

DIFFERENTIAL EFFECTS OF SELF-PACED AND DEVICE-GUIDED SLOW DEEP
BREATHING ON PHYSIOLOGICAL OUTCOMES

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LAYMAN'S ABSTRACT

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Slow breathing exercises have been used for thousands of years in practices such as yoga and meditation, and recent scientific studies demonstrate that they do reduce stress and lower blood pressure. Yet recently, device-guided breathing maneuvers that seek to replicate self-paced breathing practices have raised doubts about the effectiveness of slow breathing techniques. It is hypothesized that the act of reducing breathing rate is not the only factor contributing to stress and blood pressure reduction, and that other factors such as devices themselves and the patient's baseline personality traits and state of mind at the time of the maneuver are also important in determining the effectiveness. The present study found that changes in blood flow to the forearm, heart rate, and blood pressure were affected differently by device-guided maneuvers and self-paced maneuvers. In addition, several psychological metrics are predictive of different physiological responses to each of these practices.

ABSTRACT

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Slow breathing exercises have been used for thousands of years in yoga and meditation practices, and have been successful in reducing sympathetic nerve activity and lowering blood pressure. Recently, computerized devices aimed to replicate self-guided breathing have been used. There have been mixed results regarding the efficacy of these devices. It is hypothesized that the act of reducing breathing rate is not the only factor contributing to physiological changes, and that factors such as external aid and psychological metrics are also important in determining the responses to breathing rate changes. Therefore we compared device-guided (n=10) and self-paced (n=11) slow breathing maneuvers in young healthy males and found differences between conditions in forearm vasodilation as measured by venous occlusion plethysmography ($111.78 \pm 7.82\%$ and $96.24 \pm 5.82\%$, $p=0.067$) and changes in systolic blood pressure as measured by finometry (-3.47 ± 1.43 mmHg and -0.45 ± 2.35 mmHg, $p=0.138$). In addition, several psychological metrics are predictive of differences in physiological responses to each of these practices: trait anxiety correlates with a lack of reduction in mean arterial pressure (MAP) in the self-guided intervention ($r=0.452$, $p=0.81$); acting without awareness correlates with a lack of reduction in MAP in the self-guided intervention ($r=0.683$, $p=0.01$); and acting without awareness correlates with a reduction in MAP in the device-guided intervention ($r=-0.472$, $p=0.084$). It is concluded that these two breathing practices are inherently different breathing maneuvers, given the differences in physiological outcomes and psychological predictors.

To those who have supported me

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KEY TO ABBREVIATIONS

AHA	American Heart Association
BMI	Body Mass Index
CFQ	Cognitive Failure Questionnaire
CVLM	Caudal Ventrolateral Medulla
DBP	Diastolic Blood Pressure
DG	Device-Guided
ECG	Electrocardiogram
IRB	Institutional Review Board
FFMQ	Five-Faceted Mindfulness Questionnaire
MAP	Mean Arterial Pressure
MSNA	Muscle Sympathetic Nerve Activity
NTS	Nucleus Tractus Solitarius
pFRG	Parafacial Respiratory Group
PreBotC	PreBotzinger Complex
RSA	Respiratory Sinus Arrhythmia
RTN	Retrotrapezoidal Nucleus
RVLM	Rostroventrolateral Medulla
SBP	Systolic Blood Pressure
SDB	Slow Deep Breathing
SE	Standard Error
Self	Self-Guided Breathing

STAI-S	State Trait Anxiety Inventory – State
STAI-T	State Trait Anxiety Inventory – Trait
V_E	Total Ventilation
VOP	Venous Occlusion Plethysmography
VRG	Ventral Respiratory Group

LITERATURE REVIEW

Slow-paced breathing is a respiratory maneuver, utilized during relaxation techniques and meditative practices, which claims to promote a reduction in stress. Given its long history of use in yoga and meditation, there is an abundance of anecdotal evidence relating to stress reduction associated with slow breathing. With the potential medical applications of slow-paced breathing, such as a reduction in blood pressure in hypertensive patients, it is important to give these practices a scientific framework. This will lead to a better understanding of slow-paced breathing's viability as a method to reduce stress and how to best advise patients to achieve the maximum benefit from these techniques.

Breathing Practices

Yoga and self-paced breathing

Slow-breathing is an anchor in yoga-practices, in addition to being mindful of one's body and physical changes in body posture. Due to the positive anecdotal evidence surrounding yoga and meditative slow breathing, several studies have focused on determining efficacy of slow breathing on reducing sympathetic nervous system activity and blood pressure. It has been shown that three months of regular slow breathing practice reduces sympathetic nerve activity and increase parasympathetic nerve activity (Pal, Velkumary et al. 2004). Several studies have demonstrated a reduction in blood pressure in response to a variety of yoga slow breathing techniques (Sundar, Agrawal et al. 1984, McCaffrey, Ruknui et al. 2005, Raghuraj and Telles 2008, Upadhyay Dhungel, Malhotra et al. 2008, Bhavanani, Madanmohan et al. 2012).

Device-guided breathing

Given the positive benefits noted above, the medical device industry has attempted to extract and standardize the breathing aspect of these practices by inventing devices that coach patients through a slow breathing practice with tones and monitoring. Recently, a study concluded that device-guided breathing practices were not effective at reducing blood pressure after an eight week period in white, hypertensive male and female patients (Landman, Drion et al. 2013). Although the conclusions of that study have been challenged due to low sample sizes and potentially unsuccessful randomization (Huang and Subak 2014). Several other device-guided studies have also supported the idea that slow deep breathing is not feasible as a method to reduce blood pressure (Altena, Kleefstra et al. 2009, Anderson, McNeely et al. 2010, Hering, Kucharska et al. 2013).

On the other hand, many groups have shown a reduction in blood pressure using device-guided practices (Grossman, Grossman et al. 2001, Schein, Gavish et al. 2001, Viskoper, Shapira et al. 2003, Elliot, Izzo et al. 2004, Meles, Giannattasio et al. 2004, Schein, Gavish et al. 2009, Oneda, Ortega et al. 2010, Bertisch, Schomer et al. 2011). Similarly, several studies have concluded that muscle sympathetic nerve activity (MSNA) was significantly lower after an acute use of device-guided breathing (Hering, Kucharska et al. 2013, Harada, Asanoi et al. 2014), although this was not seen in chronic use (Hering, Kucharska et al. 2013).

Despite the evidence that self-paced breathing exercises, such as yoga breathing, lowers blood pressure and despite mixed results from device-guided studies, the American Heart Association (AHA) recently issued a position statement providing moderate support to device-guided breathing while withholding support of yoga breathing (Brook, Appel et al. 2013). Notably, however, resistance to supporting yoga breathing is based on a lack of good studies and not to

negative findings, per se. Rather than working from data that does not take into account all of the variables, it is important to perform well-controlled studies on slow breathing practices to determine whether there are significant differences between these two slow deep breathing practices and their effects on the cardiovascular system.

Physiological Mechanisms of Respiratory Control

In order to carry out useful studies of the effects of controlled breathing on heart-related functions, it is necessary to understand some of the key mechanisms by which the two physiological systems interact.

Central control of respiration

See figure 1. Inspiration and expiration involve two areas in the medulla that rhythmically generate motor efferent activity to muscles involved in respiration, the Pre-Botzinger Complex (PreBotC) and the parafacial respiratory group/retrotrapezoidal nucleus (pFRG/RTN). These two areas in the brainstem—PreBotC and pFRG/RTN—are phase-locked, or synchronized, through reciprocal excitatory coupling to ensure proper breathing patterns, or eupnoea. (Mellen and Thoby-Brisson 2012).

The PreBotC is involved in generating inspiration. This was established by observing that PreBotC activity is coupled to respiratory patterns (Smith, Greer et al. 1990, Smith, Ellenberger et al. 1991, Richter and Spyer 2001, Ramirez, Zuperku et al. 2002) and lesion studies showing that destruction of the PreBotC leads to ataxic breathing patterns (Gray, Janczewski et al. 2001). While much of the rhythmicity of the PreBotC is still unclear and debated, it is agreed upon that about half of this area in the medulla is comprised of pacemaker neurons that initiate the

inspiratory patterning. From there, inspiratory-related neural activity is relayed to premotor neurons in the rostral ventrolateral medulla (RVLM), which continues to project to the phrenic nerve (diaphragm) and intercostal nerves (external intercostal muscles) to cause contraction of these muscles and thus inhalation (Feldman and Del Negro 2006).

Under rest or light activity, the lungs will deflate through passive recoil of the ribcage and diaphragm. Under conditions that require increased ventilation or conscious breathing, active expiration will arise from a second area in the medulla, the pFRG/RTN. The pFRG/RTN also exhibits rhythmicity similar to the PreBotC, but is lesser known due to its recent identification (Mellen, Janczewski et al. 2003, Feldman and Del Negro 2006, Janczewski and Feldman 2006). Activity in the pFRG/RTN is relayed to the central ventrolateral medulla, before continuing on to the intercostal nerves, which innervate the internal intercostal muscles as well as the abdominal muscles. Contraction of both of these muscle groups results in active expiration.

Respiratory-sympathetic coupling

The central respiratory neural network is anatomically and physiologically linked to autonomic outflow to the rest of the body. The most obvious case of this is evident in variations in heart rate that align with breathing patterns, called respiratory sinus arrhythmia (RSA). RSA causes heart rate to increase during periods of inspiration and decrease during periods of expiration, allowing for more efficient gas exchange. This is modulated via changes in parasympathetic cardiac vagal outflow, causing an increase in vagal tone during expiration and a withdrawal during inhalation, controlled by pulmonary stretch afferents and the Hering-Breuer reflex (Yasuma and Hayano 2004).

Less evidently, the central respiratory neural network also acts as a “gate” for sympathetic outflow. To be more specific, the sympathetic nervous system is more active during the

inspiratory phase, and is “blocked” during the expiratory phase. An example of this was shown cleverly through stimulation of the baroreceptors by applying a baroreflex-triggering procedure of external neck pressure or suction during different phases of the respiratory cycle and observing that blood pressure responses, and therefore sympathetic responses, were greatest during inspiratory phases (Eckberg and Orshan 1977, Eckberg, Kifle et al. 1980, Wallin and Eckberg 1982, Eckberg 2003). This respiratory “gate” phenomenon is also demonstrated by observing that MSNA quiescence occurs during exhalation (Mozer, Fadel et al. 2014).

Beyond the acute effects of respiratory-sympathetic coupling on physiological outcomes mentioned earlier, there are also several relevant chronic effects. It has been shown that, when broken in tertiles based on breathing rate, those who breathe at a faster rate also have a higher MSNA burst incidence (Narkiewicz, van de Borne et al. 2006). It has also been shown that higher breathing rates are correlated with an increase in total peripheral resistance in young males (Charkoudian, Gusman et al. 2010).

The anatomical basis for the above relationships is based in central connections between the respiratory and autonomic neurons. The nucleus tractus solitarius (NTS) is a structure in the dorsolateral medulla, and is considered an integrative center for various processes related to the sympathetic nervous system, including baroreflex and chemoreflex control. The NTS can be considered the “master” regulator of sympathetic outflow through direct projections to the RVLM (Zoccal, Furuya et al. 2014).

The RVLM, found in the medulla, houses presympathetic neurons, which are the point of origin for excitatory inputs to the preganglionic sympathetic neurons that are responsible for controlling arterial pressure (Guertzenstein and Silver 1974, Ross, Ruggiero et al. 1984).

The ventral respiratory group neurons (VRG, which is where the PreBotc and BotC are located) are intermingled with the RVLM (Moraes, Bonagamba et al. 2011). An anatomical link between these two areas was established when it was discovered that some axons from the BotC lied close to presympathetic neurons in the RVLM, suggesting communication between these two areas (Sun, Minson et al. 1997). Even further, catecholaminergic neurons, or C1 neurons, in the RVLM were shown to be phase-locked with the respiratory cycle (Moraes, da Silva et al. 2013).

Psychological Correlates to Physiological Outcomes

There are many psychological metrics are used in the assessment of various traits related to personality and higher levels of brain functioning. In the current study, we focused on metrics that were deemed to be pertinent to assessing differences between conditions. These included the State-Trait Anxiety Inventory (STAI), Five Facet Mindfulness Questionnaire (FFMQ), Cognitive Interference Questionnaire (CIQ), Penn State Worry Questionnaire (PSWQ), and Cognitive Failures Questionnaire (CFQ).

The STAI is broken down into two sections, both of which assess anxiety levels within the participant. The first section, STAI-state, looks at how anxious the participant is in the current moment, and is capable of changing rapidly in response to new events. The second, STAI-trait, assesses the anxiety levels as a personality trait, meaning that it is stable through time and is subject to little, change, if any. The FFMQ assesses at various aspects of mindfulness. It is broken down into five categories: observational, or how aware the subject is of your body and your surroundings; descriptive, or how well the subject is capable of articulating their feelings and thoughts; acting with(out) awareness, or how unaware the subject is of their actions both before and during their completion; non-judgemental, or how self-critical the subject is of their

thoughts and feelings; and non-reactionary, or how capable the subject is at stepping away from their emotions and feelings without becoming overwhelmed by them.

Finally, the CIQ measures the relative amount that the subject thought of other topics during the duration of the study, including topics related to the study (“am I performing well?”) and in their everyday lives (“I need to pick up the kids after work”). The PSWQ measures the how frequently the participant worries and how much worry affects their lives. The CFQ measures the relative frequency at which the participant forgets what they are doing, how frequently they fail to notice their surroundings.

There is an increasing amount of evidence that points to the involvement of the state of mind on physiological outcomes. It has been shown that a high level of trait mindfulness is related to a reduction in blood pressure, lower IL-6 levels (Tomfohr, Pung et al. 2015), lower cortisol levels (Brown, Weinstein et al. 2012, Jacobs, Shaver et al. 2013), and lower activation of the amygdala in response to stressors (Creswell, Way et al. 2007). Taken together, these studies indicate that a person’s level of mindfulness is important to consider when discussing autonomic activity.

Another psychological trait potentially linked to autonomic activity is cognitive interference. It has been shown that a decrease in the amount of attention that can be spared, or an increase in the amount of cognitive interference occurring, results in an increase in blood pressure in response to negative, stressful images (Okon-Singer, Mehnert et al. 2014).

Specific Aim I

To determine if device-guided and self-guided breathing result in different physiological outcomes. We hypothesize that self-guided breathing and device-guided breathing are

fundamentally different practices, and thus will result in differing degrees of physiological responses, such as reduction in blood pressure. The results from pursuing this aim will help better understand if it is appropriate to use devices to assist in slow breathing maneuvers. This is especially relevant for clinicians and choosing the best intervention for their patients.

Specific Aim II

To determine if psychological metrics are correlated to a reduction in blood pressure during slow pace breathing. Psychological traits such as mindfulness and attention are important in modulating the autonomic nervous system and blood pressure. We hypothesize that various levels of mindfulness, cognitive interference and related psychological metrics will be correlated with the degree to which blood pressure is affected in both device and self-guided slow pace breathing practices. These results will be important in predicting a person's response to slow-paced breathing maneuvers based on their psychological traits. Further, it may also explain the mixed results found with device-guided studies.

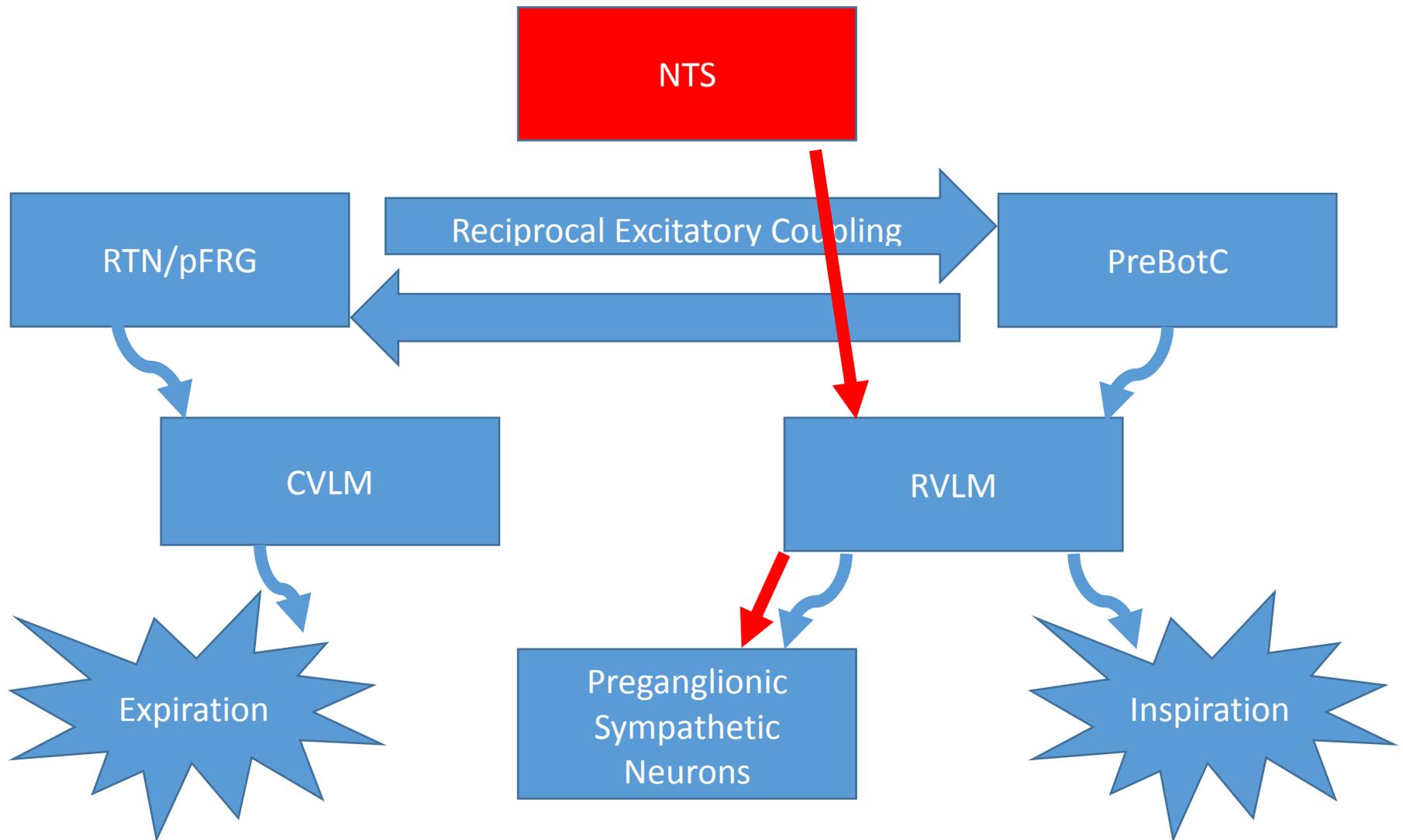


Figure 1: Central Respiratory Control Diagram.

Figure 1: Central Respiratory Control Diagram (cont'd). The PreBotzinger's Complex (PreBotC) houses inspiratory pace maker neurons. Inspiratory neurons project to the Rostroventrolateral Medulla (RVLM), which relays inspiratory signals to the diaphragm and external intercostal muscles via the phrenic and intercostal nerves, respectively, to cause inhalation. The Retrotrapezoid Nucleus/Parafacial Respiratory Group (RTN/pFRG) houses expiratory pacemaker neurons that function under active expiration situations. The expiratory neurons project to the Caudal Ventrolateral Medulla, which relays exhalation signals to the abdominal and internal intercostal muscles, triggering exhalation. The RTN/pFRG and PreBotC reciprocally excite each another, leading to phase locking and ensuring proper breathing patterns, or eupnoea. The Nucleus Tractus Solitarius (NTS) receives input from baroreceptors and chemoreceptors. These inputs are then translated to sympathetic nerve activity increases or decreases through the RVLM. The RVLM's role in modulating inspiration and sympathetic output is the reason for respiratory-sympathetic coupling.

CURRENT STUDY

Methods

Subject inclusion/exclusion

Participants in this study were young healthy males, 18-25 years of age, non-obese (BMI<30), non-hypertensive (BP <140/90), moderately active (less than 60 min, 3 times per week), and yoga/meditation naïve. Participants could not be smokers or have any chronic diseases related to the cardiovascular or respiratory systems, nor be taking any medication that could potentially alter cardiovascular function.

On the day of the study, participants were asked to refrain from caffeine or alcohol in the past 6 hours and food in the past 3 hours, and intense exercise 24 hours. Prior to arrival, participants were randomly sorted into either device-guided or self-guided breathing groups. Participants were screened and consented prior to enrollment.

Table 1: Demographics.

Demographics	Device (n=10) ±SE	Self (n=11) ±SE	P- value
Age	20.01 ±0.4	20.17 ±0.43	0.78
BMI	23.92 ±0.96	24.59 ±0.77	0.59
Heart Rate	60.2 ±2.71	63.18 ±3.30	0.5
Systolic	117.46 ±1.91	121.3 ±2.58	0.26
Diastolic	67.41 ±1.45	65.2 ±2.77	0.5
Breathing Rate	15.39 ±1.28	14.35 ±1.03	0.53

IRB approval

This study was reviewed and approved by Michigan State University's Institutional Review Board, IRB approval #: 14-691. All participants read and signed a consent form outlining their rights as a participant.

Software/hardware

All physiological data was collected and analyzed using Labchart (version 7.3.7, ADInstruments) in conjunction with Powerlab 8/35 (ADInstruments). The inductotrace, finometer, and plethysmograph (see below) were all connected to the Powerlab unit with BNC cables. ECG was connected using a BNC cable allowing communication between the main unit and the bio amp unit.

Electrocardiography (ECG)

ECG was obtained using a 4cm x 3.5cm electrode (3M Red Dot 2560). The electrodes were attached to an ADInstruments 5-lead shielded bio amp cable, which was plugged into a ADInstruments Dual Bio Amp/Stimulator unit.

Three leads were placed in total. The negative lead was positioned just under the right clavicle. The positive lead was positioned just above the left iliac crest. The ground was placed just above the right iliac crest. This positioning is known as Lead II. The skin was cleaned using an alcohol swab prior to lead attachment.

Pneumobelt

A double pneumobelt set up from Inductotrace was used to monitor chest wall and abdominal movements associated with breathing (model number 10.9000 from Ambulatory Monitoring Inc.) Appropriate transducer band sizes were determined by measuring the circumference of the

subject's body at the level of the navel and just below the axilla for the waist and chest bands, respectively. Pneumobelts were calibrated using a one-liter spirometry bag after the participant was lying down, using a nose clip to prevent nose breathing. Participants fully inflated and deflated the bag 4 times and these values were used to convert raw voltage readings to liters. Data from both calibrated belts were summed to determine total air flow.

Finometer

The finometer MIDI (Finapres Medical Systems) was used to assess beat-to-beat blood pressure continuously throughout the study. Appropriate finger cuff sizes were determined by using the cuff size guide apparatus provided by the company. The finger cuff was placed on the left hand middle finger intermediate phalanx and adjusted for proper readouts. To verify and calibrate the finometer readouts, manual blood pressure was taken two to three times before and after the study. Participants were asked to remain still. If the participant's hand or finger became cold, we provided a small heating pad.

Venous occlusion plethysmography

Forearm blood flow was assessed using Venous Occlusion Plethysmography (VOP). Two rapid cuff inflators (E20, Hokanson) were attached to one inflator air source (AG 101, Hokanson). One rapid cuff inflator was attached to a child's arm blood pressure cuff, which was wrapped around the wrist, and the other rapid cuff inflator was attached to an adult arm blood pressure cuff, which was fastened around the upper arm. The rapid cuff inflator attached to the cuff around the bicep was on an 8 sec inflate/7 sec deflate timer, while the wrist cuff stayed inflated for 4 minutes.

Mercury strain gauges (Hokanson) were used to assess the expansion of the forearm. To select the appropriate sized strain gauge, the forearm circumference was measured two cm distal from the elbow fold. This was also the location of the strain gauge when fitted onto the participant. The measured size of the forearm was then subtracted by two and the resulting value was the size of the strain gauge used (cm). The cables were taped to avoid any unnecessary movement. The strain gauge was attached to a plethysmograph (EC6, Hokanson). The plethysmograph was calibrated prior to fitting of the strain gauge to the participant.

VOP data was analyzed in Labchart by measuring the slope of each tracing during the inflation phase of the upper arm cuff over eight cycles of VOP and the individual slopes were averaged.

Study conditions

Participants in the device-guided breathing condition were instructed to synchronize their breathing to a series of tones administered through headphones, which results in a breathing rate of 6 breaths/min. Participants in the self-guided condition were instructed to reduce their breathing to a rate slower than normal, but still maintain a breathing pattern that is comfortable to them in both rate and depth. Self-guided participants also had headphones on, but no audio was played. All studies occurred in the same room with controlled temperature and humidity, dimmed lighting, and quiet conditions.

Psychological surveys

A State-Trait Anxiety Survey-State (STAI-S) was administered prior to instrumentation. Another STAI-S was administered after de-instrumentation. Following the second STAI-S, the participant was given the following surveys, in order: STAI-Trait, Five Facet Mindfulness Questionnaire,

Cognitive Interference Questionnaire, Penn State Worry Questionnaire, and Cognitive Failures Questionnaire.

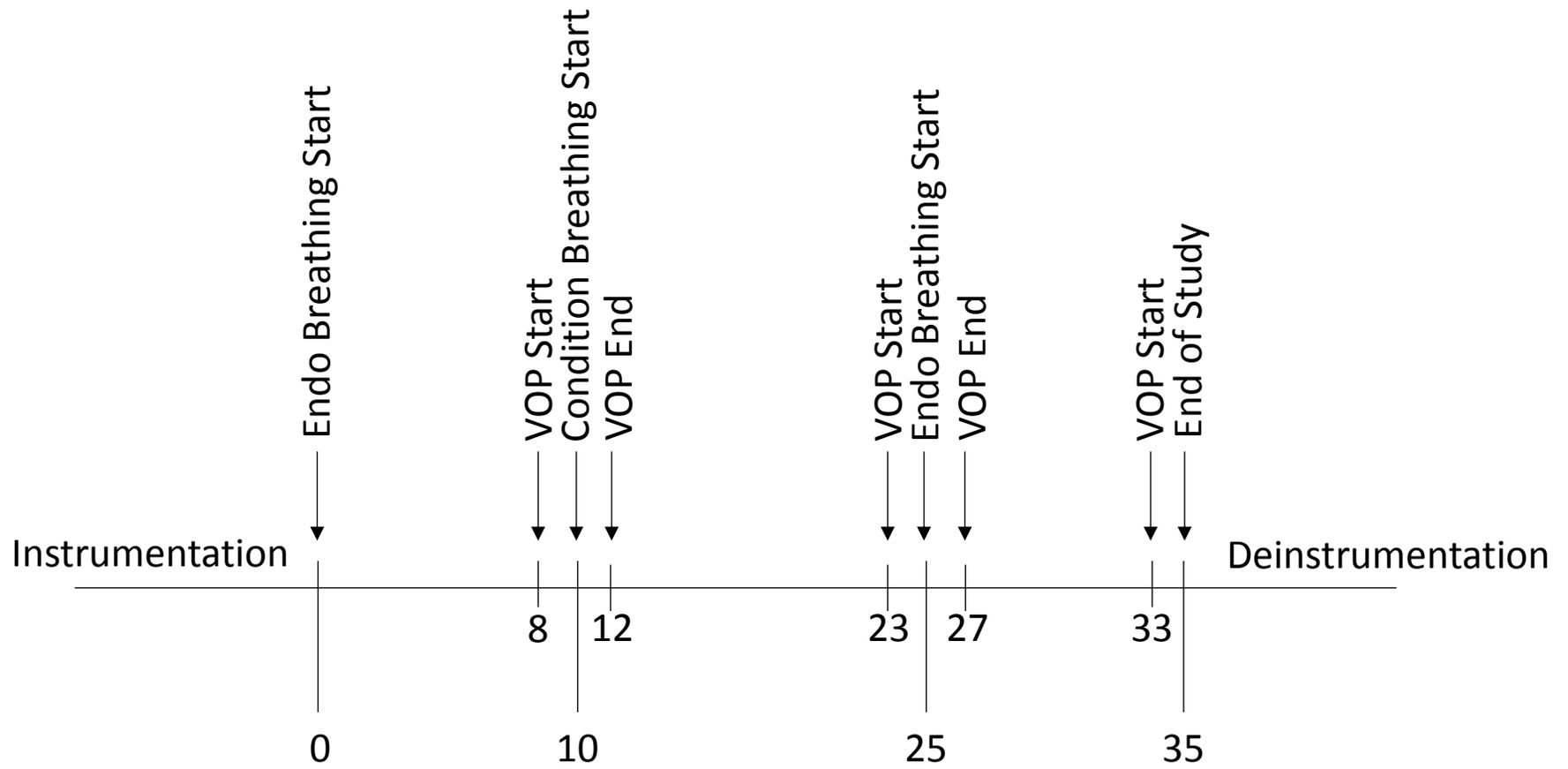


Figure 2: Timeline for studies.

Figure 2 (cont'd): Participants were screened and consented, then filled out an STAI-S, followed by intake forms. Next, the participant was instrumented with ECG, pneumobelts, finometer, and VOP equipment, and the appropriate calibrations completed. To begin data collection, the participant was asked to breathe normally for 10 minutes, then slow their breathing down for 15 minutes either by self-guiding or device-guiding, then breathe normally again for 10 minutes. Data collection stopped after the end of the second endogenous breathing, and the participant was deinstrumented and asked to fill out the remaining psychological surveys. Throughout the entirety of data collection, the ECG, pneumobelts, finometer were continuously recording data. VOP cycling began 2 minutes before the end of a condition and ended 2 minutes after the beginning of the next.

Results

Breathing Manipulation

Baseline breathing rate was not different between the self-guided and device-guided groups (14.36 ± 1.03 vs 15.39 ± 1.29 breaths per minute, $p=0.27$). Breathing was reduced to 8.36 ± 0.74 breaths per minute (BPM) in self-guided ($p < 0.01$ compared to baseline) and 6.37 ± 0.10 BPM in device-guided ($p > 0.01$ compared to baseline, $p < 0.01$ between groups). During recovery, breathing rate was lower from baseline levels in self-paced (13.25 ± 0.716 BPM, $p=.030$) and device-guided (13.67 ± 1.11 BPM, $p=0.020$, Figure 3). Recovery breathing was not significant between conditions ($p=0.378$). Breathing data represents the mean breath rate of the entire 10 or 15 min period.

During baseline, self-guided and device-guided conditions had similar tidal volumes of 0.460 ± 0.04 L and 0.42 ± 0.05 L, respectively ($p=0.31$). During condition breathing, the device-guided group had higher tidal volumes of 1.17 ± 0.116 L ($p > 0.01$ compared to baseline) compared to self-guided conditions with an average of 0.710 ± 0.0924 L ($p < 0.01$ compared to baseline, $p > 0.01$ between groups). During the recovery period, both self- and device-guided groups returned to baseline tidal volume levels of 0.47 ± 0.075 L and 0.49 ± 0.087 L, respectively ($p > 0.05$ between groups, Figure 4).

Total ventilation (V_E) was similar for both self- and device-guided breathing during baseline at 6.172 ± 0.35 L/min and 6.176 ± 0.82 L/min, respectively ($p=0.498$). V_E was increased in the device-guided condition during condition breathing at 7.41 ± 0.69 L/min ($p=0.056$ compared to baseline) compared to self-guided condition breathing at 5.430 ± 0.364 L/min ($p > 0.01$). During recovery, both groups returned to baseline ventilation levels and did not differ from each other:

5.919±0.721 (p=0.322 compared to baseline) and 6.335±1.000 (p=0.300 compared to baseline) L/min respectively (p=0.368 between conditions, Figure 5).

Specific Aim I

Aim I: To determine if device-guided and self-guided breathing result in different physiological outcomes.

Self-guided and device-guided conditions do not statistically differ (p>0.05) nor come statistically close to differing from each other (p>0.1) in any study phase (baseline, condition start, condition end, recovery start, recovery end) unless explicitly stated. Similarly, within-condition differences to baseline during study phases are either statistically insignificant (p>0.05) and/or are not statistically close to significance (p>0.1) unless explicitly stated.

Changes in systolic blood pressure (SBP) from baseline for self-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was -1.4±1.6, -3.5±2.4 (p=0.018 compared to baseline), -3.9±2.2 (p=0.003 compared to baseline), and -1.9±1.7 mmHg (p=0.0332 compared to baseline), respectively. Changes in SBP from baseline in device-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was 0.0±1.5, -0.4±2.4, -0.7±2.3, and +0.3±1.6 mmHg, respectively. During the end of condition breathing, self-guided and device-guided breathing were not statistically different (p=0.138), though the means differed by a clinically relevant 3.2 mmHg (Figure 6).

Changes in diastolic blood pressure (DBP), from baseline for self-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was +0.1±0.7, -1.8±0.8 (p=0.019 compared to baseline), -2.0±0.9, and -1.0±1.4 mmHg, respectively. Changes in DBP during device-guided breathing during the start of condition, end of condition, start of recovery,

and end of recovery was -1.8 ± 0.6 ($p=0.008$ compared to baseline), -0.6 ± 1.0 , -0.3 ± 1.0 , and -0.6 ± 1.4 mmHg, respectively. During the start of the condition, device-guided breathing had a significantly greater drop in blood pressure compared to the self-guided condition ($p=0.036$ between conditions) (Figure 7).

Changes in mean arterial pressure (MAP), from baseline for self-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was -0.4 ± 2.3 , -2.3 ± 2.0 ($p=0.004$ compared to baseline), -2.6 ± 2.1 ($p=0.013$ compared to baseline), and -1.4 ± 3.1 mmHg, respectively. Changes in MAP during device-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was -1.2 ± 2.3 ($p=0.045$ compared to baseline), -0.5 ± 1.9 , -0.4 ± 1.9 , and -0.3 ± 3.0 mmHg, respectively (Figure 8).

Change in forearm blood flow, as percentage changes from baseline, for self-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was 108.9 ± 4.2 ($p=0.030$ compared to baseline), 119.8 ± 8.0 ($p=0.018$ compared to baseline), 111.8 ± 7.8 , and 115.8 ± 9.0 %, respectively. Forearm blood flow for device-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was 112.4 ± 4.1 ($p=0.025$ compared to baseline), 103.5 ± 6.8 , 96.2 ± 5.8 , and 97.2 ± 3.5 %, respectively. During the recovery end phase, the self-guided condition had significantly higher forearm blood flow than the device-guided group ($p=0.039$) (Figure 9).

Change in heart rate, as percentage change from baseline, for self-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was 101.5 ± 1.4 , 98.7 ± 1.8 , 99.0 ± 1.5 , and 97.5 ± 1.7 %, respectively. Change in heart rate for device-guided breathing during the start of condition, end of condition, start of recovery, and end of recovery was 105.3 ± 1.7 ($p=0.007$ compared to baseline), 98.6 ± 5.2 , 99.8 ± 2.7 , and 99.2 ± 1.9 %,

respectively. At the condition start phase, device-guided breathing had a significantly higher change in heart rate than the self-guided condition ($p=0.047$) (Figure 10).

Specific Aim II

Aim II: To determine if psychological metrics are correlated to a reduction in blood pressure during slow pace breathing.

All changes in blood pressure used for testing correlation with psychological metrics are referring to a change in MAP from the average of the last two minutes of baseline to the average of the last two minutes of condition breathing.

Before the study, anxiety levels as determined by the STAI-S were not different between groups, 30.54 ± 2.23 for the self-guided group and 32.5 ± 3.08 for the device-guided group ($p=0.304$ between groups). After the study, anxiety levels decreased to 26.00 ± 1.24 for self-guided ($p=0.024$ pre vs. post) and 28.5 ± 1.81 for device-guided ($p=0.038$ pre vs. post, $p=0.131$ between groups) (Figure 11).

Trait anxiety as determined by the STAI-T was found to be highest in participants whose blood pressure did not fall in response to self-guided breathing conditions ($r=0.452$, $p=0.081$). There was no correlation found with device-guided conditions ($r=-0.301$, $p=0.199$) (Figure 12).

Cognitive failures was found to be highest in participants whose blood pressure did not respond to self-guided breathing conditions ($r=0.498$, $p=0.059$). There was no correlation found with device-guided conditions ($r=0.0923$, $p=0.400$) (Figure 13).

Observational mindfulness was found to be highest in participants whose blood pressure did not respond to device-guided breathing conditions ($r=0.405$, $p=0.123$). There was no correlation found with self-guided conditions ($r=-0.246$, $p=0.233$) (Figure 14).

Acting without awareness, a trait associated with no thinking about your actions before and during their execution, was found to correlate with a lack of response in blood pressure in self-guided participants ($r=0.683$, $p<0.01$). Acting without awareness was found to correlate with a reduction in blood pressure in device-guided participants ($r=-0.472$, $p=.084$) (Figure 15).

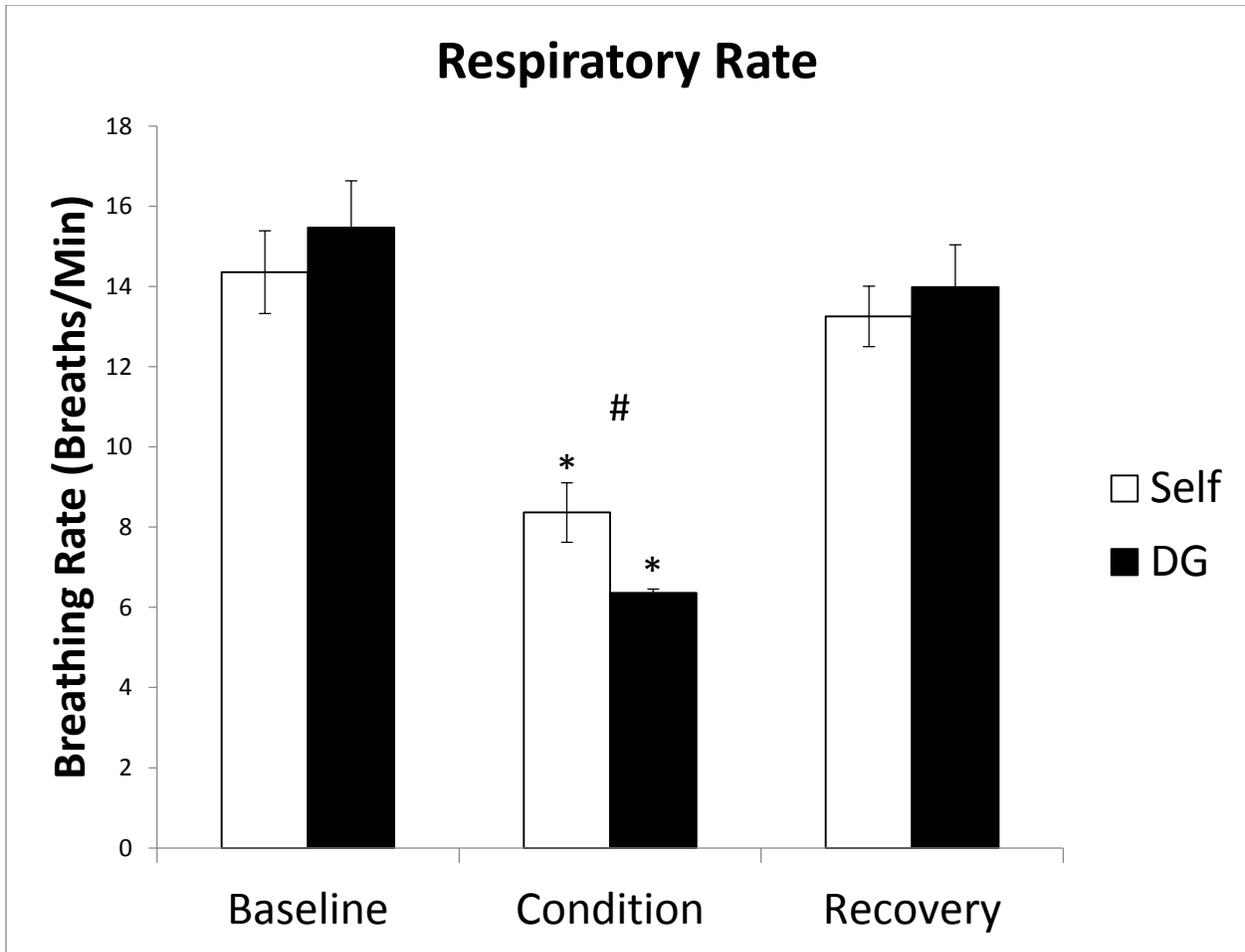


Figure 3: Respiratory Rate. Respiratory rate determined using double pneumobelts. Bars represent the mean \pm SE of each entire phase. (# indicates $p < 0.05$ between the two conditions at the given phase, * indicates $p < 0.05$ between the current phase and baseline for the same condition). Device-guided condition denoted with DG, self-paced condition denoted with Self.

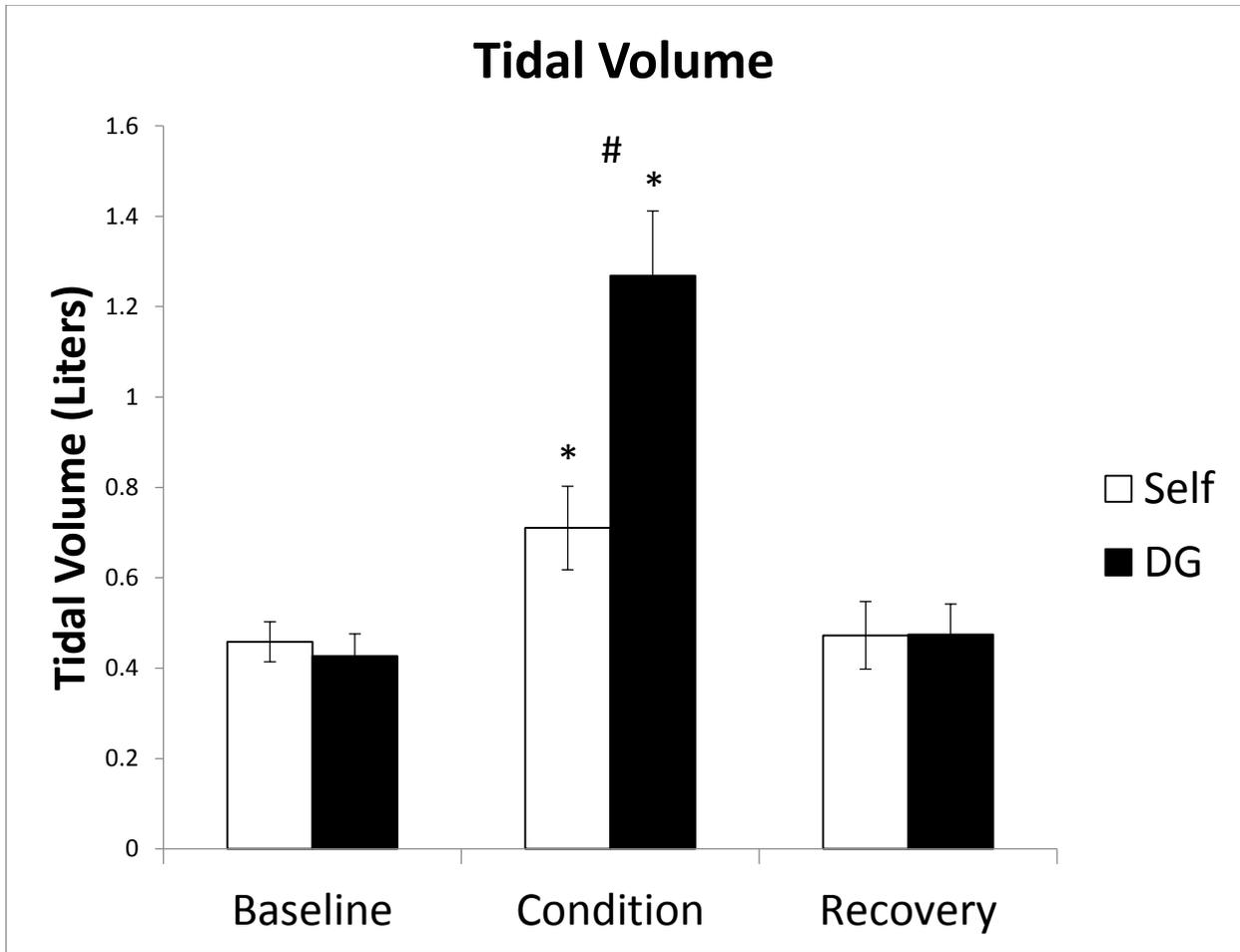


Figure 4: Tidal Volume. Tidal volumes determined using calibrated double pneumobelts. Values shown are the mean \pm SE of the entire duration of the condition. (# indicates $p < 0.05$ between the two conditions at the given phase, * indicates $p < 0.05$ between the current phase and baseline for the same condition). Device-guided condition denoted with DG, self-paced condition denoted with Self.

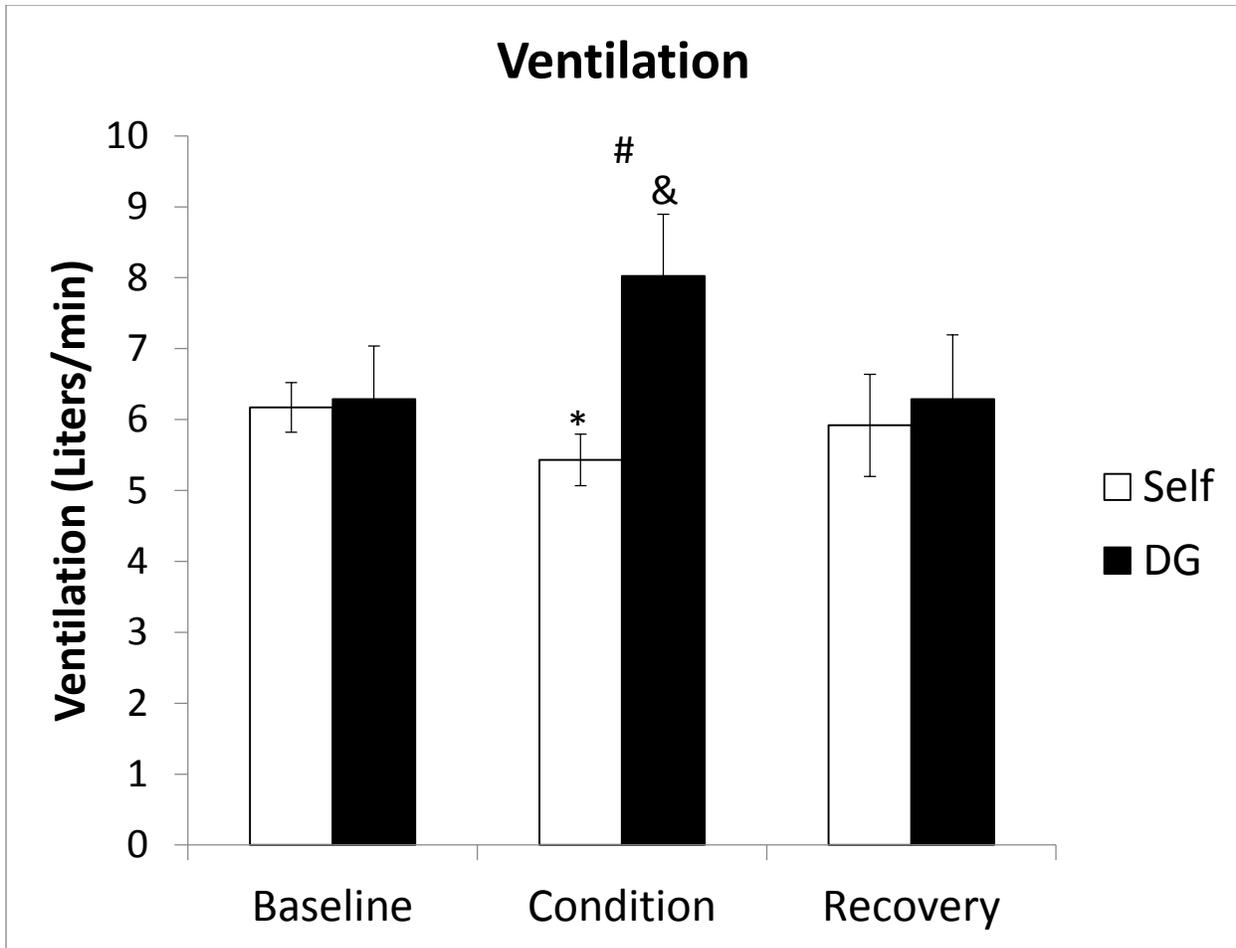


Figure 5: Ventilation. Ventilation (V_E) (breathing rate x tidal volume). Breathing rate and tidal volumes determined using double pneumobelts were manually multiplied for V_E calculation. Values shown are the mean \pm SE of the entire duration of the condition. (# indicates $p < 0.05$ between the two conditions at the given phase, * indicates $p < 0.05$ between the current phase and baseline for the same condition, & indicates $p = 0.056$). Device-guided condition denoted with DG, self-paced condition denoted with Self.

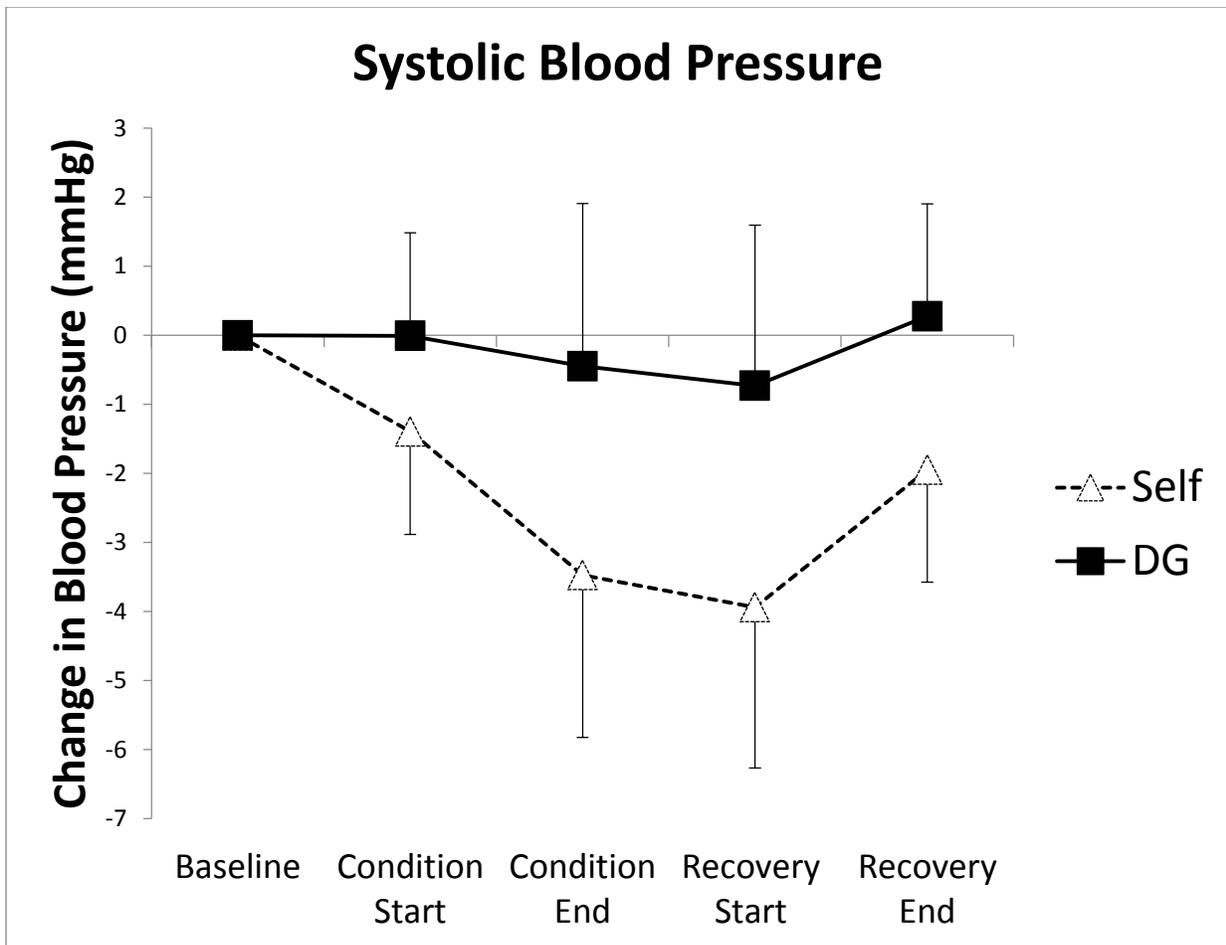


Figure 6: Systolic Blood Pressure. Systolic blood pressure determined using a finometer.

Values represented are the mean \pm SE for the two minute period at the beginning or end of each phase. Device-guided condition denoted with DG, self-paced condition denoted with Self.

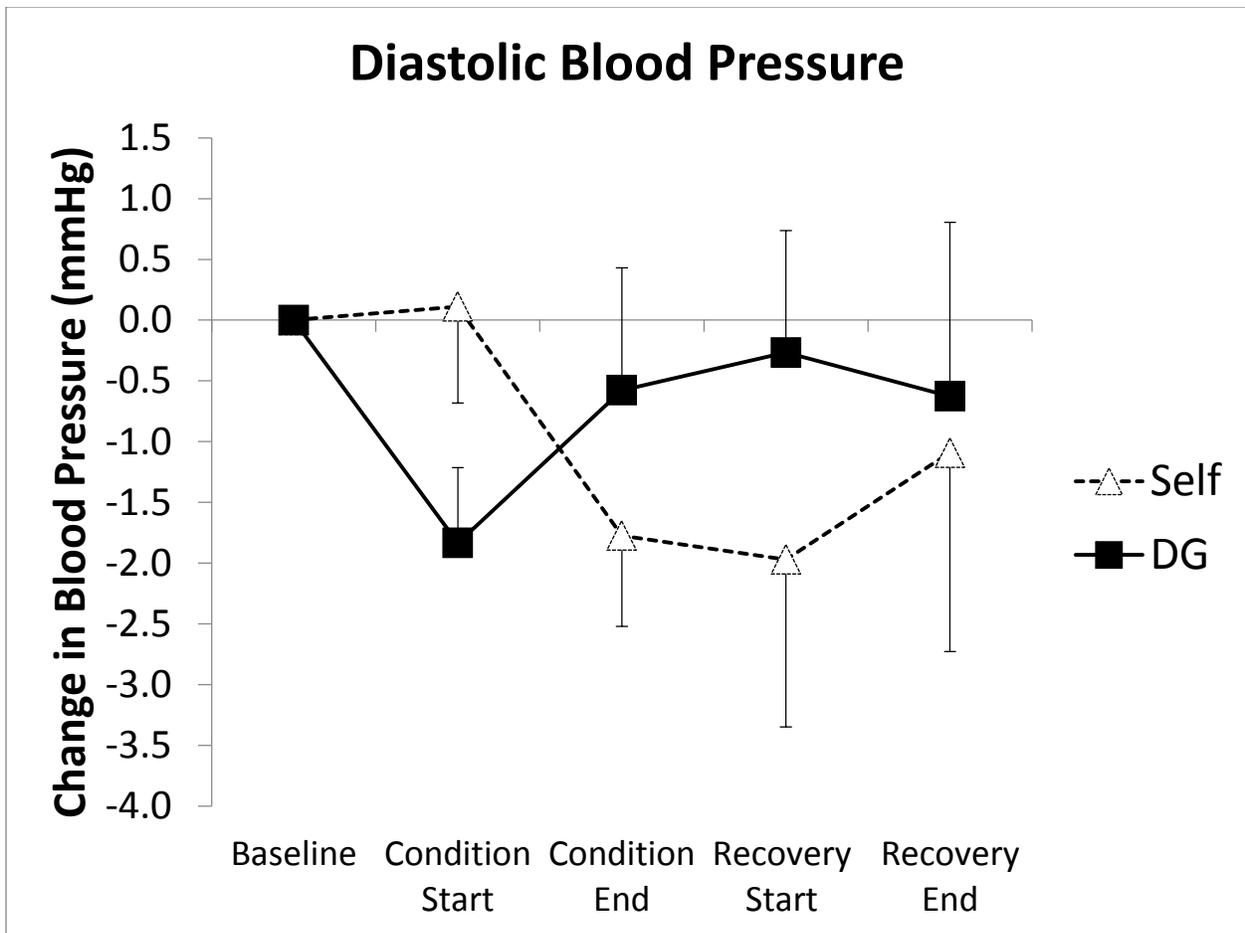


Figure 7: Diastolic Blood Pressure. Diastolic blood pressure determined using a finometer.

Values represented are the mean \pm SE for the two minute period at the beginning or end of each phase. Device-guided condition denoted with DG, self-paced condition denoted with Self.

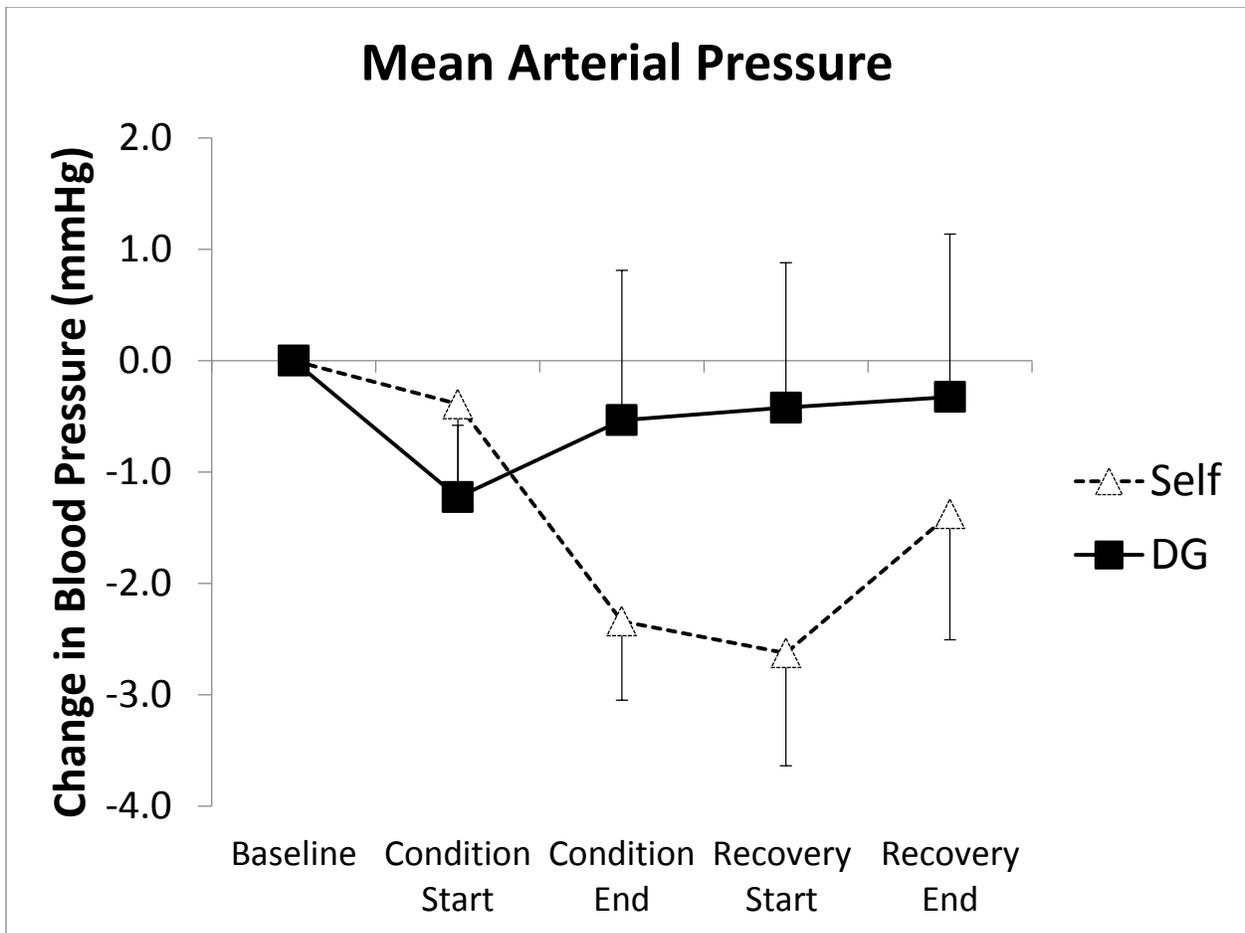


Figure 8: Mean Arterial Blood Pressure: Mean arterial pressure was calculated by adding $(2/3 \times \text{Diastolic})$ and $(1/3 \times \text{Systolic})$. Diastolic and systolic values determined using a finometer. Values represented are the mean \pm SE for the two minute period at the beginning or end of each phase. Device-guided condition denoted with DG, self-paced condition denoted with Self.

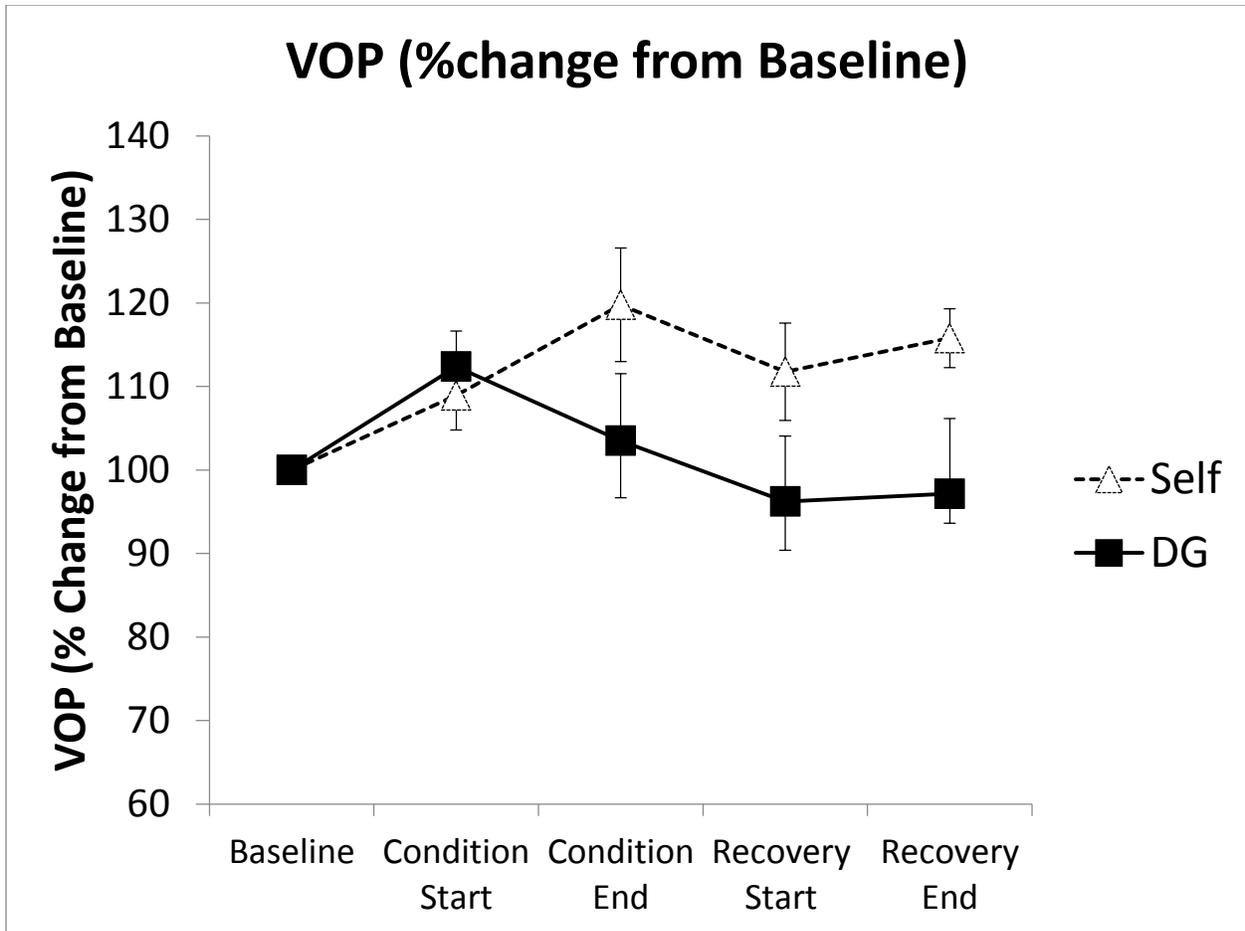


Figure 9: Forearm Vasodilation. Forearm vasodilation determined using venous occlusion plethysmography (VOP). Change from baseline was calculated by $(\text{phase}/\text{baseline}) \times 100$. Values represented are the mean \pm SE for the two minute period at the beginning or end of each phase. Device-guided condition denoted with DG, self-paced condition denoted with Self.

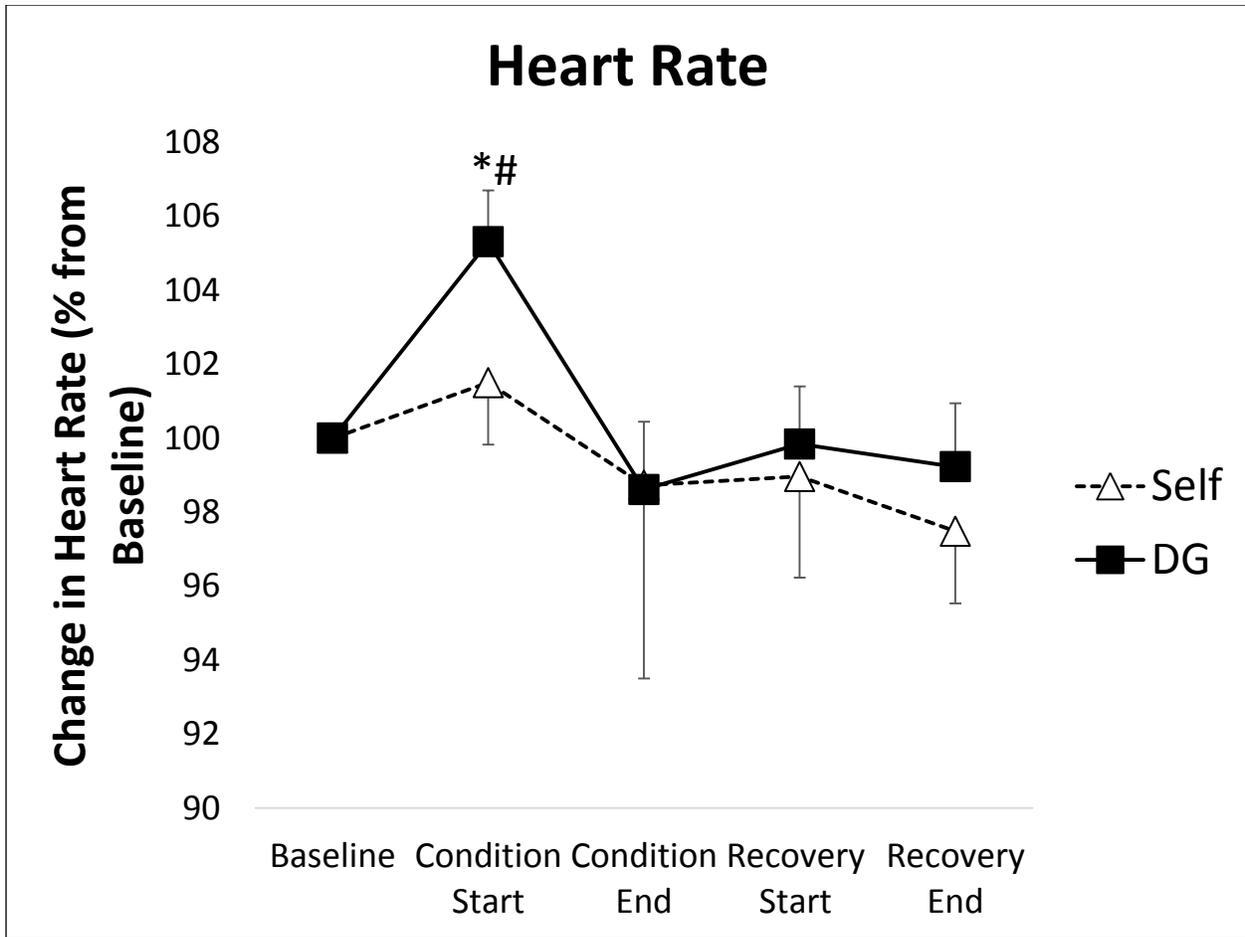


Figure 10: Heart Rate. Heart rate determined using ECG lead II. Values represented are the mean \pm SE for the entirety of the phase. (# indicates $p < 0.05$ between the two conditions at the given phase, * indicates $p < 0.05$ between the current phase and baseline for the same condition). Device-guided condition denoted with DG, self-paced condition denoted with Self.

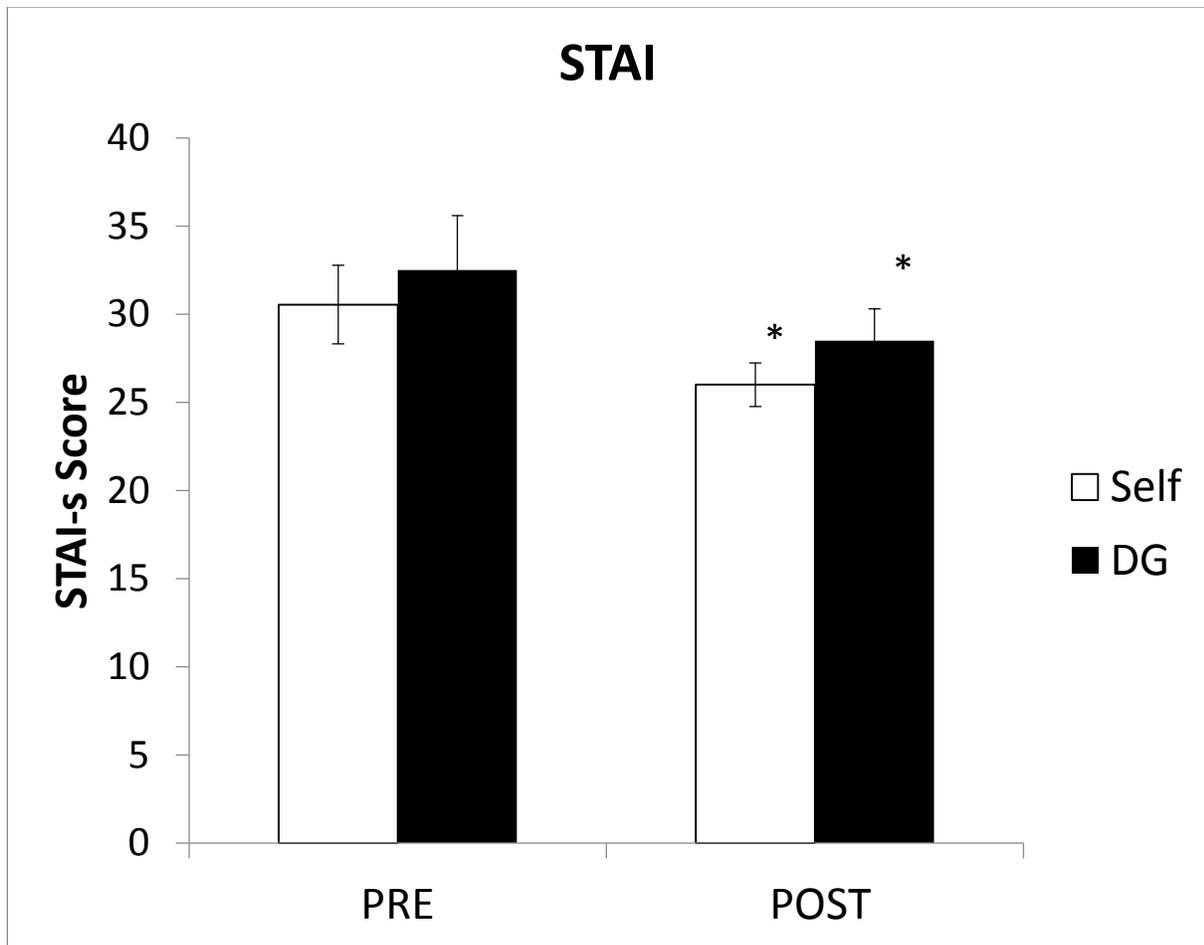


Figure 11: State Anxiety. State-Trait Anxiety Inventory – State was administered before and after the study. Values shown are the mean \pm SE. (# indicates $p < 0.05$ between the two conditions at the given time point, * indicates $p < 0.05$ between the two different time point, same condition). Device-guided condition denoted with DG, self-paced condition denoted with Self.

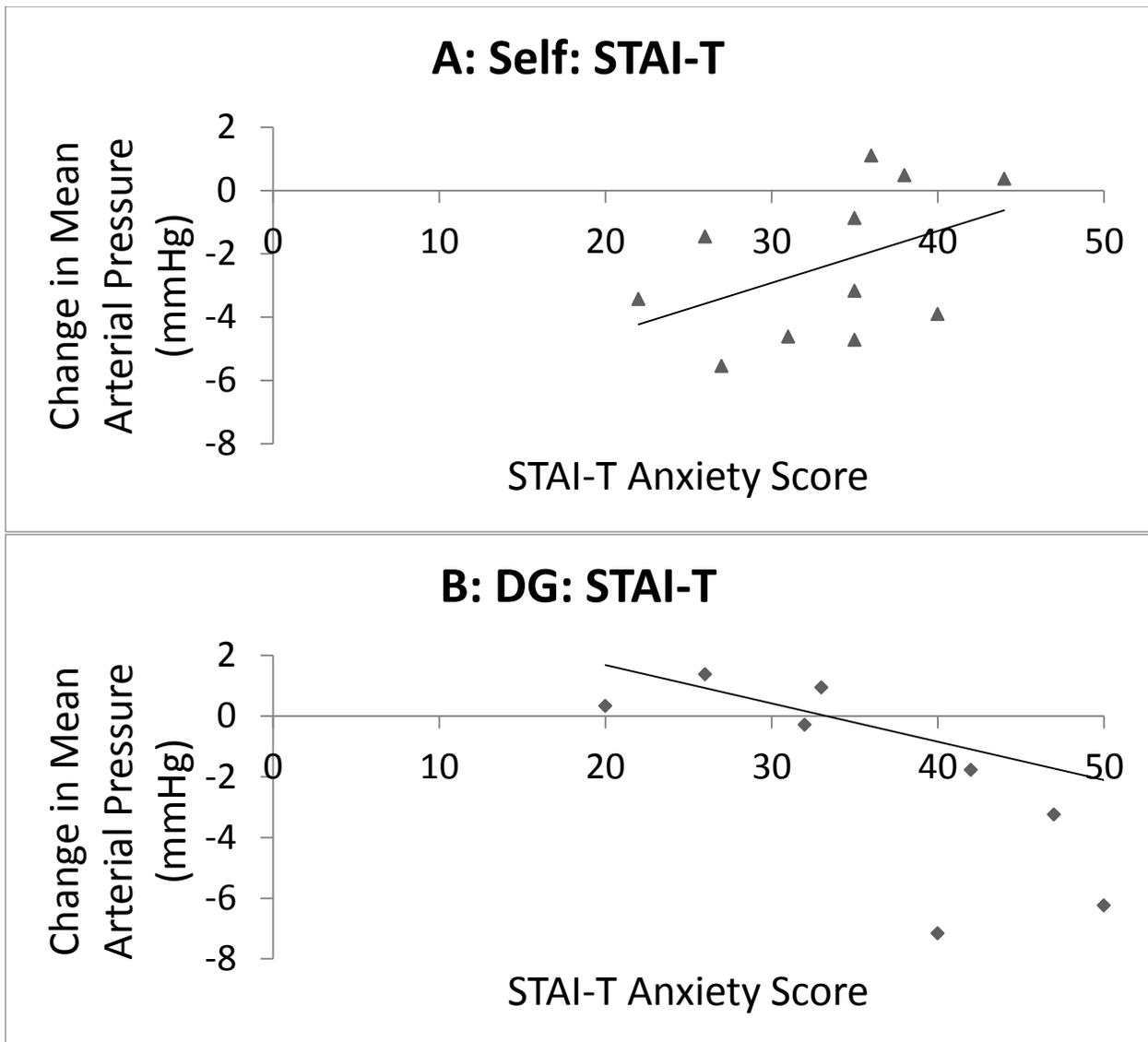


Figure 12: Trait Anxiety. STAI-T administered post-study. Pearson correlations listed below. A) STAI-T scores positively correlate with change in MAP in self-paced conditions ($r=0.452$, $p=0.081$). B) STAI-T scores weakly negatively correlate with change in MAP in device-guided conditions ($r=-0.301$, $p=0.199$). Device-guided condition denoted with DG, self-paced condition denoted with Self.

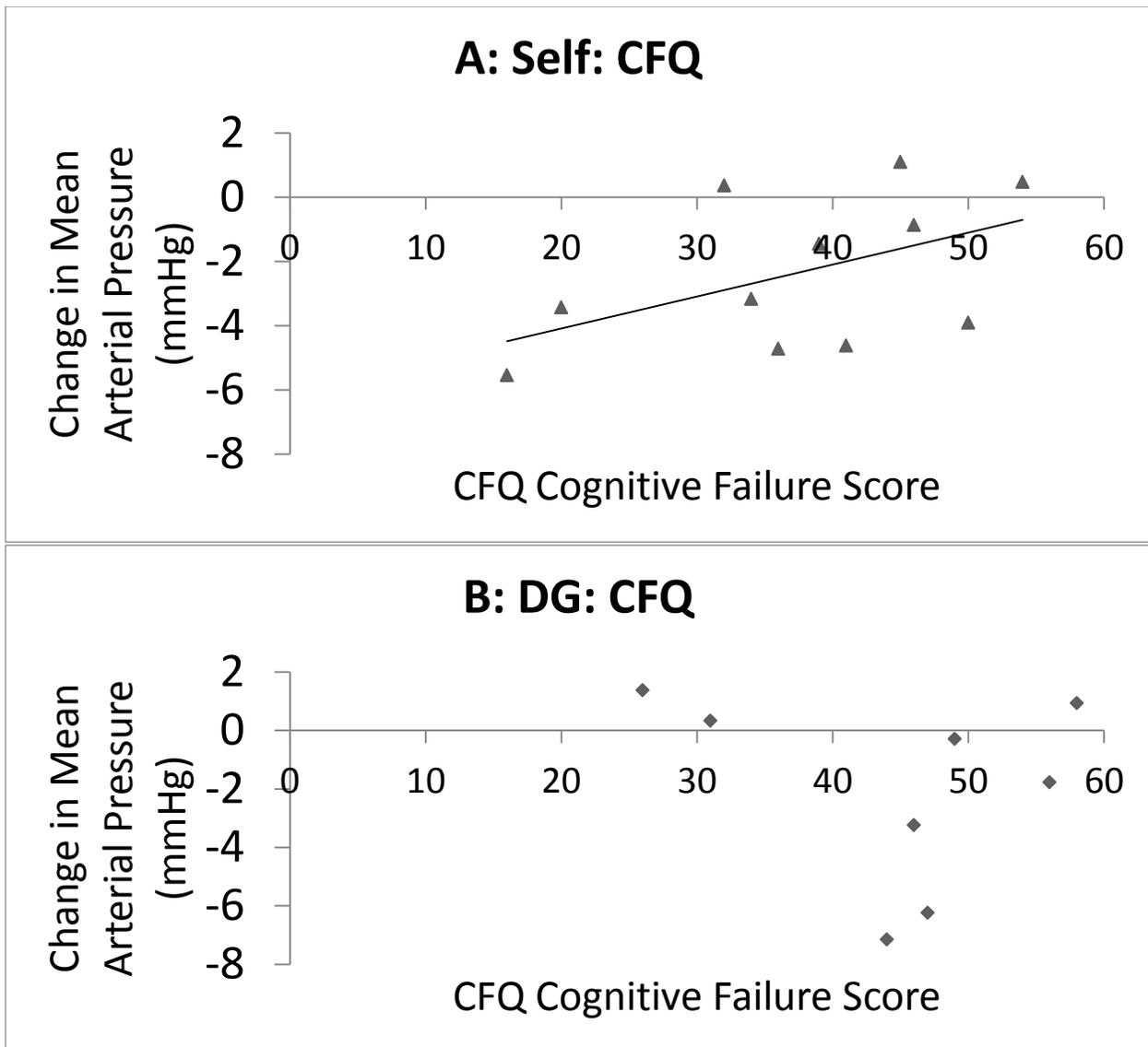


Figure 13: Cognitive Failure. Cognitive failure questionnaire (CFQ) administered post-study.

Pearson correlations listed below. A) CFQ scores positively correlated with change in MAP in self-paced conditions ($r=0.498$, $p=0.059$). B) CFQ score did not correlate with change in MAP in device-guided conditions ($r=0.092$, $p=0.400$). Device-guided condition denoted with DG, self-paced condition denoted with Self.

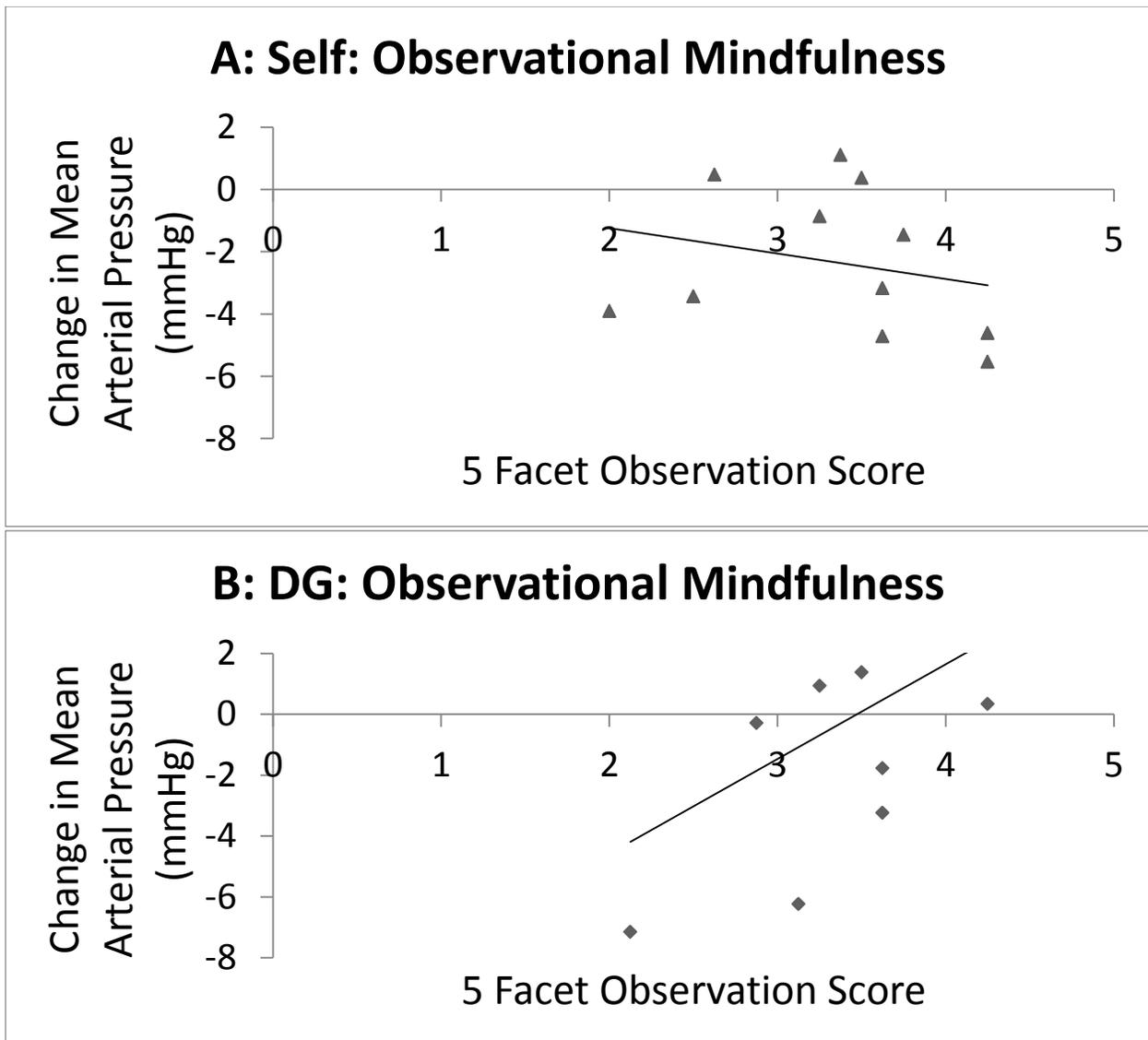


Figure 14: Observational Mindfulness. Five-facet mindfulness questionnaire (FFMQ) was administered post study. Pearson correlations listed below. A) FFMQ-observation scores weakly negatively correlated with change in MAP in self-paced conditions ($r=-0.246$, $p=0.233$). B) FFMQ-observation scores moderately positively correlated with change in MAP in device-guided conditions ($r=0.405$, $p=0.123$). Device-guided condition denoted with DG, self-paced condition denoted with Self.



Figure 15: Acting Without Awareness. Five-facet mindfulness questionnaire (FFMQ) was administered post study. Pearson correlations listed below. A) FFMQ-act without awareness scores positively correlated with a change in MAP in self-paced conditions ($r=0.683$, $p=0.010$). B) FFMQ-act without awareness scores negatively correlated with a change in MAP in device-guided conditions ($r=-0.472$, $p=0.084$). Device-guided condition denoted with DG, self-paced condition denoted with Self.

Discussion

Slow-paced breathing has been used for thousands of years and has amassed anecdotal evidence for its effects on stress reduction. Due to recent attempts to replicate self-paced breathing maneuvers with devices, and their mixed results in recent studies, we looked at the differences between self-guided and device-guided slow breathing practices and the psychological correlates. We hypothesized that slow-breathing exercises would result in a reduction in blood pressure, that self-paced slow breathing would be more effective than device-guided breathing, and that psychological metrics could explain the variability in responses to each condition. Our data supported our hypotheses. Diastolic blood pressure, systolic blood pressure and mean arterial pressure were reduced in the self-guided condition but not the device-guided condition. Trait anxiety, cognitive interference, observational mindfulness, and acting without awareness all were predictive of blood pressure responses in either device-guided or self-guided practices. In all, device-guided slow breathing and self-guided slow breathing are inherently different maneuvers with different outcomes and psychological predictors.

In both self and device-guided conditions during the slow breathing phase, breathing rate was reduced and tidal volume increased (Figure 3, 4). V_E increased during device-guided breathing, but decreased during self-paced breathing (Figure 5). The device-guided group had a lower breathing rate, higher tidal volume and higher ventilation during slow breathing than the self-paced breathing (Figures 3, 4, 5). During device-guided breathing, there is a possibility that arterial oxygen and carbon dioxide levels are altered, which could cause changes in chemoreflex function not seen in the self-guided group. This is another aspect where the two breathing practices may differ, as self-paced breathing practices allow participants to alter their blood gas levels through modulation of their breathing rate.

We attribute these differences between conditions in breathing rate, tidal volume, and ventilation to the instructions given to participants for their slow breathing phases. Device-guided participants reduced their breathing to exactly 6 breaths/min by matching to the musical tones that were played to this cadence. Subjects were breathing, either inhaling or exhaling, during the entire 5 second duration of the continuous tone played during to mark the inspiratory/expiratory phases, producing a very low standard error for breathing rate, a higher tidal volume and higher V_E . Self-paced participants varied in their slow breathing patterns based on individual preference, and were able to breathe at a comfortable depth and rate as long as it was slower than their regular breathing rate. It is arguable that the drop in breathing rate in the device-guided condition may have induced stress in some participants, preventing reductions in blood pressure. While six breaths per minute is not necessarily strenuous for most people, perhaps there is an optimal rate for each person that should be explored further, perhaps by instructing participants to first perform self-guided breathing to find a comfortable slow breathing rate, then syncing the device to this cadence.

The self-guided condition resulted in a reduction in systolic blood pressure with a persistence of this effect lasting into the recovery period, whereas device-guided breathing resulted in systolic blood pressure remaining at baseline levels throughout the entirety of the study (Figure 7), suggesting that self-paced breathing on average may be more effective in reducing blood pressure.

The reduction in diastolic and mean arterial blood pressure seen in the device-guided group at the beginning of the condition (Figures 6, 8) is likely an immediate response to the deep breathing practice that is quickly compensated for through baroreflex mechanisms over the course of condition breathing. Self-guided reductions in diastolic and mean arterial pressure do

not immediately present themselves at the onset of condition breathing, but drop during the condition breathing and persist through recovery (Figures 6, 8). This could also potentially be due to a change in baroreflex function, including a change in the operating point and/or a change in sensitivity, though this has not been confirmed and requires further investigation.

Supporting the idea of differential baroreflex influence on changes in blood pressure, there is an increase in heart rate alongside the decrease in mean arterial and diastolic blood pressure that occurs immediately after the onset of slow breathing in the device-guided group (Figure 10), which would be expected due to baroreflex functioning. In self-paced breathing, heart rate does not change instead of increasing, potentially allowing for a decrease in diastolic and mean arterial blood pressure. This is suggestive of a change in the operating point of the baroreflex, as otherwise heart rate would increase. This would need to be confirmed in future studies.

These data concerning blood pressure reductions during self-paced breathing maneuvers (Figures 6, 7, 8) support previous studies on the acute effects of yoga breathing (Raghuraj and Telles 2008, Bhavanani, Madanmohan et al. 2012), and though the current study was an acute study, its findings also support previous studies on the effects of chronic yoga breathing (Sundar, Agrawal et al. 1984, McCaffrey, Ruknui et al. 2005, Upadhyay Dhungel, Malhotra et al. 2008).

The results regarding device-guided breathing and lack of reduction in blood pressure (Figure 6, 7, 8) are difficult to compare to previous studies. This is because the present study used young, healthy men, and previous studies used older, hypertensive men (and in some cases, women). Setting this aside, our data supports previous studies that show that device-guided breathing does not reduce blood pressure (Altena, Kleefstra et al. 2009, Anderson, McNeely et al. 2010, Hering, Kucharska et al. 2013), and contradicts the studies that show a reduction in blood pressure (Grossman, Grossman et al. 2001, Schein, Gavish et al. 2001, Viskoper, Shapira et al. 2003,

Elliot, Izzo et al. 2004, Meles, Giannattasio et al. 2004, Schein, Gavish et al. 2009, Oneda, Ortega et al. 2010, Bertisch, Schomer et al. 2011).

The increase in forearm blood flow in the self-guided condition (Figure 9) indicates that there is a reduction in sympathetic outflow, resulting in a decrease in vasoconstriction in the forearm.

This is consistent with previous findings of reductions in sympathetic nerve activity in self-paced breathing practiced (Pal, Velkumary et al. 2004). There is no increase in forearm blood flow in the device-guided group (Figure 9), indicating no change in sympathetic outflow. Because forearm blood flow is determined by vasodilation and vasoconstriction, it is intimately related to sympathetic nerve activity. Thus, the lack of increase in forearm blood flow contradicts previous work that shows a decrease in MSNA activity due to device-guided breathing slow breathing (Hering, Kucharska et al. 2013, Harada, Asanoi et al. 2014).

We examined several psychological metrics in attempts to reveal predictor variables for success in reduction of blood pressure in response to these breathing practices. Both groups had a reduction in state anxiety after the study compared to before (Figure 11), despite no correlation between reduction of anxiety and reduction in blood pressure (data not shown). Trait anxiety was predictive of blood pressure reductions in both groups (Figure 12). It appears that the more anxious a person is, the worse they will respond with a self-paced practice, but they will perform better on a device-guided maneuver. We suggest that this is because highly anxious people need guidance in performing a task so that they are validated that they are doing what is being asked.

High levels of cognitive failures were predictive of poor responses in blood pressure in self-paced groups, but were not correlated with device-guided groups (Figure 13). This makes sense, as those with high levels of cognitive failures may forget to follow instructions and not adhere to the protocol, resulting in blunted results. This goes hand-in-hand with acting without awareness,

as those with higher levels of this trait are not attentive to what they are doing and thus may forget. Interestingly, those who act without awareness performed better on device-guided breathing (Figure 15), suggesting that not thinking about the protocol while doing it is best.

Higher levels of observational mindfulness, or your awareness to your body and surroundings, were prognostic of poor responses to device-guided breathing (Figure 14). We suggest that this is due to a focus shift from the body to an external device, which could be potentially invasive to a highly mindful person.

Conclusion

Our data shows that there are physiological and psychological differences between device-guided and self-paced breathing practices. This provides support to the idea that devices used to help reduce breathing rate in hopes to replicate the successes of self-paced breathing may not be appropriate in some cases. Our psychological data suggests that there are several metrics that a patient could be screened for prior to prescribing a slow-paced breathing practice in order to improve efficacy and successfully reduce blood pressure. Further studies should be done to understand the mechanisms behind the phenomenon of divergent physiological responses to these two seemingly similar maneuvers.

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