THE EFFECT OF BIOLOGICAL VARIABLES AND AUTONOMIC MEASURES ON ACUTE BLOOD PRESSURE RESPONSES TO SLOW BREATHING

Tingyu Huang, BSc

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Cardiovascular Health and Autonomic Regulation Laboratory

Department of Kinesiology and Physical Education

McGill University, Montreal

Supervisor: Charlotte Usselman, PhD

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1. Preface

1.1 Abbreviations

BMI	Body Mass Index
BP	Blood Pressure
BRS	Baroreflex Sensitivity
BSL	Baseline
CO	Cardiac Input
COi	Cardiac Output Index
CVLM	Caudal Ventrolateral Medulla
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
HF	High-Frequency
HR	Heart Rate
HRV	Heart Rate Variability
LF	Low-Frequency
MAP	Mean Arterial Pressure
NN	Normal-To-Normal (Intervals)
NTS	Nucleus Tractus Solitarius
PNS	Parasympathetic Nervous System
PP	Pulse Pressure
RRI	R-R Interval
RSA	Respiratory Sinus Arrythmia
RVLM	Rostral Ventrolateral Medulla
SB	Slow Breathing
SBP	Systolic Blood Pressure
SDNN	Standard Deviation of NN Intervals
SNS	Sympathetic Nervous System
SV	Stroke Volume
SVi	Stroke Volume Index
TPR	Total Peripheral Resistance
RMSSD	Root-Mean Square Differences of Successive RRI

1.2 Abstract

Slow breathing (SB) is recognized as a means of lowering blood pressure (BP) in hypertensive individuals. While studies in both clinical and healthy populations have demonstrated that SB decreases BP, not all studies have agreed, indicating high variability in SB-mediated BP responses. Thus, we examined autonomic, biological, and lifestyle factors as potential predictors of the BP response to SB. Young, healthy women (n=14) and men (n=16) underwent 15-min device-guided SB (RESPeRATE, Intercure Ltd.). Respiration (respiratory belt transducer, ADInstruments), heart rate (HR; 5-lead ECG), and beat-by-beat systolic BP (SBP; finger photoplethysmography, Finometer MIDI) were recorded continuously. Indices of autonomic function were spontaneous cardiovagal baroreflex sensitivity (BRS) and HR variability (HRV). Biological and lifestyle factors were established determinants of BRS and HRV (resting SBP, BMI, physical activity levels). No autonomic (up-BRS, R^2<0.01, P=0.71; RMSSD, R^2<0.01, P=0.73), biological (resting SBP, R^2=0.02, P=0.43; BMI, R^2=0.10, P=0.09) or lifestyle (physical activity, R^2=0.09, P=0.19) variables predicted SBP response to SB. However, *post hoc* analyses grouping participants based on SBP response to SB demonstrated that participants who decreased SBP tended to have higher resting SBP than those who increased SBP during SB (117±7 *vs* 111±10 mmHg, respectively, P=0.09), as well as greater increases in abdominal tidal volume during SB (31.77±25.75 *vs* 13.67±3.58 Δ%max, P=0.09). These preliminary findings suggest that both resting SBP and the respiratory response to SB may whether an individual responds favourably to SB, although this requires further study.

1.3 Résumé

La respiration lente est reconnue comme un bon moyen de réduire la tension artérielle chez les personnes atteintes d'hypertension. Bien que des études menées dans des populations cliniques et en bonne santé aient démontré que la respiration lente réduit la tension artérielle, une incohérence existe parmi les études. Celles-ci indiquent une grande variabilité de réponses à la tension artérielle vis-à-vis la respiration lente. Ainsi, la présente étude a pour objectif d'examiner des facteurs nerveux autonomes, biologiques, et de mode de vie en tant que facteurs prédictifs potentiels de la réponse de tension artérielle à la respiration lente. Les participants (n=30 dont 14 femmes et 16 hommes) etaient jeunes (âgés 18 à 34) et en bonne santé. Ils ont suivi une session de la respiration lente de 15 minutes guidée par un appareil (RESPeRATE, Intercure Ltd.). La respiration (transducteur de ceinture respiratoire, ADInstruments), la fréquence cardiaque (HR ; ECG à 5 déonsitrés), et la tension artérielle pour chaque pulsation (photoplethysmography de doigt, finomètre MIDI), ont été enregistrées continuellement. La sensibilité cardiovagale spontanée de baroreflex et la variabilité de fréquence cardiaque étaient utilisées comme index de la fonction nerveuse autonome. Les facteurs biologiques et de mode de vie suggérés d'influencer la sensibilité baroréflexe et la variabilité de la fréquence cardiaque (tension artérielle systolique au repos, indice de masse corporelle et le niveau d'activité physique) ont aussi été évalués en tant que facteurs prédictifs potentiels. Ni les index de la fonction autonome (up-BRS, R^2<0,01, P=0,71; RMSSD, R^2<0,01, P=0,73), ni les variables biologiques (tension artérielle systolique au repos, R^2=0,02, P=0,43; indice de masse corporelle, R^2=0,10, P=0,09) n'ont prédit la réponse de la tension artérielle systolique à la respiration lente. En plus, le mode de vie (activité physique, R2=0,09, P=0,19) n'a pas prédit la réponse de la tension artérielle systolique à la respiration

lente. Cependant, des analyses post hoc regroupant les participants en fonction de la réponse de la tension artérielle systolique à la respiration lente ont démontré que les participants chez lesquels la tension artérielle systolique était réduite avaient tendance à avoir une tension artérielle systolique au repos plus élevée ainsi qu'une augmentation plus importante du volume courant abdominal (31,77±25,75 vs 13,67±3,58 Δ%max, P=0,09) que ceux chez lesquelles la tension artérielle systolique avait augmentée pendant la respiration lente (117±7 vs 111±10 mmHg, respectivement, P=0,09). Ces résultats préliminaires suggèrent que la tension artérielle systolique au repos et la réponse respiratoire à la respiration lente peuvent prédire si une personne répond favorablement à la respiration lente, bien que cela nécessite une étude plus approfondie.

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1.5 Contribution of Authors

Chapter I was written by Tingyu Huang with feedback from Dr. Charlotte Usselman and graduate students from the CHARLab. Chapter II was co-written by Tingyu Huang, Fiona Howse, and Celine Chen with feedback from Dr. Charlotte Usselman. Drs. Ross Anderson, Michael Stickland, and Benoit Gentil provided feedback for Chapters I and II. Fiona Howse and Celine Chen aided in data collection and analysis.

2. Context

Device-guided slow breathing (SB) has been recommended as a non-pharmacological, adjunct treatment to lower blood pressure in individuals affected by hypertension. However, while SB is effective in decreasing blood pressure (BP) in clinical populations (84, 119, 249) as well as in healthy individuals (2, 66, 199), researchers have also noted considerable inter-individual variability in SB-mediated BP responses (39).

The sources of this variability are difficult to pinpoint, as the mechanisms by which SB reduces BP are not well established and efforts to elucidate such mechanisms remain ongoing. However, baroreflex sensitivity (BRS) and heart rate variability (HRV) have emerged as strong contenders for mechanistic contributors to SB-mediated BP responses. Baroreflex sensitivity is a measure of the baroreflex's ability to respond to acute changes in arterial blood pressure (272). The sequence method of BRS measurement involves the identification of three or more consecutive heart beats wherein systolic blood pressure (SBP) increases and R-R interval (RRI; i.e., the interval between successive heartbeats) simultaneously lengthens (i.e., up-BRS), or SBP decreases and RRI simultaneously shortens (i.e., down-BRS) (217). Heart rate variability is the measure of the variation between individual, successive heartbeats (258) and is widely reported via standard deviation of NN intervals (SDNN) and root-mean square differences of successive RRI (RMSSD) (258).

During SB, both the amplitude of BP oscillations and HRV are increased (245). It has been hypothesized that it is the synchronization of these increased BP and heart rate oscillations which result in optimization of baroreflex function and increases in BRS, hence reducing BP during SB (172, 245). Indeed, both BRS (17, 20, 102, 118, 163, 215, 235, 239, 289) and HRV (17, 102, 215, 265, 276, 289) increase during SB. However, to the best of our knowledge, no

studies have evaluated the potential roles of baseline BRS and HRV as predictors of a subsequent SB-mediated BP response.

While the specific effects of inter-individual differences in baseline BRS and HRV on BP responses to SB remain unclear, the general determinants of BRS and HRV (i.e., within non-SB contexts) have been studied extensively. Variability in BRS appears to be highly correlated to several biological factors, including SBP (62, 149, 152), and BMI (126, 148, 158, 266), and possibly influenced by physical activity levels (60, 61, 144, 197, 198, 282). Heart rate variability may be associated with SBP (271, 280), BMI (8, 124, 138), and physical activity levels (60, 61, 96, 216, 240), among others. Thus, although not yet studied, the overarching hypothesis of this thesis is that, due to the purported importance of changes in BRS and HRV in the mediation of the blood pressure response to slow breathing (172, 245), the determinants of BRS and HRV (i.e. resting systolic BP, BMI, and physical activity levels) are the likeliest candidates for variables that may predict the effectiveness of slow breathing at reducing blood pressure within a given individual.

Therefore, the primary purpose of this study was to determine whether baseline values of BRS (specifically, up-BRS) and HRV (specifically, RMSSD) are predictors of the magnitude of the change in SBP during an acute 15-minute bout of device-guided SB in young healthy individuals. We hypothesized that higher baseline values of BRS and HRV would result in greater magnitude of decrease in SBP during SB. The secondary purpose of this study was an exploratory arm, which sought to investigate whether biological and lifestyle characteristics (resting SBP, BMI, and physical activity levels) are predictors of the SBP response to SB. We hypothesized that higher resting SBP and BMI, and lower physical activity levels, would result in increased magnitude of decrease in SBP during SB. Our findings are positioned to provide

insight into which variables affect the degree to which BP is reduced during SB. Additionally, improving our understanding of the physiological variables that contribute to this variability could help optimize device-guided SB strategies to maximize their BP-lowering effects. Consequently, we anticipate that this knowledge will allow researchers and clinicians to identify specific sub-populations for whom SB would be most effective as an adjunct treatment for the management of hypertension.

3. CHAPTER I: LITERATURE REVIEW

3.1. Clinical Relevance of Blood Pressure Regulation

Arterial BP is the force of blood exerted on artery walls as it circulates throughout the body. It is most commonly expressed as two values: systolic blood pressure (SBP) and diastolic blood pressure (DBP). During systole, the contraction of the left and right ventricles of the heart, oxygen-rich blood from the lungs is pushed into the aorta and pulmonary artery, respectively. This relatively high pressure exerted on the arteries is termed SBP. In contrast, diastole refers to the period of time in which the heart relaxes, allowing blood to refill the ventricles prior to the next contraction. This relatively low pressure is termed DBP.

In healthy individuals with a functioning baroreflex, these BP values are controlled within a tight range. The American Heart Association defines normal BP as SBP less than 120 mmHg and DBP less than 80 mmHg (299). Systolic readings below 110 mmHg in men and 100 mmHg in women are considered hypotensive (50). While extreme or unexpected drops in blood pressure can result in insufficient blood flow to vital organs (120), essential hypotension is not considered a disease state (53). As such, chronic moderately low BP is generally not a concern that requires clinical intervention (53). Conversely, chronic high blood pressure is a serious condition and one of the leading risk factors for mortality worldwide (113).

Defined as a blood pressure higher than 130/80 mmHg (299), hypertension damages the body over time by continuously placing stress on the vasculature (35). Compared to normotensive individuals, even individuals with elevated BP (between 120-129 mmHg SBP (299)) are at an increased risk of a wide array of downstream health concerns (122, 132, 174, 233, 270). Unfortunately, approximately one in three people worldwide (more than 1 billion people as of 2010) are hypertensive (194). In addition, 54% of those individuals are not aware of

their condition (194). Given that high BP is often asymptomatic (1, 194), elevated BP left unattended and untreated is a major risk factor for serious health conditions including but not limited to coronary heart disease (132, 174), stroke (157), heart attack (33), and organ failure (220).

Antihypertensive medication is a common treatment for hypertension (48, 106), as 92% of patients are prescribed antihypertensive drugs (294). Although pharmacologic treatments are effective in lowering BP (300), associations between antihypertensive medications and increased risk of developing various malignancies such as breast (153), renal (52), colorectal (256), and endometrial cancers (88) have been observed. Moreover, the pharmacologic management of hypertension places an enormous financial burden on the healthcare system (300). Recent estimates place the global financial burden of high BP at US $370 billion, or about 10% of the world's overall health-care expenditure (195). Considering that medication adherence is less than 50% (210), in combination with the high rising cost of medication (182), and the increased risk for adverse effects such as cancer (52, 88, 153) and myocardial infarction (232) (for drug-specific considerations, see (45)), there has been an increase in interest regarding alternative and adjunct approaches to lowering BP. Of these non-pharmacological adjunct treatments, we are interested in slow breathing, which decreases BP through a series of complex mechanisms that continue to be elucidated (245). To better understand the impact of slow breathing on BP and the mechanisms by which it affects BP, it is necessary to first understand the basic mechanisms behind BP regulation.

Briefly, BP is controlled *via* both chronic and acute pathways. The two most predominant mechanisms of chronic BP regulation are the renin-angiotensin-aldosterone system and antidiuretic hormone. These hormonal systems regulate BP by controlling blood volume and

peripheral resistance (for details, see (55, 260)). However, the focus of this literature review and research project is the acute regulation of BP. As such, the next section will explain the primary acute mechanism through which the body balances the dynamics of BP: the baroreflex.

3.2. Acute Regulation of Blood Pressure: The Baroreflex

3.2.1 Anatomy of the Baroreflex

The arterial baroreflex is responsible for acute changes in BP regulation. This homeostatic mechanism continuously monitors BP, keeping it tightly controlled within a small range (56). The arterial baroreflex is a negative feedback loop; it responds to any changes in the system by acting rapidly to restore BP levels to a given setpoint (explained in further detail below). These changes in BP are sensed by arterial baroreceptors, which are specialized mechanoreceptors located in the arterial walls of the carotid sinus and aortic arch. These receptors fire in response to changes in arterial pressure (69). In response to physical distention of arterial walls, baroreceptors increase afferent signalling to the nucleus tractus solitarius (NTS) located in the medulla oblongata of the brainstem (57, 58). Therefore, an increase in BP increases baroreceptor firing. Conversely, a decrease in BP elicits a reduction in baroreceptor firing, which is termed baroreceptor unloading. The NTS integrates afferent baroreceptor signalling and transmits the information to other medullary centers that control autonomic outflow. Through the two efferent arms of the baroreflex, parasympathetic and sympathetic, autonomic outflow influences cardiac output, blood volume, and peripheral vascular resistance to ultimately return BP to the setpoint (173).

3.2.2 Baroreceptor Unloading

The sympathetic and parasympathetic efferent arms of the baroreflex respond in tandem to BP changes by altering the function of target systems, thus restoring BP to its original setpoint. Broadly speaking, sympathetic nervous system (SNS) activation increases BP, and parasympathetic nervous system (PNS) activation decreases BP. While the SNS innervates both

the heart and peripheral vasculature, the PNS affects only the heart with respect to BP control. The SNS and PNS respond to fluctuations in BP by acting in an inverse and complementary fashion; that is, the activity of one system increases while the activity of the other decreases simultaneously. In the following example, we examine the response to an acute increase in BP.

When arterial pressure increases, transmural pressure likewise increases, which activates the baroreceptors in the aortic arch and carotid sinus and increases the baroreceptor afferent activity converging at the NTS. Increased afferent input to the NTS excites distinct centres in the brainstem associated with the PNS and SNS (297). This results in a cascade of downstream interactions that simultaneously increase PNS outflow and decrease SNS outflow to decrease BP.

Through the cardiovagal efferent arm of the baroreflex, activation of the PNS acts to decrease BP by decreasing heart rate (HR). Increased stimulation of the nucleus ambiguus by the NTS increases PNS vagal activity (297). The PNS innervates pacemaker cells in the heart through the release of the neurotransmitter acetylcholine. The binding of acetylcholine activates a downstream pathway that results in hyperpolarization of pacemaker cells, slowing their firing (200, 247). This decreases HR and lowers cardiac input (CO), thus leading to a decrease in BP.

On the efferent sympathetic arm, activation of the NTS results in excitation of the caudal ventrolateral medulla (CVLM) (173). This inhibits the activity of the rostral ventrolateral medulla (RVLM), which is the primary site controlling SNS activity (173). Thus, when BP is high, the baroreflex mediates reduced sympathetic outflow to the sinoatrial and atrial-ventricular nodes in the heart, resulting in decreased HR. Simultaneously, reduced RVLM activation decreases sympathetic outflow to the myocardium, the contractile unit of the heart. Hence, the force of ventricular contraction is decreased, and thus stroke volume is also reduced. Additionally, the efferent sympathetic arm of the baroreflex reduces BP by targeting the smooth

muscle component of peripheral vascular beds. Reduced sympathetic outflow decreases contraction of vascular smooth muscle cells of the tunica media, the smooth muscle layer surrounding arteries and arterioles, in the splanchnic, muscle and renal vascular beds (173). This reduced contraction of smooth muscle cells results in reduced vasoconstriction (196), which results in reduced vascular resistance within the peripheral vasculature and thus a systemic reduction in TPR. Therefore, by Purcell's equation (BP = CO x total peripheral resistance (TPR)), this reduction in TPR contributes to a reduction in BP.

In summary, both branches of the autonomic nervous system act in tandem in response to an acute increase in BP in order to return BP to its setpoint. This same reflex loop occurs to maintain baseline BP in response to an acute decrease in BP (i.e. SNS activation increases while PNS activity decreases in order to increase CO and TPR). The autonomic nervous system innervates both the heart and peripheral vasculature to target the two components of Purcell's equation (CO and TPR). While the PNS only targets CO through a decrease in HR, the SNS targets both the heart (and thus CO) and peripheral vasculature (and thus TPR). The differences in PNS and SNS modulation of HR will be explored in further detail in *Section 3.4.1*.

3.2.3 Baroreflex Resetting

There are cases in which the baroreflex is reset such that a new BP setpoint is achieved. This is termed "baroreflex resetting", and the setpoint around which BP is regulated by the baroreflex mechanisms outlined above is shifted to account for the requirements of the situation. One common example of acute baroreflex resetting is during exercise (192, 230, 231). Exercise is associated with increased skeletal muscle activity, which increases the oxygen demand to active tissues (65, 114). Thus, there is a need to increase blood flow during exercise to regions of increased metabolic demand while still maintaining blood flow to vital organs. Without

baroreflex resetting, increased demands on CO in terms of blood flow to active muscles would result in hypotension. As such, there is a well-established upwards resetting of the baroreflex during exercise, which results in a new, higher BP setpoint that is graded to the level of exercise intensity (24, 192) and thus to the blood flow demands of the exercise through the delivery of sufficient oxygen to active tissues (82).

While the baroreflex setpoint returns to its original state following the termination of exercise, this is untrue in disease states such as hypertension. Instead, in individuals with hypertension, the baroreflex is chronically reset as BP increases (70, 181, 189). Although the role of the baroreflex in the development of chronic hypertension continues to be debated (169), it is hypothesized that incomplete suppression of elevated sympathetic activity due to baroreflex dysfunction contributes to the high BP levels that characterize essential hypertension (142).

Regardless of setpoint value, the baroreflex remains a rapid system of regulating BP. The speed of this reflex can be attributed to its control by the PNS and SNS. When functioning normally, the baroreflex monitors and maintains appropriate BP values for the situation. Within a research setting, in order to determine proper function of the two autonomic arms of the baroreflex, the activity of the baroreflex must be quantified. This is performed using techniques called baroreflex sensitivity analyses.

3.3. Measuring the Baroreflex: Baroreflex Sensitivity

Baroreflex sensitivity (BRS), or baroreflex gain, is a measure of the baroreflex's ability to respond to acute changes in arterial BP (272). The terms "cardiovagal BRS" and "sympathetic (vascular) BRS" refer to the two efferent branches of the baroreflex (297). Cardiovagal BRS is quantified as the change in interbeat interval (ms) per unit change in BP (mmHg) (272), whereas sympathetic vascular BRS is quantified as muscle sympathetic nerve activity (usually in number of bursts per minute) per unit change in BP (mmHg) (75). In general, researchers have observed low to no correlation between the cardiovagal and sympathetic vascular BRS (75, 150, 206, 244), indicating that cardiovagal and sympathetic vascular BRS should be studied separately. As the focus of this project is cardiovagal BRS, sympathetic vascular BRS will not be detailed in this review (for summary, see the following paper (207)).

3.3.1 Cardiovagal Baroreflex Sensitivity

Cardiovagal BRS is used as an index of autonomic control of heart rate (297). Specifically, it reflects autonomic activity on the sinus node (74). Even so, this index is almost universally used to quantify baroreflex "vigour" (272), and it is from this measure that we extrapolate and assess baroreflex function. Also termed the cardiac parasympathetic baroreflex, the cardiovagal arm is associated with the PNS and vagal nerve. It has been described primarily as the "sensitivity of baroreflex control of the heart" (43). The cardiovagal arm responds to SBP fluctuations by eliciting changes in HR to restore overall BP (136), as explained in *Section 3.2*. Parasympathetic efference from the baroreflex does not directly affect peripheral vasculature, and as such, it is possible to infer cardiovagal BRS activity solely from the observed fluctuations of HR and SBP (272). Researchers have developed diverse cardiovagal BRS measurement methods (272). As there is still debate regarding the reproducibility and limitations of the current

263 "gold standard" measure of cardiovagal BRS by the Oxford method (16, 59, 218, 275), the
264 following sections will describe the most common methods.

265 *3.3.1 Methods of Assessing Cardiovagal Baroreflex Sensitivity*

266 Classic invasive methods of cardiovagal BRS assessment use pharmacological agents
267 such as phenylephrine and sodium nitroprusside to artificially induce changes in SBP (29, 244,
268 269). In 1969, Smyth et. al first described the 'Oxford method' (269) of assessing rising
269 cardiovagal BRS. However, the original Oxford method was limited in that it was only able to
270 assess baroreflex responses to rising arterial BP. The Oxford technique was later superseded by
271 the improved 'modified Oxford technique' (86). The modified Oxford technique (244) is widely
272 considered the gold standard method of BRS assessment in humans (147) and employs the
273 infusion of vasoactive drugs, which induce increases and decreases in BP without affecting HR
274 (86, 244). This "open loop" method allows for the assessment of BRS over a wide range of SBP
275 values, well beyond the normal operating range of a human at rest. That is, lower SBP values
276 achieved following sodium nitroprusside infusion and higher SBP values achieved following
277 phenylephrine infusion together enable researchers to attain an expanded view of the sigmoid
278 BRS curve (86, 89). However, there is debate regarding the validity of this method as a gold
279 standard for BRS measurement (218). Indeed, some researchers argue that the forced increase in
280 SBP inhibits SNS activity to such an extent that sympathetic vascular BRS at high BPs is
281 difficult to quantify with this approach (75). Regardless, the modified Oxford technique has
282 provided direct insights into baroreflex dysfunction in a variety of patient populations such as
283 individuals with elevated BP (29, 98), myocardial infarction (211, 251), and mild to moderate
284 heart failure (212), among others.

While pharmacological methods of BRS assessment have helped elucidate arterial baroreflex function (219), less invasive techniques have been applied by researchers for the assessment of spontaneous baroreflex function. Beginning in the 1980s, algorithmic, non-invasive methods for quantifying BRS were first introduced (221). Spontaneous methods of BRS assessment differ from methods using pharmacological infusion in that spontaneous methods do not rely on external perturbations to the cardiovascular system (221). Instead of actively stimulating a change in SBP and measuring the resultant vagal response (i.e. known as an "open loop" method), these methods use a "closed loop" method to non-invasively determine spontaneous cardiovagal BRS from data extracted from naturally occurring fluctuations in BP and HR, typically under resting conditions. As such, spontaneous BRS measurement methods enable assessment of BRS over the range of BPs that occur naturally during daily life (125, 219). While these methods measure BRS over a narrower range of BPs compared to the modified Oxford method, their prevalence and use has risen significantly due to their increased technical feasibility in human participants (219) relative to pharmacological methods.

Spontaneous BRS measurement methods can be separated into two categories: methods in the time domain and methods in the frequency domain (147, 225). In the time domain, most approaches to BRS measurement rely on the sequence method described by Parati and colleagues (155, 217). The sequence method requires the identification of three or more consecutive heart beats wherein SBP increases and R-R interval (RRI) simultaneously lengthens (i.e., up-BRS), or SBP decreases and RRI simultaneously shortens (i.e., down-BRS) (217). A linear regression is calculated from the relationship between change in RRI (ms) and change in SBP (mmHg), and BRS is taken as the slope of the fitted line (272). While the central idea among variant methods is the same, alternative methods in the time domain differ in the

threshold determining what constitutes a valid SBP-RRI sequence as well as the specific BP measurements used in place of or complementary to SBP (272).

In the frequency domain, spectral methods are used to analyse HR and BP signals to obtain BRS measurements. The signals are mapped to the frequency domain *via* transfer function. As such, spectral methods can be used analyse specific frequency bands. The amplitude of the transfer function is taken as the measurement of BRS (272). This is analogous to the SBP-RRI slope in time domain methods (272). Frequency domain methods are based on the premise that the arterial baroreflex instigates BP oscillations that elicit RRI oscillations at the same frequency due to their closed-loop relationship (183). For a detailed review of common methods of BRS measurement employing spectral techniques, see (221) and (297).

Researchers have debated the limitations of using spectral (i.e. frequency domain) methods over non-spectral (i.e. time domain) methods (167, 297). While analyses in the frequency domain enable researchers to study specific frequency bands, which may provide additional insight into the influence of respiratory and autonomic components (214) on BRS, spectral methods generally require longer periods of data to be analysed than non-spectral methods (297). Researchers have observed high variability between spectral and non-spectral measurements of BRS (49, 175, 228). Additionally, some researchers have observed poor correlations between spectral methods and more invasive methods of BRS measurements such as those described above (13, 49, 167, 175, 227, 228, 234). However, some researchers have observed positive correlations between BRS measurements resulting from spectral methods and those resulting from the Oxford method (214, 242, 296), suggesting that spectral methods are indeed viable alternatives to pharmacological methods of BRS measurement. Nevertheless, it is

currently unclear if spectral methods of measuring BRS are valid for use across clinical populations (49, 167, 175, 228).

While the validity of both spectral and non-spectral methods as substitutes for the modified Oxford method approach to BRS measurement has been debated (167, 175, 177, 222, 228, 296) in part due to high observed systemic deviance (89) and inconsistent estimates across various methods of spontaneous BRS measurement (155), it nevertheless appears that researchers will continue to employ spontaneous methods of BRS measurement. Even now, new spontaneous methods of BRS measurement continue to be introduced (44, 165, 298). It has been suggested that spontaneous methods of BRS measurement provide insight into daily baroreflex function that is complementary to understanding generated by pharmacological methods of BRS measurement (221). Given the additional benefits of spontaneous methods of BRS measurement such as increased feasibility (219), reproducibility of results (125, 184), reduction of measurement variability (147), and ability to measure BRS in a "daily life" setting, spontaneous methods of BRS measurement can still provide important prognostic information in clinical practices.

Through the use of both pharmacological and spontaneous methods of BRS measurement, researchers have determined that BRS can be a clinically useful tool in understanding the regulation of BP in health and disease (147). Low BRS is often a predictor of cardiovascular disease. Indeed, studies have demonstrated that reduced BRS is found in individuals with hypertension (29, 79, 223), coronary artery disease (79, 115, 130), and heart failure (79, 146). Additionally, low BRS is predictive of high risk of cardiac mortality following myocardial infarction (145). In individuals with diabetes and obesity, low BRS values have also been observed (79). Indeed, significant clinical evidence supports the prognostic use of BRS.

However, current methods of BRS assessment are often affected by the substantial and detrimental effect of signal noise (286), which can be reduced by improving our understanding of the underlying signals involved BRS measurement such as HR and BP.

3.4. Cardiorespiratory Phenomena Related to Blood Pressure

The relationship between BP, HR, and respiration is termed cardiorespiratory coupling (66). Interactions between these variables result in physiological phenomena termed HRV and respiratory sinus arrythmia (RSA). These physiological phenomena and their effects are complex and bidirectional. Understanding the interactions between cardiorespiratory coupling, HRV, and RSA will be equally important for later sections of this literature review, as the premise of this research project relies heavily on insights from these physiological relationships. As such, these physiological relationships will be explained in the following sections.

3.4.1 Heart Rate Variability

While HR is defined as the average number of heart beats per minute, HRV is the measure of the variation between individual, successive heart beats. Specifically, HRV is calculated from the small differences (in milliseconds) between each RRI (188). By analysing these beat-by-beat changes, researchers can gain further insight into the underlying physiological mechanisms that govern HR and thus BP.

In healthy individuals, HR is regulated by the sympathetic and parasympathetic branches of the autonomic nervous system (258). Without autonomic efferent input, the sinoatrial node generates an intrinsic HR of 90-100 bpm; however, the relative activity of the sympathetic and parasympathetic branches of the ANS modulates this intrinsic HR (209). At rest, autonomic regulation of HR is dominated by parasympathetic activity, which decreases the intrinsic rate of the sinoatrial node and results in an average HR of 75 bpm (258). Interestingly, parasympathetic modulation of HR is faster than that of the SNS (295). While the effects of SNS stimulation appear following >5s delay, the effects of a single parasympathetic vagal efference can be observed after only one or two heartbeats (103, 205). Therefore, rapid (i.e. within 1-2 seconds)

decreases and increases in HR are caused primarily by elevations and withdrawals of parasympathetic activity, respectively. Parasympathetic and sympathetic efference are modulated by the nucleus ambiguus and RVLM medullary integration sites, respectively (173, 297). Sensory input from proprioceptors, chemoreceptors, baroreceptors, the cerebral cortex, and the limbic system is integrated at the NTS, and PNS and SNS outflow are subsequently adjusted by their respective systems to modulate HR (259) (previously explained in detail in *Section 3.2.2*). Consequently, researchers consider HRV a reflection of the net effect of PNS and SNS outflow and thus a measure of the relative activity of the PNS and SNS (258). Indeed, HRV has been used as a measure of cardiac autonomic regulation in both healthy and clinical populations, which will be discussed later in this section. The following section will summarize the common methods by which HRV is quantified.

There are two categories of methods by which HRV is analysed: frequency domain analysis and time domain analysis (253). Methods in the frequency domain are analysed by power spectral density analysis, which is similar to the spectral methods of BRS measurement described previously (see *Section 3.3*). Briefly, ECG signals are mapped to the frequency domain and separated into component waveforms (253). The amplitude of each component waveform corresponds to the power of the associated frequency band. That is, a component waveform hidden in the variability of the ECG signal can be identified by the increased power of a frequency band (253). Two frequency ranges (also termed "bands" or "components") are commonly analysed: low-frequency (LF) and high-frequency (HF) bands. The HF band ranges from 0.15-0.40 Hz and is often termed the "respiratory band" due to the significant influence of respiration on HF band power (258). Activity in the HF spectrum (i.e., the HF band) is also used as an index of vagal activity (283) due to the rapid influence of the PNS on HR regulation (258).

Additionally, vagal blockade eliminates HF activity (179, 229), indicating that HF activity is generated primarily by parasympathetic outflow. Parasympathetic association with the HF band is supported by evidence indicating that increased sympathetic innervation is correlated with decreased HF activity (31, 117, 203). Conversely, LF band activity ranges from 0.04-0.15 Hz and is believed to reflect baroreceptor activity at rest (178). However, there is still debate regarding the interpretation of the LF band (258). While some researchers suggest that LF activity represents solely cardiac SNS innervation (for review, see: (241)), many have challenged this claim and instead suggest that LF activity is associated with cardiac autonomic outflow modulated by the baroreflex (78, 97, 236, 278). Regardless, while the exact interpretation of the LF band is still being debated, the current literature generally accepts LF band activity as a marker of simultaneous activation of both the PNS and SNS (139). For an extensive review of LF band interpretation and further information on HRV analysis methods in frequency domain, see (258).

The quantification of HRV *via* time domain methods involves the application of statistical or geometric approaches to continuous ECG recordings to generate indices of various HRV components (253). Compared to frequency domain methods, time domain markers are simpler to calculate and require less computing power (258). However, time domain analyses cannot provide insight into autonomic activity or the activity of other constituent oscillatory physiological control systems (258). Markers in the time domain are based on normal-to-normal intervals (NN intervals), which are defined as RRI with abnormal R-peaks corrected or removed (47). There are myriad variables used in time domain methods, but the two most widely reported and commonly assessed are the standard deviation of NN intervals (SDNN) and root-mean square differences of successive RRI (RMSSD)(258), both of which will be used in the data

analysis of this project. Total variability of HR from both periodic and random factors is quantified by SDNN (253). Meanwhile, RMSSD reflects short term variation (i.e. beat-to-beat HR variance) and is strongly associated with parasympathetic or HF variations in HR (253, 257). Additionally, RMSSD is the most commonly used time domain measure for estimating changes in HRV mediated by the PNS (258). For a comprehensive overview of the other metrics and norms of HRV see (253, 257)).

At the intersection of heart, brain, and autonomic nervous system interactions, the many metrics of HRV are considered autonomic markers of clinical interest (253). Metrics of HRV are commonly used by researchers and clinicians as a tool for both diagnostic and prognostic applications. Indeed, researchers consider HRV a measure of the flexibility and adaptability of various regulatory systems. High HRV is believed to reflect an increased ability of the heart to adapt to both internal and external stressors (237). For example, HRV is higher in aerobically-trained athletes compared to healthy controls (9, 291). Conversely, low HRV has serious health implications and is an independent risk factor for mortality in middle-aged men (64), the elderly (281), diabetics (129), and post-myocardial infarction patients (137). Additionally, HRV is low in patients with heart failure (26, 248), brain death (134), following myocardial infarction (38) and heart transplantation (252), as well as in individuals who smoke (180), diabetic individuals (81, 243), and obese individuals (266). For an extensive review of the applications and findings of HRV in clinical populations, see (253) and (237).

While HRV can be a useful tool for investigating autonomic control of HR, the interpretation of any index of HRV is complicated by other external influences that must also be taken into consideration. Most notably, it is necessary to discuss the influence of respiration on HRV, which occurs in the form of RSA.

3.4.2 Respiratory Sinus Arrythmia

Respiratory sinus arrythmia is a non-pathological, physiological phenomenon characterized by phasic HR fluctuations that occur synchronously with respiration. Specifically, RSA refers to the shortening of RRI during inhalation and the prolonging of RRI during exhalation (302). These oscillations are directly associated with HRV fluctuations and reflect direct interactions between the respiratory and circulatory systems (277, 302). Indeed, RSA becomes pronounced at lower respiration frequencies and is associated with HF HRV spectrum power (258). This relationship between RSA and HF HRV parasympathetic activity has generated interest in the quantification of RSA among cardiovascular researchers (302). That is, by examining measures of RSA, it is possible to evaluate cardiorespiratory autonomic control mechanisms through non-invasive means (100). Indeed, RSA is generally accepted as a non-invasive index of cardiac vagal activity in physiological, psychological, and clinical research, and it is interpreted as a measure of the influence of respiration on the sinoatrial node of the heart (21, 302). Its prevalent use in research stems in part from the benefits of the non-invasive methodological approach for quantifying RSA. Interestingly, RSA is commonly used in the field of psychophysiology as a measure of parasympathetic-related behaviour such as “emotional reactivity” (36). In general, high RSA is taken as an index of good general health. In fact, athletes and frequent practitioners of yoga have higher RSA than age-matched controls (68, 110). Moreover, RSA is blunted in individuals with diabetes (168) and coronary artery disease (3). However, while the use of RSA in research is quite common, the mechanisms generating RSA and its physiological role have yet to be fully established (15, 154).

A consensus has not been reached on the precise physiological mechanisms governing RSA, but RSA is likely influenced by both neural and physiological mechanisms (245). The

individual components of RSA are difficult to study due to the complicated cyclic and interdependent nature of cardiorespiratory mechanisms (154). Nevertheless, researchers have attained a fundamental understanding of RSA mechanisms governed by the respiratory, central nervous, and cardiovascular systems (156, 226, 245). The following sections will summarize the most prevalent theories of the mechanisms of RSA.

The central theory is a prominent and well-accepted autonomic theory of RSA generation (154). The central theory involves the influence of respiration on autonomic modulation of HR, which has been termed "respiratory gating" (171). Specifically, the "gate" refers to the modulation of cardiac vagal neuron responsiveness to external input. That is, during inspiration, activation of the chemoreflex results in activation of the respiratory center in the medulla (76). This results in the hyperpolarization of cardiac vagal neurons, which subsequently become unresponsive to baroreflex stimuli, hence "closing" the gate (94). This prevents completion of the baroreflex negative feedback loop, which contributes to increased HR during inhalation. Conversely, the gate "opens" during exhalation, and HR is decreased through reduced SNS and increased PNS innervation of the heart. The central theory supports the position that RSA generation is primary modulated autonomically.

Additionally, RSA may also be modulated by afferent feedback from pulmonary stretch receptors (7). During inhalation, mechanoreceptors in the lungs slowly activate and initiate afferent autonomic feedback to the nucleus ambiguus (21, 245), resulting in reduced cardiac vagal stimulation and decreased HR (133). Interestingly, the degree of vagal withdrawal is proportional to the volume of the breath (123). Indeed, studies have demonstrated a 53% decrease in RSA of double-lung transplant patients following vagal denervation compared to

493 healthy controls, supporting the notion that afferent feedback from pulmonary stretch receptors
494 contributes to generation of RSA (273).

495 Lastly, RSA may be mechanically mediated by intrathoracic pressure changes driven by
496 diaphragm muscle activity (7, 19). During inspiration, the diaphragm contracts downwards to
497 expand the lungs, thus generating a negative pressure in the thoracic cavity (19). Briefly, these
498 changes in intrathoracic pressure can subsequently affect venous return, stroke volume, and
499 cardiac output (10, 245). During inspiration, negative intrathoracic pressure stretches the
500 sinoatrial node and increases HR (154). This contributes to a decrease in BP, which is detected
501 by baroreceptors and results in a cascade of signals that further increase HR (as described in
502 *Section 3.2*). However, research supporting the contribution of intrathoracic pressure changes on
503 the mechanisms of RSA is inconclusive, and this theory continues to be fiercely debated (77,
504 127, 128).

505 In summary, the mechanisms contributing to the generation of RSA are complex and
506 incompletely understood. There is significant evidence for both autonomic and respiratory
507 influences on the mechanisms governing RSA (19, 21, 40, 41, 76, 245, 268, 273, 305). However,
508 individual variability in RSA from respiration-based studies confounds researchers (99, 100),
509 some of whom have observed that control of RSA amplitude and control of absolute vagal tone
510 are distinct (104, 154). In addition, researchers are currently divided regarding the contribution
511 of the baroreflex to RSA due to the speed of the response (76, 154, 302). Given the complexity
512 of the processes surrounding RSA, researchers speculate that the baroreflex exerts an effect on
513 the underlying mechanisms of RSA regulation (154), although the extent remains to be
514 determined. Unfortunately, due to the complexity of the linked interactions, it is difficult to
515 separate and study the effects of these influences independently. Nevertheless, there remains

516 considerable scientific interest in the mechanisms of RSA, and increasing our understanding of
517 RSA will significantly aid in elucidating the mechanisms of slow breathing.

3.5. Slow Breathing

Slow breathing (SB) is defined as a respiration rate between 4-10 breaths per minute (bpm) (245). This is considerably lower than the rate of spontaneous breathing, which is between 12-18 bpm (14). Respiration at this reduced rate is associated with a variety of increased physiological and psychological health benefits, most notably a reduction in BP (191, 245). Decreased BP mediated by SB has been observed in healthy individuals (2, 66, 199) as well as clinical populations such as individuals with hypertension (118), type 1 and 2 diabetes (18, 249), and post-traumatic stress disorder (84). However, SB is by no means a new phenomenon. While the research and clinical interest in SB has increased relatively recently, reported benefits of SB have been observed throughout history (245).

3.5.1 Brief History of Slow Breathing

Indeed, SB has been practiced for thousands of years as a component of meditation and yoga (255). In eastern cultures, one of the first mentioned instances of SB is *pranayama* breathing and its derivatives, which refer to breath control in a yogic context (245). However, research interest in SB developed only in the mid-20th century, shortly after its introduction in western countries in the late 1800s (245). While respiration-based treatments for diseases such as asthma already existed in western cultures, these treatments have been primarily characterised as methodological relaxation exercises as their reported efficacy has been largely anecdotal. The most notable respiration-based treatments include the Buteyko and Papworth methods (coined by physiologist Konstantin Pavlovich Buteyko and doctors at the Papworth Hospital respectfully (111, 131)). Unfortunately, there is a dearth of published clinical trials investigating these early methods (34, 111). Nevertheless, following the introduction of SB in western medical communities, scientific research interest in SB has increased considerably.

As research interest in SB grew, devices were designed to facilitate SB practices. Researchers developed device-guided breathing and biofeedback devices specifically for clinical use (for review, see: (90)). As well, a subfield of tools focussed on "cardiorespiratory biofeedback" was pioneered by researchers Lehrer and Vaschillo (reviewed in: (159)). In the consumer market, the most prominent commercially available SB device is currently the RESPeRATE (Intercure Ltd., Israel) (39, 151), which induces SB by monitoring respiration in real-time and slowly reducing respiration rate via auditory cues to an individualized frequency. The RESPeRATE device has been recommended by the Food and Drug Administration and is the only commercially available SB device recommended by the American Health Association as an adjuvant treatment for hypertension (30). A systematic review and meta-analysis of the efficacy of the RESPeRATE device in lowering BP found that the RESPeRATE acutely decreases SBP by 3.67 mmHg and DBP by 2.51 mmHg (176). The effect of device-guided SB on BP has been also analysed recently by several other review papers (39, 151), indicating a growing interest in device-guided SB.

Indeed, the first decade of the 21st century has seen an emergence of literature regarding device-guided SB, SB mechanisms, and the clinical benefits of SB in both clinical populations and healthy individuals (245). Studies investigating acute BP reductions mediated by SB have observed BP reductions in populations with type 1 and 2 diabetes (18, 249), hypertension (118), post-traumatic stress disorder (84), and even healthy normotensive individuals (2, 66, 199). A recent meta-analysis of 608 participants with hypertension reported an average decrease of 4 mmHg SBP and 3 mmHg DBP by chronic practice of device-guided SB (30). However, a 2014 meta-analysis with inclusion criteria excluding studies without randomized controls observed no

significant effect of device-guided SB in patients with hypertension (287). These conflicting results indicate a need for further research regarding the long-term effects of SB.

Research investigating the mechanisms of SB is still being conducted. It has been suggested that the positive health effects of SB stem from a wide range of physiological factors. These include a shift in autonomic balance towards parasympathetic dominance (204), increased BRS (17), and increased ventilation gas exchange efficiency (245, 262). However, there currently exist multiple theories regarding the mechanisms through which SB reduces BP. The following section will summarize the most prevalent theories.

3.5.2 Proposed Mechanisms of Slow Breathing

In part due to our incomplete understanding of cardiorespiratory phenomena including RSA (previously described in *Section 3.4.2*), there currently exist multiple, possibly co-dependent theories regarding the mechanisms surrounding SB (245).

Firstly, SB may cause a shift in autonomic balance from sympathetic regulation toward parasympathetic regulation, thus contributing to a reduction in BP by pathways described in *Section 3.2* and *Section 3.4.2*. It has been suggested that decreases in SNS activity are mediated by lung stretch receptor activation. As respiration rate decreases, the accompanying compensatory increase in tidal volume results in increased activation of cardiopulmonary stretch receptors, thus reducing sympathetic activity (245). Indeed, decreases in sympathetic activity following lung inflation have been observed in artificially ventilated anesthetized cats (91). However, feasible methods for quantifying human cardiopulmonary stretch receptor activity are limited (285). Therefore, lung inflation-mediated decreases in SNS are difficult to experimentally validate in humans. Nevertheless, there is evidence to support the contribution of autonomic factors to SB-mediated decreases in BP. Studies have demonstrated acute decreases in

sympathetic outflow following SB in normotensive populations (2, 208, 254), patients with hypertension (63, 108, 202, 261), and patients with COPD (239). Therefore, it is proposed that lung inflation-mediated decreases in SNS leads to vasodilation of peripheral vasculature, thereby reducing peripheral resistance and BP (116).

It has also been suggested that the coherence of BP and HR oscillations contributes to the reduction of BP during SB (245). Specifically, researchers theorize that the synchronization of BP oscillations and respiration-mediated HR oscillations around 0.1 Hz (6 bpm) lead to the amplification of both of these oscillations (187), which lead to enhanced baroreflex efficiency (239). That is, SB entrains cardiovascular oscillations, resulting in increased BRS (20). Some researchers speculate that BP decreases during SB may result from this cardiovascular oscillation entrainment (17, 302). Indeed, SB increases both cardiovagal BRS (20, 118, 163, 235, 239) and HRV (6, 17, 102, 215, 265, 276, 289). In 1964, Angelone and Coulter first observed the maximization of HRV amplitude (i.e. maximum HR fluctuation amplitude change in BPM) in healthy men at approximately 0.1 Hz (6). Since then, investigation of HRV maximization as a mechanism of SB has been extensively explored by researchers Vaschillo and Lehrer, who coined the terms *resonance* or *resonant frequency* (162). Resonance frequency is defined as the respiration frequency at which resonance of the cardiovascular system is optimized, which is approximately 0.1 Hz in humans (162, 258). They additionally propose that resonance frequency is personalised and likely dependent on an individual's baseline anthropometric measurements, such as height, as well as biological sex (288). They have demonstrated that breathing at this personalized resonance frequency, which varies in adult humans from 4.5 to 6.5 breaths per minute, results in cardiorespiratory resonance, maximization of HRV, and increased BRS (290). Recent studies investigating the effect of SB at an individualized respiration frequency have

609 demonstrated promising but inconclusive results with respect to BP decreases (160, 213, 292).
610 Therefore, it is currently unclear to what degree resonance frequency affects BP responses to SB.
611 Further research is required investigating how synchronization of cardiorespiratory oscillations
612 associated with the vascular baroreflex control loop affects BP decreases during SB.
613 Lastly, it is hypothesized that RSA plays a central role in BP reductions during SB, but its
614 exact mechanistic contributions are currently unclear. Slow breathing at 0.1 Hz (6 bpm)
615 maximizes RSA (6, 110, 163). It is thought that maximization of RSA (i.e. the phasic respiratory
616 modulation of the aforementioned HR and BP oscillations) contributes to the coherence of these
617 oscillations and thus increased BRS (83). Additionally, increases in RSA are postulated to
618 increase the efficiency of pulmonary gas exchange (105). It is hypothesized that RSA conserves
619 energy at rest by enabling synchrony of alveolar ventilation and capillary perfusion, thus
620 increasing gas exchange efficiency (104). A large body of evidence directly supporting this
621 theory is derived from invasive studies in dogs (100, 301, 303), but recent human studies have
622 similarly concluded that RSA is associated with increased gas exchange efficiency (92, 93).
623 While promising, further investigation is required to validate the role of RSA in the mechanisms
624 of SB as understanding in the mechanisms of RSA is likewise incomplete.

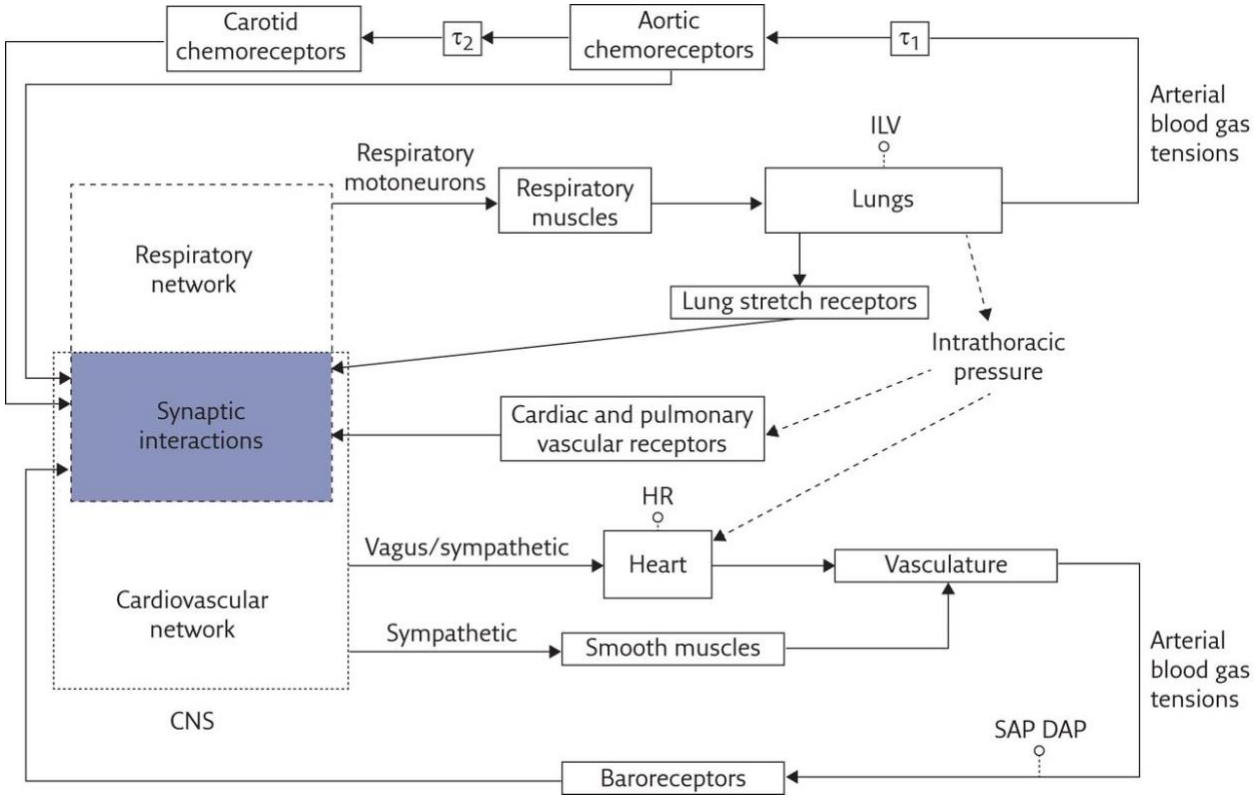


Figure 1: Many cardiorespiratory pathways are proposed to be relevant to SB-induced reductions in BP. τ: circulatory delay; ILV: instantaneous lung volume; HR: heart rate; CNS: central nervous system; SAP: systolic arterial pressure; DAP: diastolic arterial pressure (245).

In conclusion, the mechanisms governing SB are incompletely understood and clearly complex (see *Figure 1*). Whether the changes that occur to BRS and HRV during SB are a cause, consequence, or simply a correlate of SB remain to be determined. Regardless, it is clear that further research into all facets outlined in *Figure 1* is required to understand the mechanisms governing SB. Through this research study, by examining the effect of biological variables and autonomic measures on the BP responses to SB, we aim to increase current understanding in this field.

3.6. The Effect of Biological Determinants of Autonomic Measures Governing Blood Pressure Responses to Slow Breathing

While the precise pathways by which SB reduces BP continues to be elucidated, it has been speculated that BRS and HRV contribute mechanistically to SB-mediated BP reductions (245). As summarized in *Section 3.5.2*, the amplitude of BP oscillations and HRV are increased during SB (245). It has been hypothesized that the synchronization of these BP and HR waveforms increases BRS and contributes to the reduction in BP during SB (245). However, there has been little research examining baseline BRS and HRV as determinants of BP response during SB. Conversely, researchers have extensively investigated the determinants of BRS and HRV. Although this has yet to be studied, we hypothesize that the determinants of BRS and HRV may affect the degree to which BP is reduced during SB. That is, we propose that the biological determinants of BRS and HRV may contribute to the effectiveness of SB at reducing BP. As such, the following sections will summarize key findings regarding the biological determinants of BRS and HRV as well as the potential influence of these determinants on SB-mediated reductions in BP.

3.6.1 Biological Determinants of Cardiovagal Baroreflex Sensitivity

As previously discussed in *Section 3.5.2*, increased cardiovagal BRS is a probable mechanism by which BP is reduced by SB (245), as demonstrated by cardiovagal BRS increases from baseline during acute bouts of SB (18, 20, 118, 119, 163, 235, 239, 241). However, in the bulk of SB literature, BRS has been examined as a dependent outcome of SB. Consequently, there is a lack of research examining the effect of baseline cardiovagal BRS as a determinant of SB-mediated BP response. Nevertheless, researchers have investigated potential determinants of cardiovagal BRS, and we speculate that these determinants may play a role in the degree to

660 which SB reduces BP. As such, the following section will summarize the current literature
661 investigating the biological determinants of cardiovagal BRS.
662 Biological variability in spontaneous cardiovagal BRS has been attributed to age, HR,
663 SBP, DBP, body mass index (BMI), and sex (126). A study of 1100 individuals reported that
664 decreased cardiovagal BRS is associated with increased age, HR, SBP, DBP, and BMI (126).
665 Other studies have likewise demonstrated that cardiovagal BRS decreases as participants age
666 (54, 71, 98, 238, 263), HR (42, 62, 98, 152), BMI (126, 148, 158, 266), and SBP and DBP (62,
667 149, 152). While these determinants of cardiovagal BRS have been investigated and are
668 relatively well established, the effect of sex as a determinant of cardiovagal BRS is still being
669 debated. While some researchers have reported no impact of sex on cardiovagal BRS (62, 75,
670 126, 274, 275), other studies demonstrate that females present with lower cardiovagal BRS than
671 males (23, 46, 149). These varying results on the effect of sex on cardiovagal BRS may be due to
672 underlying sex differences in baroreflex control (87, 121, 135, 264). Specifically, sex differences
673 in vascular mechanics within the aortic arch and carotid sinus may contribute to sex differences
674 in cardiovagal BRS. Indeed, Klassen and colleagues examined the difference in cardiovagal BRS
675 between females and males and demonstrated that sex differences in cardiovagal BRS were
676 eliminated after controlling for vascular mechanisms of sex differences (136). That is, no sex
677 differences in cardiovagal BRS remained once the impacts of these mechanics were eliminated.
678 Nevertheless, further research is required to validate their findings. Additionally, a positive
679 relationship between physical activity and cardiovagal BRS has been demonstrated in both males
680 (144, 197, 198, 282) and females (60, 61) compared to sedentary peers. However, a few studies
681 (164, 170) as well as the aforementioned meta-analysis (126) observed no statistically significant
682 effect of physical activity levels on cardiovagal BRS when analysed in the context of a

683 multivariate model. In summary, variability in cardiovagal BRS appears to be highly correlated
684 to age, resting HR, BMI, SBP, and DBP, and is possibly affected by sex differences and physical
685 activity levels. Interestingly, researchers have observed a positive association between
686 spontaneous cardiovagal BRS and HRV (60, 61, 115, 140, 161, 163, 282). Indeed, BRS and
687 HRV appear to share many common determinants. As such, the following section will
688 summarize the biological determinants of HRV.

689 *3.6.2 Biological Determinants of Heart Rate Variability*

690 As outlined above, SB may reduce BP by achieving an optimal balance between the
691 sympathetic and parasympathetic nervous systems (i.e. higher PNS activity and/or lower SNS
692 activity (245)). As summarized in *Section 3.4.1*, HRV is used as both an indirect index of
693 autonomic outflow and an indication of overall cardiac health. As such, the determinants of HRV
694 have been well-described. However, research examining the biological determinants of HRV in
695 the context of SB remains in its infancy. Therefore, examining the determinants of HRV may
696 increase our current understanding of which variables affect SB-mediated BP responses.

697 The most well-established determinants of HRV are age and resting HR (280). Most
698 notably, a study by Tsuji and colleagues of 2722 human subjects demonstrated that age and HR
699 are strong independent determinants of HRV (280). Other studies have likewise demonstrated a
700 strong negative association between age and HRV (8, 60, 141, 143, 166, 267, 284, 304),
701 including a study examining 1208 individuals from four European countries (271), which
702 suggests that the association between age and HRV is independent of ethnic differences within
703 this region. The same study found that HR and HRV were independently similarly associated
704 with biological and lifestyle determinants (271). The role of HR as an independent predictor of
705 HRV is also supported by other studies (8, 143). In conclusion, HRV shows a strong negative

association with age and HR, indicating that those with higher HR and older age have lower HRV.

While age and HR continue to be the strongest observed determinants of HRV (304), researchers have also observed sex differences in HRV (8, 139, 166, 267, 280, 284, 304). The independent effects of sex and age on HRV were investigated by Umetani and colleagues, who observed lower HRV measures in females under 30 years of age compared to males of similar age (284). Sex differences in HRV may be due to sex differences in autonomic control (12, 46, 51). Indeed, researchers observed in a 2016 meta-analysis that, despite having higher HRs on average, females display a relative dominance of parasympathetic activity in HRV compared to males (139). However, Umetani and colleagues observed a decrease in sex differences in HRV between 30 years and 50 years of age, and there were no observed sex differences in HRV after age 50 (284). The authors suggest that higher levels of sympathetic activity in young males and the decrease in sympathetic activity with age may contribute to sex differences in HRV after age 50; however, physical activity levels were not controlled in their analyses, which may have affected observed sex differences in HRV in young populations (284). Further research investigating sex differences in HRV in populations aged 50 and over is required. In summary, it currently appears that HRV is higher in males compared to females until approximately 50 years of age, after which sex differences in HRV disappear.

Notably, the authors of the same 2016 meta-analysis investigating sex differences in HRV also observed a strong correlation between HRV and physical activity (139). However, many studies failed to report physical activity levels, thus rendering the authors unable to control for physical activity in their meta-analyses (139). Nonetheless, several other studies have observed a positive relationship between physical activity levels and HRV (60, 61, 96, 216, 240).

The mechanism behind this association is currently unclear. While it has been suggested that high physical activity levels lead to increased vagal tone (101), as demonstrated by resting bradycardia in athletes (25), some researchers have challenged this hypothesis and instead suggest that other mechanisms result in the bradycardia responsible for increased HRV in athletes (28). In the aforementioned study including 1208 individuals, weak associations were observed between HRV and lifestyle factors such as physical activity levels (271). However, the discrepancy between these results and studies observing a positive relationship between physical activity levels and HRV may be due to the presence of other confounding variables as well as non-standardized methods of quantifying physical activity levels (216). As such, further research is needed to understand the direct effect of physical activity on HRV.

Studies have demonstrated weak or unclear results regarding the associations between HRV and BMI (8, 124, 138, 279), DBP and SBP (271, 280), and ethnicity (109, 216, 271). While reduced HRV has been observed in obese subjects (124) and non-obese individuals with higher BMI (138), other studies have observed no effect of BMI on HRV (8) or no difference in HRV between underweight and overweight in healthy individuals (279). Independent analyses of DBP and SBP in a meta-analysis observed that DBP values $\geq$ 90 mm Hg and SBP values $\geq$160 mm Hg were associated with lower and higher HRV, respectively (280). However, the effects were not significant following stepwise regression analysis (280), indicating that variability in HRV was attributable to age and heart rate. Similarly, another study observed a very low independent effect of SBP on HRV (271). In regard to ethnicity, a systematic review and meta-analysis found that HRV was greater in African Americans relative to European Americans even after controlling for covariates (109). However, no difference in HRV were observed between participants from separate European countries (216, 271). Together, these data indicate that the

752 degree to which BMI, DBP, SBP, and ethnicity influence HRV is unclear, and further research is
753 required to separate and identify their effects.

754 In summary, HRV is influenced by a multitude of factors. The association between age,
755 HR, and sex on measures of HRV has been explored extensively, but the relationship between
756 HRV and variables such as physical activity levels, BMI, DBP, SBP, and ethnicity require
757 further research. While inter-individual variability in HRV may influence the degree to which BP
758 decreases during SB, some studies suggest that SB may be an effective tool to lower BP across a
759 variety of patient characteristics (67, 95).

760 Therefore, we aim to gain further insight into the mechanisms of SB-mediated BP
761 reductions by investigating the degree to which determinants of BRS and HRV affect BP
762 responses to SB. Given the proposed contributions of BRS and HRV in BP response to SB,
763 investigation of these common determinants may improve our understanding of which factors
764 influence SB-mediated BP response.

4. CHAPTER II: MANUSCRIPT

4.1. Introduction

More than one in three individuals are affected by hypertension worldwide (194). Defined as blood pressure (BP) higher than 130/80 mmHg (299), hypertension places chronic stress on the vasculature (35) leading to myriad downstream cardiovascular consequences. As such, hypertension is acknowledged as a major risk factor for serious adverse cardiovascular events including but not limited to coronary heart disease (132, 174), stroke (157), heart attack (33), and end-organ failure (220). As such, reduction of cardiovascular disease risk through the management of hypertension is a crucial public health priority.

Hypertension is frequently treated using pharmacological agents (48, 106, 294), but there are limits to this approach. While at least 92% of patients worldwide are prescribed antihypertensive medications (294), predicted medication adherence is as low as 50% within one year of treatment (22, 37, 73, 250). Moreover, an expected 19.7% of patients are resistant to antihypertensive medication (37). Recent estimates place the global financial burden of high BP at US $370 billion, or about 10% of the world's overall health-care expenditure (195). Consequently, pharmacologic management of hypertension places an enormous financial burden on the healthcare system (300). Additionally, there is some data suggesting that the most commonly prescribed classes of antihypertensive medications are associated with increased blood glucose levels (45) and risk of adverse metabolic effects (45), myocardial infarction (72, 232), cardiovascular events (32), and cancers (52, 88, 153). Together, these data support the need for non-pharmacological means of managing hypertension. As such, it is imperative to explore alternative treatment options that may be effective in lowering BP.

A promising adjunct treatment to lower BP in hypertensive individuals is device-guided slow breathing (SB; defined as a respiration rate <10 breaths/min) (245). Indeed, SB has been demonstrated to acutely decrease BP in individuals with hypertension (119), diabetes (249), and post-traumatic stress disorder, (84), as well as in healthy populations (2). Additionally, a recent 2019 meta-analysis of randomized controlled trials in patients with hypertension or prehypertension reported significant reduction in systolic blood pressure (SBP) and diastolic blood pressure (DBP) following SB interventions of at least 5 minutes per day for 3 days per week for at least 4 weeks (39). However, while many studies have validated the use of SB as an effective technique to lower BP, other studies have concluded that SB is not always effective in lowering BP (5, 151, 176). Given these conflicting findings, it may be that SB-mediated decreases in BP are not universal, and that BP responses to SB differ between individuals. Indeed, a meta-analysis of device-guided SB in populations with hypertension reported high variability in SB-mediated reductions in BP (287). However, our understanding of the potential drivers of this variability remains poor.

To understand the potential sources of variability in BP responses to SB, one can consider the physiological pathways through which SB acutely lowers BP. Prevalent theories span multiple physiological systems and include the contributions of both autonomic and cardiorespiratory pathways (245). While the mechanisms through which SB decreases BP continue to be elucidated, baroreflex sensitivity (BRS) and heart rate variability (HRV) have emerged as two strong contenders for mediators of SB-mediated BP responses. Baroreflex sensitivity is a measure of the baroreflex's ability to respond to acute changes in arterial BP (272). The sequence method of BRS measurement involves the identification of three or more consecutive heart beats wherein SBP increases and R-R interval (RRI; i.e., the interval between

successive heartbeats) simultaneously lengthens (i.e., up-BRS), or SBP decreases and RRI simultaneously shortens (i.e., down-BRS) (217). On the other hand, HRV is the measure of the variation between individual, successive heartbeats (258), a measure which is often quantified as the root-mean square differences of successive RRI (RMSSD) (258). During slow breathing, the amplitude of both blood pressure oscillations and HRV are increased (187, 239). It has been hypothesized that the synchronization of these oscillations serves to increase BRS and thereby contributes to the reduction in blood pressure (245). Indeed, slow breathing increases both BRS (17, 20, 102, 118, 163, 215, 235, 239, 289) and HRV (17, 102, 215, 265, 276, 289). However, no studies have examined the impacts of baseline BRS and HRV as determinants of a subsequent SB-mediated blood pressure response.

Given the importance of BRS and HRV in mediating SB-induced reductions in blood pressure, the determinants of BRS and HRV emerge as candidates for variables that may affect blood pressure responsiveness to SB. Indeed, the determinants of HRV and BRS have been thoroughly investigated within non-SB-related contexts. Variability in resting levels of BRS have been attributed to a number of biological and lifestyle factors, including SBP (62, 149, 152), body mass index (BMI) (126, 148, 158, 266), and, to a lesser extent, physical activity levels (60, 61, 144, 197, 198, 282). Similarly, there is evidence to suggest that variability in resting HRV is associated with measures of resting SBP (271, 280), BMI (8, 124, 138), and with physical activity levels (60, 61, 96, 216, 240). Given the proposed mechanistic contributions of BRS and HRV to the blood pressure response to SB, it is possible that the determinants of these variables may also contribute to inter-individual variability of blood pressure responses to SB.

Therefore, the primary purpose of this study was to determine whether baseline values of BRS (specifically, up-BRS) and HRV (specifically, RMSSD) are predictors of the magnitude of

the change in SBP during an acute 15-minute bout of device-guided SB in young healthy individuals. We hypothesized that higher baseline values of BRS and HRV would be associated with increased magnitude of decrease in SBP during SB. The secondary purpose of this study was an exploratory arm, which sought to investigate whether biological and lifestyle characteristics (resting SBP, BMI, and physical activity levels) are predictors of the SBP response to SB. We hypothesized that higher resting SBP and BMI, and lower physical activity levels, would be associated with greater SB-induced decreases in SBP. We anticipate that the results from this study are positioned to provide insight into which variables predict an individual's SB-mediated blood pressure response. This information could ultimately allow for the identification of sub-populations for which SB is likely to be most effective as an adjunct treatment for the management of hypertension.

4.2. Methods

Participants: We recruited non-smoking men (n=19) and women (n=18) who were free from cardiovascular, neurological, respiratory, and endocrinological diseases. Women were either eumenorrheic (cycle length: 22-30 days) or regular users of hormonal contraceptives (n=4; levonorgestrel and ethinyl estradiol) and were tested during the early follicular phase of the menstrual cycle or during the placebo phase of hormonal contraceptive use. All participants provided written, informed consent for the study. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Faculty of Medicine Institutional Review Board at McGill University (IRB Study Number: A05-M14-181).

Experimental Design: Each participant attended a familiarization visit prior to their testing day during which they practiced the SB protocol and experienced all instrumentation. Participants also completed the International Physical Activity Questionnaire (85) and a health history questionnaire to assess physical activity levels and to confirm the presence of inclusion criteria, respectively.

On the test day, participants arrived in the laboratory following a 3-hr fast and 12-hr abstention from caffeine, strenuous exercise, and alcohol. Testing occurred in a dimly lit room at an ambient air temperature of 22-25°C. All participants were tested at the same time of day (08:00 ± 1 hr) to minimize any cardiovascular effects of diurnal hormonal variations, as occurs in men (11). Participant height and weight were assessed prior to instrumentation. Participants were instrumented on a padded table and tested in the supine position.

Following instrumentation, participants rested for 10 minutes or until BP values were stable. Resting BP was reported as the mean of three separate assessments at the brachial artery *via* manual sphygmomanometry performed by a trained research assistant. Each assessment was

separated by 2 minutes of rest. Baseline (BSL) data was then recorded for 15 minutes. Following BSL, participants underwent the 15-minute device-guided SB protocol using the RESPeRATE device (Intercure Ltd., Israel). Briefly, the RESPeRATE device induced SB by monitoring baseline respiration rate during an initial calibration period, and then gradually reducing respiration rate *via* auditory musical tones that instruct the user when to inhale and exhale. Participants were instructed to inhale through the nose and exhale through the mouth. In addition, participants were informed that they should not hold their breath at any point in time, and that their eyes should remain open for the duration of the protocol.

Instrumentation: Participants were instrumented for heart rate (HR; 5-lead ECG), and beat-by-beat BP was obtained by finger photoplethysmography (Finometer Midi, Finapres, Amsterdam, The Netherlands), calibrated to the mean of 3 manual BP values. Respiratory activity was measured by two respiration belts (respiratory belt transducer, ADInstruments, Dunedin, New Zealand), one placed around the chest at the xiphoid process to monitor chest respiratory patterns, and the other placed around the abdomen at the umbilicus to monitor abdominal respiratory patterns.

Data Analyses: Mean arterial BP (MAP), SBP, and DBP were obtained from the calibrated beat-to-beat finger BP waveform, and pulse pressure (PP) was calculated as SBP – DBP. Cardiac output (CO) was assessed using the beat-to-beat finger BP signal *via* the Non-Invasive Cardiac Output algorithm (three-element Windkessel model; ADInstruments). Stroke volume (SV) was calculated as CO/HR*1000. To account for inter-individual differences in heart size, CO and SV were normalized to body surface area. That is, CO and SV were divided by body surface area to calculate cardiac output index (COi) and stroke volume index (SVi),

respectively. An estimation of body surface area using the height and weight of the participants was calculated *via* the Mosteller formula (201):

$$\text{Body surface area } (m^2) = \sqrt{\frac{Height(cm) \times Weight(kg)}{3600}}$$

Breathing mechanics were assessed *via* chest tidal volume index and abdominal tidal volume index. Tidal volume indices were calibrated by a maximal inspiratory capacity maneuver, in which two maximum inhalations and exhalations were performed prior to the SB protocol. The maximum value for each of the chest and abdominal signals across both inhalations was assigned a value of 100% and the minimum value across both exhalations was assigned a value of 0%. Then, two-point calibrations were applied to each of the chest and abdominal signals wherein the absolute maximum inspiratory capacity was 100% and the absolute maximum expiratory capacity was 0%.

Spontaneous cardiovagal BRS was assessed via the sequence method using Ensemble software (Elucimed, Wellington, New Zealand), which identified sequences of three or more consecutive heartbeats in which R-R interval (RRI) and SBP simultaneously increased (up sequences). A minimal coefficient of correlation between changes in SBP and RRI was required to validate a sequence ($r^2 > 0.8$). BRS was quantified as the mean slope between SBP and RRI.

HRV was assessed by the root mean square of successive R-R intervals (RMSSD) as determined *via* Ensemble (Elucimed, Wellington, New Zealand).

Physical activity levels were quantified as total weekly metabolic expenditure (MET-mins/week) of all low, moderate, and vigorous activities.

Data from the last 10 minutes of BSL were used to determine resting BRS and HRV, as large time bins (e.g. 3 mins or greater) have been recommended to maximize the accuracy of these quantifications of BRS and HRV (147). To quantify responses to SB, the last 5 minutes of

SB were extracted within each participant, in line with data published from our laboratory which indicated that the final 5-mins of RESPeRATE-guided SB were associated with the achievement of steady state respiratory patterns (2). To calculate relative responses to SB, data from the final 5-mins of BSL measures were extracted to enable the calculation of delta values (i.e., mean of the last 5-mins of SB – last 5-mins of BSL). Responses to SB were defined in terms of cardiorespiratory responses, that is, changes in CO_i, HR, SV_i, PP, respiration rate, and chest and abdominal tidal volume indices.

Statistical Analyses: The effects of resting BRS and HRV on the magnitude of change in BP during SB were analyzed *via* simple linear regressions (P-value to reject = 0.05; P-values <0.15 were considered trending; R-value to reject = 0.8; GraphPad Prism 8 (La Jolla, California)). Effects of biological and lifestyle variables of interest (i.e., resting SBP, BMI, physical activity levels) on BP responses to SB were also assessed *via* simple linear regressions (P-value to reject = 0.05; P-values <0.15 were considered trends; R-value to reject = 0.8; GraphPad Prism 8 (La Jolla, California)). Cardiorespiratory responses to SB were analyzed *via* 2-tailed paired t-tests (alpha set to 0.05; P-values <0.15 were considered trending).

Post hoc analyses of key cardiorespiratory variables (i.e., COi, PP, and chest tidal volume index) were performed to investigate whether the change in work performed by the heart (i.e., CO_i), the relationship between the change in SBP and DBP (i.e., PP) or change in respiratory mechanics (i.e., chest tidal volume index, a non-invasive technique which offers similar sensitivity towards changes in tidal volume as spirometry (186)) predict the change in SBP during SB. The relationships between the changes in these cardiorespiratory variables and the change in SBP were analyzed *via* simple linear regressions (P-value to reject = 0.05; P-values

<0.15 were considered trending; R-value to reject = 0.8; GraphPad Prism 8 (La Jolla, California)).

Finally, *post hoc* analyses were conducted to categorize participants as responders or non-responders to the SB stimulus. Based on data demonstrating that a change in SBP as low as 2 mmHg has been shown to be clinical meaningful (224), "responders" were defined as participants whose decrease in SBP from baseline to SB was greater than 2 mmHg, and "non-responders" were defined as participants whose increase in SBP from baseline to SB was greater than 2 mmHg. A 2-way ANOVA was used to analyse how the SBP response differs between responders and non-responders. Further analyses on responder groups were performed *via* 2-tailed unpaired t-tests to determine whether autonomic (i.e., resting up-BRS and RMSSD), biological (i.e., resting SBP and BMI), lifestyle (i.e., physical activity), and cardiorespiratory responses (i.e. CO_i, HR, SV_i, PP, respiration rate, and chest and abdominal tidal volume indices) to SB differed between groups. The cardiorespiratory variables investigated were expanded to evaluate if additional determinants thought to affect SBP, such as the components of CO_i (HR and SV_i) (190, 246), and additional determinant of breathing mechanics (abdominal tidal volume index and respiration rate) (2, 293) differ between responders and non-responders. All data are reported as mean ± standard deviation (SD) with an alpha value set to 0.05. Given our small sample size (due to restrictions on human data collection resulting from the COVID-19 pandemic during the execution of this master's thesis), we considered P-values < 0.15 as statistical "trends".

4.3. Results

Baseline characteristics: The participants were young (22 ± 2 years of age; range: 20-29) with a lean mean BMI (23 ± 3 kg/m^2; 17-28). Seven participants (n = 3 men and n = 4 women) were excluded from data analyses due to the onset of adverse symptoms during the SB protocol (light headedness, general malaise) (n=3), failure to stay awake during the SB protocol (n=2), or hypertensive baseline BP (n=2). On average, participants performed physical activity at least 3 times a week and had a weekly metabolic expenditure of 4410 ± 2321 MET-mins/week (1106-12558). The participants were normotensive (resting SBP of 112 ± 9 mmHg (91-127); resting DBP of 70 ± 7 mmHg (57-81)).

Baseline data and responses to SB: Participants' resting up-BRS and RMSSD were 59 ± 26 ms (21-122) and 29 ± 12 ms/mmHg (8-68), respectively. Cardiorespiratory measures at BSL (i.e., during the last 5-mins of BSL) and during SB are reported in *Table 1* and the magnitude of the change in SBP is reported in *Figure 1A*. When all participants were considered together, SBP, DBP, PP and HR remained unchanged from baseline (*Table 1*). In contrast, MAP, CO_i and SV_i tended to be increased during SB (*Table 1*). Respiration rate was reduced and both chest and abdomen tidal volume indices were increased with SB (all P<0.01; *Table 1*).

Autonomic, biological, and lifestyle variables as predictors of the SBP response to SB: Neither autonomic (i.e., resting up-BRS or RMSSD), resting SBP, or lifestyle (i.e., physical activity levels as quantified by MET-mins/week) variables predicted the magnitude of the SBP response to SB (*Table 2*). A trend towards a weak positive correlation (R^2 = 0.10, P = 0.09) was observed between BMI and the magnitude of the SBP response to SB (*Table 2*).

Cardiorespiratory responses to SB as predictors of the SBP response to SB: Because autonomic, biological, and lifestyle variables failed to account for the change in SBP during SB,

977 we sought to determine whether cardiorespiratory responses to SB predicted the magnitude of
978 SB-induced SBP change. The SB-induced change in chest tidal volume index (21 ± 18 Δ%max
979 (2.4 - 67 Δ%max) was not correlated with the change in SBP (R^2 = 0.01; P = 0.70). However, we
980 observed a weak but significant positive correlation between the change in CO_i and the SB-
981 induced change in SBP, as well as a strong positive relationship between the change in PP and
982 the change in magnitude of SBP during SB (*Figure 2*).
983 *SB responders versus non-responders:* As a result of the high degree of variability in both
984 the magnitude of the SBP response and the time course of changes in SBP during SB (*Figure 1A*
985 *and 1B*, respectively), a categorical analysis comparing responders and non-responders was
986 performed to investigate whether autonomic, biological, or lifestyle variables or
987 cardiorespiratory responses to SB varied between SB responders and non-responders. By design,
988 the responders and non-responders differ in their SBP response to SB. No main effect of group
989 (responders *vs* non-responders) or condition (BSL *vs* SB) was observed. However, we observed a
990 trending interaction between the effect of group and the condition on SBP (*Figure 3*). As
991 expected, responders exhibited a decrease in SBP from BSL while non-responders exhibited an
992 increase in SBP from BSL (*Figure 3*). In addition, responders tended to have greater resting SBP
993 than non-responders (*Figure 3*).
994 Resting up-BRS was similar between responders and non-responders (28 ± 8 ms/mmHg
995 vs 29 ± 18 ms/mmHg, respectively, P = 0.94), as was resting RMSSD (67 ± 28 ms *vs* 55 ± 20
996 ms, P = 0.28). In addition, both BMI (22 ± 3 kg/m^2 *vs* 24 ± 2 kg/m^2, P = 0.99) and physical
997 activity levels (4276 ± 1151 MET-mins/week *vs* 3579 ± 1513 MET-mins/week, P = 0.46) were
998 similar between responders and non-responders.

SB-induced changes in HR, chest tidal volume index, and respiration rate were similar between groups (*Table 3*). However, we observed a trend towards greater increases in abdominal tidal volume index in responders relative to non-responders (*Figure 4*). Also, SB-mediated increases in CO_i and SV_i tended to be smaller in responders than non-responders (*Figure 4*), and the PP response to SB differed between groups in that a net negative change in PP was observed in responders while a net positive change was observed in non-responders (*Figure 4*).

4.4. Discussion

The aim of this study was to investigate potential contributors to inter-subject variability in BP responses to device-guided SB across a cohort of healthy normotensive individuals. Resting values of autonomic function, which have been proposed to be strong mediators of the BP response to SB, did not appear to predict SBP responses to SB. Similarly, the biological and lifestyle variables which determine BRS and HRV did not predict the SBP response to SB. Rather, cardiovascular responses to SB (i.e., COi and PP) appeared to predict the extent to which an individual would demonstrate a favourable reduction in SBP during a bout of SB. Further supporting this finding was a subgroup analysis of SB responders *versus* non-responders which demonstrated that responders were characterized by higher resting levels of SBP than non-responders. This analysis also demonstrated that unfavourable SBP responses to SB may result from smaller increases in abdominal respiration than SB responders, which leads to relatively high SB-induced increases in COi, SVi, and PP. We speculate that SB responders may be characterized by a preponderance towards abdominal *versus* chest breathing during SB, which limits cardiac loading, and thus enables SB-induced reductions in SBP in these individuals. Together, these data suggest that cardiorespiratory responses to SB (i.e. abdominal breathing, COi, SVi, PP) may play an important and poorly recognized role in mediating favorable SBP responses to SB.

Contrary to our hypothesis, resting autonomic factors (i.e., up-BRS and RMSSD), and the related biological (i.e., resting SBP and BMI) and lifestyle (i.e., physical activity) determinants of these outcomes, did not predict the magnitude of the SBP response to SB. While we observed a trending positive relationship between participants' BMI and the magnitude of the SBP response to SB, the correlation was weak ($R^2 = 0.14$) (107). That is, despite trending statistical

significance (P = 0.09), the low Pearson coefficient (R^2 = 0.14) indicates a highly variable relationship between BMI and SBP response to SB. As such, BMI is not predictive of the SBP response to SB. To the best of our knowledge, no studies have looked at autonomic factors as predictors of the SB-mediated SBP response. Previous investigations have suggested that autonomic factors are contributors to SB-mediated decreases in BP (17, 302), in that SB has been shown to increase both BRS (17, 20, 102, 118, 163, 215, 235, 239, 289) and HRV (17, 102, 215, 265, 276, 289). However, our findings indicate that neither resting indices of cardiovagal BRS or HRV are viable predictors of an individual's SBP response to SB. In other words, despite the established role of changes in cardiovagal BRS and HRV as mediators of BP responses to SB (17, 302), our data suggest that inter-individual differences in resting indices of autonomic function cannot account for the variability in the SBP responses to SB.

Conversely, we observed that magnitude of SBP response to SB may instead be dependent on resting SBP values. That is, we observed that SB responders tended to present higher resting SBP than non-responders. Indeed, it is possible that inter-individual variability in SBP at rest may contribute to producing the variability in SBP responses to SB (*Figure 1A*) in a normotensive and healthy population. A previous study within our laboratory observed consistent decreases in SBP during the last five minutes of SB in a similar young and healthy population (2). However, the discrepancy in our laboratory may be attributed to elevated resting SBP values compared to our similar young and healthy population (117.5 ± 9.5 *vs* 113.0 ± 10.0 mmHg, respectively) (2). In alignment with this observation, another study in a young and healthy population observed variability in BP responses to SB (66). Although the BP response to SB was reported as MAP, a large range in the percent change in MAP during SB from baseline (-9.1–1.2%) was observed, indicating considerable variability in BP response to SB (66). Notably,

the individual with the greatest BP increase had the lowest resting BP (66). Overall, these data suggest that SB may lower BP more effectively in individuals with slightly higher resting SBP and variability in resting SBP may attribute to variable SBP response to SB.

The present study also demonstrated that those individuals who increased their abdominal breathing during SB also exhibited the greatest SB-induced reductions in SBP. Previous studies have demonstrated that the interactions between abdominal *vs* chest breathing (i.e. breathing type) and inspiration *vs* expiration (i.e. breathing phase) affect venous return, a determinant of SV (193) and ultimately SBP (185). Inspiration leads to an increase in venous return to the right atrium, which subsequently decreases SV of the left ventricle and therefore SBP (193). Moreover, chest breathing throughout inspiration has also been demonstrated to increase venous return (193). The reverse occurs during expiration, during which venous return to the right atrium decreases, leading to increases in SV of the left ventricle and thus SBP (245). In contrast, abdominal breathing during expiration leads to larger increases in venous return (193). Notably, in our study we observed that responders to the bout of SB tended to have larger increases in abdominal breathing compared to non-responders, whereas chest breathing was similar between groups. Additionally, the breathing pattern of the RESPeRATE involves doubling the expiration to inspiration time (80). As such, abdominal breathing will be a greater determinant of changes in hemodynamic outcomes than chest during device-guided SB. In line with these findings, responders tended to maintain COi and SVi throughout SB, while non-responders tended to increase COi and SVi. Cardiac output, the product of SV and HR, is a determinant of SBP (185). Therefore, the increases in SBP observed in non-responders during SB may result from increases in SVi *via* increased venous return. Overall, the larger increases in abdominal breathing observed in responders may be responsible for the decreased SV, and therefore SBP *via*

increased venous return during expiration. The reverse is true for non-responders as the smaller increase in abdominal breathing observed may be responsible for the increased SV, and therefore SBP *via* decreased venous return during expiration.

Additionally, changes in SV may account for the changes in PP during SB. Indeed, responders experienced narrowing of PP while non-responders experienced widening of PP and larger increases in SVi, which may attribute to the differing SBP response to SB. That is, despite similar baseline measures of DBP between groups, responders decreased SBP, and non-responders increased SBP during SB. Interestingly, SV is directly correlated with PP, in that the widening of PP in young, and healthy participants is suggested to be associated with increases in SV (4). Through this association, elevated PP has emerged as an important risk factor for the development of cardiovascular disease (27). More specifically, the widening of PP due to increased SBP while DBP remains within the normal range, has been associated with increased risk of heart disease (112). As such, SB is a promising adjunct treatment because of its effects on narrowing PP *via* the decrease in SBP and maintenance of DBP, a reliable indicator of cardiovascular health. Together, these findings suggest that variability in the SBP and PP response to SB may be mediated by changes in SV, which arise from respiratory mechanisms.

4.4.1. Methodological Considerations

The findings presented in our study should be taken in the context of the following limitations. First, the number of variables we were able to include in our regression analysis was limited by our small sample size (n=30), which was the product of an unforeseen and drastic limitation to the data collection period for this thesis due to restrictions related to the global COVID-19 pandemic. While previous studies have established other variables including age (8, 54, 60, 71, 98, 141, 143, 166, 238, 263, 267, 271, 280, 284, 304), resting HR(8, 42, 62, 98, 143,

152, 271, 280), diastolic BP (62, 149, 152, 271, 280), ethnicity (109, 216, 271) are determinants of the autonomic outcomes explored in this study, additional regressions would have been underpowered due to the small sample size. Thus, a larger sample size is necessary to adequately evaluate additional related biological factors. Additionally, the initial pandemic restrictions required that we restrict ourselves to assessing individuals at low risk for the adverse effects of COVID-19. Given our resultant population of young, healthy, lean, and normotensive individuals, the variability in age, BMI, physical activity and resting SBP was limited. However, we would still assert that this data set allows for meaningful conclusions, as it is helpful to first evaluate potential predictors of the SBP response to SB in the absence of cardiovascular dysfunction, as is often observed in clinical populations including individuals with hypertension. That is, we believe that it is important to understand "normal" physiology before attempting to elucidate the effects of the pathophysiology (i.e., hypertension), which introduces even further variability. That being said, further research is clearly necessary to test the validity of our findings in a hypertensive population.

4.5. Conclusions

Our study provided insight on the efficacy of SB in a young, healthy, and normotensive population. That is, the SBP response to SB may be determined by cardiorespiratory mechanisms, as opposed to measures of resting autonomic function. In addition to the lower respiration rate induced during device-guided SB, the type of breathing performed appears to play an important role on the effectiveness of the SB-mediated SBP response. As SB has been recommended as an adjunct treatment for hypertension, it may be that maximizing abdominal rather than chest breathing would be preferable to optimize the benefits of SB (i.e. SBP reduction). Given that SB appears to be most effective in young, healthy, and normotensive

individuals with higher resting SBP, SB is likely to be a promising therapy for the prevention and treatment of hypertension.

4.6. Tables and Figures

Table 1: Cardiorespiratory responses to slow breathing (SB).

	Baseline	SB	P value
SBP (mmHg)	113 ± 10 (91 –130)	114 ± 10 (90 – 129)	0.36
DBP (mmHg)	70 ± 8 (50 – 81)	70 ± 8 (50 – 81)	0.19
MAP (mmHg)	87 ± 7 (68 – 100)	89 ± 7 (71 – 101)	0.14
PP (mmHg)	44 ± 8 (26 – 68)	44 ± 9 (27 – 71)	0.61
HR (bpm)	63 ± 7 (51 – 84)	64 ± 8 (52 – 82)	0.37
SV_i (mL/m^2)	49 ± 8 (36 – 66)	51 ± 7 (35 – 61)	0.12
CO_i ($L/min/m^2$)	3.1 ± 1.2 (2.0 - 4.8)	3.2 ± 0.6 (2.0 – 4.3)	0.07
Respiration Rate (breaths/min)	11 ± 4 (5 – 19)	5.1 ± 0.9 (4.2 – 9.4)	<0.01
Tidal volume index – Chest (%max)	14 ± 9 (5 – 48)	37 ± 21 (13 – 89)	<0.01
Tidal volume index – Abdomen (% max)	24 ± 17 (5 – 76)	46 ± 20 (7 – 82)	<0.01

*, $p<0.05$. Data are mean ± standard deviation (range). CO_i, cardiac index; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure SBP, systolic blood pressure; SV_i, stroke volume index.

Table 2: Baseline autonomic and biological variables as predictors of the magnitude of the systolic blood pressure response to slow breathing.

	R^2	P value
RMSSD	<0.01	0.73
Up-BRS	<0.01	0.71
SBP	0.02	0.43
BMI	0.10	0.09
Physical Activity	0.09	0.19

BMI, body mass index; RMSSD, root mean squared of successive RR interval differences; SBP, systolic blood pressure; up-BRS, baroreflex sensitivity up sequences.

Table 3: Slow breathing induced changes in cardiorespiratory measures in responders and non-responders.

	Responders	Non-responders	P value
HR (Δbpm)	1.29 ± 5.41 (-9.69 – 8.59)	1.59 ± 4.41 (-4.54 – 8.00)	0.89
Respiration rate (Δbreaths/min)	-5.78 ± 3.77 (-11.48 – 0.11)	-7.21 ± 4.44 (-14.16 – (-1.22))	0.45
Chest tidal volume index (Δ%max)	36.25 ± 23.50 (2.42 – 67.23)	16.25 ± 10.13 (4.82 – 38.87)	0.21

Data are mean ± standard deviation (range). HR, heart rate.

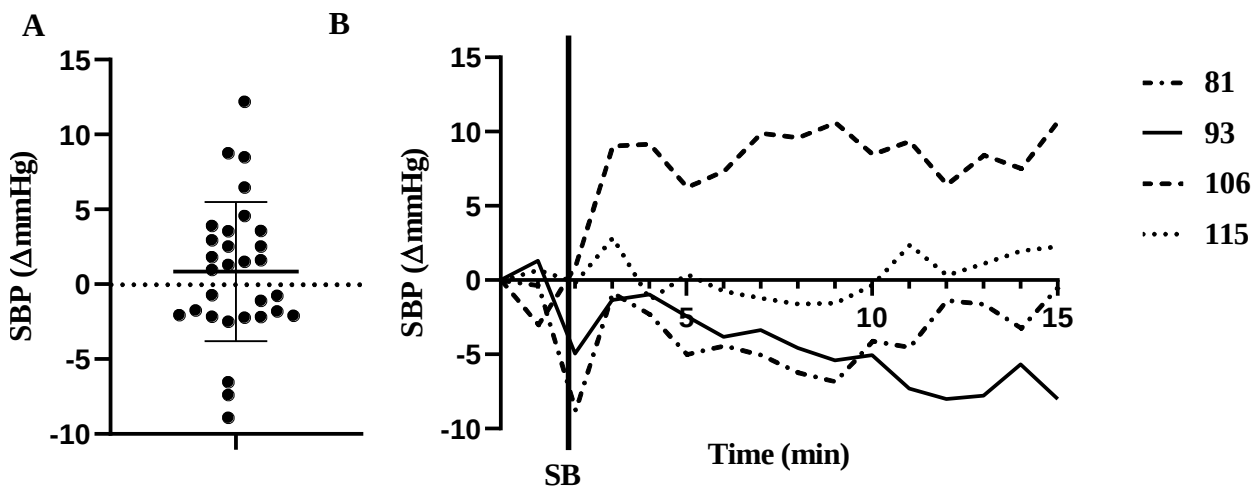
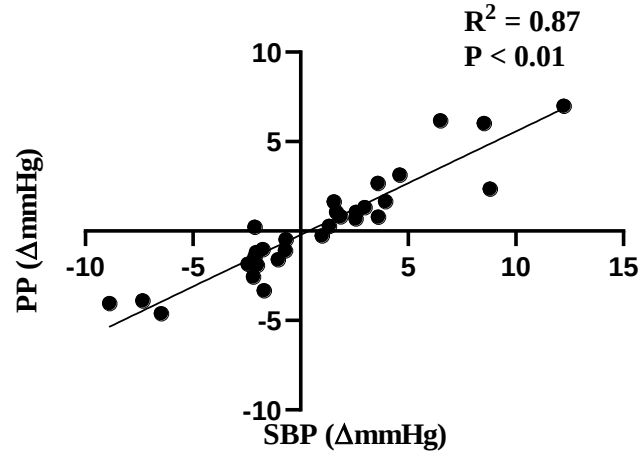


Figure 1: Systolic blood pressure (SBP) response (A) and time course (B) during slow breathing varied across participants. A. We observed a large degree of variability in the SBP response to SB (0.8 ± 4.7 ΔmmHg (-8.9 - 12.2 ΔmmHg)). **B.** Four participants were selected to represent four distinct sub-groups of SBP time course throughout the 15-minute bout of SB. Participant 93 represents a classic responder, while participant 106 represents a classic non-responder. Participant 115 represents the sub-group of participants in whom SBP did not change markedly throughout SB. Participant 81 represents a sub-group of participants who initially responded to SB (i.e., experienced a reduction in SBP), but this response was no longer observed towards the end of the SB intervention.



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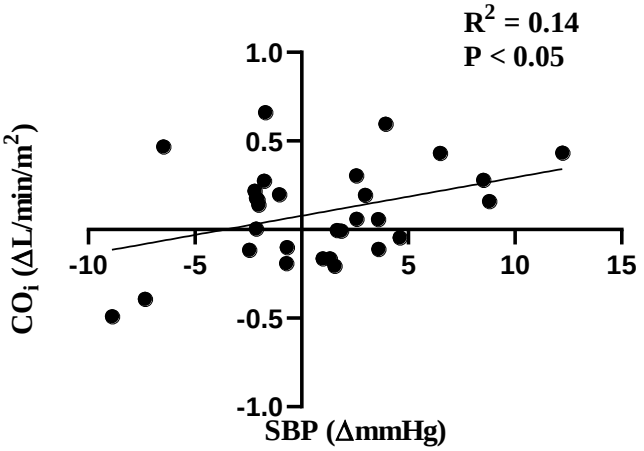

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Figure 2: Change in pulse pressure (PP) and cardiac index (CO_i) are strongly and weakly correlated, respectively, with the magnitude of systolic blood pressure (SBP) change during slow breathing. A positive relationship between the change in pulse pressure (0.27 ± 2.88 ΔmmHg (-4.59-7.00 ΔmmHg)) and the change in SBP during SB **(**0.8 ± 4.7 ΔmmHg (-8.9 – 2.2 ΔmmHg) was demonstrated (R^2=0.87; P<0.01). A weak but significant positive correlation between the change in COi (0.10 ± 0.27 ΔL/min/m^2 (-0.49 – 0.66 ΔL/min/m^2) and the SB-induced change in SBP was demonstrated (R^2 = 0.14; P = 0.04).

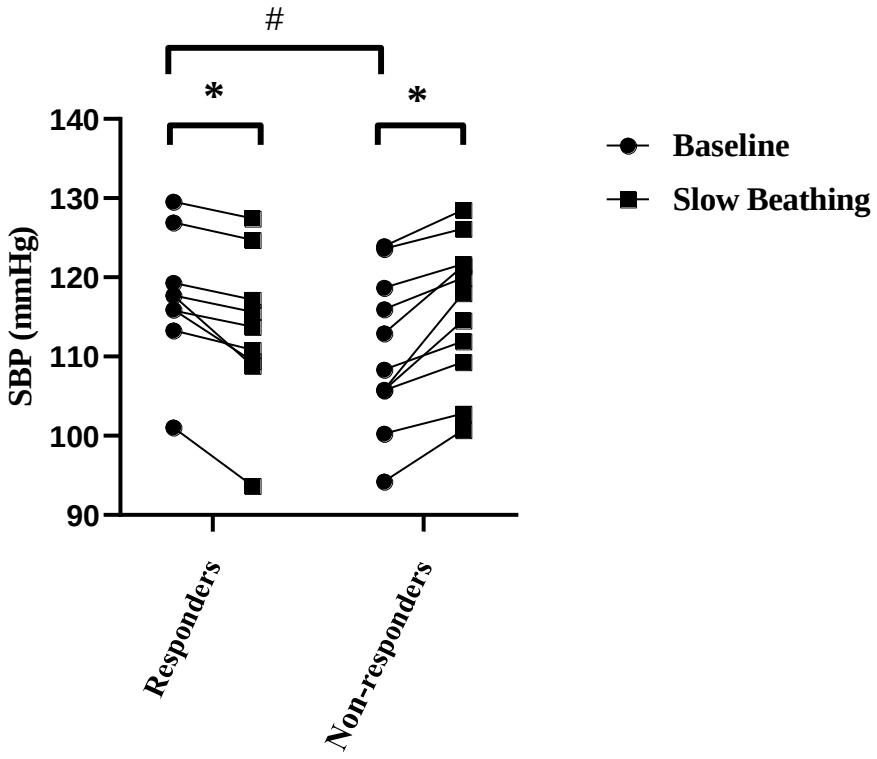
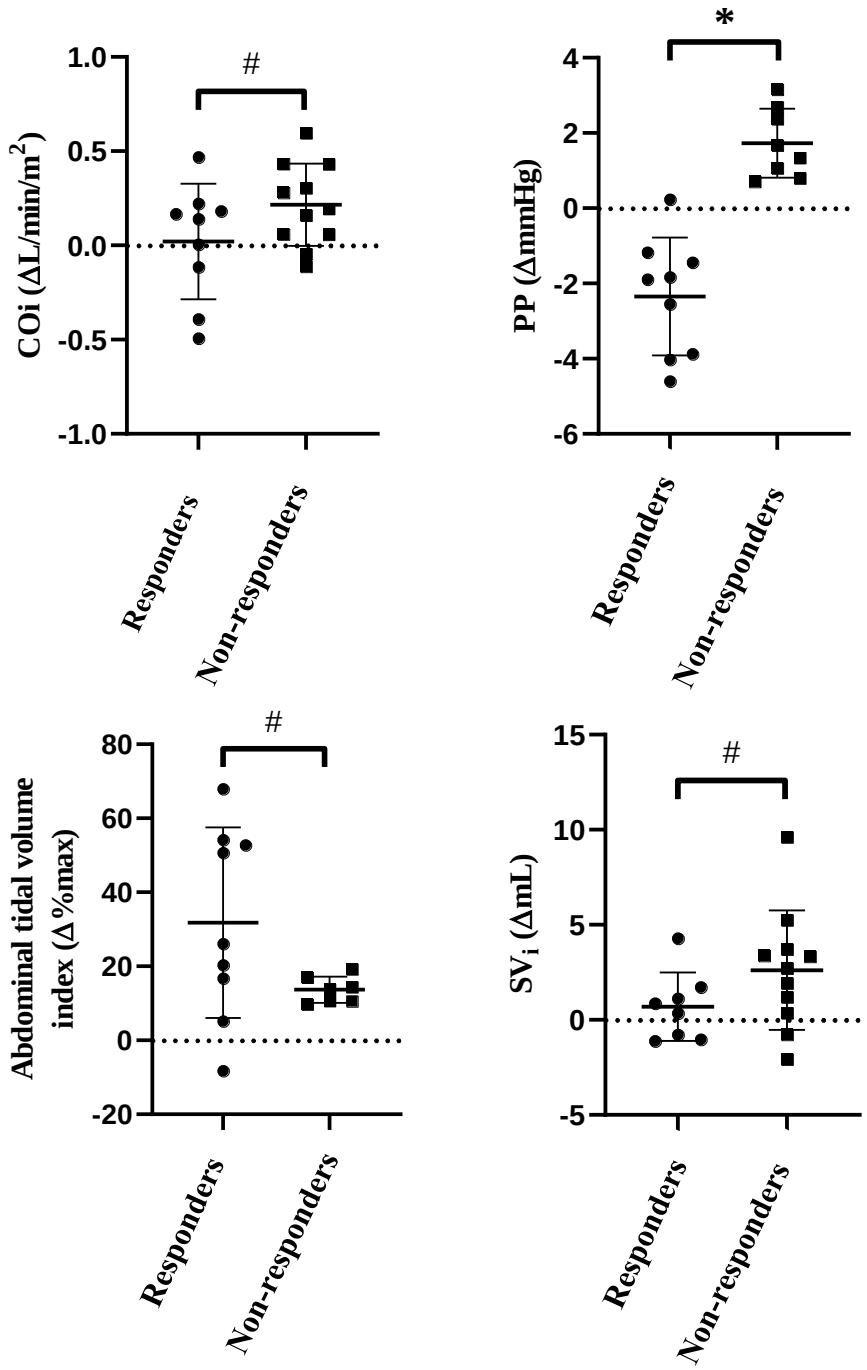


Figure 3*:* Systolic blood pressure (SBP) differed between responders and non-responders. *, P < 0.05; #, P <0.10. No main effect of group (responders *vs* non-responders; P=0.43) or condition (BSL *vs* SB; P=0.80) was observed. However, we observed a trending interaction between the effect of group and the condition on SBP (P=0.11). As expected, in responders SBP was decreased during SB relative to baseline (114 ± 10 mmHg *vs* 117 ± 7 mmHg, respectively; P<0.01), while in non-responders SBP was increased relative to baseline (116 ± 10 mmHg *vs* 111 ± 10 mmHg; P<0.01). Responders tended to demonstrate greater resting SBP than non-responders (117 ± 7 mmHg *vs* 111 ± 10 mmHg, respectively, P=0.09).



1157 **Figure 4: Changes in cardiorespiratory variables differed between responders and non-**
1158 **responders.** The change in pulse pressure (PP) response differed between responders and non-
1159 responders (-2.35 ± 1.56 *vs* 2.21 ± 1.67 mmHg, respectively; P<0.01). The change in cardiac
1160 index (CO_i) tended to differ between responders and non-responders (0.02 ± 0.31 *vs* 0.22 ± 0.22
1161 ΔL/min/m^2; P=0.12), respectively. The change in stroke volume index (SVi) response tended to
1162 differ between responders and non-responders (0.69 ± 1.80 *vs* 2.61 ± 3.15 ΔmL/m^2; p=0.14),
1163 respectively. The change in abdominal tidal volume index response tended to smaller increase in
1164 responders compared to non-responders (31.77 + 25.75 Δ%max *vs* 13.67 ± 3.58 Δ%max;
1165 P=0.09), respectively.

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