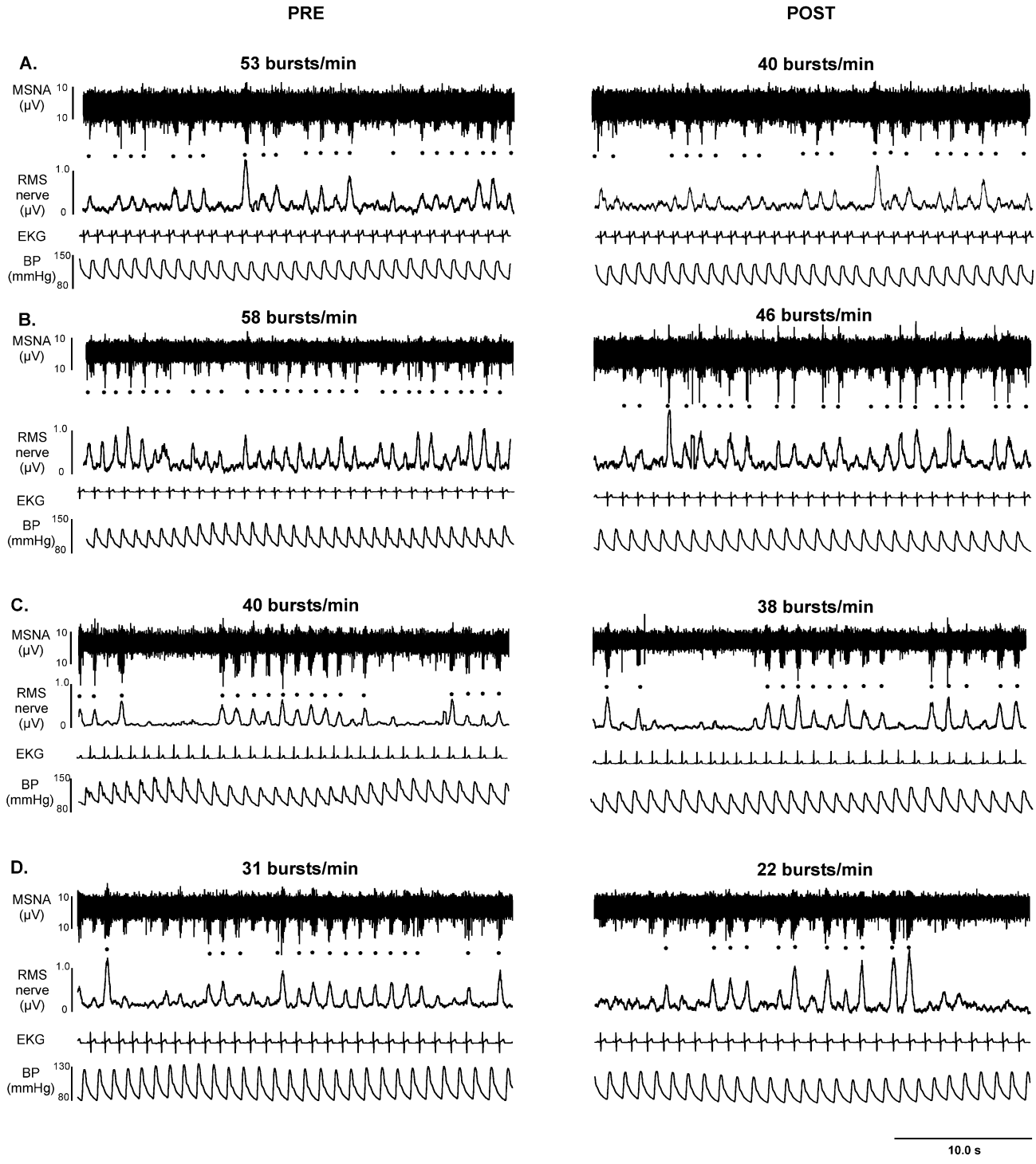

Figure 4. Representative recordings (30 s) of muscle sympathetic nerve activity (MSNA), blood pressure (BP), electrocardiogram (EKG) and chest wall motion obtained from four participants pre – post 6 weeks high intensity IMST. Asterisks (*) indicate sympathetic bursts included in the bursts/min count. Mean voltage neurograms shown in the root mean square (RMS) nerve trace were used to quantify the number of sympathetic bursts (bursts/100hb).

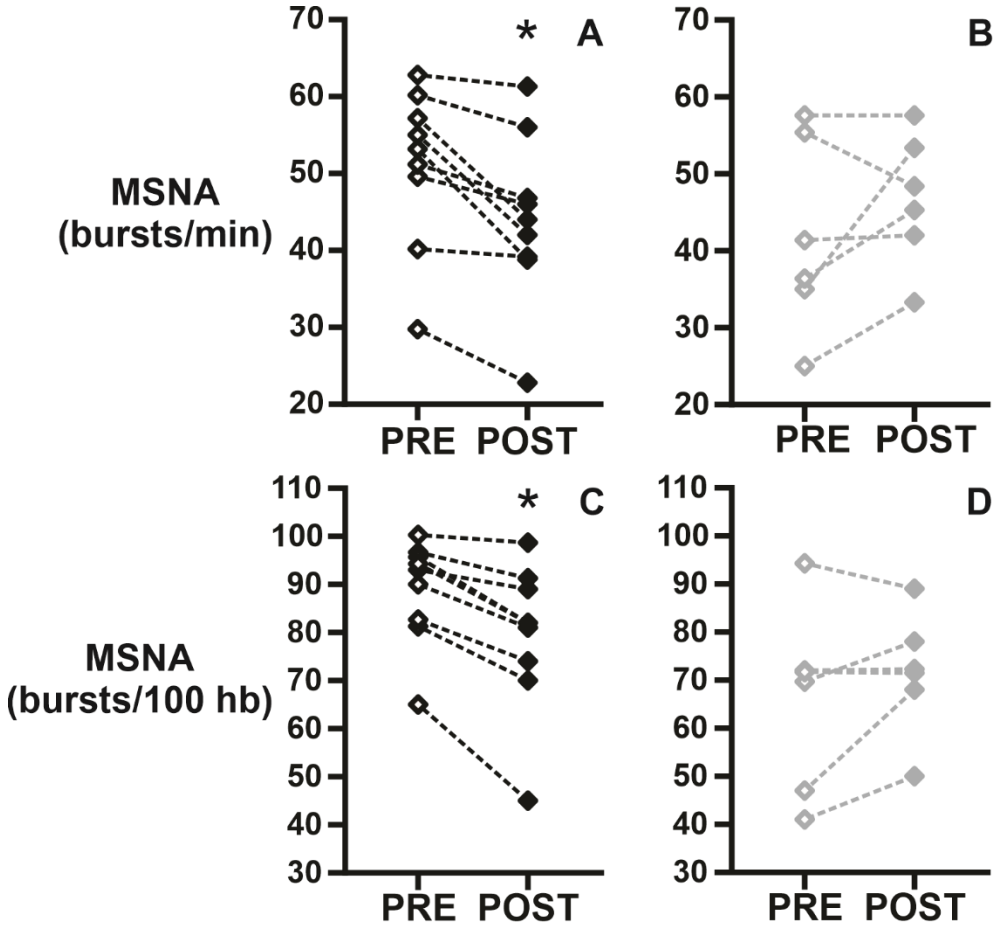

Figure 5. In-laboratory measures of resting muscle sympathetic nerve activity (MSNA). Panels A-B. Individual measures of MSNA bursts/min^{-1} and MSNA bursts/100 heart beats (Panels C-D), pre (week 1) and post (week 6) high intensity IMST (black symbols) (n=9) or control (gray symbols) (n=6) groups. Asterisks indicate significant differences in average values pre-post intervention ($p<0.05$).

Study 3 References

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SUMMARY AND FUTURE DIRECTIONS

The goal of the studies presented in this dissertation is to address additional key questions regarding IMST and effects on blood pressure. The studies entailed acute studies in healthy young adults and intermediate (6 week) duration studies in older adults with OSA.

Acute cardiovascular effects of IMST. Study 1 was the first to record MSNA, heart rate, blood pressure during 5 sets of 6 high intensity, low volume IMST "breaths." The results of the study reveal IMST-related increases heart rate and reductions in MSNA with no effect on blood pressure. Importantly, MSNA recovery trajectories are different for young women *versus* men with IMST related suppression for both sexes, but with suppression persistent throughout Recovery in young women. In view of these findings, future studies should focus on the difference in MSNA suppression between young women and men in Recovery. Key in determining the role sex plays in modulating the effects of IMST would be to assess within sex differences in MSNA over a longer period of Recovery i.e., 10 minutes. A second consideration is the potential role for menstrual phase and/or oral contraceptives to impact MSNA both acutely and in Recovery. A third and necessary control experiment is to establish what the acute effects of sham-training (15%PI_{max}) may have on the same cardiovascular parameters.

IMST and dynamic cardiovascular function. Study 2 comprises a respiratory muscle fatigue protocol pre *versus* post 6 weeks IMST to evaluate the effects of the intervention on the dynamic cardiovascular response. In the context of respiratory fatigue, the results of Study 2 show 6 weeks IMST-related improvements in respiratory endurance and blood pressure. IMST-related improvements in respiratory endurance have been reported

previously (77) however, these studies do not include an assessment on respiratory muscle work pre *versus* post. Conversely, respiratory muscle work is reported in the context of whole-body exercise to exhaustion (see for example reference 26). As outlined, for Study 2 the respiratory circuit comprised a mouthpiece attached to a non-rebreathing valve and inspiratory pressures detected via a tube attached to a bespoke respiratory valve. Using this set-up, we obtained estimates of inspiratory muscle work per breath (mmHg/s) by integration of inspiratory pressures generated on successive inspiratory efforts. When inspiratory muscle work is plotted against the %change in heart rate or blood pressure, pre-IMST analyses reveal a moderate, positive relationship. This relationship is abolished following six weeks IMST, a phenomenon not reported previously in studies of this kind.

Although it was my intention to recruit equal numbers of women and men, our efforts to recruit additional participants was cut short by the pandemic. Equal numbers of women and men would add strength to the findings and may provide additional valuable insights on potential effects of sex.

IMST and MSNA in OSA. In Study 3, we measured resting blood pressure and MSNA in older adults diagnosed with obstructive sleep apnea with the intent to identify if MSNA (one mediator of vascular resistance) is lowered following six weeks IMST. In individuals who performed IMST at 75% of their PI_{max}, we reported significant reductions in daytime blood pressure *and* overnight systolic blood pressure concomitant with reductions in resting MSNA. Therefore, results from Study 3 indicate that declines in MSNA play a key role in lowering daytime resting systolic blood pressure. However, regulation of blood pressure and vascular resistance is not solely determined by sympathetic drive to

vascular smooth muscle. Future studies should aim to assess other mediators of vascular resistance including nitric oxide-driven vasodilation and vascular compliance. Finally, our studies to date have tested the intermediate effects of IMST on cardiovascular health and function. Additional studies will be necessary to determine whether the trajectory of BP continues to decline with even longer interventions (i.e. 8, 12 and 24 weeks).

There is much more work to be done to understand the physiological effects of IMST on potential mediators of vascular resistance in healthy and patient populations. The work contained in this dissertation provides an initial framework for these studies and contributes additional de novo findings on the potential for abbreviated forms of respiratory training to bring about rapid improvements in cardiovascular health.

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