EFFECTS OF SIX WEEKS INSPIRATORY MUSCLE STRENGTH TRAINING ON RESPIRATORY MUSCLE ELECTROMYOGRAPHY

By

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Abstract

Inspiratory muscle strength training (IMST) holds promise as a non-pharmacologic treatment that can improve respiratory muscle strength and reduce blood pressure in hypertensive adults. There is a gap in knowledge regarding the specific respiratory mechanisms that gives rise to these favorable outcomes. Here, I explore the effect of IMST on respiratory muscle fatigue, blood pressure, and heart rate in recreationally active men and women. Four subjects underwent a 6-week intervention comprising 30 breaths a day 5 days a week with a respiratory muscle fatigue protocol pre and post intervention. Pre-post intervention measures consisted of resting blood pressure, heart rate, and surface electromyographic (EMG) recordings of the scalene, parasternal, and oblique muscles. The effects of 6 weeks IMST on respiratory muscle fatigue were evaluated in the context of a fatigue protocol and by assessment of the centroid frequency of EMG power spectrum. My preliminary results in 4 subjects failed to show definitive results. I hypothesize that confounding factors, namely individualized breathing strategies contributed considerable variation to the patterns of respiratory muscle activation exhibited by our participants and invalidating pre-post comparisons of EMG power. Future studies should control for breathing patterns within subjects (i.e. across pre-post fatigue protocols) to ensure consistent patterns of respiratory muscle activation. Additionally, care should be taken to ensure uniformity of instructions across both fatigue protocols and daily IMST i.e., use of a diaphragmatic breathing pattern, to minimize between subject differences in respiratory muscle activation.

Introduction

As the prevalence of hypertension grows worldwide, novel approaches are needed to combat what may become a public health epidemic (Brook et al., 2013; Mancia et al., 2013). Previous experiments in our lab have shown the efficacy of IMST as a novel approach to combating hypertension (Vranish, & Bailey, 2015; Delucia, & Bailey, 2018). Apart from its ability to lower

systolic and diastolic blood pressure over a short time course (4–6 weeks) and the abbreviated format of the daily training (~5 min day), there are several other features of IMST that set it apart from traditional aerobic exercise (i.e. running or cycling). First, IMST is performed in stationary standing and the individual's breathing rate is constrained to 10–12 breaths min. This is in contrast to traditional forms of aerobic exercise such as cycling, running or swimming that engage arms and/legs and for which breathing rates may reach upwards of 45 breaths per minute. (Harms et al., 1998). Third, the inspiratory pressures generated by a healthy adult performing IMST typically range between −50 and −70 mmHg (Inbar, Weiner, Azgad, Rotstein, & Weinstein, 2000; Romer, McConnell, & Jones, 2002; Vranish, & Bailey, 2015) easily exceeding the inspiratory pressures generated in tidal breathing (approximately −2.5 mmHg; Walls, Laine, Kidder, & Bailey, 2013), deep or yogic breathing (approximately −5 mmHg; Landman et al., 2014; Vranish, & Bailey, 2015) and high-intensity aerobic exercise (95% maximal oxygen uptake, approximately −22 mmHg; Harms et al., 1998; Johnson, Aaron, Babcock, & Dempsey, 1996).

Despite the important benefits of IMST on cardiovascular health (Vranish, & Bailey, 2015; Delucia, & Bailey, 2018), the mechanisms underlying the improvements in blood pressure are not well understood. Based on previous findings in our laboratory, one plausible explanation is that IMST conditions respiratory muscles thereby reducing their susceptibility to fatigue. Any reduction in fatigue might be the result of a reduction in blood flow "stolen" or redirected to the respiratory musculature from limb musculature. That is, respiratory muscle conditioning may increase the individual's capacity to generate larger (i.e., negative) inspiratory pressures *and* modulate increases in sympathetic activation in skeletal muscles of the leg (Sheel et al., 2001; St Croix, Morgan, Wetter, & Dempsey, 2000).

Accordingly, in these experiments I assessed the effects of IMST on blood pressure and sympathetic nervous system regulation in the context of a respiratory muscle fatigue task. We recruited healthy young adults to undergo 6 weeks of daily IMST and assessed the effects of this training on subjects' maximum inspiratory pressure (MIP), blood pressure, centroid frequencies of respiratory muscle (scalene, parasternal, and oblique muscles) electromyographic activity and measures of autonomic nervous system function i.e., plasma norepinephrine and muscle sympathetic nerve activity (MSNA), pre-post the intervention.

Methods

Ethical approval. Individuals were recruited from the student population at the University of Arizona to participate in a 6-week IMST protocol. Subject were screened prior to participation to select for non-smokers, absent history of hypertension, respiratory, neuromuscular or cardiovascular disease via an electronic survey. Experimental procedures were approved by the Human Subjects Protection Program at the University of Arizona. Subjects gave their written informed consent before participation.

General procedures. Commercially available software (WinspiroPRO 6.4, Medical International Research, New Berlin, WI, USA) was used to assess pulmonary function including measures of forced expiratory volume ($FEV_{1.0}$), forced vital capacity (FVC) and $FEV_{1.0}$ /FVC. An $FEV_{1.0}$ /FVC $\geq$ 80% of predicted was considered acceptable for inclusion in the study. Measures of systolic (SBP) and diastolic blood pressure (DBP) were determined via an automated sphygmomanometer (CT40; Suntech Medical, Morrisville, NC, USA.) at the brachial artery.

Blood pressures were obtained while subjects rested quietly with arms and back supported and their feet squarely on the ground for 5 minutes. Three measures, taken on alternating arms, were averaged to obtain the individual's SBP and DBP.

Venous blood draws were performed at intake and study close. Blood was collected from the antecubital region following 30 min 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, 1,500 RPM, 15 min) and the plasma frozen at −80°C. Plasma samples were obtained to assay circulating dopamine, epinephrine, and norepinephrine content. Importantly, pre training and post training blood draws were performed at the same time of day for each subject and, in accordance with previously published methods, subjects fasted overnight and were instructed to refrain from caffeine and over-the-counter pain or allergy medications for the 12 hours leading up to the blood draw.

Respiratory Fatigue Protocol. Subjects performed a respiratory fatigue protocol at intake and again after 6 weeks of IMST. Subjects performed the protocol while seated in an upright position, in a dental chair and breathed via a two-way non-rebreathing valve (2600 series; Hans Rudolph, Shawnee, KS, USA) used for IMST (see Intervention below). A tube attached to the non-rebreathing valve was coupled to a pressure transducer (Omegadyne Inc., Sunbury, OH, USA) to detect airway opening pressure and to a pneumotachometer (PNT series 4183; Hans Rudolph, Shawnee, KS, USA) to measure inspiratory and expiratory airflows. Lead II ECG was sampled continuously and recorded beat to beat changes in SBP via an automated finger cuff pressure transducer (ccNexfin; Bmeye, Amsterdam, The Netherlands).

For both fatigue protocols, subjects initially rested quietly for 5 minutes in a seated position. During this time, we calculated the subject's rest breathing frequency. Each subject then performed three maximal inspirations from residual lung volume with the average of these breaths was used to calculate their MIP. Next, each subject completed a respiratory fatigue protocol in which they were required to inspire to a target pressure (65% of their MIP). The target pressure was displayed via a projector onto a screen immediately in front of the subject. The subject was instructed to breathe in to reach the target pressure and then to breathe out. Simultaneously, an auditory tone was used to guide breathing frequency—an ascending tone indicated inspiration and a descending tone indicated expiration. For all subjects, the tone frequency was adjusted to match each subject's rest breathing rate but with longer durations for inspiration than expiration i.e., thus the duty cycle or the ratio of inspiratory time to total respiratory cycle duration was set to 0.7/1.0.

Whereas each subject was instructed to breathe in time with the auditory tones and to use the visual feedback to adjust the levels of the inspiratory pressure they were generating for the duration of the protocol, no additional instructions were provided in regard to their breathing. The protocol was terminated when the subject failed to attain their target pressure on three consecutive breaths or, was unable to sustain the effort required to perform the task.

Throughout the fatigue protocols, we recorded surface, whole muscle electromyographic (EMG) recordings of the parasternal, scalene, and oblique muscles. The skin surface was initially abraded and cleaned with alcohol swabs prior to electrode placement and secured with 3M Transpore surgical tape (3M, St. Paul, MN, USA) and shielded biopotential electrodes (Shielded Biopotential Electrodes; AD Instruments, Sydney, Australia) were placed on the skin directly

overlying each muscle. Intercostal EMG was recorded bilaterally in the second intercostal space immediately adjacent to the sternum (Duiverman et al., 2003) with a ground over the left clavicle. Scalene EMG was recorded at the right scalene with a ground electrode on the right clavicle. To identify correct EMG placement of the scalene muscle, subjects were instructed to rotate head left and produce a sharp inspiratory sniff (Falla et al., 2001; Segizbaeva 2013). Oblique EMG was recorded on the abdomen ~2 cm laterally from the umbilicus (Martin, & De Troyer, 1982) on the right side with a ground electrode placed on the right iliac crest. To confirm correct placement for the oblique subjects were instructed to exhale forcefully. EMG signals were amplified (P511 AC amplifier, Astromed) and filtered (100-3000 Hz; x100 parasternal), (100-1000 Hz; x 200 scalene), and (100-1000 Hz; x100 oblique), according to previously established methods (Duiverman et al, 2003) (Falla et al., 2001) (Martin, & De Troyer, 1982). Two of the four subjects performed a maximal activation of the oblique muscle at the start and end of the fatigue protocols. That maximal activation provided a maximum EMG value against which to normalize oblique EMG recorded in the fatigue protocol, thus, subjects completed a Valsalva maneuver i.e., forced exhalation against a closed glottis with maximal abdominal contraction.

We also obtained intraneural recordings of muscle sympathetic nervous activity (MSNA), an index of limb muscle vasoconstriction from the right peroneal nerve via microneurography (Vallbo et al., 1979) from each subject in each of the fatigue protocols. Multiunit postganglionic muscle sympathetic nerve recordings were made using low impedance (250 kΩ) (Vallbo, Hagbarth, Torebjork & Wallin, 1979) tungsten microelectrodes (FHC, Bowdoin, ME, USA). The neural signals were passed to a preamplifier and differential amplifier and recorded (500 Hz-2kHz; x20,000) (Powerlab, AD Instruments, Sydney, Australia). Muscle sympathetic bursts

subsequently were identified by visual inspection of the filtered neural signal and RMS processed signal (Macefield, 2013).

EMG and nerve signals were output to a data acquisition device (Powerlab, AD Instruments, Sydney, Australia). All EMG signals were displayed online using analysis software (Labchart, AD Instruments, Sydney, Australia).

IMST Intervention. As previously detailed (Delucia, & Bailey, 2018), subjects completed 6 weeks of supervised training in the laboratory. To determine each individual's training level, subjects initially performed a maximal inspiration from residual lung volume against a constant resistance via a mouthpiece connected to a pressure transducer to detect pressure generated at the mouth (Omegadyne Inc., Sunbury, OH, USA). The average of three trials yielded each subject's maximum inspiratory pressure or MIP.

After establishing the individuals MIP, each subject undertook in laboratory training 5 days a week for 6 weeks with training pressures set to 65% of MIP. Training was performed on a two-way non-rebreathing valve (2600 series; Hans Rudolph, Shawnee, KS, USA) fitted with a mouthpiece and a flow limitation end cap on the inhalation port. The flow limitation cap provided a constant, near-maximal inspiratory resistance, and restricted airflow to a pin-hole leak sufficient to permit detection of pressure at the mouth (Hanly et al., 2007). A tube attached to the device was coupled to a pressure transducer (Omegadyne Inc., Sunbury, OH, USA) to detect airway opening pressure. The pressure signal was sampled at 500 Hz, digitized, stored using a Cambridge Electronic Design 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK), and displayed on a computer monitor to provide visual feedback to subjects.

We obtained measures of resting blood pressure, spirometry, and maximal inspiratory pressure (MIP) at intake, and each week for 6 weeks of the intervention (see below) and again at study close. Measures obtained for each subject were performed at the same time and day.

Data Analysis

EMG. All data were analyzed using Labchart software and built-in analysis modules. To determine the development of respiratory muscle fatigue during the fatigue protocol, centroid frequency (f_c) of the EMG power spectra for each muscle was assessed in the pre and post-fatigue protocols (Segizbaeva et al., 2013). Mouth pressure was used to differentiate the inspiratory and expiratory phases of each breath cycle. Subsequently, EMG signals corresponding to the inspiratory phase of the breath cycle were analyzed over consecutive breaths for the duration of the trial. The average centroid frequency (Hz) was determined for each muscle using built-in analysis modules in Labchart (Mean Power Frequency). Because the total number of breaths generated by each subject over the course of the fatigue trial varied (range 105-230), we determined the average centroid frequency for the initial 5% and terminal 5% of the total breath number for each of the respiratory fatigue protocols (see Tables 4 & 5). To assess differences in oblique muscle activation pre-post 6 weeks IMST, EMG values were normalized with respect to maximal EMG activity (% maximum). Thus, oblique EMG activity recorded in the initial 5% and terminal 5% of each fatigue trial were averaged and expressed as % of max EMG.

Plasma Catecholamines. Plasma samples were analyzed via quantitative high-performance liquid chromatography (Associated Regional and University Pathologists - ARUP Laboratories,

Salt Lake City, UT). The plasma samples are awaiting analysis and therefore these data are not reported.

Results

Four subjects (3 females and 1 male) completed 6 weeks of IMST and both pre and post fatigue assessments. Results for systolic and diastolic blood pressures are shown in Figures 1 and 2, respectively and tabulated data are presented in Tables 1 and 2 (see Tables p. 17-19). Two of four subjects exhibited a reduction in SBP pre-post intervention of 9.4± 4.6 mmHg while two subjects exhibited an increase in SBP of 6.7 ± .42 mmHg. Only one subject exhibited a reduction in DBP pre-post training of 11.73 mmHg. In three subjects there was no significant effect of training on DBP (1.85±1.06 mmHg).

Weekly measures of heart rate are shown in Table 3. In two of four subjects, IMST resulted in significant reductions in heart rate (11.9± 2.08 bpm). Conversely, two other subjects exhibited IMST-related increases in heart rate of 7.63± 1.54 bpm.

Tables 4 and 5 report results for the average centroid frequency for each of the three muscle groups obtained at the start and end of each of the fatigue protocols pre versus post intervention. Figure 3 depicts the averages for each of the three muscle groups (start and end) of the fatigue protocol for each subject pre and again post intervention. Contrary to our expectations, results for centroid frequency were inconsistent across all muscle groups. Specifically, subject 1 exhibited a smaller shift to a lower frequency pre-post in the parasternal muscles and subject 2 exhibited a larger shift to the lower frequency range pre-post in all three muscle groups. Subject 3 exhibited a shift to the higher frequency range pre-post in the parasternal muscles and subject 4 exhibited a shift to the lower frequency range in both the parasternal and scalene muscles pre-post.

Tables 6 and 7 present data on the net change in average centroid frequency (i.e., pre-post). Pre-intervention, only one subject exhibited a decrease in all three muscle groups. Two others exhibited an increase in the centroid frequency across all three muscle groups in the pre-intervention protocol. Post intervention, one subject exhibited a decrease in all three muscle groups, however this was not the same subject who exhibited the decline pre-intervention. However, in general, results for centroid frequency post training were more consistent than for pre-training, with all subjects exhibiting a decrease in the centroid frequency in at least one muscle group.

Tables 8 and 9 report relative activation levels in the oblique muscle (% maximum of EMG) in the initial 5% and terminal 5% of the fatigue protocol, pre-post 6 weeks IMST for Subjects 1 and 2. Subject 1 exhibited a decline in oblique muscle activation over the course of the pre fatigue protocol but after 6 weeks IMST oblique activation increased over the time course of the post fatigue protocol. Conversely, Subject 2 exhibited an increase in activation over the course of the pre intervention protocol but a decline in the post intervention protocol.

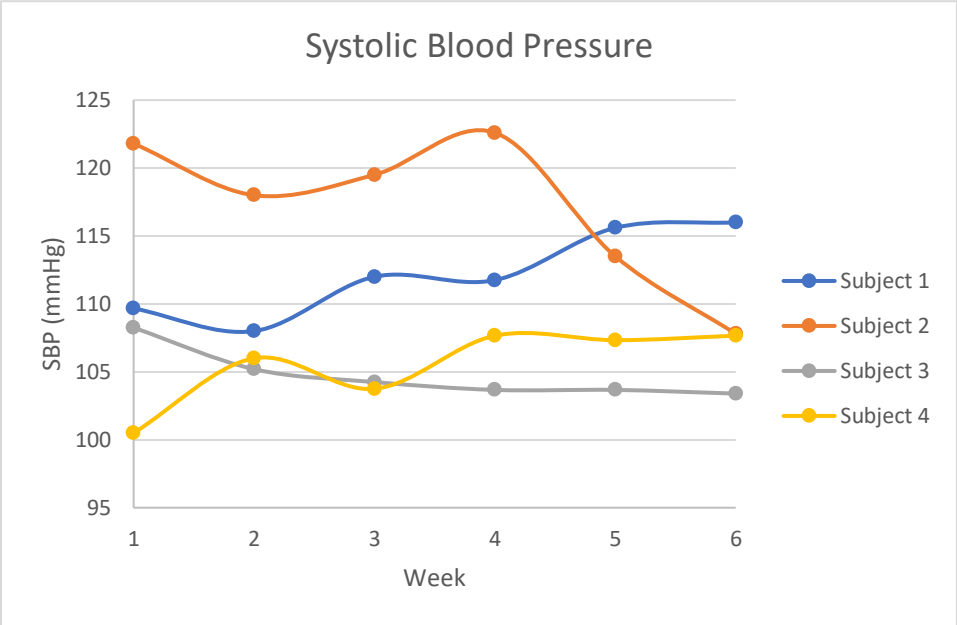

Figure 1. Shows the systolic blood pressure (SBP) on a weekly basis for each subject.

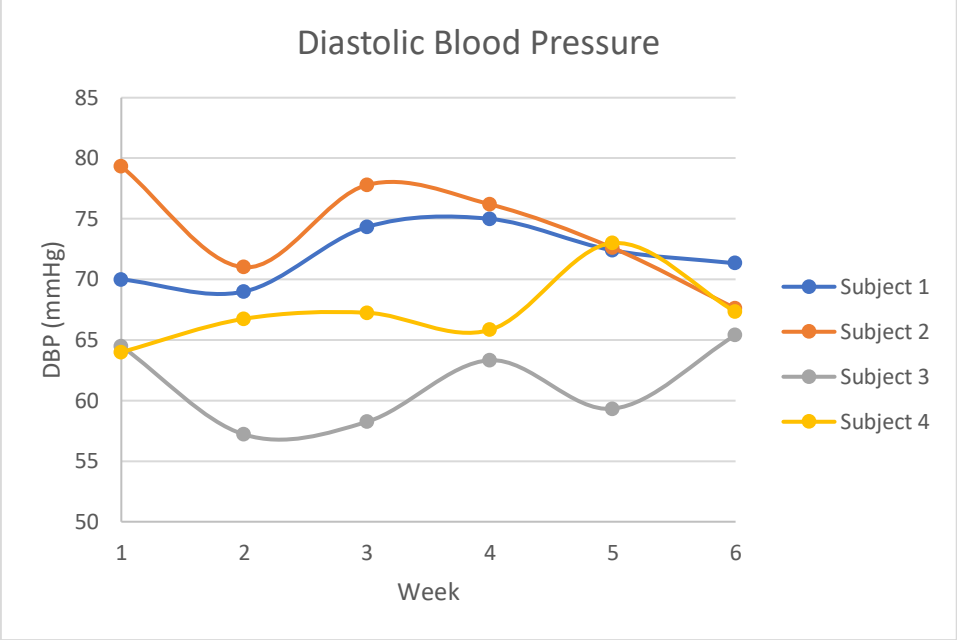

Figure 2. Shows the diastolic blood pressure (DBP) on a weekly basis for each subject.

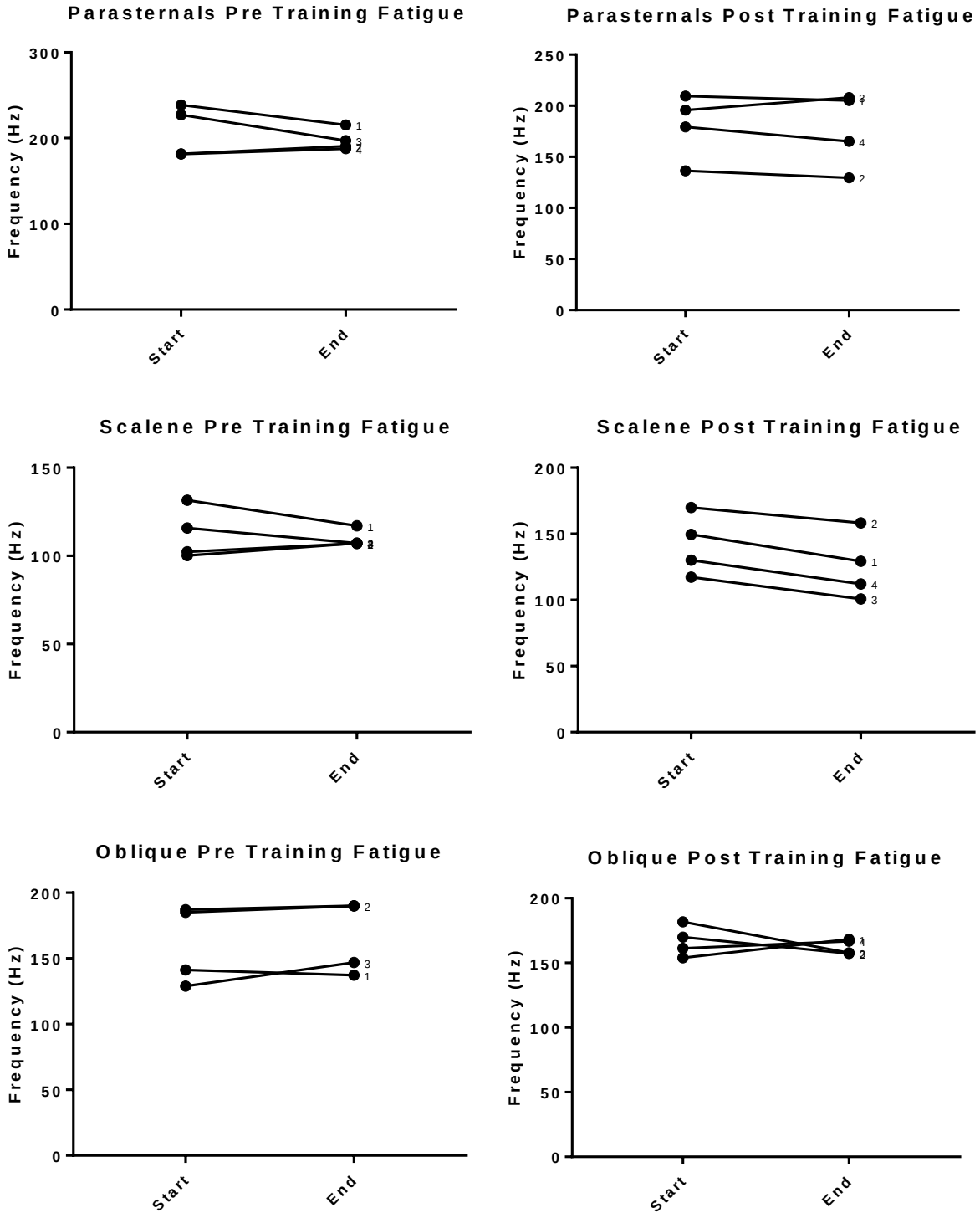

Figure 3. Depicts the average centroid frequency (Hz) for each subject in the parasternal, scalene, and oblique muscles of the start (initial 5%) and end (terminal 5%) of each fatigue protocol, pre and post 6 weeks IMST.

Discussion

Main Findings. The goal of this work was to assess the effects of 6 weeks IMST on respiratory muscle fatigability, while monitoring the effects of training on subjects' heart rate, blood pressure and sympathetic nervous system activation. To do this, I recruited four recreationally active men and women from the student population at the University of Arizona and supervised their daily IMST sessions over 6 weeks. After the 6 weeks intervention, measures of blood pressure and heart rate showed no clear directional change (i.e., either an improvement, or decline) for the group, rather individual subjects exhibited both increases and decreases in blood pressure, heart rate, and respiratory muscle EMG activation pre vs post intervention. We consider these indeterminate results likely are the result of a small sample size (N=4) and unanticipated heterogeneity in performance of the fatigue protocols (see detail below). My results provide important new insights into the limitations of original experimental protocol and ways in which the protocol could be improved / refined.

Blood Pressure and Heart Rate. Pre-post measures of blood pressure varied both within and between subjects. Our laboratory previously has reported that healthy (normotensive) adults who perform inspiratory muscle training (IMST) each day for 6 weeks exhibit declines in systolic (SBP) and diastolic blood pressure (Vranish, & Bailey, 2015, 2016; Delucia, & Bailey, 2018). However, three of the subjects exhibited increases in either SBP, DBP, or both of the measures. These results are explained by outlier blood pressure readings obtained during the course of the six-week intervention. For example, blood pressure readings obtained at week 1 and week 6 for subject 2 varied by 14 mmHg while those obtained at weeks 2-4 varied by ≤ 4.5 mmHg. A similar phenomenon is seen in measures of heart rate indicative of generally inconsistent observations. The unreliable nature of the readings is attributed to the following factors: a)

subjects were provided an insufficient duration of rest or subjects were not "quiet" before cardiovascular measurements were obtained; and b) inadequate (or excess) hydration or caffeine intake impacted BP and HR, as the body compensates for lack of blood volume by increasing heart rate and vasoconstricting. Therefore, decreases or increases in BP and HR seen pre-post intervention could be attributed to confounding factors outside of the intervention.

Respiratory Muscle Fatigability. A shift to a lower frequency in muscle activation is an accepted indicator of muscle fatigue, which is defined as the "inability to produce or sustain a desired force or power" (Gandevia, 2001). As muscles fatigue under a workload, a switch to a lower frequency of myoelectric signals occurs (Aldrich, 1983; Gross et al., 1979) signaling both a decline in motor unit firing rates and a reduction in the conduction velocity of the muscle fibers. Initially, we had hypothesized that after a six-week regimen of IMST subjects would demonstrate decreased fatigue compared to their pre training protocol results. Accordingly, we predicted a smaller shift to the lower frequency range. However, an increased resistance to fatigue across all three muscle groups was not consistently in evidence. The net change in frequency from the start to end of the initial and final fatigue protocol varied widely within and between subjects. In some subjects, the mean centroid frequency for a given muscle increased in pre training assessment and decreased in the post training assessment or vice versa. We had expected the centroid frequency to shift lower in both pre and post intervention protocols with the smallest shift anticipated in the post intervention protocol after 6 weeks of IMST. Yet, only one subject exhibited the trend we had expected and in only one muscle group.

Activation patterns of respiratory muscles are highly individualized and change depending on how subjects are instructed to breathe (Ramsook et al., 2016). Although in this case subjects

were provided very general instructions about how to perform the task (see Methods: Respiratory fatigue protocol), how each individual attained the target pressure presumably varied. Furthermore, subjects may have implemented different strategies and/or engaged different muscle groups in the initial fatigue protocol than for the post training fatigue protocol. These **two** sources of variability i.e., between subject and within subject variability likely contributed to the variation in average centroid frequencies observed in each of the fatigue protocols.

I hypothesize that the mixed outcomes from our three muscle groups are in part due to differences in how subjects performed the two fatigue protocols. Because subjects were performing IMST each day, it is likely that some/all subjects adapted to the training and improved upon their technique for generating the requisite large inspiratory pressures. Results from Subjects 2 and 3- who show a shift to a lower frequency in the oblique muscles in the post training fatigue protocol but no evidence of a decline in centroid frequency in the initial fatigue protocol-support this hypothesis. That is, these individuals appear to have attained larger i.e., more negative inspiratory pressures after 6 weeks IMST largely by increasing activation of the oblique muscles of the abdomen to compensate for a fatiguing diaphragm (Romer 2008 and Abraham 2002). Thus, the greater activation of the oblique muscles post assessment attributable to 6 weeks IMST may be the result of an increase in voluntary (i.e., cortical) drive to abdominal muscle and evidenced as an **increase** in the average centroid frequency in the oblique muscle pre-post intervention.

A comparison of the oblique EMG activation as a percentage of maximum (%max) at the start and end of the fatigue protocol and pre-post 6 weeks IMST could provide some assistance in this regard. For those subjects in which we obtained maximum maneuvers, one subject showed

evidence of increased oblique activation (%max) pre to post 6 weeks IMST however, another subject exhibited a decline in oblique activation (%max) pre to post 6 weeks IMST.

A similar phenomenon i.e., alteration in the patterns of respiratory muscle activation, also may explain observed increases in the centroid frequency of the scalene muscles in the post fatigue protocol and why in general, there is no clear direction for the shifts in centroid frequency among our subjects. If we are to gain insights into the issue of IMST and its effects on respiratory fatigue it will be imperative to control for this potential confound and impose stricter guidelines to subjects for breathing during the 6 weeks of training and especially in each of the fatigue protocols. Requiring subjects to isolate the diaphragm as the main driver of the ventilatory effort would also help the end goal; to ensure within-subject breathing uniformity.

Tables and Table Legends

		Subject #			
		1	2	3	4
SBP (mmHg)	Week 1	109.67	**121.8**	**108.25**	100.5
	Week 2	108	**118**	**105.2**	106
	Week 3	112	**119.5**	**104.25**	103.75
	Week 4	111.75	**122.6**	**103.67**	107.66
	Week 5	115.6	**113.5**	**103.67**	107.33
	Week 6	116	**107.8**	**103.4**	107.66

Table1. Average systolic blood pressure (SBP; mmHg) for each subject over 6 weeks of training. Bolded values indicate subjects in whom resting SBP declined pre-post 6 weeks IMST.

		Subject #			
		1	2	3	4
DBP (mmHg)	Week 1	70	**79.33**	64.5	64
	Week 2	69	**71**	57.2	66.75
	Week 3	74.33	**77.8**	58.25	67.25
	Week 4	75	**76.2**	63.33	65.83
	Week 5	72.4	**72.6**	59.33	73
	Week 6	71.33	**67.6**	65.4	67.33

Table 2. Average diastolic blood pressure (DBP; mmHg) for each subject over 6 weeks of training. Bolded values indicate subjects in whom resting DBP declined pre-post 6 weeks IMST.

		Subject #			
		1	2	3	4
HR (bpm)	Week 1	81	**83**	66.25	**73.167**
	Week 2	69.83	**70.8**	67.2	**70.83**
	Week 3	87.66	**67.33**	68	**67.4**
	Week 4	84.75	**69**	59	**77.83**
	Week 5	84.2	**69.16**	62.25	**78.16**
	Week 6	90.167	**69**	72.33	**63.33**

Table 3. Average heart rate (HR; beats per minute) for each subject over 6 weeks of training. Bolded values indicate subjects in whom resting HR declined pre-post 6 weeks IMST.

Pre	Cf Parasternal (Hz)		Cf Scalene (Hz)		Cf Oblique (Hz)	
Subject	Start Trial	End Trial	Start Trial	End Trial	Start Trial	End Trial
1	238.71	215.31	131.55	117.10	141.28	137.22
2	181.62	190.59	102.33	107.02	187.06	190.11
3	227.15	197.22	115.84	107.23	128.86	146.91
4	181.34	187.53	100.13	107.13	185.14	189.99

Table 4. Average centroid frequencies (Hz) for the parasternal, scalene, and oblique muscles in the initial 5% and terminal 5% of the fatigue protocol pre-intervention.

Post	Cf Parasternal (Hz)		Cf Scalene (Hz)		Cf Oblique (Hz)	
Subject	Start Trial	End Trial	Start Trial	End Trial	Start Trial	End Trial
1	209.53	205.19	149.61	129.31	153.90	168.32
2	136.26	129.54	169.86	158.28	169.86	157.23
3	195.80	207.93	117.32	100.68	181.70	157.74
4	179.18	165.11	130.10	112.05	161.20	166.80

Table 5. Average centroid frequencies (Hz) for the parasternal, scalene, and oblique muscles in the initial 5% and terminal 5% of the fatigue protocol post-intervention.

Pre 6 weeks IMST	ΔCf Parasternal (Hz)	ΔCf Scalene (Hz)	ΔCf Oblique (Hz)
Subject #			
1	23.4	14.45	4.06
2	8.97	4.69	3.05
3	29.93	8.61	18.05
4	6.19	7	4.85

Table 6. Net change in the average centroid frequencies (Hz) for the parasternal, scalene, and oblique muscles (i.e., cf in initial 5% - cf in terminal 5%) of the fatigue protocol pre-intervention. Note that values in red indicate a net decline in the centroid frequency and values in black indicate a net increase in the centroid frequency.

Post 6 weeks IMST	ΔCf Parasternal (Hz)	ΔCf Scalene (Hz)	ΔCf Oblique (Hz)
Subject #			
1	4.34	20.3	14.42
2	6.72	11.58	12.63
3	12.13	16.64	23.96
4	14.07	18.05	5.6

Table 7. Net change in the average centroid frequencies (Hz) for the parasternal, scalene, and oblique muscles (i.e., cf in initial 5% - cf in terminal 5%) of the fatigue protocol post-intervention. Note that values in red indicate a net decline in the centroid frequency and values in black indicate a net increase in the centroid frequency.

Subject 1	% Maximum (initial 5%)	% Maximum (terminal 5%)
Pre	1.02	0.99
Post	1.11	1.22

Table 8. Depicts the % maximum of oblique EMG activation for Subject 1 at the start and end 5% of the fatigue protocol, pre-post 6 weeks IMST.

Subject 2	% Maximum (start trial)	% Maximum (end trial)
Pre	1.58	1.61
Post	1.28	1.19

Table 9. Depicts the % maximum of oblique EMG activation for Subject 2 at the start and end 5% of the fatigue protocol, pre-post 6 weeks IMST.

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