EVALUATING CARDIORESPIRATORY RESPONSES TO SLOW BREATHING IN YOUNG HEALTHY INDIVIDUALS: FITBIT "RELAX MODE" VERSUS RESPERATE.

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August, 2020

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A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science in Kinesiology and Physical Education.

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PREFACE

Abstract

Device-guided slow breathing has been used to reduce blood pressure (BP) in clinical populations. The Fitbit's "Relax Mode" (FB) and the RESPeRATE (RESP) are commercially-available devices which guide users through bouts of slow breathing (SB). However, it remains to be established which device is most effective in reducing BP. Therefore, the purpose of this study was to test the null hypothesis that there would be no difference in the SB-induced reductions in BP between the FB and RESP. We tested 7 young individuals (22±1yr; 6 women) with a normal body mass index (24±2 kg/m^2). Blood pressure was measured using finger photoplethysmography (Finometer Midi), which was then used to calculate stroke volume (SV). We observed that the relative decrease from baseline in systolic BP induced by the RESP (-1.5±2.2 mmHg) was greater than that elicited by the FB (+1.5±1.8, $P<0.05$) when both devices were used according to manufacturer-recommended conditions. Interestingly, the FB resulted in increases in SV (+3.6±2.9 ml) which were not observed with the RESP (+0.3±3.9 ml; $P<0.05$), which likely limited the BP-lowering effects of the FB relative to RESP. Likewise, when duration of exposure was matched between devices, the RESP reduced mean arterial pressure, systolic BP, and diastolic BP to a greater extent than the FB (main effect of device, $P<0.05$). These preliminary data indicate that the RESP device may be more effective than the FB device in reducing BP, supporting use of the RESP device in the management of hypertensive disorders.

Résumé

L’utilisation d’appareils guidant la respiration lente permet de réduire la pression artérielle (PA) dans les populations cliniques. Le Fitbit “Mode Relax” (FB) et le RESPeRATE (RESP) sont des appareils disponibles sur le marché qui guident les utilisateurs à travers des épisodes de respiration lente (SB). Cependant, il reste à déterminer quel appareil est le plus efficace pour réduire la PA. Par conséquent, le but de cette étude était de tester l'hypothèse nulle selon laquelle la réduction de la PA par la SB ne serait pas différente entre le FB et le RESP. Nous avons testé 7 jeunes individus (22±1 an; 6 femmes) avec un indice de masse corporelle normal (24±2 kg/m^2). La PA a été mesurée en utilisant la photopléthysmographie au niveau du doigt (Finometer Midi), et a ensuite été utilisée pour calculer le volume systolique (SV). Nous avons observé que la diminution relative de la PA systolique induite par le RESP (-1,5±2,2 mmHg) était supérieure à celle provoquée par le FB (+1,5±1,8, $P < 0,05$) lorsque les deux appareils étaient utilisés conformément aux recommandations du fabricant. De plus, le FB a entraîné une augmentation de la SV (3,6±2,9 ml) qui n'a pas été observée avec le RESP (0,3±3,9 ml; $P < 0,05$), ce qui a pû limiter les effets de réduction de la PA du FB par rapport au RESP. De même, lorsque la durée d'exposition a été appariée entre les appareils, le RESP a réduit la PA moyenne, la PA systolique et la PA diastolique de manière plus importante que le FB (effet principal de l’appareil, $P < 0,05$). Ces données préliminaires indiquent que l’appareil RESP pourrait être plus efficace que l’appareil FB pour réduire la PA, soutenant l'utilisation de l’appareil RESP dans la gestion des troubles d’hypertension.

Acknowledgments

I would like to thank my supervisor, Dr. Charlotte Usselman for instilling in me a great base and appreciation for research. From starting as an undergraduate student to completing my masters, I have gained numerous skills which I will carry with me for the rest of my life. I thank you for always supporting my path and for being a great mentor.

Thank you to my co-supervisor, Dr. Dennis Jensen who always pushes the scientific envelope and provides a unique perspective. Your willingness to always make time and provide excellent feedback was extremely appreciated.

All of the undergraduate and graduate students in the CHARLab have been instrumental in my journey by providing great support and by making the lab a great environment. Celine Chen and Fiona Howse were extremely helpful with data analysis. Seasy Huang was vital for piloting, data analysis and for continuing the slow breathing movement.

Lastly, I would like to thank my family and friends for their constant love and support. Your encouragement and positivity have helped me achieve and overcome any obstacle that stood in my way.

This project was financially supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada and a Canada Graduate Scholarship-Master's from the Canadian Institute of Health Research.

Contribution of Authors

All chapters were written by Evan D. Jette. Drs Charlotte Usselman and Dennis Jensen provided advisory feedback for all chapters. Drs. Jenna Gibbs and Celena Scheede-Bergdahl provided feedback for chapters one and two. Celine Chen and Fiona Howse aided with baroreflex sensitivity and heart rate variability analysis, respectively.

CHAPTER 1: LITERATURE REVIEW

1 Hypertension

Arterial blood pressure (BP) is defined as the force of circulating blood within large arteries (1). Adequate BP is necessary for survival as perfusion of tissues needs to be maintained both acutely and chronically (2). According to the 2017 revisions to the American Heart Association's (AHA) BP guidelines, normal BP is now defined as a systolic BP less than 120 mmHg and a diastolic BP less than 80 mmHg (3). Conversely, stage 1 hypertension is now classified as a systolic value of 130-139 mmHg or a diastolic value of 80-89 mmHg, and stage 2 hypertension is classified as a systolic value of at least 140 mmHg or diastolic value of at least 90 mmHg.

These recent adjustments to the AHA guidelines were made with the understanding that even small increases in BP are associated with future adverse cardiovascular events (4). As such, individuals with hypertension are at an increased risk for stroke, myocardial infarction, kidney failure, and retinopathy (5). This is concerning as the prevalence of hypertension in Canada, as assessed according to the 2017 AHA guidelines, is 42% (6). Aged individuals are most at risk as the prevalence of hypertension increases substantially after the age of 60 years (7). Clinically, the burden of hypertension is expected to increase considerably with the aging population that Canada faces (8).

Mechanistically, there are several reasons why individuals suffer from hypertension. Some important factors include the over-activation of the renin angiotensin aldosterone system (RAAS), which results in the chronic elevation of blood volume through an increase in sodium and water reabsorption (see (9) for review). Over-activation of the sympathetic nervous system can also lead to pronounced vasoconstriction, thereby increasing total peripheral resistance and

BP (see (10) for a full review). Finally, and particularly relevant to this thesis project, diminished baroreflex sensitivity (BRS) is a strong contributor to hypertension (11). Essentially, the baroreflex cannot buffer changes in BP adequately in hypertensive individuals with reduced BRS.

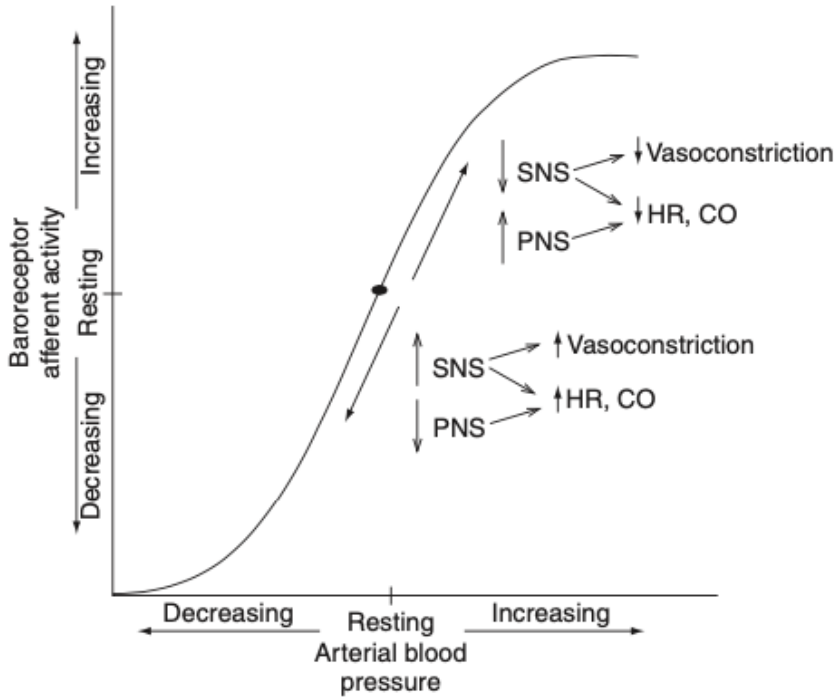
Therefore, the baroreflex is an important mechanism to target considering that traditional antihypertensive pharmacotherapies (i.e. beta blockers, ACE inhibitors) primarily target the RAAS (9). Therefore, slow breathing (SB; see *section 3*) may be an adjunctive non pharmacological therapy which may help reduce BP in part through the activation of the baroreflex (12).

2 Acute Regulation of Blood Pressure: Baroreflex

Overall, the baroreflex is a critical mechanism for the regulation of BP on an acute basis (13). The baroreflex functions to maintain BP within a certain range which can be defined as the normal "set point". Therefore, if BP values are too high the baroreflex will work to lower them and *vice versa* if BP values are too low.

Changes in BP are sensed by arterial baroreceptors (mechanical stretch receptors) located in the arteries of the neck (carotid sinus) and the heart (aortic arch) (14, 15). When BP increases beyond a certain threshold, baroreceptors sense an increase in mechanical stretch which then triggers an increase in afferent neural activity to the brainstem (specifically, the nucleus of the solitary tract; NTS) via the vagus and glossopharyngeal nerves (16, 17). This information is integrated within the NTS, and results in alterations to the level of efferent parasympathetic ("rest and digest") and sympathetic ("fight or flight") neural activity. Consequently, a decrease in sympathetic activity reduces heart rate (HR), and total peripheral resistance via a reduction in vasoconstriction of the peripheral vasculature (16, 18). An increase in parasympathetic activity

reduces (HR) via increased acetylcholine release at the sinoatrial and atrioventricular nodes (16, 18). Combined, these effector mechanisms result in a reduction in BP towards the normal set point. Conversely, when BP values are too low, baroreceptor activity is diminished which results in an increase in sympathetic activity and a decrease in parasympathetic activity, which in turn increases vasoconstriction, HR, and BP as shown in Figure 1.1 (16, 18). As such, the baroreflex is a negative (inhibitory) feedback loop, which can monitor and maintain appropriate BP values when functioning optimally.



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157 **Figure 1.1. Relationship between arterial blood pressure, baroreceptor afferent activity,**

158 **and cardiovascular autonomic outflow.** When blood pressure is below a certain set point,

159 baroreceptor afferent activity decreases which results in an increase in sympathetic activity and a

160 decrease in parasympathetic activity thereby increasing vasoconstriction, heart rate and cardiac

161 output. When blood pressure is above a certain set point, baroreceptor afferent activity increases

162 resulting in a decrease in sympathetic activity and an increase in parasympathetic activity

163 thereby decreasing vasoconstriction, heart rate and cardiac output. The set point is indicated by

164 the black circle on the sigmoidal curve. SNS, sympathetic nervous system; PNS,

165 parasympathetic nervous system; HR, heart rate; CO, cardiac output. Figure from Sved et al.

166 2009 (16).

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2.1 Baroreflex Sensitivity

Baroreflex sensitivity (BRS) is a marker of how well the baroreflex buffers acute changes in BP (19). Specifically, cardiovagal BRS reflects the ability of the baroreflex to modulate HR in response to changes in systolic BP, which is largely the result of parasympathetic effector mechanisms (20). Baroreflex sensitivity can also be quantified in terms of peripheral vascular responsiveness, which describes the magnitude of change in muscle sympathetic outflow for a given change in diastolic BP. This branch of the baroreflex is summarized elsewhere (see (21) for a review). In this project (and section) we will focus exclusively on cardiovagal BRS.

As alluded to in *section 1*, individuals with hypertension operate with (a) reduced cardiovagal BRS, and (b) they do so at a higher set point (Figure 1.2) which further contributes to their elevated BP (11, 13, 22). Reduced BRS can be attributed to lower vascular compliance in individuals with hypertension (11). Although the exact mechanism which reduces vascular compliance in people with hypertension remains unknown, it is likely that factors such as the composition of the arterial wall (type of collagen fibers) and reduced endothelium derived compounds (nitric oxide) play a role (23). Regardless, reduced baroreceptor stretch per unit rise in BP results in less BRS activation, therefore resulting in a smaller afferent response, and accordingly a weakened efferent response. In terms of alterations to the baroreflex set point in people with hypertension, the range of BP values that can be defined as normal may be altered. The set point can change depending on the task at hand or in certain pathologies. For example, during exercise a higher BP is needed to ensure adequate oxygen delivery to the working muscles (24). Therefore, the baroreflex set point is reset to a higher level which is now considered the new normal.

Baroreflex sensitivity can be quantified through the use of various techniques. Intrusive methods such as the use of vasoactive drugs (i.e. the modified Oxford technique) and the neck chamber technique are available to conduct comprehensive analyses of BRS under a wide range of BPs (see (25) for a full review). Briefly, in the modified Oxford technique the injection of phenylephrine (a vasoactive drug) causes an increase in BP without directly affecting HR (25, 26). This is followed by an injection of sodium nitroprusside (a vasodilator drug) which lowers BP without directly affecting HR (26). As such, BRS is assessed by measuring the change in the HR response to changes in BP induced by the injection of phenylephrine and sodium nitroprusside. Conversely, the neck chamber technique allows for selective activation or deactivation of carotid baroreceptors by altering external pressure to the neck region (25). For example, a positive pressure applied by the neck chamber is sensed by baroreceptors, which detect transmural pressure, as a decrease in BP and thus elicits a reflex response to increase BP and HR. While these techniques are useful and provide detailed information regarding baroreflex function, non-intrusive techniques such as the sequence method have been developed to examine the function of the baroreflex under normal physiologic conditions (25, 27).

The sequence technique makes use of HR and BP data to determine cardiovagal BRS. The standard electrical firing pattern of the heart is measured by a 3- or 5- lead electrocardiogram (ECG). The duration of a given heartbeat is quantified based on the distance between the “R” peaks of the “QRS” complex of an ECG waveform. Therefore, the quantitative measurement of BRS via the sequence method is dependent on the number of heartbeats in which increases or decreases in R-R interval and systolic BP occur at the same time (27). Thus, BRS can be obtained from the slope of the fitted line representing the relationship between the change in R-R interval (milliseconds) and the change in systolic BP (mmHg). All computed

slopes are averaged to obtain the BRS. As such, gain in BRS indicates a greater capacity of the baroreflex to modulate changes in HR in response to a change in systolic BP. Therefore, a steeper slope is indicative of increased BRS. Conversely, individuals with low BRS (< 3 ms/mmHg) post-myocardial infarction (28) or with heart failure (29) are at an increased risk for cardiac mortality. As such, BRS can be an important prognostic tool for cardiovascular health in certain clinical populations.

Advantages for the use of the sequence method include automation and standardization of identifying sequences which reduces measurement variability (25). Additionally, it allows the distinct measurement of up (concurrent increases in systolic BP and R-R interval) and down (concurrent decreases in systolic BP and R-R interval) sequences (25). The up sequences reflect mainly vagal activation whereas down sequences reflect vagal inhibition (30). Therefore, the delineation of up *versus* down sequences can reveal whether baroreceptors demonstrate specificity in response to increases or decreases in BP under a variety of clinical and physiological conditions. Slow breathing (SB) may be an effective means of increasing BRS and thus reducing BP (12). Interestingly, previous SB studies have found only increases in the up sequences (31-33).

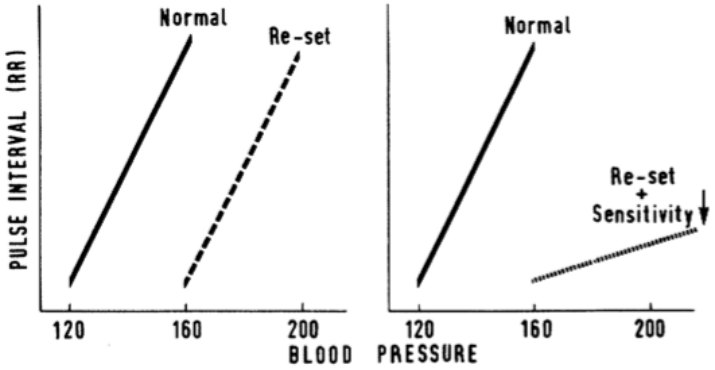

Figure 1.2. Alteration of baroreflex function. (Left) Resetting of the baroreflex to a higher set point without a change in sensitivity as seen during exercise. The slope of the relationship between blood pressure and pulse interval is not changed. (Right) In addition to resetting of the baroreflex to a higher set point, there is a decrease in sensitivity as commonly seen in individuals with hypertension. Adapted from Bristow et al. 1969 (11).

3 Slow Breathing

Slow breathing has been defined as a respiration rate between 4-10 breaths per minute (34). At these breathing frequencies, a number of benefits have been documented including: increased respiratory efficiency, a shift from sympathetic to parasympathetic dominance, and an augmentation in BRS (12, 34-36). As a result of those changes, increased heart rate variability (HRV; see *section 4.1* for further details) and reduced BP are evident (12, 34).

In fact, SB is an effective method of reducing BP in people with hypertension (37), type 2 diabetes (38), post-traumatic stress disorder (39), and even healthy individuals with normal BP (31). Chronic implementation of SB has been successful in reducing systolic BP and diastolic BP in non-diabetic and diabetic hypertensives by 3.7 and 2.5 mmHg, respectively, as per a meta-analysis (40). These changes exceed the generally accepted threshold for a clinically meaningful reduction in BP of 2 mmHg (41, 42). However, a more recent meta-analysis with stricter inclusion criteria (i.e. excluding any study without an randomized control) found no clear evidence of BP reductions following chronic SB in non-diabetic and diabetic hypertensives (43). As such, further research on the long-term use of SB is warranted.

Acute bouts of SB have demonstrated significant and immediate reductions in systolic BP. Both hypertensive and normotensive individuals who performed 30 seconds of SB have shown reductions in systolic BP of 3.9 and 3.4 mmHg, respectively (44). Additionally, we recently demonstrated a mean reduction in systolic BP of 3 mmHg in both normotensive men and women during 15 minutes of SB (31).

There are numerous ways to perform SB. Pranayama yoga breathing is a form of SB which has been practiced for thousands of years (45). This method involves conscious inhalation, retention, and exhalation of a given breath. However, one of the major drawbacks is that it may

be difficult for naïve participants to become accustomed to. Without a coach, an individual may breathe both at an inappropriate respiration rate and tidal volume. As such, individuals may become hypocapnic or hypercapnic, both of which can result in dizziness and breathlessness (46). Additionally, newer techniques such as HRV-biofeedback have been developed. This technique requires an extensive protocol done in a laboratory setting to assess and ensure a specific breathing frequency (see (47) for the full methodology). Essentially, software and equipment are needed to ensure that HR and respiration rate are in phase with each other. As such, clinical utility of this technique is limited because the protocol and equipment are not readily available for consumer use. Therefore, device-guided SB has emerged as a suitable alternative in which participants are coached through acute bouts of SB via a technological device. This ensures that participants are lowering their breathing frequency to a therapeutic level and in an appropriate manner. Currently, there are numerous devices on the market, including SB smartphone applications, wearable technology, and other devices (see *section 6* for more details).

4 Respiratory Sinus Arrythmia

A major component of the pathway which governs the beneficial effects of SB involves respiratory sinus arrythmia (RSA). Specifically, RSA is HRV in synchrony with respiration, such that HR increases with inspiration and decreases with expiration (48). The exact phase relationship between HR and respiration is dependent on the specific breathing frequency (49). However, to fully understand RSA, one must be familiar with HRV.

4.1 Heart Rate Variability

Heart rate variability is defined as the variation over time between R-R intervals. It has been suggested that HRV reflects the heart's ability to adapt to certain challenges such as

exercise or stressors (50, 51), with reduced HRV associated with increased risk of cardiac mortality (52) especially in individuals who suffered a myocardial infarction (53). In these individuals, it has been hypothesized that decreased HRV correlates with increased sympathetic tone, which may predispose an individual to ventricular fibrillation (53). Additionally, reduced HRV has been linked with autonomic neuropathy in diabetic patients (54). In fact, reduced HRV has been used to detect autonomic neuropathy prior to the onset of symptoms such as postural hypotension, gastric fullness, and hypoglycemic unawareness (55, 56). Therefore, HRV can be seen as an important prognostic and diagnostic tool in certain diseased populations.

The quantification of HRV is a surrogate measure to assess overall cardiac health. Specifically, it quantifies the activity of the autonomic nervous system responsible for regulating cardiac activity (50). An ECG is an accurate and precise method to record HR patterns. This is because the shape and timing of the waveform is easily detected by software algorithms capable of discriminating normal from ectopic (abnormal) beats (57). Afterwards, power spectrum analysis and time domain analysis can be completed (see (58) for a full review on the methodology).

Power spectrum analysis (or frequency domain analysis) estimates the distribution of absolute or relative power into distinct frequency bands. Power is defined as the signal energy found within a frequency band. Of importance are the low frequency (LF) and high frequency (HF) bands. The LF band, previously called the “baroreceptor range”, reflects baroreceptor activity at rest. The LF band ranges from 0.04-0.15 Hz and represents a combination of parasympathetic and sympathetic activity. However, at respiration rates less than 8.5 breaths per minute, the LF band is largely vagally- (i.e. parasympathetically-) mediated (51, 59). Conversely, the HF band ranges from 0.15-0.40 Hz and is called the respiratory band as it corresponds with

variations in HR related to the respiratory cycle during quiet breathing (51, 59). The HF band exclusively reflects parasympathetic activity.

Time domain analysis includes measurements of normal-to-normal (NN) intervals, defined as R-R intervals with ectopic beats excluded. These measurements are easy to calculate and are highly reproducible if the recording periods are of the same length (51). Of major interest is the standard deviation of NN intervals (SDNN). This measurement is highly correlated with LF power spectrum analysis. It is also parasympathetically-mediated via RSA, especially during SB protocols (51, 59). Additionally, the root mean square of the successive differences between adjacent R-R intervals (RMSSD) reflects vagally-mediated changes in HRV. It is correlated with the HF band and is less affected by changes in respiration rate (60).

4.2 Origins and Mechanisms of Respiratory Sinus Arrythmia

The mechanisms that generate RSA are still unclear; however, it is believed that both central and peripheral factors play a role (34, 61). The central pathway involves both the NTS and the nucleus ambiguus, which generate cardiorespiratory rhythms via a neural "pacemaker". The most well-established theory in relation to the "pacemaker" is termed "respiratory gating". Closing of the gate occurs during inspiration, while opening of the gate occurs with expiration. As such, cardiac vagal preganglionic neurons are hyperpolarized during inspiration due to acetylcholine post-synaptic inhibition (62). Therefore, HR increases during inspiration and decreases during expiration.

Additionally, pulmonary stretch receptors play a vital role in the generation of RSA (34). These mechanoreceptors located in the lungs send autonomic afferent information to the nucleus ambiguus (63, 64). During inspiration, pulmonary stretch receptor activity is enhanced, which then decreases cardiac vagal activity thereby increasing HR (65). However, the degree of cardiac

vagal withdrawal is dependent on the level of lung stretch as influenced by the size (volume) of the breath. Interestingly, double-lung transplant patients with intact hearts but vagal denervation demonstrate a 53% reduction in RSA in comparison to control subjects (63). This confirms the obligatory role of vagal feedback from the pulmonary stretch receptors in the genesis of RSA.

Other factors such as baroreceptor activity may play a minor role in RSA amplitude. Experiments which stimulate baroreceptors via neck suction in humans have demonstrated maximum vagal excitation during expiration but minimal activity during inspiration (66). This suggests that the baroreflex may decrease HR during expiration further contributing to RSA. However, the involvement of the baroreceptors in relation to the genesis of RSA is still up for debate (67).

Lastly, mechanical factors driven by respiration such as changes in intrathoracic pressure, venous return, stroke volume and cardiac output may also affect RSA. Specifically, these changes may stretch the sinoatrial node thereby increasing HR during inspiration (68). As such, RSA is diminished but present in heart transplant patients indicating a necessary role for non-neural factors (69). Therefore, it is evident that the genesis and factors involved with RSA are multifactorial and complex.

5 Effects of Slow Breathing on Blood Pressure

During SB, a couple of different mechanisms and pathways occur which result in changes in BP. Firstly, SB is accompanied by a reduction in respiration rate, which is offset by an increase in tidal volume to maintain normal minute ventilation (37). This leads to an increase in cardiopulmonary stretch receptor activation, which reduces sympathetic activity (70). Although it is difficult to measure in humans, anesthetized and artificially ventilated cats have demonstrated reduced sympathetic tone in response to lung inflation or electrical stimulation of

the vagus nerve (71). This suggests that inflation of the lungs during SB can induce vasodilation of vascular beds, therefore reducing total peripheral resistance and BP provided there are no changes in cardiac output.

Acetylcholine release and hydrolysis at the cardiac level are optimised at a respiration rate of six breaths per minute, thereby maximizing RSA (72). Importantly, RSA results in periodic oscillations in BP which entrain the baroreflex. An increase in BP is observed during expiration, while BP decreases during inspiration (73). Additionally, spontaneous fluctuations in BP titled “Mayer waves” oscillate at 0.1 Hz (equivalent to 6 breaths per minute) and occur due to the baroreflex (74). Therefore, during SB these “Mayer waves” synchronize with BP oscillations that arise due to RSA (70). This results in an increase in BRS (34). Thus, SB improves the capacity of the baroreflex to buffer changes in BP.

Combined, both the cardiopulmonary stretch receptors and the baroreflex play a role in reducing BP during SB as shown in Figure 1.3 (12). Given that cardiopulmonary stretch receptor activity is not feasible to quantify in humans, this paper will focus on direct measurement of the baroreflex.

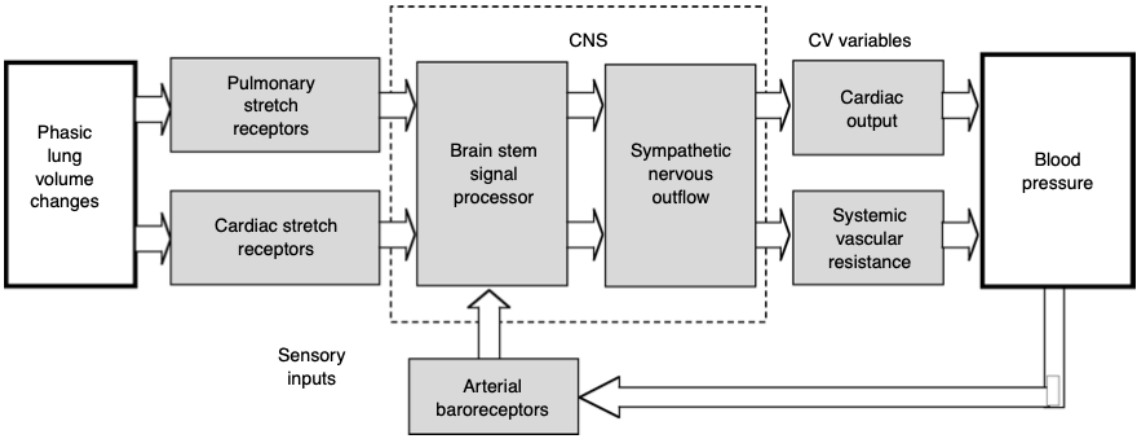

Figure 1.3. Schematic illustrating the modulating effects of phase lung volume changes on blood pressure, with focus on reflex mechanisms. CNS, central nervous system; CV cardiovascular. Diagram from Izzo et al. 2008 (70).

6 Slow Breathing Devices

Respiratory sinus arrhythmia is an important aspect of SB and the two variables of interest which can be used to quantify RSA are respiration rate and HRV. During SB, respiration rate decreases and HRV increases resulting in an increase in RSA (34). However, different SB devices target separate aspects of RSA.

The RESPeRATE device (Intercure Ltd., Israel) is approved by the Food and Drug Administration (FDA) and recommended by the AHA as an adjunct treatment to reduce BP for people with hypertension (75). This device analyzes respiration rates via an elastic strap and a breathing sensor placed around the chest or abdomen depending on individual breathing preference (Figure 1.4). It then guides the user to reduce their respiration rate to 4-6 breaths per minute over the course of a SB session (typically 15 minutes) through the use of musical tones. This device has been used extensively in other research studies; however, the BP lowering effects have been mixed. Specifically, performing SB for 10-15 minutes daily over an 8-week period has resulted in 8-15 mmHg and 4-10 mmHg reductions in both systolic and diastolic BP, respectively, in individuals with hypertension (76-78). However, not all studies have found prolonged BP reductions (79-81).

In contrast to the RESPeRATE device, the Fitbit Charge 2 has a feature called “Relax Mode” (Fitbit Inc., San Francisco, CA), which does not monitor respiration rate. Instead, it monitors HRV and delivers instructions for SB in the form of visual cues on the screen and vibrations of the device (Figure 1.5). This device has not been widely studied and further research is warranted regarding the effectiveness of this stimulus in reducing BP.

Given these key differences in the way that the RESPeRATE and Fitbit monitor physiological outcomes and deliver SB instructions, it is likely that users of these devices will

achieve different respiration rates using each device, which has the potential to affect RSA and thus the BP-lowering outcomes of each device.

Therefore, the **purpose** of this MSc thesis research was to compare the BP-lowering effects of two SB devices: RESPeRATE and Fitbit. To conduct a comprehensive analysis of their efficacies, we evaluated both devices in healthy normotensive adults in a supine position at rest under manufacturer-recommended conditions, with matched duration of exposure, and also during a period of acute hypertension induced via the cold pressure test (CPT), an established sympatho-excitatory stressor. We **hypothesized** that both devices would be equally effective at reducing BP under all experimental conditions.

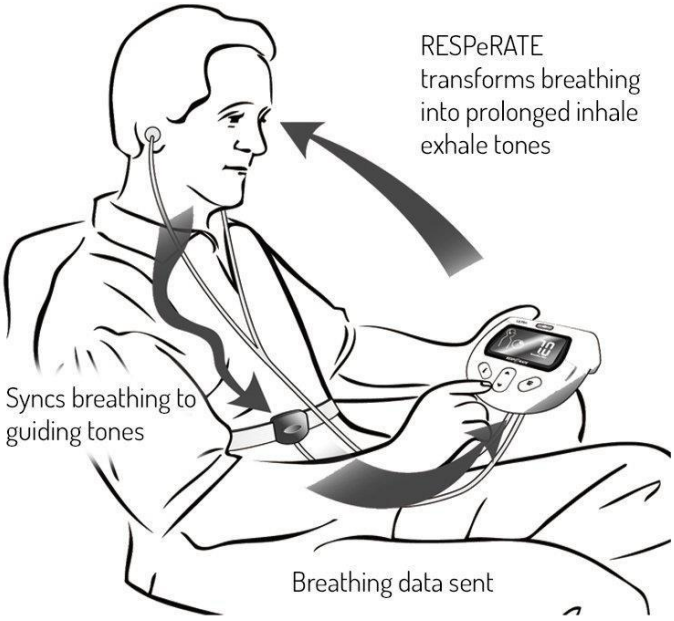

Figure 1.4. Diagram of an individual using the RESPeRATE device to perform slow breathing. Image from Intercure Ltd., Israel.

Figure 1.5. Fitbit Charge 2 demonstrating the visual cue on screen during an exhalation.

Image from Fitbit Inc., San Francisco, CA.

CHAPTER 2: MANUSCRIPT

7 Introduction

In Canada, 42% of adults present with hypertension as defined according to the 2017 American Heart Association guidelines (6). This is important clinically as even moderate elevations in blood pressure (BP) can contribute to the development of future cardiovascular events (5). Therefore, the effective management of BP is a significant public health priority.

Typically, hypertension is addressed with pharmacological therapies; however, they are not always effective in resolving hypertension. For example, suboptimal adherence to anti-hypertensive medication occurs in 50% of patients within one year of beginning treatment (82). Moreover, it is estimated that 12-18% of hypertensive individuals suffer from drug-resistant hypertension (83). However, when effective and used consistently, anti-hypertensive medication typically reduces systolic BP (SBP) by 9 mmHg, which exceeds the generally accepted threshold for a clinically meaningful reduction in BP of 2 mmHg (41, 42). Still, in some individuals this may not be enough to resolve hypertension completely, particularly in individuals with a SBP of 140 mmHg or greater (84). Given that 31% of the global adult population are classified as having a SBP of at least 140 mmHg or a diastolic BP (DBP) of at least 90 mmHg (85), it is clear that non-pharmacological adjunct treatments are urgently needed to improve the management of hypertension.

A simple technique that has shown promise to effectively lower BP in hypertensive individuals over several weeks is slow breathing (SB) (75-78, 86). Typically, SB is defined as a technique which prolongs the respiration period such that breathing frequency is reduced from a normal rate of 15-20 breaths/min to 4-10 breaths/min (34). Acute bouts of SB have been shown to reduce SBP in untreated hypertensive individuals by up to 8 mmHg (37), while performing a

daily 15-min bout of SB for 8-weeks has been shown to lead to sustained reductions in SBP of 5 mmHg in treated and untreated hypertensive individuals (76). Finally, we have demonstrated that SB is equally effective in lowering SBP by 3 mmHg in young healthy men and women (31).

Due to the promising nature of SB as a non-pharmacological adjunct anti-hypertensive therapy, devices which aim to guide a user through bouts of SB have been made commercially available. The FDA-approved RESPeRATE (RESP) device, which is recommended by the American Heart Association as an adjunct treatment to lower BP in hypertensive individuals (75), analyzes a user's breathing frequency and uses musical tones to gradually reduce respiration rate and then maintain a steady state of 4-6 breaths/min, typically for a period of 15 minutes. In contrast, some models of the Fitbit (FB) include a function titled "Relax Mode" which analyzes a user's heart rate variability (HRV) to help generate a customized breathing pattern. The FB device then guides the user into a SB pattern through the use of visual and vibrational cues. However, information from the manufacturer is limited, such that the extent and utility of HRV in generating a customized breathing pattern remains unknown. Additionally, it has been previously demonstrated that a customized breathing pattern based upon HRV metrics may not be necessary in order to reduce BP (87). Other relevant differences between the RESP and FB devices include the use of different physiological cues to generate a custom SB pattern for each user (i.e. RESP: respiration rate; FB: HRV). Respiration rate and HRV are the principal components of respiratory sinus arrhythmia (48), which is hypothesized to play a primary role in the SB-induced reductions in BP (34, 46). In this phenomenon, HRV is synchronized with respiration whereby the R-R interval of the electrocardiogram signal is shortened during inspiration and prolonged during expiration (48). This physiological synchrony acts to augment baroreflex sensitivity (BRS), thereby improving the capacity of the baroreflex to buffer changes

in BP (34), and lowering BP both during an acute bout (31, 37, 87) and chronically, when performed repeatedly over several weeks (75-78, 86). Thus, the targeting of different yet equally critical components of respiratory sinus arrhythmia in the RESP and FB devices (i.e. respiration rate *versus* HRV, respectively) may result in differences in the efficacy of these devices in lowering BP. However, to the best of our knowledge, there have been no direct comparisons of the effectiveness of these devices in lowering BP.

As such, the purpose of this study was to compare the acute BP-lowering effects of the RESP and FB devices. To conduct a comprehensive analysis of their efficacies, we evaluated both devices under three different conditions: i) when used as intended by the manufacturer, ii) when matched in duration of exposure, and iii) during an acute sympatho-excitatory stress, the cold pressor test (CPT), in order to simulate a hypertensive state. In all comparisons we tested the null hypothesis, such that there would be no difference in the magnitude of fall in BP from baseline elicited by the two devices.

8 Methods

Participants: We recruited 7 healthy individuals (1 man and 6 women) who were young (mean±SD; 22±1 yr), healthy and non-obese (body mass index 24±2 kg/m^2). Women were eumenorrheic (cycle length: 22-30 days; n=4) or regular users of hormonal contraceptives (n=1 drospirenone and ethinyl estradiol; n=1 levonorgestrel and ethinyl estradiol). None of the participants were smokers, pregnant, and/or reported any endocrinopathy, neurological, respiratory, or cardiovascular diseases, as assessed by a Health History Questionnaire. All participants took part in the study after providing written, informed consent. This study conformed to the guidelines in the Declaration of Helsinki and was approved by the Faculty of Medicine Institutional Review Board at McGill University (IRB Study Number A00-M12-20A).

Experimental Design: Prior to testing, participants attended the lab for a familiarization session, during which they experienced all instrumentation and protocols, including being trained to perform SB using the RESP device (RESPeRATE, Intercure, Israel) and the FB (Fitbit Charge 2 "Relax Mode"; Fitbit Inc., San Francisco, CA). To determine percent body fat, participants entered an air displacement plethysmograph where body fat percentage was assessed in duplicate (BOD POD, Life Measurement Instruments, Concord, CA).

On testing days, participants arrived at the laboratory at 8:00am following an overnight fast and having abstained from caffeine, strenuous exercise, and alcohol for at least 12 hours. Women were tested during the early follicular phase of the menstrual cycle (i.e. days 1-7) or during the placebo phase of oral contraceptive use. Testing took place in a dimly lit room at an ambient air temperature of 22–25°C. Upon arrival, participants were asked to void their bladders. Participants were positioned supine on a padded table. Following instrumentation, and a 10-min period of quiet rest which allowed for the stabilization of BP values (88), resting blood pressure was assessed at the brachial artery just proximal to the antecubital fossa via manual sphygmomanometry by a single trained researcher (3 values separated by 2-mins each). Afterwards, we collected 10-min of "true baseline" data (i.e. the resting state prior to any perturbations). Participants then executed the following 3 protocols in randomized order (see Figure 2.1). Each SB protocol consisted of 15-min of device-guided SB, with a CPT in the final 3-min (i.e. 12-min of SB alone, followed by 3-min of SB+CPT). The default setting of the RESP device includes 15-min of SB, thus the participants simply followed the device-recommended patterns during exposure to RESP. However, the FB device guides the user through up to 5-min of SB. Therefore, in order to match the duration of exposure to the RESP, FB-guided SB was repeated 3 times in a row (i.e. 3 x 5-min = 15-min). Given that the FB requires a calibration

period between each subsequent repetition, a coaching period was implemented in order to ensure that the participants continued to practice SB wherein the researchers assessed the participant's SB respiration rate and then coached them to continue that respiratory pattern until the FB's calibration period had been completed. Finally, participants also completed a CPT under normal conditions (i.e. without SB). The CPT is a commonly used sympatho-excitatory stimulus which can elevate SBP levels acutely by 16 mmHg in normotensive individuals (89). It involves a participant submerging their hand, above their wrist, in ice cold water (~4°C) from one to seven minutes (90, 91). Thus, in a randomized order, each participant performed i) CPT alone, ii) FB then FB+CPT, and iii) RESP then RESP+CPT. Each protocol was preceded by a 3-min baseline period and followed by a recovery period of at least 3-min, or until BP and hand skin temperature returned to baseline values, usually approximately 10-min (Figure 2.1).

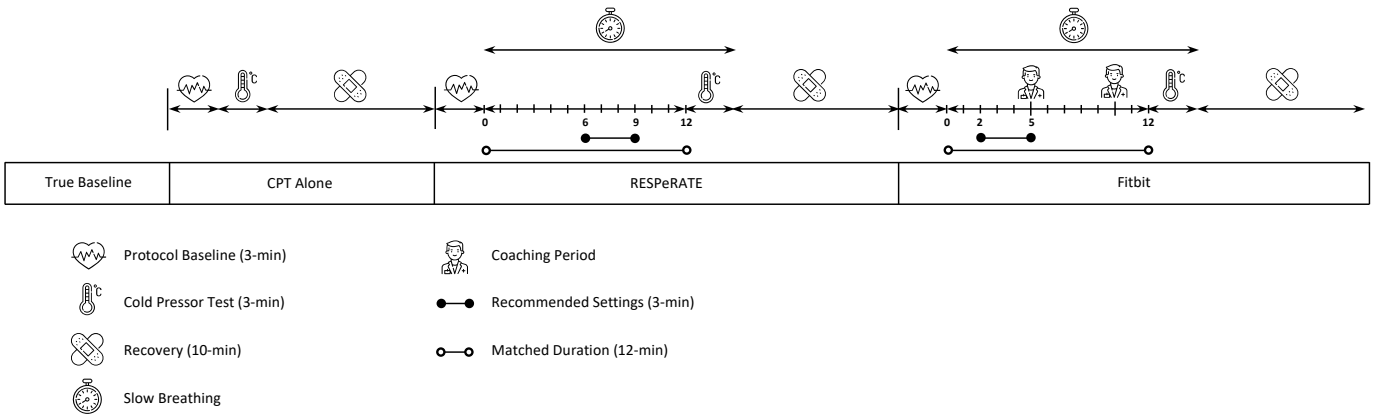

Figure 2.1. Example of Experimental Protocol Timeline. True baseline was a 10-min recording period before any of the protocols started. The order of cold pressor test (CPT) Alone, RESPeRATE and Fitbit protocols were randomized for each participant. All protocols started with a 3-min baseline protocol and ended with a recovery period which lasted around 10-mins in duration. The CPT Alone protocol involved the participant performing the CPT for 3-min. The RESPeRATE and Fitbit protocol required the participant to perform 15-min of slow breathing, i.e. 12-min alone and 3-min with a CPT. However, the Fitbit required coaching periods at minutes 5 and 10 which occurred during device calibration. For data analyses, data to assess responses to the recommended settings were selected as minutes 6-9 of the RESPeRATE and minutes 2-5 of the Fitbit as these time points allowed each device to reach a steady state in terms of respiration rate while conforming to the manufacturers intended use (closed circles), and to assess responses with matched duration minutes 0-12 were extracted for both the RESPeRATE and the Fitbit (open circles).

Instrumentation: Participants were instrumented for beat-to-beat BP (finger photoplethysmography; Finometer Midi, Finapres, Amsterdam, The Netherlands), heart rate (HR; 5-lead ECG, 1000 Hz sampling rate), and respiration rate (respiratory belt transducer, ADInstruments, Dunedin, New Zealand). All BP values were calibrated to the mean of three resting values (manual sphygmomanometry). Hand temperature was verified to return to baseline levels via a surface skin temperature probe (ADInstruments, Dunedin, New Zealand).

Data analysis: Systolic BP, DBP, and mean arterial BP (MAP) were obtained from the beat-to-beat BP signal. Pulse Pressure (PP) was calculated as SBP minus DBP. Cardiac output (Q; $L \cdot min^{-1}$) was calculated beat-to-beat from the finger BP signal offline using the Non-Invasive Cardiac Output algorithm (three-element Windkessel model; ADInstruments). Total peripheral resistance (TPR; $mmHg \cdot L^{-1} \cdot min^{-1}$) was calculated as MAP/Q. Stroke volume (SV; ml) was calculated as Q/HR*1000.

Spontaneous cardiovagal baroreflex sensitivity (BRS) was assessed via the sequence method (27, 92). We used Ensemble software (Elucimed, Wellington, New Zealand) to identify sequences of three or more consecutive heartbeats in which the period between R-R intervals and SBP changed simultaneously (i.e. both increased or decreased). A minimal coefficient of correlation between changes in SBP and changes in R-wave to R-wave (R-R) interval were required to validate a sequence ($r^2 > 0.8$) (93). Given the relatively slow HR of participants during baseline and SB, we expected that the effects of the baroreflex on the R-R interval would be observed within the same cardiac cycle; we therefore chose not to apply a lag of one beat to the dataset (92). Cardiovagal BRS was assessed separately for up-sequences (in which SBP and R-R interval increase) and down-sequences (in which SBP and R-R interval decrease), given the

638 literature supporting a propensity of SB to enhance up-sequences without an effect on down-
639 sequences (31, 32).

640 To obtain indirect indices of autonomic outflow to the sinoatrial node, HRV time domain
641 measures included standard deviation of normal-to-normal intervals (i.e. R-R intervals with no
642 artifact; SDNN) and root mean square of successive R-R intervals (RMSSD). Additionally,
643 frequency domain measures included both low frequency (LF, 0.05-0.15 Hz) and high frequency
644 (HF, 0.15-0.4 Hz) spectral density. All HRV metrics were assessed using Ensemble software
645 (Elucimed, Wellington, New Zealand).

646 *Statistical analysis:* To compare the BP-lowering effects of RESP and FB under
647 manufacturer-recommended conditions (i.e. 15-min for RESP and 5-min for FB), we extracted 3-
648 min of steady state data following the initial acclimatization period which occurred at the onset
649 of any SB protocol (i.e. FB minutes 2-5, RESP minutes 6-9). Hemodynamic responses to SB
650 were assessed as deltas such that the SB response was expressed relative to the preceding
651 baseline period. To compare the hemodynamic effects of SB between devices, we conducted 2-
652 tailed paired T-tests. Conversely, to compare the effects of the devices on BRS and HRV, we
653 analyzed absolute values *via* 2-way repeated measures ANOVA (factor 1 = Device, 2 levels =
654 FB, RESP; factor 2 = Condition, 2 levels = baseline, SB).

655 To compare the BP-lowering effects of RESP and FB with matched duration of exposure
656 (i.e. 15-min of each), hemodynamic variables were averaged into 3-min time bins and the first
657 12-min of SB (i.e. prior to the commencement of CPT) within each device were extracted and
658 expressed as deltas (i.e. SB – baseline). We compared these responses using 2-way repeated
659 measures ANOVAs (factor 1 = Device, 2 levels = FB, RESP; factor 2 = Time, 4 levels = minutes
660 0-3, 3-6, 6-9, and 9-12). However, to compare the effects of the devices on absolute values of

BRS and HRV, the 10-min of true baseline (i.e. the baseline period from the beginning of the test session; see Figure 2.1) and minutes 2-12 of SB were extracted for analysis and assessed using a 1-way repeated measures ANOVA (3 levels: true baseline *vs* RESP *vs* FB).

Finally, to compare the BP-lowering effects of RESP and FB devices during the CPT, we first calculated the relative changes in all variables during exposure to CPT alone. We then subtracted this value from the relative changes that we observed during CPT+SB for each device in order to determine whether the magnitude of increase in BP was attenuated by SB (and whether this effect differed between RESP and FB). Two-tailed paired T-tests were used to compare the MAP, SBP and DBP responses. Also, 2-tailed paired T-tests were used to compare the BP responses between the CPT alone and the FB, as well as the CPT alone and the RESP.

All statistical analyses were performed using GraphPad Prism 8 (La Jolla, California) with a 0.05 alpha value. All data are reported at mean ± standard deviation.

9 Results

Participant characteristics, including baseline hemodynamic and autonomic characteristics, are presented in Table 2.1. By design, participants were classified as having a moderately lean body fat percentage (women: 26 ± 5%; man: 20%) as per the BODPOD guidelines. They were also classified as normotensive according to the 2017 revisions to the American Heart Association's BP guidelines (3).

Responses to Manufacturer-Recommended Settings. A steady state of SB in terms of respiration rate was achieved (Figure 2.2). Therefore, minutes 2-5 for the FB device and minutes 6-9 for the RESP device were selected to compare the SB-induced changes in steady state hemodynamic and autonomic responses between devices. The relative decrease in respiration rate from baseline was not different between devices (Figure 2.3). Likewise, relative changes in

MAP and DBP were not different between devices (Figure 2.3), nor were changes in PP, HR, Q, or TPR (Table 2.2). However, the SB-induced decrease in SBP was greater with RESP than FB by an average of 3 mmHg (Figure 2.3), and SV increased to a greater extent during FB compared to RESP-guided SB (Table 2.2). Cardiovagal BRS during up-sequences was enhanced during SB, whereas BRS during down-sequences was unaffected by SB (Figure 2.4). However, we observed no effect of device or device-by-SB interactions in BRS during either up- or down-sequences. Finally, while analysis of HRV demonstrated a main effect of SB on SDNN and LF power, both of which were increased by SB, we observed no main effect of device or device-by-SB interaction in any components of HRV (Table 2.3).

Responses to SB with Matched Duration. When the duration of SB was matched between devices by extending the duration of FB-guided SB to 15-mins, the relative decrease in respiration rate throughout SB was not different between devices (Figure 2.5). However, RESP resulted in greater relative reductions in MAP, SBP, DBP compared to FB over 12-mins of device-guided SB (Figure 2.5). Although changes in PP, HR, Q, and TPR were similar between devices, the FB device resulted in greater relative increases in SV compared to the RESP device (Table 2.4). Analysis of HRV revealed a main effect of SB on SDNN and LF power (Table 2.6), with no main effect of device. Cardiovagal BRS during down-sequences was unaffected by either device, whereas BRS during up-sequences increased as a main effect of SB with the FB device. However, there was no significant difference between the two devices in BRS during either up- or down-sequences (Table 2.5).

Effect of SB on Responses to the Cold Pressor Test. Participants performed SB and the CPT concurrently during minutes 12-15 of the SB protocols (Figure 2.2). Increases in BP following the CPT alone (MAP +7.2 ± 5.8 mmHg; SBP +7.2 ± 7.4 mmHg, ; DBP +6.0 ± 4.4

mmHg) were not significantly different from the BP responses during RESP+CPT (MAP +9.1 ± 5.3 mmHg, P=0.1; SBP +7.2 ± 5.6 mmHg, P=0.9; DBP +8.2 ± 5.3 mmHg, P=0.06). However, the increases in BP following the CPT alone were significantly lower than those of the FB+CPT (MAP +10.3 ± 5.8 mmHg, P<0.05; SBP +9.2 ± 7.3 mmHg, P<0.05; DBP +8.8 ± 4.8 mmHg, P<0.05). Importantly, there were no differences in the effects of SB on the BP responses to the CPT between the two devices (Figure 2.6).

10 Discussion

These preliminary data suggest that the RESP device is better able to reduce SBP than the FB device when the devices were assessed under manufacturer-recommended conditions in young, normotensive individuals. Moreover, when the duration of SB for FB was extended in order to match the duration of exposure between devices, RESP remained more effective than FB, reducing MAP, SBP and DBP to a greater extent than FB. In order to ascertain why RESP elicited greater reductions in BP than FB, we assessed a number of hemodynamic and autonomic outcomes known to be acute determinants of BP. We observed that FB elicited increases in SV during SB, which were not observed with RESP, suggesting a mechanism by which the SB-induced reductions in BP are limited when guided by the FB device. Together, these data suggest that the RESP device may be a more suitable choice for device-guided SB with the goal of acute lowering of BP. Whether RESP remains more effective than FB when used over time (e.g. following a 12-week SB intervention), or when used in hypertensive individuals, remain to be established.

To the best of our knowledge, this study was the first to compare BP responses to device-guided SB between two commercially-available devices, the RESP and FB. We did so by first investigating the BP responses of each device under manufacturer-recommended conditions; that

is, 15-min of SB using RESP *versus* 5-min of SB using FB. We selected data following each device's acclimatization period to ensure that participants had reached a steady state respiration rate. In this analysis, we observed that RESP elicited greater reductions in SBP than FB, although MAP and DBP were reduced to a similar extent between devices. While these data indicate that the RESP device evoked a stronger BP-lowering effect than the FB device, it may also be that this was simply a reflection of the shorter SB period that was evoked through the manufacturer-recommended conditions of FB. Indeed, although there is some evidence to support acute reductions in SBP (-3.4 mmHg) and DBP (-1.2 mmHg) following just 30-sec of SB in normotensive participants (44), another study which exposed participants to only 3-min of SB failed to elicit a significant reduction in SBP (+2 mmHg) or DBP (0 mmHg) (94). Conversely, SB of at least 10-min in duration was accompanied by reductions in SBP (-3.2 and -4.6 mmHg) and DBP (-1.3 and -2.1 mmHg) (31, 87). Therefore, while the evidence is not universal, it is likely that the duration of exposure to SB is an important factor for the BP-lowering effect associated with SB, and an exposure of greater than 3-min may be necessary to achieve a statistically significant drop in BP from baseline. To address this, we conducted subsequent analysis at matched durations of the two devices by extending exposure to the FB device. However, we once again observed that RESP evoked a stronger BP-lowering effect than FB, as evidenced by relatively greater reductions in MAP, SBP and DBP from baseline.

From a respiratory perspective, respiration rate did not differ between the two devices under the recommended settings or when matched for duration. Therefore, it is unlikely that one device was more successful in reaching an optimal resonance frequency than the other. Resonance frequency, which can be defined as a 0-degree phase shift between HR and respiration rate, and a 180-degree phase shift between BP and respiration rate, is believed to

753 stimulate the baroreflex on each breath and thus increase vagal activity (73). Resonance
754 frequency occurs at a specific respiration rate (~6 breaths/min) but which is likely optimized
755 when personalized to each individual's HRV to account for inter-individual differences in HR
756 (95), and, when achieved, has been hypothesized to maximize the BP-lowering effects of SB
757 (73). However, other studies have demonstrated that breathing at resonance frequency compared
758 with breathing at resonance frequency +1 breath/min resulted in no differences in the eventual
759 SB-induced reductions in BP (87). Thus, achieving resonance frequency may not be a
760 requirement for the significant lowering of BP with SB. Even if RESP and FB devices had
761 resulted in small (yet non-significant) differences in respiration rate in the present study, it
762 appears unlikely that this would account for the differences in the BP-lowering effects that we
763 observed between the RESP and FB.
764 From an autonomic standpoint, HRV and cardiovagal BRS gave insight into the
765 autonomic mechanistic pathways of SB-induced reductions in BP. Our results are in accordance
766 with the SB literature such that SDNN and LF power increased with both devices, both during
767 the manufacturer-recommended settings and when matched in duration (96, 97). Increases in
768 SDNN during a single bout of SB are primarily influenced by respiratory sinus arrhythmia, and
769 moderated by parasympathetic activity (59). Therefore, the observed increases in SDNN with
770 both devices may be interpreted as an increase in parasympathetic outflow to the heart during
771 SB. Although LF power can contain both sympathetic and parasympathetic information,
772 increases associated with LF power during SB may be attributed primarily to vagal activity (59).
773 As such, the increase in LF power elicited by both devices during SB may be interpreted as an
774 increase in parasympathetic activity. Furthermore, our observation of SB-induced increases in
775 BRS during up-sequences is in alignment with the current literature (31-33). Up-sequences occur

776 during concurrent increases in SBP and R-R interval (25), and reflect the activity of the
777 baroreflex as it responds to an acute increase in BP by decreasing HR and also reducing total
778 peripheral resistance via a reduction in vasoconstriction of the peripheral vasculature (16). Thus,
779 an increase in BRS during up-sequences provides further evidence for a SB-mediated increase in
780 parasympathetic activity with both FB and RESP devices (16, 18). Taken together, both HRV
781 and BRS indicate a shift towards an increase in parasympathetic activity which reflects a more
782 optimal sympatho-vagal balance (i.e. defined as more parasympathetic outflow and/or less
783 sympathetic outflow) (34). This is clinically relevant as poor sympatho-vagal balance that is
784 shifted toward increased sympathetic outflow and/or reduced parasympathetic outflow is
785 associated with hypertension and diabetes (98). However, while it remains clear that SB in
786 general is advantageous in terms of eliciting a positive increase in parasympathetic activity, we
787 observed no significant differences between RESP and FB devices in their effects on HRV or
788 BRS. This finding persisted across both the manufacturer-recommended condition and when the
789 devices were matched for duration, and thus we are unable to attribute the differences in the BP
790 effects to an autonomic mechanism.
791 Interestingly, we observed that FB-guided SB was associated with increases in SV which
792 were not observed with the RESP device. However, there were no differences in PP between the
793 two devices, which was unexpected as it has been suggested that a change in PP is proportional
794 to a change in SV (99). However, a different study demonstrated that PP and SV are nonlinear,
795 such that changes in PP are smaller than changes in SV (100). In other words, alterations in PP
796 may underestimate changes in SV, which may explain why we observed no apparent differences
797 in PP between the two devices. Importantly, SV is a primary determinant of SBP, such that an
798 increase in SV may result in an increase in SBP (101). Thus, it may be that increases in SV

during FB-guided SB resulted in significantly smaller reductions in SBP when compared to RESP-guided SB, although the exact mechanism by which SV increased to a greater extent during FB compared to RESP-guided SB remains unknown. Interestingly, during spontaneous inspiration increases in lung volume and decreases in intrathoracic pressure are associated with increases in right atrial venous return and decreases in left ventricular SV (34, 70, 102). These changes are reversed during expiration such that right atrial venous return decreases and left ventricular SV increases. However, the changes in venous return are dependent on the participant's breathing pattern, such that thoracic breathing increases venous return during inspiration, whereas diaphragmatic (abdominal) breathing results in larger venous return throughout expiration (103). Therefore, it may be that the participants' breathing pattern (diaphragmatic *vs* thoracic) and/or changes in lung volume and intrathoracic pressure played a role in the observed differences in SV, and, in turn, BP, between FB and RESP-guided SB. While many studies have investigated the effects of SB on BP, very few if any have looked at the role that SV may play. Therefore, future studies should examine SB-mediated changes in SV and the mechanisms associated with them in order to improve our understanding of the complex cardiorespiratory responses that are associated with the BP-lowering effects of SB.

Another notable difference between the devices which may have contributed to our findings centres around the experience of SB using RESP *versus* FB. Namely, the RESP device plays musical tones in order to guide the participant through SB, whereas the FB device uses vibrations directed to the wrist as well as visual cues to guide the user. Previous studies examining the RESP device have included comparisons to the effects of either a sham device which provided musical tones without performing SB, or a Walkman to expose the user to relaxing music. These studies have demonstrated that both control measures are effective in

eliciting reductions in BP (i.e. without exposure to SB) (79, 81, 104). Although the exact mechanisms by which listening to music reduces BP remains poorly understood, it is hypothesized that music may reduce sympathetic activity and trigger the release of endorphins which enhance a sense of well-being (105). Therefore, it is indeed possible that the musical tones may have contributed to the greater BP reductions observed during RESP compared to FB-guided SB.

To increase the clinical relevance of our study, which was conducted in healthy, young, non-obese normotensive adults, we induced an acute hypertensive state by the CPT to determine whether RESP and FB differed in their ability to reduce acute hypertension. However, contrary to our expectation, neither device was effective in mitigating the BP response to the CPT. We observed no significant difference between RESP and FB in reducing BP during the CPT, and moreover, the FB device appeared to exacerbate the BP response to the CPT. Although we are not aware of any studies which have examined the potential of SB to mitigate the BP and/or cardiovascular hemodynamic response to an acute sympatho-excitatory stimulus such as the CPT, our results are in alignment with a previous study which found that BP responses to a CPT were not affected by 8-weeks of SB (106). Thus, it may be that the pressor response evoked by the CPT is too potent to be mitigated or overcome by SB.

10.1 Methodological Considerations

One of the biggest limitations to this study was the relatively small sample size. Unfortunately, COVID-19 abruptly halted data collection after 7 participants had completed our study due to safety concerns. Given that 1 out of the 7 participants was a man, our results may be skewed towards women until both sexes are tested equally. However, we have previously shown that SB is equally effective in reducing BP in both men and women (31). Therefore, these results

must be seen as preliminary until further data collection can be completed. Prior to beginning the study, we had conducted sample size calculations based on our pilot data, which demonstrated a large effect size of 0.80 in the differences between RESP and FB devices in the BP responses to SB (FB appeared to be more effective than the RESP in lowering BP), as well as a desired power of 0.8 and type I error of 0.05. As such, we calculated that we would require a sample size of 17 participants to observe a difference between RESP and FB devices. Accordingly, in order to effectively evaluate our hypothesis in both men and women, we will seek to continue data collection at the earliest possible opportunity.

Given that the cardiorespiratory responses to SB are complex and multifactorial, we were unable to evaluate all of the potential mechanistic pathways involved with SB in order to pinpoint the reason for the larger reductions in BP elicited by the RESP compared to FB-guided SB. Specifically, it is possible that the typical increases in tidal volume associated with SB (34, 37), then activated pulmonary stretch receptors to induce vagal withdrawal and potentially contribute to the BP-lowering response (12). Therefore, it is indeed possible that the RESP device activated pulmonary stretch receptors to a greater extent than the FB device which could therefore partially account for the different BP responses.

Lastly, although the coaching periods that we implemented for the FB device in order to match the duration of exposure were necessary in order to make a meaningful time comparison to the RESP device, they may have been a source of variation as the participants had to switch cues from the device to the researcher. Given that there is some doubt as to whether very short (i.e. 3-min) bouts of SB are effective in reducing BP, a more ideal approach would be an extension of the duration of SB as guided by the FB in order to facilitate direct comparisons between the two devices.

11 Conclusion

Our novel and preliminary study demonstrated that the RESPeRATE device acutely reduced blood pressure to a greater extent than the Fitbit "Relax Mode" in healthy, young and non-obese normotensive individuals, most of whom were women. The RESPeRATE device was better able to acutely reduce blood pressure than the Fitbit device, both under manufacturer-recommended conditions and when the two devices were matched for duration. Although the exact mechanism(s) mediating this difference are poorly understood and require further examination, greater Fitbit-induced increases in stroke volume may have prevented the reductions in blood pressure, specifically systolic blood pressure, that we observed with the RESPeRATE device. Given that the results of this study revealed potentially meaningful differences in the cardiorespiratory response to SB guided by RESPeRATE and Fitbit devices, we believe that the FDA-approved RESPeRATE device may be better suited than the Fitbit device to lower blood pressure, and perhaps also to treat hypertension in clinical populations at risk for adverse cardiovascular health outcomes. However, our results are directly generalizable only to young, healthy, non-obese, normotensive individuals. Therefore, further research with pre-hypertensive and hypertensive participants is necessary.

12 Tables and Figures

Table 2.1. Participant baseline characteristics.

Height (cm)	169 ± 9
Weight (kg)	68 ± 6
Body Fat %	25 ± 6
MAP (mmHg)	81.9 ± 4.9
SBP (mmHg)	109.7 ± 5.1
DBP (mmHg)	64.1 ± 3.9
PP (mmHg)	45.6 ± 5.1
HR (bpm)	60.2 ± 11.4
Q (L/min)	4.6 ± 1.1
SV (ml)	77.4 ± 17.6
TPR (mmHg/L/min)	18.8 ± 4.4
Respiration Rate (breaths/min)	10.2 ± 2.5
BRS down (ms/mmHg)	40.6 ± 19.0
BRS up (ms/mmHg)	37.1 ± 19.6
LF (ms^2)	5500 ± 8800
HF (ms^2)	2300 ± 1900
SDNN (ms)	0.095 ± 0.051
RMSSD (ms)	91 ± 48

Data are mean ± standard deviation. MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HR, heart rate; Q, cardiac output; SV, stroke volume; TPR, total peripheral resistance; BRS, baroreflex sensitivity; LF, low frequency power; HF, high frequency power; SDNN, standard deviation of the NN interval; RMSSD, root mean squared of successive RR interval differences

Table 2.2. Changes in hemodynamic measures during Fitbit and RESPeRATE recommended settings.

	RESPeRATE	**Fitbit**	***P* value**
PP (mmHg)	-0.2 ± 1.5	1.7 ± 2.0	0.2
HR (bpm)	1.3 ± 1.7	1.6 ± 1.2	0.7
Q (L/min)	0.1 ± 0.3	0.3 ± 0.1	0.1
SV (ml)	0.3 ± 3.9	3.6 ± 2.9	<0.05
TPR (mmHg/L/min)	-0.8 ± 1.6	-1.8 ± 1.8	0.1

Data are mean ± standard deviation. PP, pulse pressure; HR, heart rate; Q, cardiac output; SV, stroke volume; TPR, total peripheral resistance.

Table 2.3. Heart rate variability measures analyzed under Fitbit and RESPeRATE recommended settings

Measures	Fitbit		RESPeRATE		Condition	Device	Condition x Device
	BSL	SB	BSL	SB			
SDNN (ms)	0.10 ± 0.06	0.14 ± 0.04	0.11 ± 0.05	0.14 ± 0.04	<0.05	0.9	0.6
RMSSD (ms)	110 ± 62	120 ± 52	100 ± 42	110 ± 37	0.5	0.8	0.7
LF Power (ms^2)	2200 ±2400	4600 ± 2500	2400 ± 3000	4800 ± 2800	<0.05	0.9	0.9
HF Power (ms^2)	1800 ± 1600	900 ± 610	980 ± 1100	830 ± 490	0.07	0.4	0.2

Data are mean ± standard deviation. SDNN, standard deviation of the NN interval; RMSSD, root mean squared of successive RR interval differences; LF, low frequency power; HF, high frequency power. BSL, baseline; SB, slow breathing.

Table 2.4. Hemodynamic measures during Fitbit and RESPeRATE with matched duration of exposure.

		PP (mmHg)	HR (bpm)	Q (L/min)	SV (ml)	TPR (mmHg/L/min)
Minutes 1-3	Fitbit	1.1±1.4	0.7±1.1	0.2±0.1	1.5±2.1	-0.7±0.6
	RESPeRATE	0.9±1.4	0.7±1.1	0.2±0.1	1.4±1.3	-1.2±0.7
Minutes 4-6	Fitbit	1.5±1.8	1.6±1.4	0.3±0.2	3.7±3.3	-1.8±2.0
	RESPeRATE	0.7±1.0	1.6±1.2	0.3±0.2	2.0±1.9	-1.5±1.6
Minutes 7-9	Fitbit	0.8±2.0	0.8±2.1	0.3±0.3	4.0±4.6	-1.5±2.6
	RESPeRATE	-0.2±1.4	1.3±1.6	0.1±0.3	0.3±3.7	-0.8±1.5
Minutes 10-12	Fitbit	0.1±2.3	1.9±1.9	0.2±0.3	2.2±6.0	-1.3±2.4
	RESPeRATE	-0.6±1.6	1.3±1.7	0.1±0.3	0.5±3.3	-0.5±1.4
P value	Time	0.1	0.5	0.6	0.8	0.8
	Device	0.2	0.9	0.09	<0.05	0.3
	Time x Device	0.9	0.7	0.7	0.4	0.5

Data are mean ± standard deviation. PP, pulse pressure; HR, heart rate; Q, cardiac output; SV, stroke volume; TPR, total peripheral resistance.

Table 2.5. Baroreflex sensitivity measures during Fitbit and RESPeRATE with matched duration of exposure.

	Baseline	Fitbit	RESPeRATE
BRS down sequence (ms/mmHg)	40.6 ± 19.0	42.2 ± 24.0	40.8 ± 28.8
BRS up sequence (ms/mmHg)	37.1 ± 19.6	49.9 ± 22.5 *	43.9 ± 14.1

Data are mean ± standard deviation. *$P < 0.05$ Fitbit *vs* baseline. BRS, baroreflex sensitivity

Table 2.6. Heart rate variability measures during Fitbit and RESPeRATE with matched duration of exposure.

	Baseline	Fitbit	RESPeRATE
SDNN (ms)	0.10 ± 0.05	0.14 ± 0.04 *	0.13 ± 0.04 *
RMSSD (ms)	91 ± 48	120 ± 58	110 ± 39
LF Power (ms^2)	5500 ± 8800	14000 ± 7500 *	14000 ± 7600 *
HF Power (ms^2)	2300 ± 1900	3000 ± 2500	2600 ± 1500

Data are mean ± standard deviation. *$P < 0.05$ Fitbit or RESPeRATE *vs* baseline. SDNN, standard deviation of the NN interval; RMSSD, root mean squared of successive RR interval differences; LF, low frequency power; HF, high frequency power.

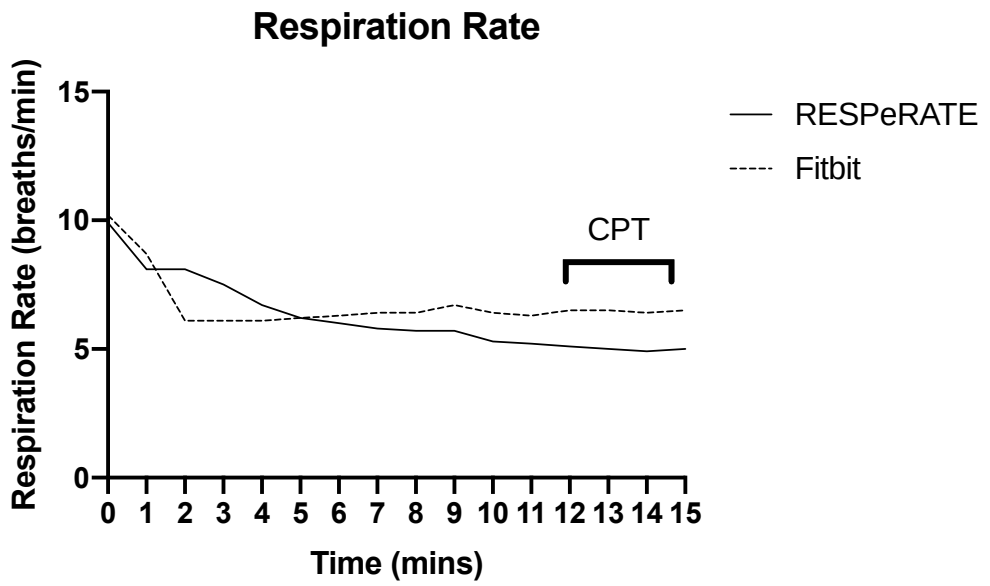

Figure 2.2. Effect of RESPeRATE (RESP) and Fitbit (FB) on respiration rate. The RESPeRATE achieved a steady state of slow breathing after 6-mins. The Fitbit achieved a steady state of slow breathing after 2-mins. CPT, cold pressor test.

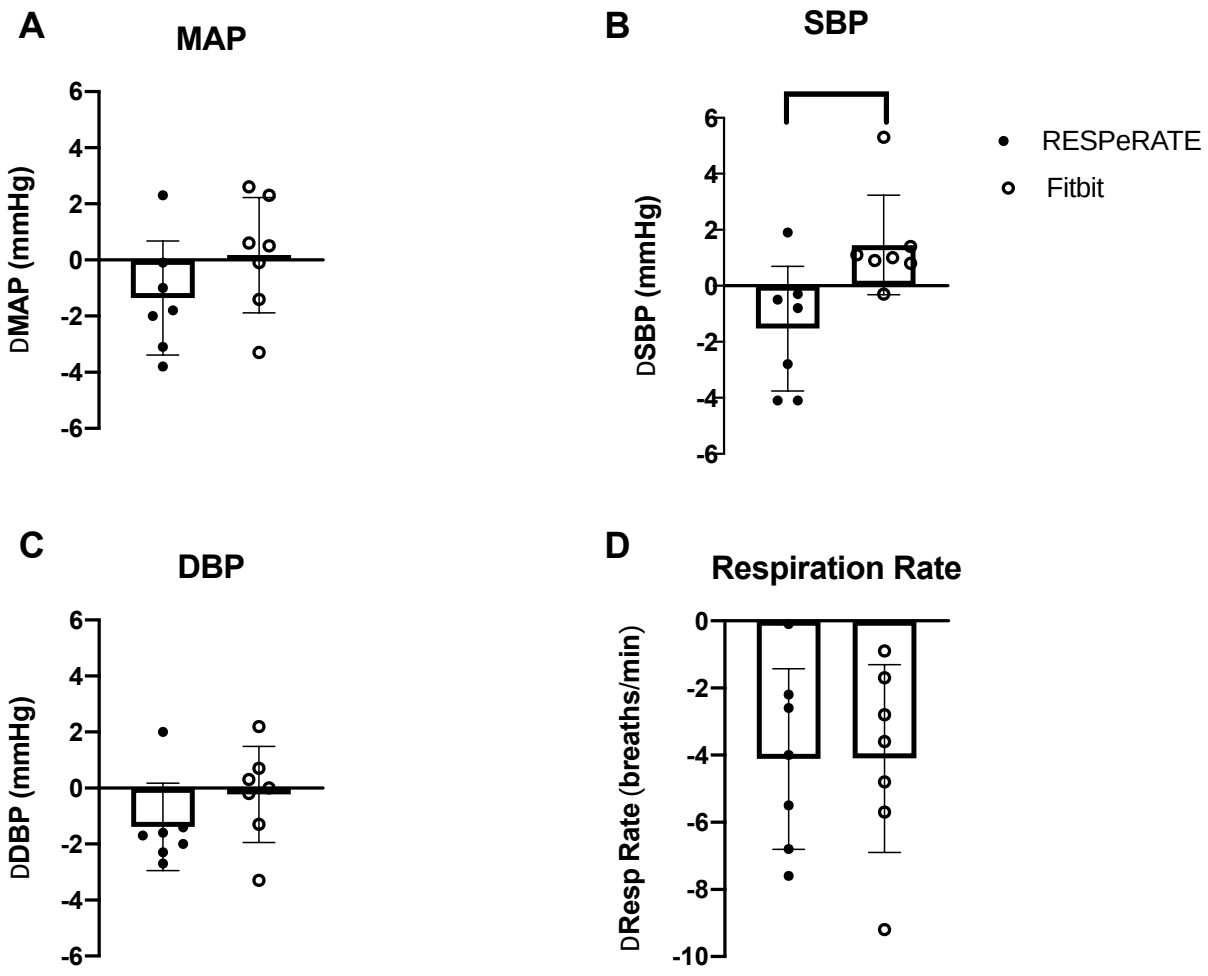

Figure 2.3. Effect of RESPeRATE (RESP) and Fitbit (FB) on blood pressure and respiration rate as per the manufacturer recommended settings. (A) Mean arterial pressure (MAP) did not differ between devices, $P = 0.1$. (B) Systolic blood pressure (SBP) decreased significantly with the RESP in comparison to the FB, $P < 0.05$. (C) Diastolic blood pressure (DBP) did not differ between devices, $P = 0.08$. (D) Respiration rate did not differ between devices, $P = 0.9$; individual data shown, Data are mean ± standard deviation, $n = 7$.

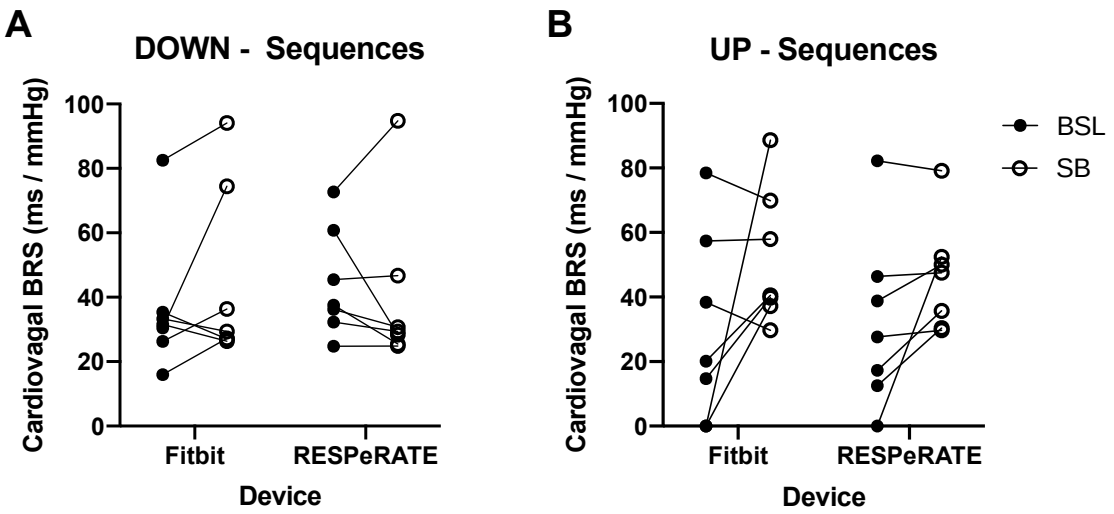

Figure 2.4. Effect of slow breathing on cardiovagal baroreflex sensitivity (BRS) using the Fitbit (FB) and the RESPeRATE (RESP) under manufacture recommended conditions. (A) Cardiovagal BRS in response to hypotensive stimuli (DOWN-sequences) was unchanged in both Fitbit and RESPeRATE. Effect of slow breathing (SB), $P = 0.6$; effect of device, $P = 0.9$; SB X Device, $P = 0.2$. (B) Cardiovagal BRS in response to hypertensive stimuli (UP-sequences) increased in both Fitbit and RESPeRATE. Effect of SB, $P < 0.05$; effect of device, $P = 0.9$; SLOWB X Device, $P = 0.6$; individual data shown. Repeated Groups: $n = 7$. BSL, baseline.

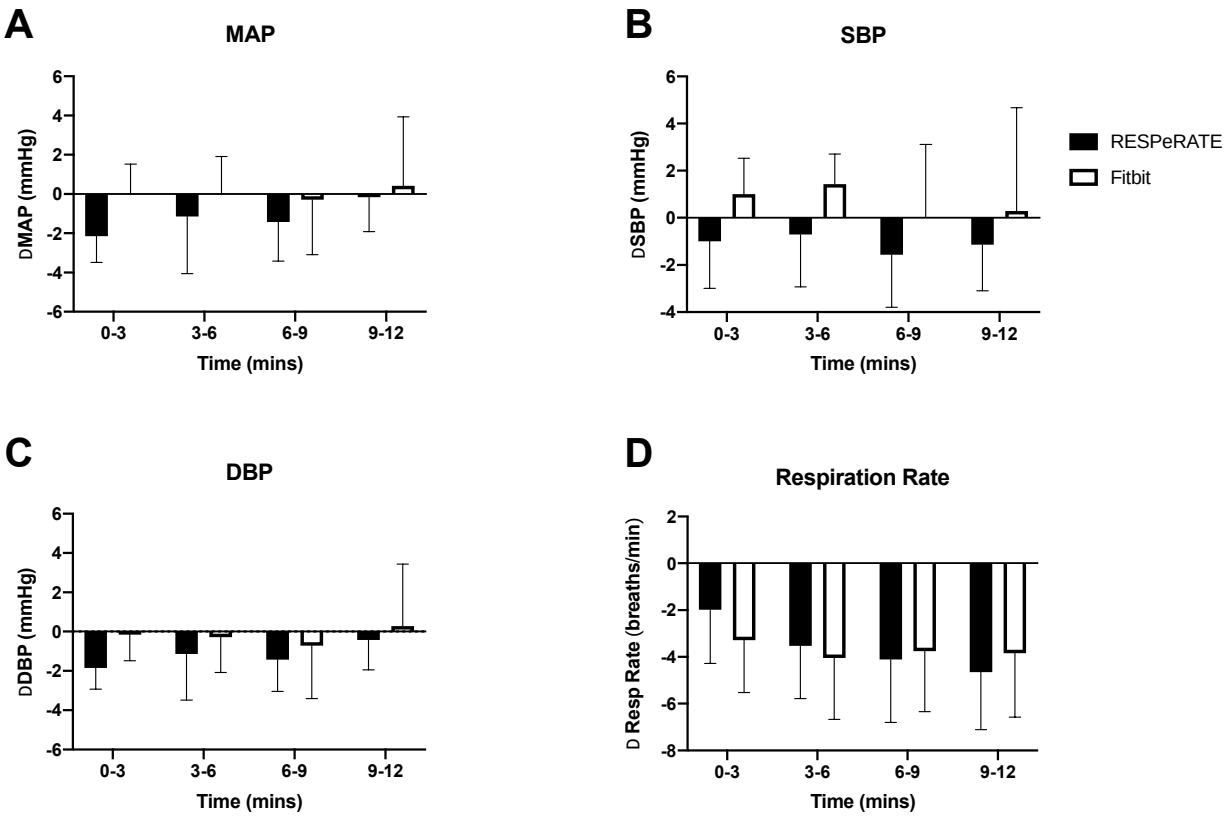

Figure 2.5. Effect of RESPeRATE (RESP) and Fitbit (FB) on blood pressure and respiration rate matching for duration. (A) Mean arterial pressure (MAP) differed significantly between devices (main effect of device; $P = <0.05$), no main effect of Time ($P = 0.7$), no Device x Time interaction ($P = 0.7$). (B) Systolic blood pressure (SBP) differed significantly between devices ($P = <0.05$), no main effect of Time ($P = 0.6$), no Device x Time interaction ($P = 0.9$). (C) Diastolic blood pressure (DBP) differed significantly between devices ($P = <0.05$), no main effect of Time ($P = 0.7$), no Device x Time interaction ($P = 0.8$). (D) Respiration rate (resp rate) did not differ between devices ($P = 0.6$), no main effect of Time ($P = 0.6$), no Device x Time interaction ($P = 0.1$). Data are mean ± standard deviation.

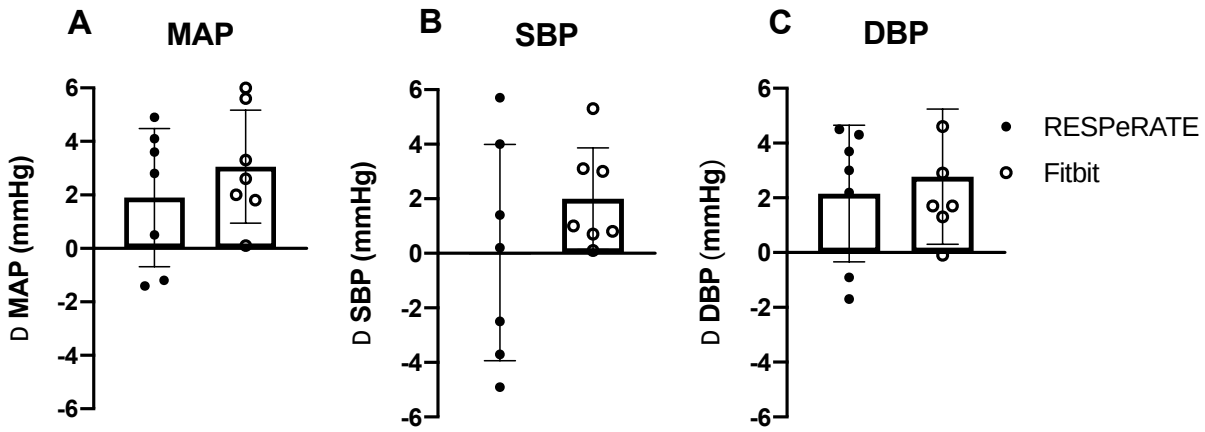

Figure 2.6. Effect of RESPeRATE (RESP) and Fitbit (FB) on blood pressure during a cold pressor test. (A) Mean arterial pressure (MAP) did not differ between devices, $P = 0.4$. (B) Systolic blood pressure (SBP) did not differ between devices, $P = 0.3$. (C) Diastolic blood pressure (DBP) did not differ between devices, $P = 0.6$; individual data shown. Repeated Groups: $n = 7$. Data were analyzed as the delta of CPT+SB minus the delta of CPT alone. CPT, cold pressor test; SB, slow breathing. Data are mean ± standard deviation.

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