ACUTE CARDIOVASCULAR RESPONSES TO SLOW AND DEEP BREATHING

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By

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ABSTRACT

Slow and deep breathing (SDB) has long been regarded as a nonpharmacological method for dealing with several physiological and emotional imbalances, and widely used for relaxation purposes. There is, however, limited understanding of the putative mechanisms by which SDB acutely impacts the cardiovascular and autonomic systems to elicit chronic adaptations. The present thesis explored how the manipulation of breathing pattern and intrathoracic pressure during SDB could further the understanding of the regulatory mechanisms that underpin the acute cardiovascular response to SDB. This thesis makes an original contribution to the existing knowledge by reporting a previously undescribed inversion of normal within-breath (inspiration *vs.* expiration) left ventricular stroke volume (LVSV) pattern for breathing frequencies < 8 breaths·min^{-1}. This finding might reflect the influence of a lag between enhanced right atrial filling and right ventricular stroke volume during inspiration, and its expression in left ventricular stroke volume; this lag results from the time required for blood to transit the pulmonary circulation. Furthermore, blood pressure variability (BPV) was reduced significantly at the lowest breathing frequencies, likely due to the involvement of baroreflex mediated responses. The pattern of responses was consistent with the buffering of respiratory-driven fluctuations in left ventricular cardiac output ($\dot{Q}$) and arterial blood pressure (ABP) by within breath fluctuations in heart rate (*fc*), i.e., respiratory sinus arrhythmia (RSA) (Chapter 4). Chapter 5 demonstrated that magnifying negative intrathoracic pressure with inspiratory loading during SDB increased inspiratory pressure-driven fluctuations in LVSV and *fc,* and enhanced $\dot{Q}$, independently of changes in V_T and f_R. The data support an important contribution to the amplification of RSA, during SDB, of previously underappreciated reflex, and/or 'myogenic', cardiac response mechanisms. The findings in Chapter 6 confirmed that inspiratory loading during SDB amplified the effects observed with un-loaded SDB (reported in chapter 5). In contrast, expiratory loading increased ABP and attenuated RSA, LVSV and $\dot{Q}$ during SDB. A lower RSA for higher ABP, supports the presence of a formerly underappreciated contribution of sinoatrial node stretch to RSA, and throws into question the clinical benefits of expiratory resisted SDB, particularly in hypertensive populations. In conclusion, the findings of the present thesis provide novel information regarding the mechanisms contributing to acute cardiovascular response to SDB. These new insights may contribute to the development of more

effective SDB interventions, geared towards maximising the perturbation to the cardiovascular control systems.

“You know that our breathing is the inhaling and exhaling of air. The organ that serves for this is the lungs that lie round the heart, so that the air passing through them thereby envelops the heart. Thus, breathing is a natural way to the heart. And so, having collected your mind within you, lead it into the channel of breathing through which air reaches the heart and, together with this inhaled air, force your mind to descend into the heart and to remain there.”

Nikephoros the Monk

(XIII century Orthodox monk and spiritualist)

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ABP	Arterial blood pressure
Ach	Acetylcholine
ANGII	Angiotensin II
ANP	Atrial natriuretic peptide
BPV	Blood pressure variability
BPV_{HF}	Blood pressure variability high-frequency power
BPV_{LF}	Blood pressure variability low-frequency power
BPV_{TOT}	Blood pressure variability total power
BRS	Cardiac baroreflex sensitivity
BRS_{Freq}	Cross-spectral cardiac baroreflex sensitivity gain
BRS_{up}	Sequence baroreflex sensitivity positive sequence gain
BRS_{down}	Sequence baroreflex sensitivity negative sequence gain
BRS_{Seq}	Average sequence baroreflex sensitivity gain
BTPS	Body temperature and pressure, saturated with water vapour
CBF	Cerebral blood flow
CSF	Cerebrospinal fluid
DBP	Diastolic blood pressure
EF	Expiratory flow resisted slow and deep breathing
ET	Expiratory threshold loaded breathing slow and deep breathing
DF	Simultaneous inspiratory and expiratory flow resisted slow and deep breathing
DGB	Device-guided breathing
DT	Simultaneous inspiratory and expiratory threshold loaded slow and deep breathing
fc	Heart rate
fc_E	Heart rate during expiration
fc_I	Heart rate during inspiration
FEV_1	Forced expiratory volume in one second
FFT	Fast Fourier transform
f_R	Respiratory frequency
FRC	Functional residual capacity
FVC	Forced vital capacity

HF	High frequency
HRV	Heart rate variability
HRV_{HF}	Heart rate variability high frequency power
HRV_{LF}	Heart rate variability low frequency power
HRV_{TOT}	Heart rate variability total power
IF	Inspiratory flow resisted slow and deep breathing
IL	Inspiratory resisted slow and deep breathing
IT	Inspiratory threshold loaded slow and deep breathing
LBNP	Lower body negative pressure
LBPP	Lower body positive pressure
LF	Low frequency
LVSV	Left ventricular stroke volume
$LVSV_E$	Left ventricular stroke volume during expiration
$LVSV_I$	Left ventricular stroke volume during inspiration
ΔSV	Within-breath left ventricular stroke volume variation
MAP	Mean arterial pressure
MIF	Mean inspiratory flow
MSNA	Muscle sympathetic nerve activity
P_aCO_2	Partial pressure of carbon dioxide in arterial blood
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide
P_aO_2	Partial pressure of oxygen in arterial blood
P_E	Expiratory pressure
P_{Emax}	Maximal expiratoy pressure
P_I	Inspiratory pressure
PP	Pulse pressure
PSD	Power spectral density
PulTT	Pulmonary transit time
$\dot{Q}$	Cardiac output
$\dot{Q}_I$	Inspiratory cardiac output
$\dot{Q}_E$	Expiratory cardiac output
$\Delta\dot{Q}$	Within-breath cardiac output variation
RESP	Instantaneous lung volume
RMSSD	Square root of the mean squared differences of successive normal-to-normal RR intervals
RSA	Respiratory sinus arrhythmia

RVSV	Right ventricular stroke volume
SASRs	Slowly adapting pulmonary stretch receptors
SBP	Systolic blood pressure
SD	Standard deviation
SDB	Slow and deep breathing
SDNN	Standard deviation of normal-to-normal RR intervals
SD1	Standard deviation of the dispersion of successive RR intervals perpendicular to the identity line of the Poincaré plot
SD2	Standard deviation of the points along the identity line of the Poincaré plot
SEM	Standard error of the mean
SNA	Sympathetic nerve activity
SSf_R	Semi-spontaneous breathing at a fixed breathing frequency
SSV$_T$	Semi-spontaneous breathing at a fixed tidal volume
T_E	Expiratory phase duration
T_I	Inspiratory phase duration
T_I/T_{TOT}	Duty cycle
T_{TOT}	Total breath duration
TPR	Total peripheral resistance
UL	Un-resisted slow and deep breathing
$\dot{V}_A$	Alveolar ventilation
$\dot{V}_D$	Dead space ventilation
$\dot{V}_E$	Minute ventilation
VLF	Very low frequency
V_T	Tidal volume
V'	Respiratory flow rate

CHAPTER 1 – GENERAL INTRODUCTION

1-1 Study context

There is currently a body of evidence suggesting that the daily practice of slow and deep breathing (SDB), over a period of 2 months or more, may have antihypertensive effects in individuals with high blood pressure (Cernes and Zimlichman, 2015). Similarly, a very recent meta-analysis, limited to randomised controlled trials including a variety of SDB exercises, has shown significant reductions in resting heart rate (*fc*) and ABP, following at least 2 weeks of training, in patients with cardiovascular disease (Zou, Zhao, Hou et al., 2017).

The use of SDB has been historically associated with meditation and yoga, and traditionally believed to convey a healing effect. Controlled breathing patterns have been a common thread in the integrative physiology and psychophysiology research fields for the past four decades. Early studies employed a respiratory stimulus as a perturbation to the cardiovascular neural control systems, in an attempt to understand the interaction between respiratory and cardiovascular control (Dornhorst, Howard and Leathart, 1952b, Angelone and Coulter, 1964, Eckberg, Kifle and Roberts, 1980, Eckberg, 1983). There are also early reports of SDB interventions using biofeedback, yoga, meditation or a combination of these techniques, resulting in improvement in relevant clinical outcomes, in particular reduced arterial blood pressure (ABP) (Patel, 1975, Patel and North, 1975, Blackwell, Bloomfield, Gartside et al., 1976, Surwit, Shapiro and Good, 1978, Messerli, Decarvalho, Christie et al., 1979, Patel, Marmot and Terry, 1981, Sundar, Agrawal, Singh et al., 1983). However, many of these studies were conducted in combination with pharmacological therapies, and/or applied more than one SDB technique. Furthermore, in most studies, the respiratory pattern was not controlled tightly.

The work of an Israeli research team has been prominent in this field, using a SDB device that they invented (RESPeRATE, Intercure, Ltd., Lod, Israel). Their research has generated a series of studies that supported the antihypertensive effect of so-called device guided breathing (DGB) (Grossman, Grossman, Schein et al., 2001, Rosenthal, Alter, Peleg et al., 2001, Schein, Gavish, Herz et al., 2001, Viskoper, Shapira, Priluck et al., 2003, Elliott, Izzo, White et al., 2004, Schein, Grossman, Rosenthal et al., 2005, Schein, Gavish, Baevsky et al., 2009), but the effectiveness of RESPeRATE is still questioned (Mahtani, Nunan and Heneghan, 2012). Whilst studies conducted by other research groups have reported similar outcomes (Meles,

Giannattasio, Failla et al., 2004, Bae, Kim, Choe et al., 2006, Anderson, McNeely and Windham, 2010, Bertisch, Schomer, Kelly et al., 2011, Hering, Kucharska, Kara et al., 2013, Howorka, Pumprla, Tamm et al., 2013, Shantsila, Adlan, Lip et al., 2015), other studies have found no significant antihypertensive effect of DGB (Logtenberg, Kleefstra, Houweling et al., 2007, Altena, Kleefstra, Logtenberg et al., 2009, Landman, Drion, van Hateren et al., 2013). A meta-analysis and systematic review conducted in 2012 showed no significant anti-hypertensive effects of SDB with RESPeRATE when only randomised controlled trials were considered and those trials sponsored by, or involving, the manufacturers of the device excluded (Mahtani et al., 2012).

Notwithstanding, studies using other methods of SDB, have also resulted in significant positive clinical outcomes in participants other than those with hypertension. For example, Lehrer and coworkers demonstrated that the use of respiratory sinus arrhythmia (RSA) biofeedback therapy (a technique that drives participants to breathe at the low f_R corresponding to maximum amplitude of heart rate oscillations) results in improvements in cardiac baroreflex sensitivity (BRS), in people with asthma (Lehrer, Vaschillo, Vaschillo et al., 2003) and hypertensive individuals (Lin, Xiang, Fu et al., 2012). The same technique of daily training has been reported to increase HRV and decrease blood pressure variability (BPV) in women diagnosed with fibromyalgia (Hassett, Radvanski, Vaschillo et al., 2007) and in prehypertensive individuals (Lin et al., 2012). The use of SDB techniques employing a specific, fixed f_R of 6 breaths·min^{-1}, have also been suggested to lead to sustained reductions in ABP (Mourya, Mahajan, Singh et al., 2009, Jones, Sangthong and Pachirat, 2010, Sangthong, Ubolsakka-Jones, Pachirat et al., 2016), improvement in cardiac autonomic function (Wang, Li, Xu et al., 2010, Jones, Sangthong, Pachirat et al., 2015), elevation of BRS (Lin et al., 2012) and to reduce the exercise pressor response (Mourya et al., 2009, Jones et al., 2015), in hypertensive or prehypertensive individuals. Improvement of HRV indices, cardiac autonomic balance and reductions in ABP were also reported in healthy individuals, after one month of daily deep breathing at 6 breaths·min^{-1} (Tharion, Samuel, Rajalakshmi et al., 2012). Other observations include the reduction of central sympathetic outflow, but with no change in BRS, arterial stiffness, cardiac function and structure (Shantsila, Adlan, et al., 2015), after 8 weeks of DGB using RESPeRATE.

Overall, the reported acute physiological changes associated with SDB interventions have included, but are not limited to:

- reductions in indices of muscle sympathetic nerve activity (MSNA) in both healthy and hypertensive individuals with a single session of DGB (Oneda, Ortega, Gusmao et al., 2010, Hering et al., 2013, Shantsila, Adlan, Lip et al., 2014a, Shantsila, Adlan, Lip et al., 2014b). A similar effect had been observed previously in chronic obstructive pulmonary disease (COPD) patients (Raupach, Bahr, Herrmann et al., 2008);
- reduction of chemoreflex sensitivity with breathing at 6 breaths·min^{-1} in healthy controls performing yoga or paced breathing (Spicuzza, Gabutti, Porta et al., 2000, Bernardi, Gabutti, Porta et al., 2001);
- increase of BRS in hypertensive (Joseph, Porta, Casucci et al., 2005), pulmonary (Raupach et al., 2008) and cardiac heart failure patients (Bernardi, Porta, Spicuzza et al., 2002), as well as healthy individuals (Lehrer et al., 2003, Radaelli, Raco, Perfetti et al., 2004);
- augmented HRV and/or improved cardiac autonomic balance (Lehrer, Sasaki and Saito, 1999, Anderson, McNeely and Windham, 2009, Chang, Liu and Shen, 2013, Chang, Liu, Li et al., 2015);
- increased tolerance to orthostatic stress (Lucas, Lewis, Sikken et al., 2013);
- reduced end tidal CO_2 ($P_{ET}CO_2$; Anderson et al., 2009).

Based on some of these results, several presumed mechanisms have been advanced to explain a potential anti-hypertensive and cardioprotective effect of SDB interventions. These include changes in central chemosensitivity accompanied by alterations in acid-base regulation and renal regulation of sodium (Gilbert, 2003, Anderson et al., 2009); re-conditioning of cardiac baroreflex control of ABP (Vaschillo, Lehrer, Rishe et al., 2002, Lehrer et al., 2003); increased blood flow to internal organs (Fonoberova, Mezic, Buckman et al., 2014), and alteration of sympathetic baroreflex regulation (Fonoberova et al., 2014). Nonetheless, these potential links between the acute and chronic response to SDB lack experimental proof and are mostly founded on mathematical models of the cardiovascular system or based on a series of compound indices that are thought to provide information about autonomic and reflex cardiovascular regulation. Furthermore, the direct impact of SDB upon the most traditional measures of cardiovascular function still lacks detailed characterisation, as a large number of assumptions are based upon studies in animal models, or spontaneously breathing human beings.

Collectively, there is currently compelling and intriguing evidence suggesting the existence of relevant acute responses, and chronic adaptations, to SDB interventions. However, the mechanisms connecting the reported acute effects and the longer-term responses to the regular practice of SDB remain controversial and require further examination. Furthermore, the acute modulatory effect of SDB upon key haemodynamic variables, such as left ventricular stroke volume (LVSV), cardiac output ($\dot{Q}$) and ABP, remain insufficiently understood, and should be regarded as an essential stepping stone in the process of identifying the putative acute mechanisms that might underpin chronic health benefits of the regular practice of SDB. Thus, studying the characteristics of the respiratory stimulus by manipulating rate and depth during SDB may provide new insights into the acute cardiac and systemic response, and presumed regulatory mechanisms stimulated by SDB. Additionally, the integration of small respiratory resistances to conventional SDB will lead to a better understanding of the contribution of a key feature of SDB, *viz.*, intrathoracic pressure changes. These mechanistic insights may lead to improvement in the effectiveness of SDB interventions by facilitating manipulation of these factors to optimise the acute stimulus provided by SDB.

To this end, this thesis seeks to understand the acute effects of SDB upon a range of cardiovascular variables that might underpin the stimulus to the chronic lowering of ABP.

Specific aims of this thesis were:

1. To understand the independent effects of breathing frequency (f_R) and tidal volume (V_T) on the cardiovascular and autonomic response to SDB (Chapter 4).
2. To explore the increasingly negative intrathoracic pressure to the magnitude of cardiovascular respiratory modulation elicited by SDB (Chapter 5).
3. To provide insight into the cardiac and systemic haemodynamics in response to SDB combined with different modalities of resisted breathing (Chapter 6).

These three integrative intervention studies were performed at the Department of Life Sciences – College of Health and Life Sciences, Brunel University London, from October 2013 to October 2015.

CHAPTER 2 – LITERATURE REVIEW

2-1 Introduction

This chapter will provide a synopsis of the relevant literature concerning the interactions between the respiratory and cardiovascular systems in the context of the acute response to slow and deep breathing (SDB). Section 2-2 describes the mechanisms behind the acute regulation arterial blood pressure (ABP) as these are likely involved in the direct haemodynamic modulatory effects of SDB. Section 2-3 provides a summary of the known mechanical interactions between the lungs and the heart. Section 2-4 focuses on the description of the known cardiovascular rhythms that are present in both ABP and *fc*, as well as in sympathetic nerve activity, and culminates with a description of how synchrony of such rhythmicities can potentiate the effects of SDB. This leads into section 2-5, where a summary of the current understanding of the acute effects of SDB is provided. Finally, this chapter of the thesis is summarised in section 2-6 and closes with the study aims and hypotheses section 2-7.

2-2 Afferent influences upon acute control of blood pressure

2-2.1 Arterial baroreceptors

The principal arterial baroreceptors located in the carotid arteries and the aortic walls play a focal role in the reflex response to acute cardiovascular perturbations. Both sets of mechanoreceptors consist of unencapsulated nerve endings situated in the medial-adventitial arterial border of the aortic arch and carotid sinus (Mancia and Mark, 1983, Milnor, 1990) and operate as the sensors of a negative feedback control system (Sagawa, 1983, Milnor, 1990). Variations in arterial blood pressure (ABP) result in deformation of the baroreceptors thereby modulating afferent neuronal traffic. These afferent impulses are relayed centrally within the nucleus tractus solitarius, in the medulla oblongata (Milnor, 1990). A decrease in ABP results in reduced afferent firing leading to a decrease in parasympathetic nerve activity and enhanced sympathetic nerve activity (SNA). The converse occurs when ABP is elevated, with the stretch of the baroreceptors augmenting afferent traffic, thus triggering a reflex-mediated increase in parasympathetic nerve activity and reduction of SNA, as depicted in Figure 2-1 (Fadel, 2008). Therefore, acute regulation of blood pressure in mammals is mainly accomplished through modulation of sympathetic and parasympathetic nerve activity to the heart and

sympathetic outflow to the peripheral vasculature (Thames and Kontos, 1970, Eckberg, Fletcher and Braunwald, 1972, Eckberg, 1977, Abboud, Eckberg, Johannsen et al., 1979). Accordingly, the combined influences of cardiac output (Q̇; as the product of heart rate and left ventricular stroke volume) and vasomotor tone determine ABP at any given moment. Despite the constant adjustment of these variables it has been shown that arterial baroreceptor stimulation has minimal impact upon LVSV at rest (Ogoh, Fadel, Monteiro et al., 2002, Ogoh, Fadel, Nissen et al., 2003, Kim, Deo, Vianna et al., 2011) making heart rate (*fc*) the main factor controlling Q̇. Furthermore, the changes in vascular resistance (determined by vasomotor tone) account for the majority of the baroreflex-mediated ABP response in resting human males (Ogoh et al., 2002, Keller, Wasmund, Wray et al., 2003, Ogoh et al., 2003), while women show a dominant contribution of Q̇ (via *fc*), particularly to hypertensive stimulus (Kim et al., 2011).

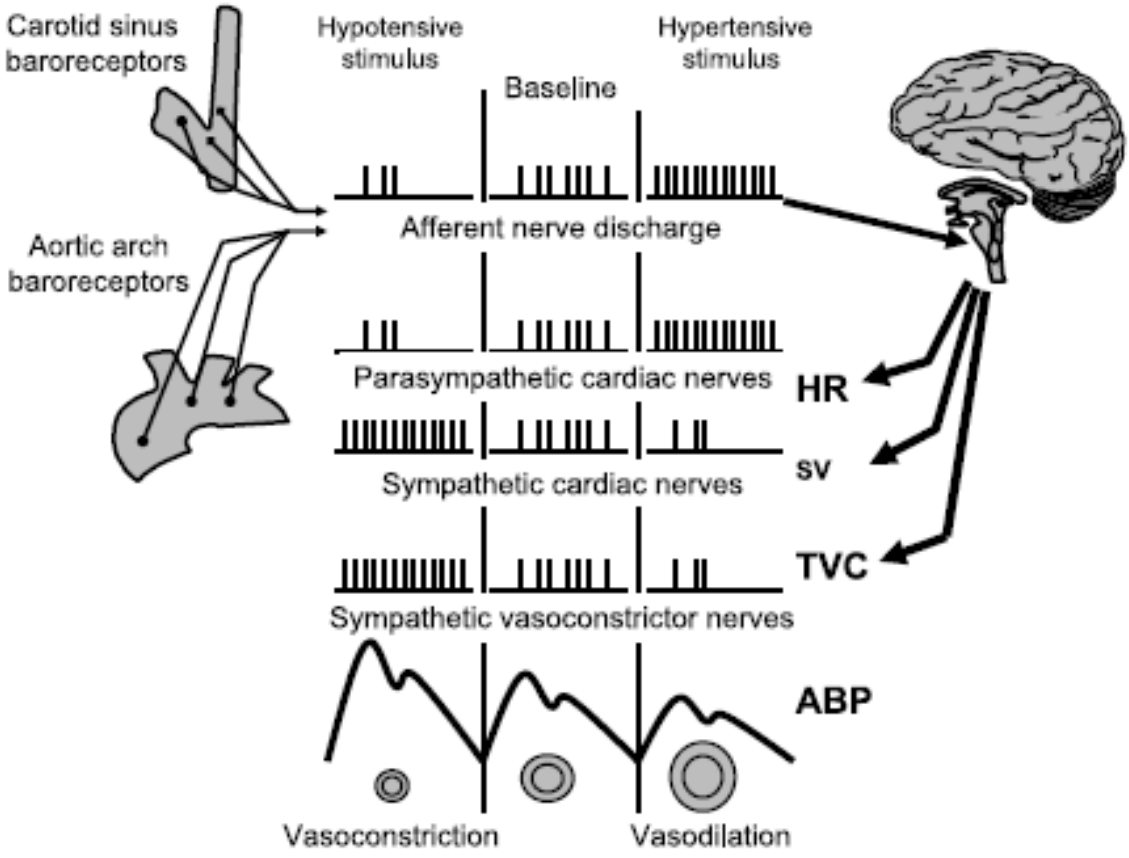


Figure 2-1 – Schematic representation of the neural baroreflex response to hypotensive and hypertensive stimuli. The alteration of arterial blood pressure (ABP) is sensed by carotid and aortic baroreceptors leading to changes in afferent baroreceptor nerve firing. Stimulation with hypertensive stimulus leads to increased neural input to the brainstem, resulting in increased parasympathetic efferent activity to the heart and decreased sympathetic outflow to the vasculature and heart. Conversely, a sudden drop in ABP (hypotensive stimulus) results in decreased parasympathetic nerve activity and increased sympathetic efferent discharge. HR – heart rate; SV – stroke volume; TVC – total vascular conductance. From Fadel (2008).

Differences between carotid and aortic arterial baroreceptor response

The diffuse network of sensing structures localised in the arterial walls has been the object of extensive research, particularly during the 1960s and 1970s. The review by Kirchheim (1976) considers in detail all aspects of baroreceptor reflexes, including the receptors themselves. Notwithstanding, structurally and functionally the two most important systemic arterial baroreceptors are those located in the aortic arch and carotid sinus (Milnor, 1990). Whilst the presence of different sensorial sites might appear redundant as ABP is similar in both regions, under certain conditions that might not necessarily be the case.

Important for the context of this thesis, the aortic arch baroreceptors are susceptible to variations in aortic transmural pressure caused by respiratory changes in intrathoracic pressure, while the carotid sinus baroreceptors, due to its extra-thoracic location, are only affected by systemic arterial pressure (Angell James, 1971). Furthermore, each set of receptors seems to possess a specific operating range, suggesting that for the same variation in ABP a change in afferent discharge might only arise from one sensorial region. In brief, animal studies have demonstrated that carotid baroreceptors operate over a wider range, above and below normal ABP levels, while aortic baroreceptors operate only at higher pressures (Hainsworth, Ledsome and Carswell, 1970, Pelletier, Clement and Shepherd, 1972). However, when these receptors were stimulated at normal pulsatile pressures, both no differences (Angell James and de Burgh Daly, 1970) or increased carotid reflex-mediated vascular response relative to that of aortic arch receptors (Dampney, Taylor and McLachlan, 1971), were reported. Nonetheless, Angell James and de Burgh Daly (1970) also demonstrated that the vasodilatory response produced by a rise in mean carotid sinus pressure was significantly greater than that secondary to a similar rise in aortic arch pressure.

Collectively, this might suggest that aortic baroreceptors are functionally more important in providing buffering to ABP rises than falls, while suggestion that the isolated carotid sinus baroreflex response might be better tailored to buffer reductions in ABP (Mancia and Mark, 1983). Nevertheless, the isolated stimulation of aortic and carotid baroreflexes is only achieved in non-physiological, experimental conditions; in ‘real life’, a combined stimulation is integrated centrally leading to a summated response (Kendrick, Matson and Lalley, 1979, Sagawa, 1983).

2-2.2 Other baroreceptors involved in the cardiovascular and autonomic responses

Atrial stretch receptors

The atria also contain a series of unencapsulated nerve endings, mostly concentrated in the atria-venous junctions. These are responsive to the stretch of the atria, caval and pulmonary veins (Coleridge, Hemingway, Holmes et al., 1957), triggering a reflex tachycardia (Ledsome and Linden, 1964, Goetz, 1965, Ledsome and Linden, 1967), but no appreciable inotropic (Furnival, Linden and Snow, 1971) or vasomotor effects (Carswell, Hainsworth and Ledsome, 1970). Notwithstanding, atrial stimulation inhibits renal nerve activity and the secretion of vasopressin, renin and cortisol, thus promoting diuresis (Carswell et al., 1970, Drinkhill, Hicks, Mary et al., 1988, Drinkhill and Mary, 1989). This response pattern was first described in 1915 when Francis Bainbridge reported a cardiac acceleration in response to rapid increases in venous return, induced by rapid intravenous infusions (Bainbridge, 1915). Since then, the response to atrial stretch is commonly referred to as Bainbridge reflex.

In human beings, this response pattern was only fully confirmed in the 1950s, when it was established that central venous pressure could be increased with no concurrent rise in ABP, by passively elevating the legs of supine human volunteers, thus isolating the atrial stretch response from that of the arterial baroreflex (Roddie and Shepherd, 1956, Roddie, Shepherd and Whelan, 1957). Despite the original ascription to a vagal autonomic reflex, more recent research has supported the existence of an intracardiac myogenic mechanism, capable of inducing a positive chronotropic response to atrial stretch, beyond that associated with a reflex response (Quinn and Kohl, 2012). Even though most evidence supporting this theory arises from isolated hearts or cardiac tissue, isolated sino-atrial nodes, fibres or even single cells, at least two studies involving denervated individuals (animal and human) provide strong support for the existence of an intracardiac myogenic mechanism mediating chronotrpoic changes (Cooper and Kohl, 2003, Quinn and Kohl, 2012). Specifically, data from denervated, anaesthetised, open-chested dogs showed an instantaneous increase in *fc*, even in the presence of cholinergic and adrenergic blockade, when exposed to graded stretch of the sino-atrial node (Brooks, Lu, Lange et al., 1966). Furthermore, in heart transplanted human beings (i.e. denervated hearts) a small degree of RSA is still present, which might be

explained by sino-atrial node stretch induced by respiratory-driven fluctuations in venous return (Bernardi, Keller, Sanders et al., 1989, Taha, Simon, Dempsey et al., 1995).

Other cardiac baroreceptors

Over the last century, extensive research has examined the anatomical, physiological and functional characteristics of a series of reflexogenic areas in the heart and large vessels. A detailed description of these baro- and chemoreceptors is beyond the scope of this thesis. Nonetheless, it is pertinent to highlight the existence of both ventricular and coronary artery receptors. The first seem to not be readily activated by changes in ventricular filling, being more responsive to chemical stimuli, suggesting a minor role in normal cardiovascular control. The latter, correspond to nerve endings in the proximal regions of the coronary arteries and are stimulated by changes in coronary pressure. When stimulated, these receptors trigger a vasomotor response analogous to that of the arterial baroreceptors. However, the coronary baroreceptors seem to operate at much lower pressures and have a more prolonged effect upon sympathetic efferent activity than the carotid arterial baroreceptors, leading to the assumption that they might be relevant in the cardiovascular response to hypotension and ‘longer’ term control of blood pressure. A detailed description of these (and other) cardiovascular mechano-reflexes can be found in a comprehensive review and a recent symposium report produced by Roger Hainsworth (1991, 2014).

Pulmonary artery receptors

Distension of the pulmonary trunk and its bifurcation with the right and left pulmonary arteries, stimulates myelinated vagal nerve afferents (Coleridge and Kidd, 1960, Coleridge, Kidd and Sharp, 1961) triggering reflex vasoconstriction, augmented respiratory drive and increased renal sympathetic nerve activity (McMahon, Drinkhill, Myers et al., 2000, Moore, Hainsworth and Drinkhill, 2011). The first studies conducted in open chested dogs suggested that these baroreceptors were only stimulated at unphysiologically high distension pressures (Coleridge and Kidd, 1960, Coleridge, Coleridge, Dangel et al., 1973, McMahon et al., 2000). However, more recent work from Moore and colleagues demonstrates that closing the chest, delivering phasic negative intrathoracic pressures and applying pulsatile pressures to distend the pulmonary artery, all prompt a reflex response at pressures perfectly

within the physiological range (Moore, Hainsworth and Drinkhill, 2004b, a, Moore et al., 2011).

Overall, the reflex response observed during stimulation of pulmonary artery receptors opposes that of the systemic baroreceptors. Evidence that these two reflexes interact strongly, with the stimulation of low-pressure pulmonary artery receptors altering the operating range of carotid baroreceptors and *vice-versa,* suggests a significant role of the pulmonary artery baroreflex in the control of the cardiovascular system (Moore et al., 2011). This effect is thought to be more physiologically relevant during dynamic exercise, hypotensive states and hypoxic stress (Moore et al., 2011, Fadel and Raven, 2012, Hainsworth, 2014). Importantly, for the context of this thesis, it is believed that these receptors are sensitive to changes in transmural pulmonary artery pressure, as demonstrated by increased vagal afferent activity during inspiration (Moore et al., 2004b, a). This suggests that respiratory interventions altering intrathoracic pressure, and thus affecting transmural pressure, possibly trigger a physiological response from these afferents.

2-2.3 Chemoreceptor stimulation and regulation of blood pressure

The chemoreflexes exert remarkable influences upon breathing regulation (Clark and von Euler, 1972, Duffin, 1990), as well as on cardiovascular control (de Burgh Daly and Scott, 1958, Heistad, Abboud, Mark et al., 1975b, Karim, Hainsworth, Sofola et al., 1980, O'Regan and Majcherczyk, 1982, Honig, 1989, Marshall, 1994, Gates, Bartels, Downey et al., 2009). Chemoreflex physiology is extremely complex, and for the ease of its understanding, these reflex responses are usually classified as either peripheral or central, based on the location of the primarily stimulated chemoreceptive structures.

Peripheral chemoreceptors are mostly located in the aortic and carotid bodies (Marshall, 1994). The peripheral chemoreceptors are mainly responsive to hypoxic stimulation, but might also be modulated by hypercapnia (Duffin, 1990). Central chemoreceptor location and structure is still not fully established but is believed to lie in the ventral surface of the medulla oblongata (Mitchell, Loeschcke, Massion et al., 1963, Loeschcke, 1973, Bruce and Cherniack, 1987). Central chemoreceptors respond indirectly to elevations in P_aCO_2 in the brain circulation, as its diffusion from

the cerebral capillaries into the cerebrospinal fluid (CSF) liberates H^+ ions, which reduce CSF pH (Mitchell et al., 1963, Leusen, 1972, Loeschcke, 1982). Figure 2-2 depicts a model of the chemoreflex control of ventilation.

In healthy adults undergoing SDB the stimulation of the peripheral chemoreceptors by alteration of P_aO_2 is likely negligible, particularly if minute ventilation ($\dot{V}_E$) is unchanged. Decreased chemoreflex responsiveness to hypoxia (and hypercapnia) has been observed with SDB, which is possibly due to improvement of the ventilation/perfusion ratio, leading to improved O_2 saturation of arterial blood (Bernardi, Spadacini, Bellwon et al., 1998, Spicuzza et al., 2000, Bernardi et al., 2001, Nepal, Pokharel, Khanal et al., 2013). Furthermore, P_aCO_2 impacts the responsiveness to hypoxic stimuli (and *vice-versa*), in such a way that at a P_aCO_2 of 35-40 mmHg, the ventilatory response to hypoxia is absent above a P_aO_2 of 50 mmHg (Mohan and Duffin, 1997, Duffin, 2005). Nonetheless, reductions in $\dot{V}_E$ with SDB might increase P_aCO_2 and trigger a chemoreflex response consisting primarily of a stimulus to increase ventilation (increased f_R or V_T).

While the dominant chemoreflex response to hypercapnia is to induce hyperpnoea, there is a clearly defined cardiovascular impact that translates into an increase in SNA (Somers, Mark, Zavala et al., 1989a, Oikawa, Hirakawa, Kusakabe et al., 2005, Gates et al., 2009), leading to a rise in ABP. Nonetheless, the negative feedback loop between breathing and cardiovascular control promotes an attenuation of the chemoreflex-driven cardiovascular response, in such a way that the increased activation of pulmonary stretch afferents inhibits the sympathetic response triggered by chemoreflex stimulation (Somers et al., 1989a, Somers, Mark, Zavala et al., 1989b). Furthermore, the interaction between chemoreflex and cardiovascular baroreflex control has been established, with baroreflex stimulation by raised blood pressure blunting the chemoreflex response (Heistad, Abboud, Mark et al., 1974, Heistad, Abboud, Mark et al., 1975a, Somers, Mark and Abboud, 1991). An effect of both peripheral and central chemoreflex activation upon baroreflex cardiovascular regulation has also been demonstrated recently in human beings (Cooper, Bowker, Pearson et al., 2004, Cooper, Pearson, Bowker et al., 2005). Notwithstanding, later evidence points to the absence of any meaningful impact of mild hypercapnia (5 mmHg above eucapnia) upon baroreflex regulation of *fc* and ABP (Simmons, Manson and Halliwill, 2007).

In the context of SDB, a reduced chemoreflex influence is expected if resting levels of P_aCO_2 and P_aO_2 are unaltered due to the maintenance of $\dot{V}_E$. Moreover, as mentioned previously, increased lung inflation with SDB is expected to limit chemoreflex response. Nonetheless, some studies have reported acute decreases in end tidal CO_2 ($P_{ET}CO_2$) during and after SDB (Anderson et al., 2009), while others have reported that SDB is associated with a reduction chemoreflex sensitivity to both hypercapnia and hypoxia (Spicuzza et al., 2000, Bernardi et al., 2001).

From a methodological point of view, changes in P_aCO_2 alter HRV, independent of f_R and V_T (Al-Ani, Forkins, Townend et al., 1996, Sasano, Vesely, Hayano et al., 2002, Poyhonen, Syvaoja, Hartikainen et al., 2004) and might possibly impact the cardiac response to SDB. It is therefore important to clarify the extent to which acute responses to SDB are attributable to changes in P_aCO_2.

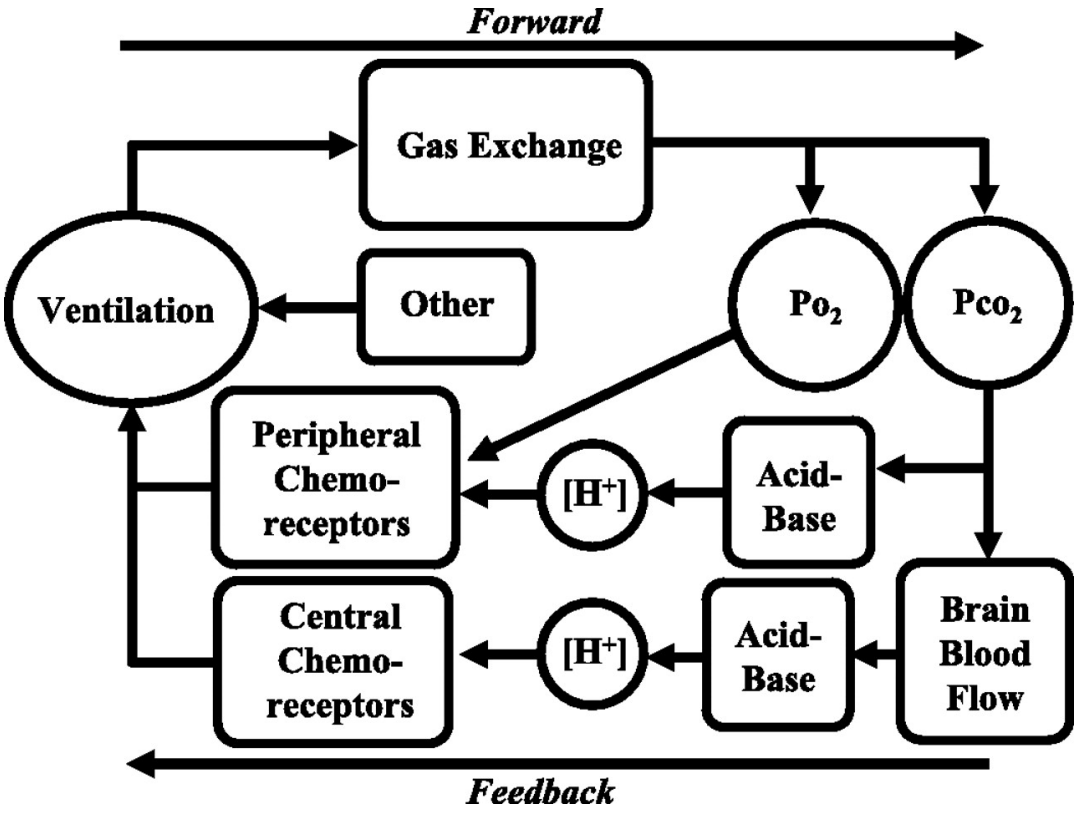


Figure 2-2 – Model of respiratory chemoreflex control of ventilation. Pulmonary ventilation controls P_aCO_2 and P_aO_2, the forward part of the loop. The concentration of hydrogen ions ([H+]) and P_aO_2 control ventilation via the respiratory chemoreflexes in the feedback part of the loop. Ventilation is also affected by drives to breathe which are independent of chemoreflexes. From Ainslie and Duffin (2009).

2-2.4 Short term hormonal control of blood pressure

The acute change of ABP prompts variations in the concentration of certain hormones over the course of minutes to hours; these changes act principally via alteration of vasomotor activity, i.e. by altering peripheral resistance, but they also affect $\dot{Q}$. Overall, adrenal medulla catecholamines, atrial natriuretic peptide (ANP) and angiotensin II (ANGII) can contribute to the immediate reflex response to baroreceptor stimulation. Accodingly, SDB might influence one or all of these hormonal pathways.

Adrenal medulla catecholamines

Throughout periods of increased sympathetic activity, the adrenal glands release catecholamines (adrenaline and noradrenaline) and enhance the adrenergic response as observed in the fight-or-flight response. However, evidence regarding an association between acute baroreflex stimulation and plasma catecholamine levels suggests that the latter are not changed significantly during moderate baroreflex-driven sympathetic responses (Mancia, Leonetti, Picotti et al., 1979, Mancia, Ferrari, Gregorini et al., 1983). Instead, the engagement of low pressure cardiopulmonary receptors, as observed in orthostatic challenges and submaximal aerobic exercise, seems necessary for significant rises in circulatory levels of these hormones (Mancia et al., 1979, Mancia et al., 1983, Grassi, Gavazzi, Cesura et al., 1985, Floras, Vann Jones, Hassan et al., 1986). In the context of acute responses to SDB, there has been at least one report of reduced plasma noradrenaline, following 15 min of SDB, in healthy human volunteers (Wehrwein, Johnson, Charkoudian et al., 2012).

Atrial natriuretic peptide (ANP)

The stretch of the atrial walls promotes the release of ANP, triggering an ABP decrease by inducing peripheral vasodilatation, promoting diuresis and natriuresis, while also inhibiting renin and aldosterone secretion (de Bold, 1985). However, the short half-life of this hormone, together with the slow rate of its release by increased atrial distention, suggests that significantly higher ANP plasma concentrations might only be achieved after sustained elevation of venous return lasting several minutes, while saturation of plasma levels might only occur after 1 hour (Anderson, Donckier, Payne et al., 1987, Anderson, Maxwell, Payne et al., 1989, Tanaka, Sagawa, Miki et al., 1991). It is not clear if the practice of SDB leads to acute increases in

circulating ANP, but the addition of a small inspiratory resistance can potentially facilitate its release (Anderson et al., 1989).

Renin-angiotensin axis

The biosynthesis of the vasoconstrictor hormone ANGII results from the elevation of the plasma levels of the hormone renin. Renin is released from the kidneys' juxtaglomerular apparatus and acts as an enzyme in the synthesis of ANGII. Since the rate of renin release is the limiting factor in the production of ANGII, the concentration of plasma renin determines the presence of ANGII in the circulation (Reid, 1992). Numerous factors can affect renin release into the circulation (Laragh and Sealey, 1992). Importantly, the plasma concentration of renin is augmented in situations of heightened sympathetic nerve efferent activity and increased levels of circulating catecholamines (Reid, Morris and Ganong, 1978, Laragh and Sealey, 1992), and inhibited by atrial stimulation (Annat, Grandjean, Vincent et al., 1976, Anderson et al., 1987, Anderson et al., 1989).

The ANGII promotes a potent vasoconstrictor effect, leading to a rapid rise in ABP and reduction of *fc*, with a concurrent decrease in $\dot{Q}$ (Debono, Leegde, Mottram et al., 1963, Laragh and Sealey, 1992). Apart from the direct vasomotor effect, the ANGII acts centrally upon the baroreflex control of *fc*, allowing ABP to increase without a simultaneous bradycardic response. At the same time, ANGII enhances sympathetic activity, presumably easing neurally-mediated catecholamine release (Ferrario, 1983, Reid, 1992). In the context of acute responses to SDB, the potential change in plasma levels of renin and/or ANGII has not yet been established. However, due to the inhibitory effect of ANP, the depressing effect of SDB upon SNA during inspiration and the aforementioned reduction in plasma catecholamines with SDB, it is plausible to expect an acute reduction in the activity of the renin-angiotensin axis during, or immediately following, SDB interventions.

2-2.5 Summary

This section of the literature review briefly summarises the known afferent and efferent signals thought to be involved in the acute regulation of ABP during SDB interventions. Furthermore, it provides a brief summary of the most important hormonal mechanisms involved in the regulation of ABP, which in theory can also be implicated in the chronic response to SDB. Nonetheless, a substantial portion of

this extant literature arises from studies that employed reductionist models, using isolated stimuli, in quadrupeds.

The integrated human response is highly complex, as the integration of the information arising from these receptors occurs within a series of structures located in the brainstem. The nucleus tractus solitarius seems to play a fundamental integrative role, relaying vagal sensory information from cardiorespiratory afferents and organs of the gastrointestinal tract (Andresen and Kunze, 1994, Lawrence and Jarrott, 1996, Travagli, 2007, Dergacheva, Griffioen, Neff et al., 2010). Co-localisation of respiratory and cardiovascular neurones can also be found in other structures, such as the rostral ventrolateral medulla, nucleus ambiguous and ventral respiratory group (Spyer, 1994, Dergacheva et al., 2010).

However, this thesis does not seek to explore the neural control mechanisms, or the neural structures, involved in determining the acute cardiac and vascular response to SDB. Instead, it aims to characterise the response itself, attempting to further clarify the direct mechanical impact, as well as the integrated reflex response, to SDB.

2-3 Mechanical effects of breathing – heart-lung interactions

The existence of a respiratory-driven cardiovascular response to SDB is inherently determined by the direct mechanical effects of the varying intrathoracic pressure upon preload, afterload and SV of the right and left sides of the heart. The following section will describe the mechanics underpinning these interrelationships. Crucial to the understanding of this section is to note that the changes in cardiac pressures herein described, are always relative to respiration and not relative to the cardiac cycle. While the latter takes place in every single heart beat and are inherent to the cardiac cycle (Figure 2-3), the former occurs over the course of several heart beats (Figure 2-4).

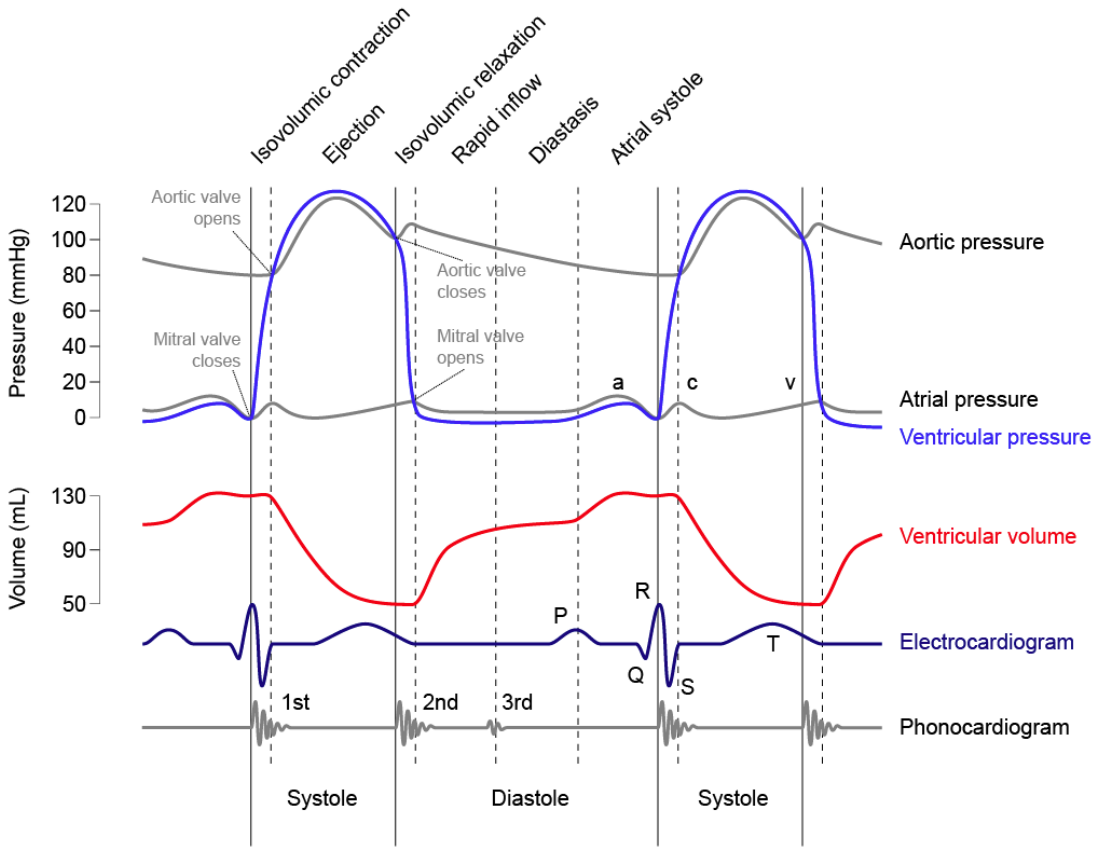


Figure 2-3 – The Wigger's diagram of the cardiac cycle demonstrating the normal relationships between heart chamber pressures, volumes and sounds, relative to the ECG events, for the left side of the heart. a - atrial contraction, c - small increase in atrial pressure from the mitral valve bulging into the atrium after closure, v - passive atrial filling. In the electrocardiogram: P wave - atrial depolarisation, QRS complex - ventricular depolarisation, and T wave - ventricular repolarization. From Hagen-Ansert (2017).

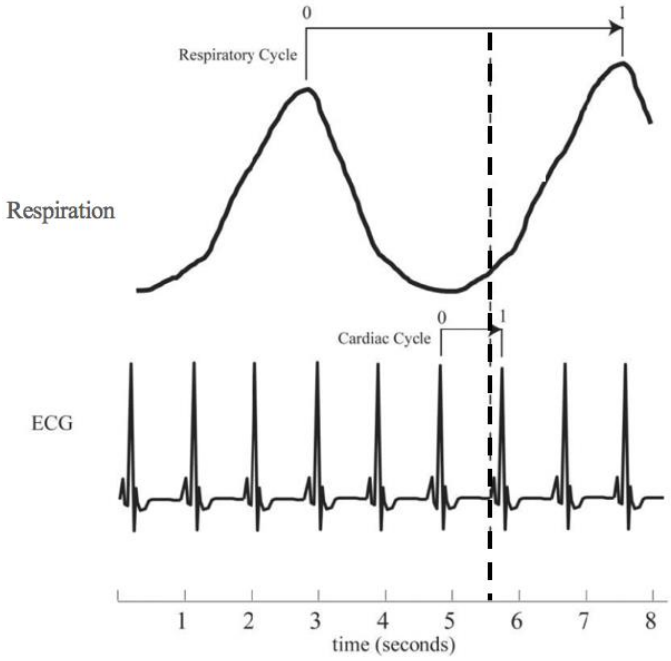


Figure 2-4 – Reporting changes in cardiac pressures relative to the respiratory cycle instead of the cardiac cycle implies that these may take place over the course of several heart beats. For example, for an individual breathing at 6 breaths·min^{-1} (10s cycle) and heart rate of 60 beats·min^{-1} each respiratory phase (inspiration/expiration) will contain, on average, 5 cardiac cycles. Adapted from Thompson and McVeigh (2006).

2-3.1 Impact of breathing on right ventricular preload

The location of the right atrium within the thorax leads to decreased right atrial pressure throughout inspiration, relative to atmospheric pressure (Permutt and Wise, 1986). As indicated by Guyton's work, the atrial pressure is, in effect, the back pressure to systemic venous return (Guyton and Adkins, 1954, Guyton, 1955, Guyton, Lindsey and Kaufmann, 1955, Guyton, Lindsey, Abernathy et al., 1957). Inspiration thus results in an augmentation of the pressure gradient between mean systemic pressure and right atrial pressure, thus accelerating blood flow towards the right atrium (Shabetai, Fowler, Fenton et al., 1965). Both right atrial pressure and venous pressure (systemic pressure in the peripheral veins) are minimal, with right atrial pressure oscillating between slightly above or below a mean value of 0 mmHg and venous pressure only being a few mmHg higher, in the supine position. Thus, the pressure gradient is small in absolute terms (<10 mmHg), which implies that small, respiratory-induced changes in either right atrial pressure or venous pressure can result in large relative changes to this pressure gradient, and thus significant alteration of venous return to the right atrium (Klabunde, 2011). According to the Frank-Startling Law, under normal conditions, increased right ventricular preload leads to an inspiratory increase in right ventricular stroke volume (RVSV) and pulmonary arterial flow (Figure 2-5) (Brecher and Hubay, 1955, Shabetai et al., 1965, Robotham, Rabson, Permutt et al., 1979).

Notwithstanding, venous return might be limited by the collapse of the venae cavae, if the right atrial pressure decreases substantially below atmospheric pressures (Guyton and Adkins, 1954). In other words, collapse occurs if extravascular pressure exceeds intravascular pressure, which tends to occur with deep inspiratory movements, particularly in the region where the venae cavae enters the thoracic cavity (Amoore and Santamore, 1994). Furthermore, the breathing pattern (thoracic vs. diaphragmatic) might impact venous return not only by altering vessel transmural pressure, but also by increasing the intra-abdominal pressure due to the descent of the diaphragm into the abdominal cavity (dominant in diaphragmatic breathing), leading to inferior vena cava compression (Willeput, Rondeux and De Troyer, 1984, Miller, Pegelow, Jacques et al., 2005b, Kimura, Dalugdugan, Gilcrease et al., 2011). As a consequence, diaphragmatic breathing has been demonstrated to lead to an inversion of the phase relation for venous return, relative to breathing, compared to thoracic breathing (Miller, Pegelow, et al., 2005b). In other words, for comparable lung excursions, venous return is augmented throughout inspiration with thoracic

breathing, while with diaphragmatic breathing, the venous return is larger throughout expiration.

In summary, respiratory induced fluctuations in intrathoracic pressure alter the pressure gradient for venous return to the heart, thus leading to respiratory phase-dependent changes in right ventricular filling, as illustrated in Figure 2-5. The magnitude of the variation of venous return can be limited by collapse of the venae cavae, and/or compression, determined by the respiratory depth and the pattern of breathing (thoracic vs. diaphragmatic), which can promote an opposite respiratory phase-dependency for venous return (Figure 2-6).

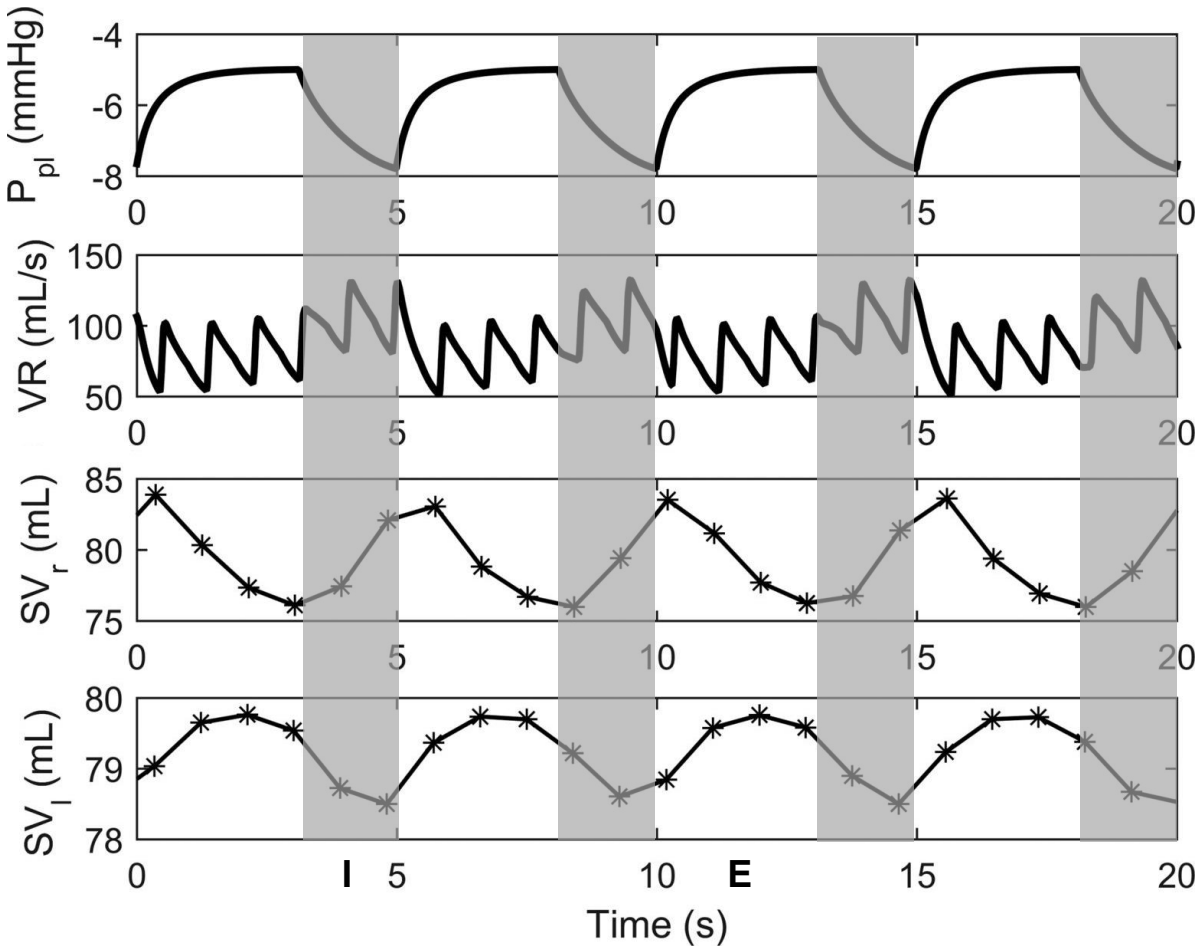


Figure 2-5 – Illustration of the mechanical effects of spontaneous respiration on cardiovascular function. Notice how venous return and right ventricular stroke volume increase with a more negative pleural pressure associated with inspiration (I; shaded) and concurrent fall throughout expiration (E; white). P_{pl} – Pleural pressure; VR – venous return; SV_r – right ventricular stroke volume, and; SV_l – left ventricular stroke volume. Adapted from Albanese, Cheng, Ursino et al. (2016).

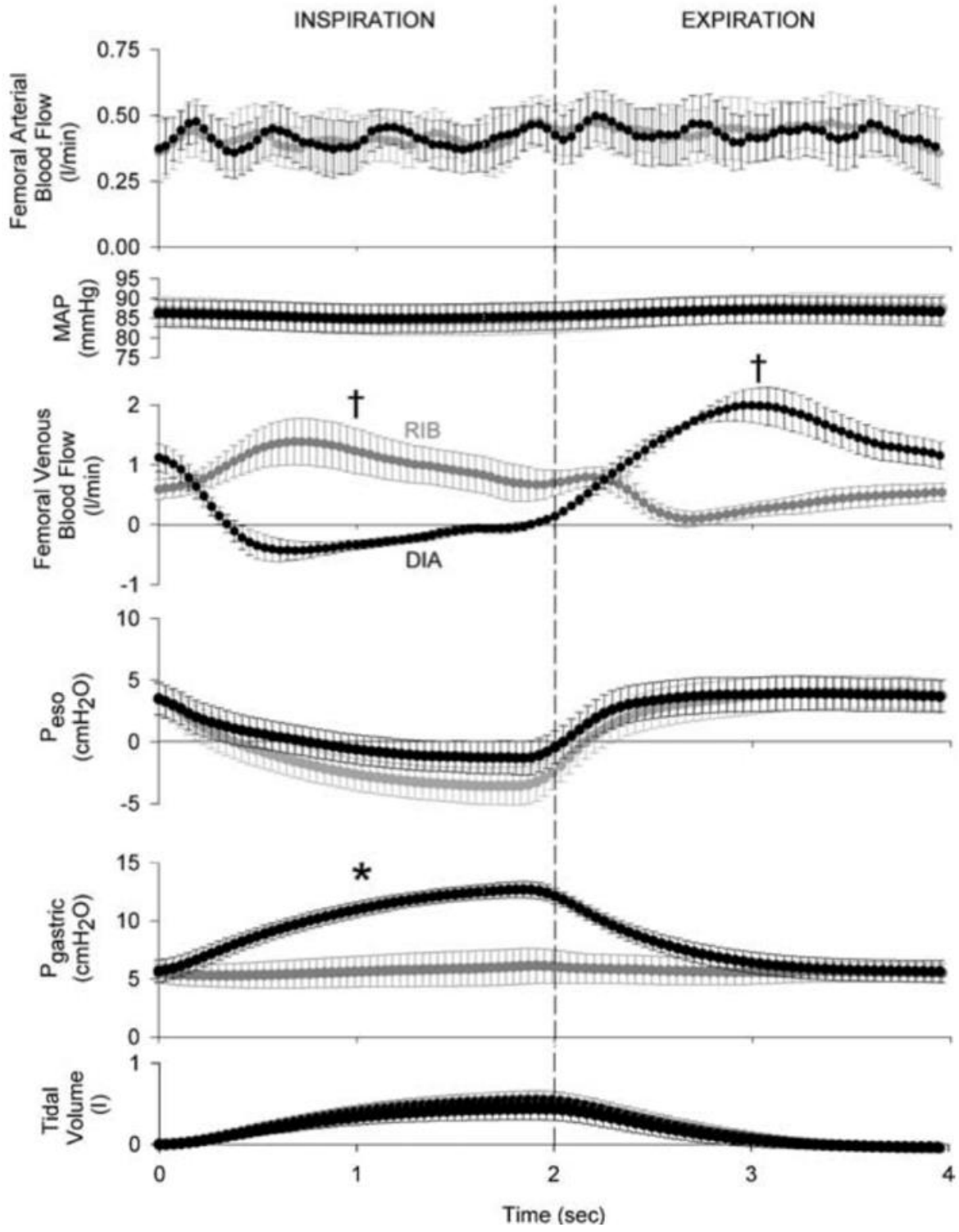


Figure 2-6 – Effects of thoracic or diaphragm breathing patterns on femoral arterial inflow, mean arterial pressure (MAP), femoral venous outflow, intrathoracic pressure (P_{eso}), intra-abdominal pressure ($P_{gastric}$) and tidal volume. Despite no discernible effect of breathing pattern on arterial inflow, femoral venous return (an indicator of overall venous return) is facilitated during a thoracic (RIB) inspiration and impeded during a diaphragmatic inspiration, with these modulatory effects being reversed during the ensuing expiratory phase of the breath. Grey circles - thoracic breathing; black circles - diaphragm breathing; †, $P < 0.01$ and *, $P < 0.05$ for comparisons of ribcage vs. diaphragm. Adapted from Miller, Pegelow, et al. (2005b).

2-3.2 Impact of breathing on right ventricular afterload

The relationship between lung vessel resistance and lung volume is extremely complex. The existence of both intra-alveolar and extra-alveolar vessels produces a coalesced effect of lung expansion stemming from the dissimilar response of each of these types of vessels to lung inflation. The overall relationship between lung volume and pulmonary vascular resistance is best described by a 'U' shaped relationship with a nadir at functional residual capacity (Figure 2-7; [West and Luks, 2016]). Increases in lung volume compress the lumen of intra-alveolar vessels, while also promoting the widening of the extra-alveolar vessels by increasing the radial interstitial forces ('parenchymal pull'). In healthy individuals, the net result is a small increase in pulmonary vascular resistance with increasing tidal breathing. This increase is more marked with larger inspiratory excursions, as the stretch across the diameter of the intra-alveolar vessels contributes to significant reduction of vessel calibre, and thus increased resistance (Permutt and Wise, 1986, Hamzaoui, Monnet and Teboul, 2013, West and Luks, 2016).

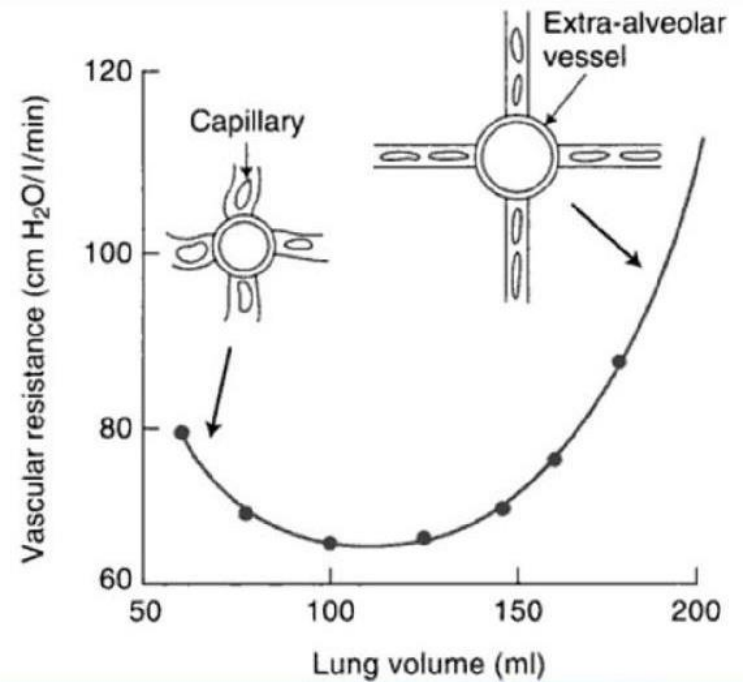


Figure 2-7 – Effect of lung volume upon pulmonary vascular resistance. Low lung volumes result in increased resistance as extra-alveolar vessels become narrow. Inflation beyond tidal breathing promotes stretching of the intra-alveolar capillaries with reduction of their calibre, contributing to increased resistance. Therefore, resistance is lowest at normal, tidal breathing volumes. From West and Luks (2016).

2-3.3 Impact of breathing on left ventricular preload

Evidence of a significant buffering of respiratory related fluctuations in systemic venous return occurring at the right ventricle and lungs has been reported previously in dogs and in computer based simulations of the human cardiovascular system (Hoffman, Guz, Charlier et al., 1965, Santamore and Amoore, 1994). These accounts are suggestive of a reduced respiratory influence upon left ventricular preload. However, data from healthy human beings suggests there is no significant difference between the within-breath amplitude of RVSV and LVSV. These data indicate that increased systemic venous return to the right side of the heart with inspiration is translated into similar-sized variation in blood flow to the left ventricle (Elstad, 2012). According to Elstad and colleagues, in spontaneously breathing individuals, RVSV and LVSV are 180º out of phase relative to the respiratory cycle, i.e. increased RVSV during inspiration only impacts preload to the left ventricle during the ensuing expiration (as illustrated in Figure 2-5). Overall, this suggests there is a decreased left ventricular preload during inspiration and increased left ventricular output during expiration, although it is not clear how SDB might influence this phase relationship.

2-3.4 Impact of breathing on left ventricular afterload

The inspiratory decrease in intrathoracic pressure also impacts left ventricular afterload, as the pressure surrounding the extra-thoracic compartment remains constant, or increases. Thus, during inhalation, a higher ejection pressure needs to be generated by the left ventricle to overcome the relatively higher afterload (Robotham, Lixfeld, Holland et al., 1978, Robotham et al., 1979, Scharf, Brown, Saunders et al., 1979). Moreover, during spontaneous breathing, intra-abdominal pressure increases due to the descent of the diaphragm, increasing the intraluminal pressure of major arterial vessels, particularly the abdominal aorta and consequently the impedance to left ventricular output (Karam, Wise, Natarajan et al., 1984, Robotham, Wise and Bromberger-Barnea, 1985). However, studies in both animals and healthy human beings seem to indicate that, when breathing spontaneously, LVSV is dominated by the respiratory effects upon preload, rather than afterload (Robotham, Stuart, Doherty et al., 1988, Scharf, 1995). Finally, a compressive effect of lung expansion (at high lung volumes) upon the heart has been hypothesised to impact left ventricular afterload (Robotham, Badke, Kindred

et al., 1983), although its role during spontaneous breathing in healthy individuals has been questioned (Scharf, Brown, Warner et al., 1989).

In summary, respiration induces cyclic changes in cardiac and great vessel transmural pressure, thus producing respiratory phase-related oscillations in blood flow through the major vessels (Figure 2-8) that influence cardiac preload and afterload.

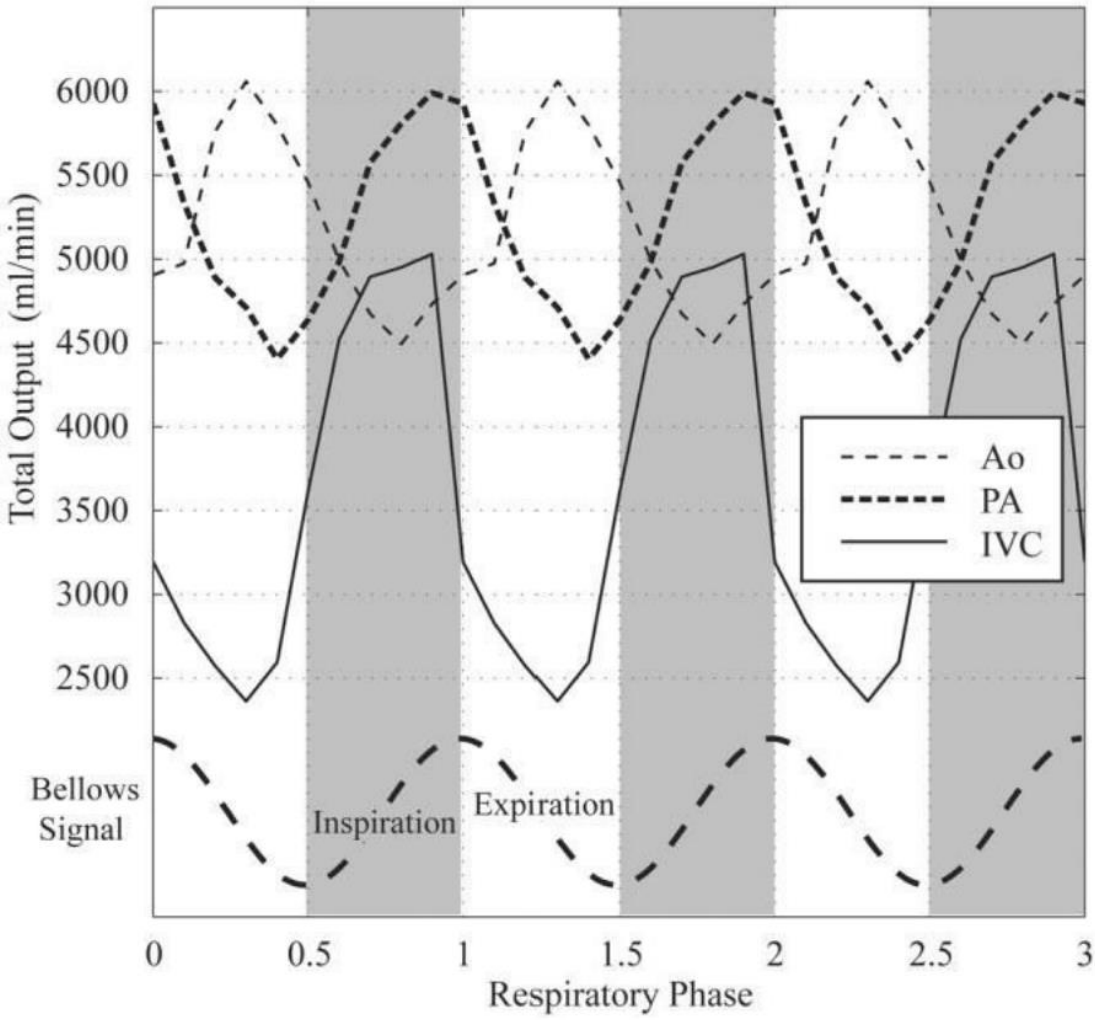


Figure 2-8 – Variation in great vessel blood flow per minute as a function of respiratory phase. IVC – inferior vena cava, PA – pulmonary artery and, Ao – aorta. From Thompson and McVeigh (2006).

2-3.5 Other factors influencing lung-heart interactions

Interventricular dependence phenomenon

The right and left ventricles are intimately interconnected by the presence of a shared pericardial casing, as well as a common septum, and myocardial fibres, which convey an obligatory interdependence between the two cardiac chambers. The shared anatomy implies that the mechanical events of one ventricle influence the behaviour of the other ventricle, as demonstrated by Janicki and Weber (Janicki and Weber, 1980, Weber, Janicki, Shroff et al., 1981). Previously, Dornhorst and colleagues had hypothesised about the impact of cardiac interdependence upon lung-heart interaction but suggested it to be of minor importance in healthy individuals, despite its relevance in certain conditions like cardiac tamponade (Dornhorst, Howard and Leathart, 1952a). Later studies explored the interdependence phenomenon and highlighted that an increase in right ventricular volume augmented both diastolic and systolic elastance (tendency to recoil) in the left ventricle, effectively impeding left ventricular filling, as well as reducing end diastolic volume.

In other words, increased inspiratory right ventricular filling pushes the septum towards the left ventricle increasing transmural pressure for a given left ventricular volume, thereby inhibiting left ventricular filling and LVSV (Bove and Santamore, 1981, Olsen, Tyson, Maier et al., 1985, Slinker and Glantz, 1986, Amoore and Santamore, 1989). However, this is contested by studies demonstrating similar values of RVSV and LVSV (Santamore and Amoore, 1994, Elstad, 2012). Although the interdependence phenomenon is most frequently examined from the perspective of how right ventricular filling affects left ventricular function, the phenomenon is bi-directional. Furthermore, experimental evidence has shown that between 20 and 40% of RVSV results from left ventricular contraction (Santamore, Lynch, Meier et al., 1976, Janicki and Weber, 1980, Slinker and Glantz, 1986, Santamore and Dell'Italia, 1998).

Pulmonary transit time

Another example of interdependence between the right and left side of the heart is the delay between the increase in right ventricular ejection and a consequent increase in LVSV, i.e. the pulmonary transit time. Before arriving at the left atrium, blood pumped from the right ventricle must transit the pulmonary circulation.

Pulmonary transit time is in the order of several seconds at rest, between 5 and 17 s (Blumgart and Weiss, 1927a, Zavorsky, Walley and Russell, 2003), which interposes a delay between changes in RVSV and LVSV (Dornhorst et al., 1952b, Amoore and Santamore, 1989). This delay might dictate that, at certain respiratory frequencies (f_R), both ventricles might be completely in phase or completely out of phase with respect to their stroke volume, which could either amplify or limit the influence of any septal interdependence (Hamzaoui et al., 2013). The combination of these two forms of interventricular dependence likely underpins the reported 180º-phase relationship between RVSV and LVSV, in spontaneously breathing healthy human beings (Elstad, 2012). However, it is not clear how, or if, this relationship is affected by SDB.

2-4 Cardiovascular oscillations

2-4.1 Historical perspective

Blood pressure oscillations

The existence of respiratory fluctuations in ABP has long been identified with the first records tracing back to Stephen Hales in 1733. Later, in 1760 Albrect von Haller was the first to note rhythmic fluctuations also occurring in heart rate (*fc*). However, neither Hales nor Haller made assumptions regarding a link between respiratory activity and cardiovascular fluctuations (Leake, 1962). The first accounts of respiratory-related oscillations in ABP came in the mid-1800s with Traube (and later Hering) observing slow oscillations at around seven cycles per minute when artificial respiration was interrupted in vagotomised dogs and cats. These oscillations ceased after two or three minutes of respiratory arrest, which led both Traube and Hering to postulate that they were linked to a strong respiratory centre discharge modulating the rhythmic activity of a vasomotor centre (Killip, 1962). Just a few years later, in 1876, Mayer described ABP oscillations in spontaneously breathing rabbits that were slower than breathing frequency (f_R), but that matched those described previously by Traube and Hering. In 1882, Fredericq reviewed the work of Mayer, Hering and Traube and concluded that the oscillations described by Traube and Hering occurred at respiratory frequency, while Mayer's waves were slower than the respiratory rhythm (Larsen, Tzeng, Sin et al., 2010).

The respiratory variations in ABP were explored in further detail in a seminal paper by Dornhorst, Howard and Leathart (1952b). In a series of small experiments in human volunteers, published in a single article, Dornhorst and colleagues demonstrated that the amplitude of ABP oscillations increased with decreasing f_R, while the phase relation between respiration and ABP was also altered by a change in f_R. Furthermore, the respiratory-driven variation in ABP seemed to be enhanced in the upright posture (compared to supine), while apnoea elicited regular ABP waves at a rate of around six cycles per minute, confirming the previous accounts of Mayer. Another important observation by Dornhorst and colleagues was that the respiratory modulation of LVSV was induced by respiratory driven fluctuations in intrathoracic pressure, which influenced right atrial filling, and that passive inflation of the lungs resulted in an 180° phase inversion between respiration and LVSV. Moreover, the relationship between respiration and LVSV also seemed to show a frequency dependent phase pattern, which the authors attributed to pulmonary transit time, i.e. the time required for blood to transit between the right and left sides of the heart. Nonetheless, the most significant finding was perhaps the identification of a synchronisation between respiratory driven variations in LVSV and those observed in peripheral resistance, when breathing at around 6 breaths·min^{-1} (0.1 Hz). It was suggested that at this f_R, the oscillations of LVSV and peripheral resistance were timed in such a way that they reinforced each other, thus creating a resonant effect (see also paragraphs dedicated to coherence and entrainment in section 2-4.5).

Earlier, in 1951, two different studies by Arthur Guyton reported vasomotor oscillations in anaesthetised, hypovolemic dogs that underwent progressive denervation; they concluded that so-called Mayer waves were sympathetically mediated (Guyton, Batson, Smith et al., 1951, Guyton and Harris, 1951). These findings were expanded by Preiss and Polosa (1974), who identified a synchrony between sympathetic preganglionic nerve activity patterns and the occurrence of the Mayer waves, in anaesthetised or otherwise decerebrated cats. One subsequent study from the same group supported the existence of temporal associations between respiratory rhythm, sympathetic outflow and ABP oscillations (Preiss, Kirchner and Polosa, 1975).

The body of evidence that rose from these (and other) studies in animals gave support to the theory that fluctuations occurring at the respiratory frequency must

involve the presence of a central mechanism modulating sympathetic cardiovascular activity, and synchronised with breathing. The slower Mayer waves were suggested to stem directly from sympathetically mediated vascular resistance oscillations (Cohen and Taylor, 2002).

Heart rate oscillations

The study of heart rate (*fc*) and heart rate variability (HRV) received less attention initially than that of ABP. However, a series of relevant accounts regarding the existence of oscillation in *fc* and the driving mechanisms were made throughout the 19th century. Around 1845, Ernst and Eduard Weber showed that *fc* was depressed by vagus nerve activity, while Albert Bezold demonstrated in 1867 that other nerves (sympathetic efferents) innervating the heart had cardio-acceleratory properties (Hurst, Fye and Zimmer, 2005). Herman Stannius (1852) established that heart rhythmicity and automaticity originated in the sino-atrial node, while Czermak's (1866) report of cardiac deceleration is response to increased pressure applied to the carotid sinus preceded Heinrich Hering's 1924 description of the cardiac baroreceptor reflex (Leake, 1962, Fleming, 1997, Fye, 2000, Larsen et al., 2010).

As is the case with ABP, two distinct rhythms have been identified in *fc*. The first has a cycle duration of approximately 10 s (0.1 Hz), i.e. like the previously described Mayer waves in ABP. This rhythm has been attributed to various mechanisms, but most recently has been assumed to reflect an integrated response to baroreflex-mediated fluctuations in sympathetic outflow to the vasculature and in parasympathetic and sympathetic cardiac activity (De Boer, Karemaker and Strackee, 1987, Di Rienzo, Parati, Radaelli et al., 2009).

A second oscillation of *fc* occurs at the respiratory frequency (f_R) and is thought to represent both mechanically-driven central blood volume changes, and autonomic neural fluctuations that are synchronised with breathing (Cohen and Taylor, 2002). These respiratory-synchronous swings in *fc* are termed respiratory sinus arrhythmia (RSA) and though they have received considerable research interest over the last decades, the exact mechanisms underpinning the origin(s) and amplitude of RSA remain elusive (see section 2-4.3 for a description of current theories relating to the mechanisms and function of RSA).

In 1936, Anrep, Pascual and Rössler, conducted a series of elegant experiments in dogs, seeking to clarify the mechanism underlying RSA (Anrep, Pascual and Rossler, 1936a, b). This was the first serious attempt to systematically test the existing theories of the time. There were, and remain, three 'schools of thought' in relation to the origin(s) of RSA:

1. lung-originated reflex mechanism, (initially proposed by Edwald and Heinrich Hering);
2. central interaction between cardiac and respiratory control centres (defended by Traube, Fredericq and Heymans);
3. based on Francis Bainbridge's work, changes in atrial filling and ABP occurring with changes in intrathoracic pressure.

Anrep et al.'s (1936a, b) experiments provided a unifying perspective, suggesting that RSA was due to a combination of factors, namely: 1) a direct inhibitory influence of central respiratory neurones upon cardiac vagal activity; and, 2) a reflex cardiac response to mechanical inflation of the lung.

The mechanisms responsible for RSA in human beings were explored in detail in a later series of studies conducted by Freyschuss and Melcher (1976a, b, c), who attributed RSA to 1) a reflex response from cardiopulmonary receptors to variations in venous return with breathing, and; 2) pulmonary stretch reflexes. Studies over the 40 years since the work of Freyschuss and Melcher have elucidated many of the factors that influence the magnitude of RSA (see section 2-4.3).

2-4.2 Oscillations in blood pressure

Oscillations occurring at 0.1Hz – Mayer waves

The current, prevalent theory suggests that Mayer waves are the result of resonances in the baroreflex control loop, occurring at 0.1 Hz in human beings (Julien, 2006). Within this conceptual framework, it has been argued that the existence of fixed time delays in the vascular baroreflex control loop lead to the production of resonant, self-sustained oscillations in ABP. The amplitude of Mayer waves is thus determined both by the sensitivity of the sympathetic branch of the baroreceptor reflex, and the strength of the triggering perturbations (De Boer et al., 1987, Julien, 2006).

Finally, some researchers have proposed an additive role of reflex (neurogenic) and myogenic processes. According to the proposers of the 'myogenic' theory, rhythmic oscillations in blood vessels are generated by pacemaker cells in the vascular smooth muscle contracting in response to variations in intravascular pressure, thereby contributing to the generation and amplitude of Mayer waves (Johnson, 1991, Stefanovska and Bracic, 1999a, b, Stefanovska, Bracic and Kvernmo, 1999).

Oscillations occurring at the respiratory frequency – Traube-Hering waves

Mechanical effects of respiration partly explain the respiration-coupled blood pressure oscillations, while the vagally induced RSA might also contribute (Eckberg and Sleight, 1992, Karemaker, 1999). Recent evidence also supports the notion that the respiratory fluctuations in ABP are, at least partly, the consequence of respiratory mediated changes in sympathetic outflow to the periphery; thereby suggesting central respiratory-sympathetic coupling as a putative mechanism for the Traube-Hering waves (Simms, Paton, Pickering et al., 2009, Towie, Hart and Pickering, 2012, Shantsila, McIntyre, Lip et al., 2015).

2-4.3 Oscillations in heart rate

Oscillations occurring at the Mayer wave frequency

One increasingly popular theory states that these 0.1Hz frequency oscillations reflect a resonant behaviour of the baroreflex control loop, determined by inherent, fixed delays in the sympathetic control loop (De Boer et al., 1987). This would suggest that 0.1Hz oscillations in *fc* are the direct consequence of the ABP fluctuations at the same frequency. However, this only seems to be observed consistently in situations where the sympathetic vascular outflow is high (e.g. head-up tilt, or standing). Typically, under other conditions, low-frequency ABP and *fc* oscillations show wide inconsistencies in coherence (i.e. spectral correlation) (Taylor and Eckberg, 1996, Hamner, Morin, Rudolph et al., 2001, Cohen and Taylor, 2002).

Respiratory sinus arrhythmia

The physiology underlying the interrelationship of breathing and heart rate variability (HRV) has been known for many years. Its best-known manifestation is the phenomenon of respiratory sinus arrhythmia (RSA), which is the broadly accepted

term describing the oscillations of the RR interval of the ECG occurring at a frequency similar to respiration. This phenomenon has been suggested to have an important teleological function, either by, 1) improving the efficiency of gas exchange by matching the alveolar ventilation with pulmonary perfusion throughout the respiratory cycle (Yasuma & Hayano, 2004), 2) reducing workload of the heart while maintaining normal levels of blood gases (Ben-Tal et al., 2012), 3) counteracting respiratory variations in LVSV, $\dot{Q}$ and ABP (Toska and Eriksen 1993; Elstad et al. 2001; Elstad, 2012; Elstad et al. 2015). Notwithstanding the existence of these theories, the precise underlying mechanisms and function of RSA, if any, remain unclear.

Currently, there are four mechanisms proposed as potential generators of RSA:

1) Reflex inhibition of the cardioinhibitory centre by the slowly adapting stretch receptors (SASR) in the lungs;
2) Central irradiation of inhibitory impulses from the respiratory centres to the cardioinhibitory centre;
3) Increased filling of the right atrium during inspiration, which activates mechanoreceptors at the junction of the great veins with the right atrium, increasing sympathetic activity to the sinus node (Bainbridge reflex); and
4) a change in sensitivity of the arterial baroreflex occurring in phase with the respiration.

The following section describes the evidence supporting each of the four theories:

RSA as a manifestation of vagal afferent input from lung mechanoreceptors

The first hypothesis arises from some of the first studies conducted on RSA, in the late 1930s. Anrep and colleagues (Anrep et al., 1936a, b) concluded that the mechanisms responsible for RSA included a reflex inhibition arising from the activation of mechanoreceptors in the lungs.

More than two decades later, de Burgh Daly and Scott (1958) described the cardiovascular responses to stimulation of the carotid arterial chemoreceptors in

anaesthetized dogs and showed that the primary bradycardic response was not seen when breathing increased in response to the chemostimulation, but ocurred when ventilation was controlled. They attributed the overriding of this primary reflex response to the activation of the lungs' mechanoreceptors, more particularly SASRs. The SASRs are vagal afferents believed to lie in the airway smooth muscle (Schelegle and Green, 2001, West and Luks, 2016). According to Adrian's classic paper (Adrian, 1933), the SASRs are primarily responsive to changes in lung volume, are inhibited by deflation and tend to adapt slowly to sustained inflation (hence the nomenclature). The stimulation of these receptors triggers cardio-acceleration; the so-called Hering-Breuer reflex. Importantly, the stimulation of SARs promotes slowing of breathing frequency (f_R) by extending expiratory time. However, unlike other mammals, which show a reflex response at resting lung volumes (Widdicombe, 1961b), respiratory pattern in human beings is not altered unless V_T exceeds 1 L (Iber, Simon, Skatrud et al., 1995). However, more recent evidence shows that afferent vagal feedback from SASR does modulate breathing in healthy adults, particularly when the perception of chest wall movements is suppressed (BuSha, Judd, Manning et al., 2001, BuSha, Stella, Manning et al., 2002).

The involvement of SASRs in RSA was supported by the research of Haymet and McCloskey (1975), reporting inhibition of both baroreceptor and chemoreceptor effects on *fc* during inspiration. Furthermore, Gandevia and colleagues (Gandevia, McCloskey and Potter, 1978) indicated that, in dogs, this inhibition is abolished by denervation of the lungs. These authors also reported that the level of inhibition was dependent upon the rate of lung inflation and that this inhibition only occurred during the inspiratory phase of breathing.

Relevant to the context of this thesis, the reflex increase in *fc* with lung expansion has direct implications for the acute chronotropic response to SDB, as studies in both lung denervated dogs and human beings showed almost complete abolition of RSA in the absence of afferent feedback from the lungs, thereby lending support to an obligatory contribution from lung vagal feedback to the generation of RSA (Anrep et al., 1936a, Taha et al., 1995). Furthermore, both the rate of change of lung volume (Davis, Fowler and Lambert, 1956) and increasingly negative intrapleural pressures (Widdicombe, 1961a, Davenport, Frazier and Zechman, 1981) are known to

increase the afferent discharge from the SASRs. Collectively, these data supported the hypothesis that excitation of lung SASRs underpinned RSA.

RSA resulting from a central generator

The findings of some of the aforementioned studies also provided support for the hypothesis of a central inhibitory impulse underpinning the generation of RSA (point 2 above). Studies conducted in dogs demonstrated the existence of an inhibitory effect of inspiration upon baro- and chemo-receptor reflex effects upon *fc*, leading to a bradycardia during the expiratory phase of breathing (Davidson, Goldner and McCloskey, 1976). Said bradycardia occurred even when ventilation was temporarily stopped and the baro- and chemo-receptor stimuli were delivered during the inspiratory phase of the neural respiratory cycle (Gandevia et al., 1978). Studies in human beings confirmed the modulation of vagal responsiveness to arterial baroreceptor stimulation (Eckberg and Orshan, 1977, Eckberg et al., 1980). The observation of the maintained *fc* rhythms within the typical respiratory band, even during apnoea and concomitant absence of respiratory movements, has provided yet more evidence to support a role for central irradiation of inhibitory impulses from the respiratory center in the generation of RSA (Hirsch and Bishop, 1981, Kollai and Mizsei, 1990).

The influence of the respiratory control centre is not limited to its effect upon cardio-vagal motoneurones; it also modulates the activity of sympathetic motoneurones. It appears that vagal cardio-motoneurones are inhibited during the inspiratory phase, but are mildly activated during expiration (Gilbey, Jordan, Richter et al., 1984, Richter and Spyer, 1990). On the other hand, sympathetic neurones exhibit high inter-species variability in their pattern of discharge, which may account the variety of confounding findings across the literature (Eckberg, Nerhed and Wallin, 1985, Richter and Spyer, 1990). That said, sympathetic outflow demonstrates a systematic pattern of respiratory modulation, in that excitation in one phase of respiration, is accompanied by inhibition in the opposite respiratory phase (Richter and Spyer, 1990). In human beings, inhibition of the sympathetic motoneuron discharge seems to occur mostly during inspiration, whilst increased sympathetic activity is observed particularly in late-expiration (Eckberg et al., 1985, Seals, Suwarno, Joyner et al., 1993, St. Croix, Satoh, Morgan et al., 1999).

These, and other observations gave way to the well-known ‘respiratory gate’ theory proposed by Dwain Eckberg (Eckberg, 2003), which expanded on the ‘respiratory gating’ concept introduced by Lopes and Palmer, almost 30 years earlier (Lopes and Palmer, 1976). The technological breakthroughs occurring during the three decades between Lopes and Palmer’s provocative work and Eckberg’s review allowed a deeper comprehension of the interrelation between autonomic cardiovascular and respiratory control. According to Eckberg, these shed light on the ‘respiratory gate’ concept. Eckberg’s theory postulates that the respiratory modulation of *fc* can be best described as a ‘gate’ consisting of inspiratory interneurones (probably located within the nucleus tractus solitarius; a brainstem structure that receives and relays vagal afferent feedback from the cardiovascular system and other structures in central nervous system), which control the passage of impulses into the nucleus ambiguous[1] (Lopes and Palmer, 1976, Eckberg, 2003).

The first of Eckberg’s new concepts was that evidence of respiratory activity could be found imprinted upon human autonomic signals. It is known that as breathing rate decreases the RR intervals fluctuations (RSA) increase, as does the total spectral power (Hirsch and Bishop, 1981, Eckberg, 1983, Saul, Berger, Chen et al., 1989, Song and Lehrer, 2003). Simultaneously, important but smaller fluctuations have also been detected in ABP and SNA (Badra, Cooke, Hoag et al., 2001). Together with previously reported evidence regarding the responsiveness of vagal motoneurones to baroreceptor stimulation (Eckberg and Orshan, 1977, Eckberg et al., 1980), these findings led Eckberg to define the ‘respiratory gate’ as a sinusoidally varying, permanently open gate (Eckberg, 2003) that regulates both vagal and sympathetic motoneurone responsiveness to external inputs, particularly of baroreflex origin (Eckberg et al., 1985, Eckberg, 2003, Rothlisberger, Badra, Hoag et al., 2003).

According to Eckberg and colleagues (Eckberg, 2003, Rothlisberger et al., 2003), not only does respiration gate muscle sympathetic nerve activity, but it also determines the timing of spontaneous baroreflex sequences through a cascade of events:

1) the respiratory gate opens, and sympathetic bursts appear;

[1] Structure in the brainstem that is responsible for vagal efferent traffic to the heart.

2) the sympathetic bursts increase ABP, triggering baroreflex RR interval prolongations;
3) the increase of ABP silences sympathetic motoneurones, ABP falls, leading to RR interval shortening.

Importantly, the Rothlisberger et al. (2003) study suggested that respiration does not affect the cardiac baroreflex sensitivity (BRS), as BRS was similar during spontaneous breathing and apnoea.

The magnitude of respiratory gating of neural outflow depends critically on the level of stimulation. This is supported by evidence showing abolition of respiratory modulation of vagal motoneurone response with intense baroreceptor stimulation (Eckberg and Orshan, 1977), or pharmacologically elevated ABP, leading to diminished RSA (Goldberger, Ahmed, Parker et al., 1994). Similar findings arose from experiments exploring graded passive upright tilt and its effects on SNA; they observed that significant inspiratory-expiratory differences of sympathetic outflow in the supine position and lower tilt angles disappear when moving to an upright posture (Cooke, Hoag, Crossman et al., 1999).

Despite not ruling out the contribution of other sources of variability, the persistence of RR interval fluctuations at the respiratory frequency in the absence of intrathoracic pressure variations (apnoea), in both human (Hirsch and Bishop, 1981, Kollai and Mizsei, 1990) and animal (Shykoff, Naqvi, Menon et al., 1991) models, favours the argument around the contribution of a central mechanism towards the timing and amplitude of respiratory related variations in *fc*. Furthermore, conventional mechanical ventilation (with phasic tidal lung inflation) reduces respiratory RR fluctuations, much like what is observed for high-frequency jet ventilation (continuous lung inflation with minute V_Ts), strengthening the case for a central generator of RSA (Koh, Brown, Beightol et al., 1998).

RSA resulting from atrial stimulation

A third putative mechanism for the generation of RSA suggests it is underpinned by the stimulation of mechanoreceptors located mainly at the junctions of the great veins with the right atrium. A more in-depth characterisation of these atrial baroreceptors has been previously provided in section 2-2.2. The first evidence of

the existence of such afferents came from the studies of Francis Bainbridge, who demonstrated that the infusion of saline or blood into the jugular vein of the anesthetised dog produced tachycardia (Bainbridge, 1915). Similar cardiac acceleration was secondary to increased venous return accompanying inspiration, in anesthetised cats and dogs (Bainbridge, 1920); this phenomenon was later to become known as the Bainbridge reflex.

The Bainbridge reflex was studied throughout the 20th century, with subsequent researchers confirming its existence and clarifying its anatomical and physiological features, mostly in dogs (Coleridge et al., 1957, Ledsome and Linden, 1964, 1967, Horwitz and Bishop, 1972, Vatner, Boettcher, Heyndrickx et al., 1975). Later studies demonstrated a species-dependency of the Bainbridge reflex, suggesting a much-attenuated magnitude of response in human beings than had been previously observed in dogs (Boettcher, Zimpfer and Vatner, 1982). A more dominant arterial baroreceptor mechanism and lower baseline vagal tone in human beings, thus precluding substantial vagal-withdrawal-mediated increases in *fc*, are the likely factors behind the less potent response observed in humans (Boettcher et al., 1982). Interestingly, in supine, conscious individuals, undergoing graded increases in central blood volume (within physiological range), the baroreflex vagal-mediated decrease in *fc* was dominant during the initial increases in blood volume but was suppressed when central blood volume increased further. This was consistent with the existence of a mild Bainbridge reflex in human beings and suggested a potential role in controlling short-term fluctuations of *fc*, and thus RSA (Barbieri, Triedman and Saul, 2002).

Despite the supporting evidence of the existence of cardiac modulation in response to changes in central blood volume, and potentially by respiratory-driven changes in venous return, it is still unclear if the dominant mechanism underpinning change in *fc* is of reflex origin (Bainbridge reflex) or, mainly myogenic. Studies in heart transplanted individuals (with no vagal innervation of the heart) demonstrated the existence of respiratory modulation of *fc* (RSA), likely linked to a direct mechanical stretch of atrial walls, induced by respiratory variations in venous return (Bernardi et al., 1989, Taha et al., 1995).

RSA resulting from baroreflex mechanisms

The fourth and final mechanism proposed to underlie the generation of RSA is related to the arterial baroreflex. The deformation of mechanosensitive baroreceptors provides afferent neural information to the medullary centres, via the vagus nerve, resulting in increases or decreases in the outflow of efferent sympathetic nerve traffic to the peripheral vasculature and heart, as well as efferent vagal traffic to the heart. A more detailed description of the mechanisms of action has been provided previously in section 2-2.1.

The idea that RSA could be mainly determined by respiratory driven alterations in ABP, transiently affecting *fc* by stimulation of the aortic arch and carotid baroreceptors, was first suggested by Schwheitzer in 1937, and later supported by a 1961 paper from Koepchen and colleagues (*cit by* de Burgh Daly, 1986). These early studies, performed in isolated and perfused carotid sinuses, garnered little support, primarily because they failed to explain why during slow breathing, tachycardia could occur in the presence of an increase in ABP (de Burgh Daly, 1986). However, the discovery of a within-breath variation in the responsiveness of cardiac vagal motoneurones to incoming baroreceptor afferent traffic (Haymet and McCloskey, 1975, Eckberg and Orshan, 1977, Eckberg et al., 1980) shed new light on the matter, and re-ignited interest in the role of the baroreflex in the generation and amplitude of RSA.

Significant advances resulted from the mathematical modelling work of De Boer et al. (1987), and later TenVoorde, Faes, Janssen et al. (1995), in which they advocated that ABP was altered mechanically by the act of breathing, with an immediate response from *fc* (within the same heart beat), determined by a fast-acting cardiac vagal baroreflex loop.

This hypothesis gained support from studies using neck suction to stimulate the carotid baroreceptors in conjunction with paced breathing (Piepoli, Sleight, Leuzzi et al., 1997, Keyl, Dambacher, Schneider et al., 2000). Piepoli and colleagues reported that RSA could be either mimicked or suppressed through the stimulation of arterial baroreceptors with neck suction. When delivering continuous sinusoidal neck suction at the frequency of respiration during apnoea, they managed to reproduce oscillations in ABP, thus supporting the idea of a mechanical origin of respiratory oscillations in ABP. In the same study, similar stimulation of a non-

baroreflex area (thigh) had no measurable effect on RSA, confirming the carotid baroreceptors as the source of the resulting RSA. The contribution of a baroreflex mechanism to the generation and amplitude of RSA was also demonstrated by the attenuation of RSA when carotid stimulation was delivered at a phase of the respiratory cycle that would counteract the normal phase relation between respiration and ABP (Piepoli et al., 1997). This was further supported by Keyl et al.'s data (2000), demonstrating that neck suction at 0.2Hz produced a similar time lag between systolic blood pressure (SBP) change and RR interval alteration to that observed with paced breathing at 0.25Hz.

What does determine respiratory sinus arrhythmia?

All things considered, the existence of a single, unifying mechanism controlling the generation and magnitude of RSA seems unlikely. In a highly interesting point/counterpoint paper in the Journal of Applied Physiology, both Dwain Eckberg and John Karemaker (with some valuable input to the discussion being provided from other expert members of the scientific community) defended their differing views regarding the main determinant(s) of RSA (Eckberg, 2009a, b, Julien, Parkes, Tzeng et al., 2009, Karemaker, 2009a, b, c). Eckberg strongly advocated that central gating was sufficient to explain respiratory frequency RR interval fluctuations and that the latency between ABP changes and parallel RR interval changes, as defined by cross-spectral phase, was too short for meaningful baroreflex responses to be mounted. Contrarily, Karemaker championed the important role of the baroreflex in the generation and the magnitude of RSA and argued that if all RSA is due to central modulation alone, similar-sized oscillations would be expected in both systolic (SBP) and diastolic blood pressures (DBP). Karemaker went on to argue that while a large respiratory variability in SBP exists, DBP varies very little, thus arguing for an extremely quick cardiovagal-baroreceptor loop, in line what he and others had previously demonstrated with mathematical models (De Boer et al., 1987). In the end, both acknowledged the existence of an undefined blend of baroreflex mechanism and central gating in the generation of respiratory frequency RR interval fluctuations, with Karemaker also pointing out the importance of other reflex input mechanisms. These included those resulting from the effects of respiration on venous return and the resulting pressure changes inside the heart chambers, which are known to have a modulating effect upon autonomic outflow (Karemaker, 2009c). Nonetheless, apart from the intricacy of the underlying physiology here discussed, and some concessions with regards to the existence of multiple mechanisms

underpinning RSA, the fact remains that no one has yet managed to provide unequivocal evidence in support of a unifying model, if in fact, one exists, underpinning respiratory-related fluctuations in RR interval.

2-4.4 Oscillations in sympathetic nervous activity

Historically, ever since it has been possible to obtain direct recordings of sympathetic nerve activity (SNA), evidence of respiratory modulation has been reported (Okada and Fox, 1967, Hagbarth and Vallbo, 1968, Preiss et al., 1975, Gerber and Polosa, 1978, Eckberg et al., 1985). Despite the evidence that sympathetic outflow is not uniform across the different vascular beds, it is accepted that cardiac, splanchnic, renal and muscle sympathetic nerve activities (MSNA) are strongly influenced by baroreceptor and chemoreceptor stimulation (Hart, Head, Carter et al., 2017). Thus, the measurement of MSNA provides a convenient indicator of sympathetic vasoconstrictor outflow to these vascular beds (Wallin, Esler, Dorward et al., 1992, Wallin, Thompson, Jennings et al., 1996). Also, relevant for the foregoing discussion, MSNA shows high inter-individual variability, but very low intra-individual variability (Fagius and Wallin, 1993).

Respiratory modulation of MSNA

In healthy individuals, vagally mediated lung stretch reflex is the primary mechanism through which MSNA is modulated by respiration (Seals, Suwarno and Dempsey, 1990, Seals et al., 1993), with the degree of lung inflation affecting the magnitude of the respiratory modulation. However, the latter is not observed in lung denervated individuals, which despite showing modulation of MSNA at the respiratory frequency, did not demonstrate a potentiation of this effect at elevated tidal volumes (Seals et al., 1993)

A contribution of baroreceptor afferent stimulation by respiratory induced changes in ABP has also been advocated as a contributor to the fluctuations in MSNA, within the respiratory frequency band (St. Croix et al., 1999). During spontaneous breathing, MSNA usually reaches a nadir in late inspiration and peaks during expiration (Dempsey, Sheel, St Croix et al., 2002). However, the modulatory influence of respiration upon MSNA seems to be attenuated in some disease conditions, including hypertension (Fisher, Reynolds, Farquhar et al., 2010, Fisher,

McIntyre, Farquhar et al., 2011), and to a lesser extent in chronic heart failure (Goso, Asanoi, Ishise et al., 2001). Furthermore, while older people tend to have higher MSNA than their younger counterparts, the pattern of respiratory inhibition of MSNA does not show substantial age-related modifications (Shantsila, McIntyre, et al., 2015). As mentioned earlier in section 2-4.2, the respiratory modulation of MSNA likely represents a causative relationship, with the presence and amplitude of ABP fluctuations occurring in parallel with respiration.

Fluctuations in MSNA occurring at 0.1Hz

Reported oscillations in SNA at a frequency close to that of the so-called Mayer waves in ABP has led to the assumption that MSNA oscillations driven by a central oscillator could be responsible for the ABP Mayer waves (Julien, 2006). Studies in animal models have demonstrated that fluctuations in SNA persist even when ABP oscillations are abolished, and are thus independent of baroreflex control (Preiss and Polosa, 1974, Grasso, Rizzi, Schena et al., 1995). Further evidence of a possible link between a central modulator of SNA and Mayer waves was shown in vagotomised, baroreflex-denervated, anesthetised cats, where oscillations in both medullary neurones and cardiac SNA were detected at the Mayer wave frequency (Montano, Gnecchi-Ruscone, Porta et al., 1996, Montano, Cogliati, da Silva et al., 2000).

There is some support for a centrally originated rhythm in SNA that is independent of baroreflex afferent signals but only under strict experimental conditions. Data from human beings where, under physiological conditions, the arterial baroreflex is the dominant ABP regulatory mechanism, shows a necessary modulation of ABP Mayer waves by reflex mechanisms (Julien, 2006). This can be demonstrated clearly by sino-aortic deafferentation, where the complete removal of baroreflex afferent input abolishes Mayer waves despite the presence of oscillations in vasomotor outflow (Di Rienzo, Parati, Castiglioni et al., 1991, Julien, Zhang, Cerutti et al., 1995, Mancia, Parati, Castiglioni et al., 1999). Thus, sinusoidal baroreflex stimulation occurring at 0.1Hz, as a consequence of resonances in the baroreflex control loop triggering an ABP rhythm, could be the main driver of 0.1Hz fluctuations in SNA (Malpas, Leonard, Guild et al., 2001).

Importantly, the magnitude of SNA outflow to each vascular bed will be determined by said organ's baroreceptor sensitivity and will be delivered with a fixed time delay in relation to the previous ABP perturbation. The combination of the delays from the different vascular beds, including the skeletal muscle vasculature, kidney, gut and lungs, implies that the vascular resistance response following the change in ABP can amplify said oscillations, instead of buffering them, thereby contributing to the 0.1 Hz fluctuations in ABP by a mechanism other than a central oscillator (Malpas et al., 2001).

2-4.5 Coherence and entrainment

The previous points in this section have highlighted the presence of rhythmicity in cardiovascular signals occurring at the respiratory frequency (f_R), but also at lower frequencies, which in humans, tend to occur with a periodicity *circa* 10s (0.1 Hz). The alignment of the different rhythms when breathing at a frequency close to 0.1Hz (6 breaths.min^{-1}) is thought to produce synchronisation, entrainment (of respiration, ABP and *fc*) and resonance (likely from the baroreflex control of ABP and *fc*, leading to amplification of the oscillations in these physiological variables). This phenomenon is sometimes referred to as 'coherent breathing' (McCraty and Tomasino, 2004, Elliot and Edmonson, 2006). 'Coherent breathing' normally results in smooth, sinusoid patterns in vascular and cardiac rhythms, which translates into a very high-amplitude peak in the low frequency (LF; 0.04-0.15 Hz) band of the HRV and BPV power spectrum (McCraty and Tomasino, 2004).

The mechanism behind this amplification of the cardiovascular rhythmicity is believed to be linked to the resonance characteristics of the vascular baroreflex control loop. Resonances are typical of some physical systems, more specifically those that comprise fixed delays (Grodins, 1963, cited by Lehrer, 2013). These result in very high amplitude oscillations at a specific frequency (resonant frequency) when a stimulus is applied with the same or similar frequency. Furthermore, existing oscillations at other frequencies are completely suppressed in the presence of resonant fluctuations (Lehrer, 2013).

While ABP and *fc* oscillations are irregular and small during spontaneous breathing, these become much larger and regular when breathing at the resonant frequency, or close to it. Although the mechanism proposed by Lehrer, Vaschillo and others to

explain the effects of breathing at resonant frequencies is likely an over-simplification (Lehrer, Vaschillo and Vaschillo, 2000, Vaschillo et al., 2002), the existence of a slow breathing frequency, which amplifies cardiovascular oscillations, is undeniable, and might be of clinical relevance. According to Vaschillo and colleagues (2002), resonant breathing is characterised by:

1) Respiration and *fc* with a 0º phase relation (completely in phase);
2) ABP and *fc* with an 180º phase relation (completely in antiphase).

The authors associated the aforementioned phase relations to a ~5 s delay within the cardiac baroreflex loop. They also argued that this interpretation was strengthened further by evidence that the ABP resonant frequency lays at even lower frequencies, corresponding to the time delay of the vascular baroreflex loop (Vaschillo et al., 2002). Furthermore, Vaschillo reasoned that the mechanical effect of breathing triggers a decrease in *fc* that reduces blood flow and therefore ABP, thus promoting a stimulus for *fc* to increase. Despite being physiologically plausible, it has been previously demonstrated that RSA only significantly contributes to the amplitude of ABP variations when sympathetic tone is suppressed (Saul, Berger, Albrecht et al., 1991).

Postural changes impact autonomic activity and can, therefore, change the contribution of RSA to ABP fluctuations, particularly when considering variations in systolic blood pressure (SBP) in the supine position (Elstad, Toska, Chon et al., 2001). Therefore, any causality between RSA and ABP must be interpreted with care. Moreover, others have reported a quicker time course of the action (2 s of time delay followed by 2s of time constant for the maximal response to be attained) of the sympathetic baroreflex control of the vasculature (TenVoorde et al., 1995, van de Vooren, Gademan, Swenne et al., 2007) than the 5 s delay proposed by Vaschillo. Thus, it is likely that Vaschillo's explanation is deficient by being unable to explain how and if other mechanisms producing rhythmic oscillations in *fc*, ABP and SNA (described earlier in this section) contribute to, or buffer, the amplitude of the observed resonant behaviour.

2-4.6 Summary

The advent of new mathematical methods has permitted the study of cardiac, respiratory and vascular fluctuations in the frequency domain and to better

understand the phase relationships between the different rhythms. Also, the widespread use of methods like the neck pressure/suction chamber and the application of lower body positive/negative pressure or tilt techniques have permitted selective manipulation of inputs to cardiovascular receptors and to gradually change autonomic tone, in human beings. Furthermore, the development of minimally invasive ways of assessing MSNA, and the reliable noninvasive measurement of beat-to-beat ABP, LVSV and $\dot{Q}$, has permitted *in vivo* studies in human beings, which were only previously possible in animal models. Taken together, the research using these techniques has furthered the understanding of cardiovascular rhythms and their interaction with breathing, but overall, the exact underlying mechanisms remain somewhat elusive. Figure 2-9 represents one of the many attempts in the literature to provide an integrated picture of the existing oscillations affecting ABP fluctuations.

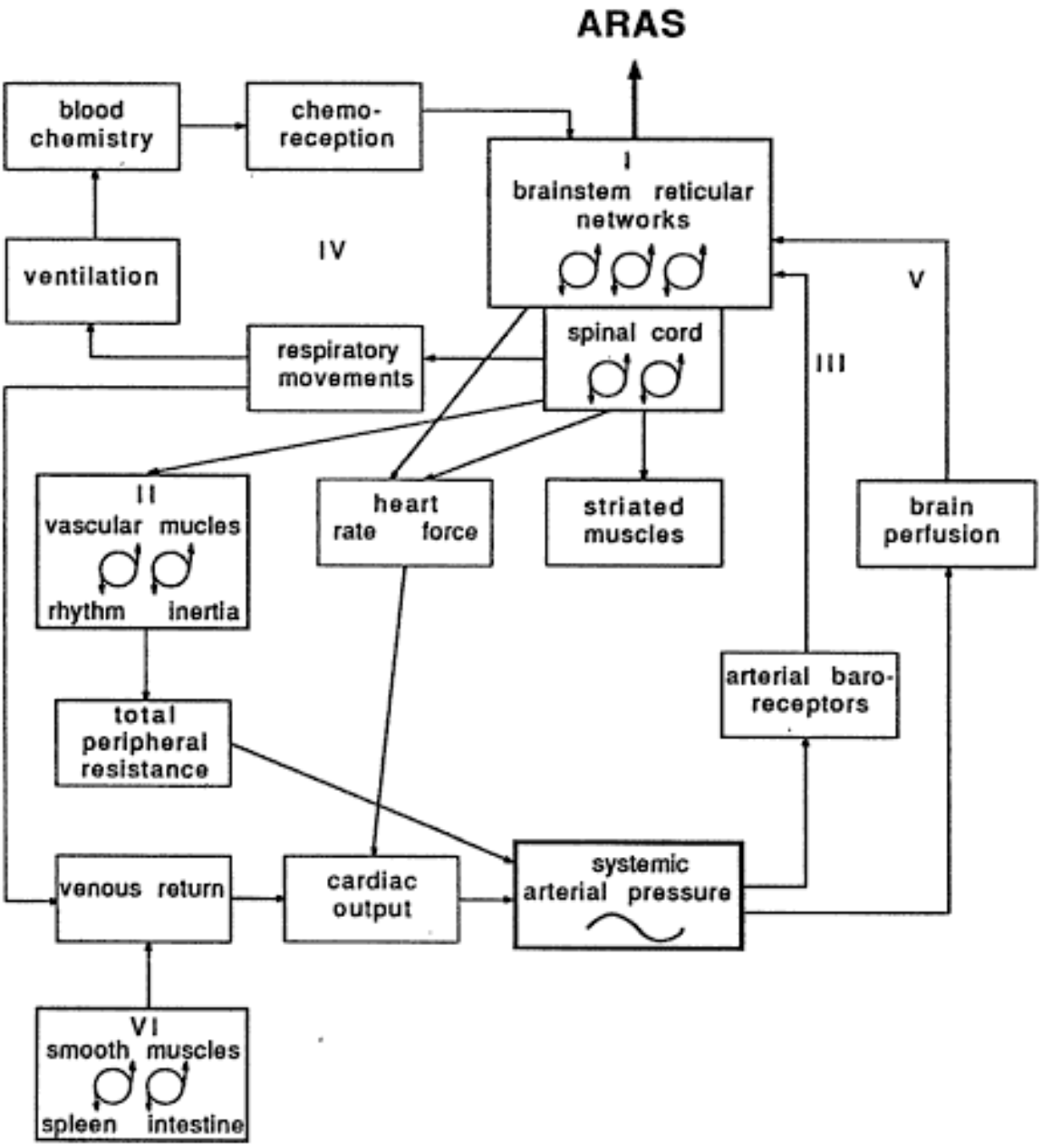


Figure 2-9 – Scheme of the main known oscillations affecting arterial blood pressure fluctuations. Circles reflect local oscillators in the central nervous system or smooth muscle system (myogenic mechanisms) Arrows represent feedback systems that oscillate under certain conditions. ARAS – Ascending reticular activating system (series of nuclei in the brainstem of which the reticular formation is the most relevant); III – Baroreceptor circuit; IV – Chemoreceptor circuit; V – Brain ischemic circuit. From Koepchen (1991).

2-5 Acute effects of variations in breathing pattern and intra-thoracic pressure upon the cardiovascular system

2-5.1 Effects of breathing pattern

The promise of potential clinical applications of SDB interventions stems mostly from the outcome of studies where SDB was administered daily for several weeks. Some of the relevant findings from such studies were summarised in the introductory section of this thesis (Chapter 1 – General Introduction). However, little is known about the link between the acute cardiovascular responses taking place during SDB and the long-term adaptations it might stimulate. Furthermore, a significant portion of the existing body of literature has focused specifically on measures of RSA, cardiac baroreflex sensitivity (BRS), heart rate variability (HRV) and blood pressure variability (BPV), as well as MSNA. Much less attention has been devoted to the actual within-breath modulation of LVSV, $\dot{Q}$ and ABP, and how changing the characteristics of the SDB stimulus might impact the respiratory modulation of cardiovascular parameters, and therefore alter the stimuli to some of the afferent signals described above; particularly to the arterial baroreceptors. Nonetheless, there is a significant body of research that provides a better understanding of what is currently known regarding the acute cardiovascular impact of SDB, which is summarised in the following sections. This summary is not intended to be comprehensive, but is a focused account of what is considered to be the most pertinent evidence. A more detailed description of each study can be found in table format in Appendix I.

The seminal paper of Dornhorst and colleagues (1952b), was one of the first to show the existence of a phase relation between respiration and ABP that was altered by the reduction of f_R. A similar finding was made a few years later for the relationship between respiration and *fc*, or more precisely, RSA (Angelone and Coulter, 1964). In their study, Angelone and Coulter reported maximal values of RSA at approximately 6 breaths·min^{-1}. Many others have reported a similar behaviour of RSA (or HRV), with a tendency for maximisation close to 6 breaths·min^{-1} (Hirsch and Bishop, 1981, TenVoorde et al., 1995, Song and Lehrer, 2003, Vaschillo, Vaschillo and Lehrer, 2006). The response of RSA, and other quantifiers of HRV, to the alteration of $f_{R,}$ is characterised by individualised curves (Hirsch and Bishop, 1981, Vaschillo et al., 2006), which have been claimed to represent individual variations in resonant frequency location (Lehrer, 2007). These individual variations

are independently related to height and gender, thus possibly reflecting inter-individual differences in the volume of the vasculature (Vaschillo et al., 2002, Lehrer, Vaschillo, Lu et al., 2006). However, the influence of f_R has not been studied in a systematic way, i.e. such that potential confounders as the simultaneous changes in V_T (see also below) and P_aCO_2 are controlled; neither have the cardiac haemodynamic and blood pressure responses to changes in f_R been studied systematically, i.e. studied simultaneously and over a comprehensive range of slow f_Rs.

An important gap in the literature is how changes in tidal volume (V_T) can simultaneously, and independently, influence the acute cardiovascular response to SDB. Most of the studies cited above failed to control for the increase in V_T that accompanies the decrease in f_R, which arises automatically to maintain minute ventilation ($\dot{V}_E$) and arterial CO_2 levels (P_aCO_2). While it has been argued that the impact of the V_T upon some variables, particularly RSA, is reduced (believed to only account for a small percentage of the overall variation of RSA during SDB, against a much larger contribution arising from the change in f_R (Hirsch and Bishop, 1981, Brown, Beightol, Koh et al., 1993, Pinna, Maestri, La Rovere et al., 2006)) the differentiation of the independent effects of both f_R and V_T is fundamental to a better understanding of the acute cardiovascular effects of SDB.

Currently, a direct positive relationship has been established between V_T and, 1) RSA magnitude (Hirsch and Bishop, 1981, Eckberg, 1983, Cooke, Cox, Diedrich et al., 1998); and 2) the amplitude of within-breath changes in MSNA (Seals et al., 1990, Seals et al., 1993). Importantly, it seems that the depth of breathing impacts the cardiac responsiveness to baroreflex stimulation; as demonstrated by increased inspiratory inhibition of efferent cardiac vagal activity at higher V_Ts, leading to diminished cardiac baroreflex responses to changes in ABP during inspiration (Eckberg and Orshan, 1977). The impact of V_T upon RSA and MSNA is thought to relate to either the stimulation of SASRs (Freyschuss and Melcher, 1976c, Seals et al., 1990), or to a direct mechanical effect of the change of intrathoracic pressure upon venous return during inspiration, leading to fluctuations in ABP (Dornhorst et al., 1952b, Freyschuss and Melcher, 1976a, b).

Lowering f_R has also been shown to increase the amplitude of ABP oscillations, i.e. blood pressure variability (BPV) (TenVoorde et al., 1995, Cooke et al., 1998,

Vaschillo et al., 2002). However, at least one account has shown a reduction in the amplitude of within-breath fluctuations of SBP when f_R is lowered (Sin, Galletly and Tzeng, 2010). These changes in the within-breath amplitude of *fc* and ABP oscillations seem to occur without significant alteration of the mean values of said variables, thus suggesting the absence of acute autonomic adjustments. This construct is reinforced by unaltered mean MSNA during SDB, despite significant within-breath modulation of MSNA (Seals et al., 1990, Limberg, Morgan, Schrage et al., 2013).

Perhaps surprisingly, there appear to be no relevant studies exploring the influence of V_T upon ABP or BPV, either during spontaneous breathing or during SDB. Furthermore, where studies have reported measures of BPV in response to SDB (TenVoorde et al., 1995, Cooke et al., 1998, Vaschillo et al., 2002, Sin et al., 2010) they failed to control for the potential effect of increased V_T upon both preload (increasing venous-return) and afterload (altering aortic transmural pressure), that would be associated with a decrease in f_R. It is accepted that increasing V_T impacts both preload and afterload, which would be predicted to increase the amplitude of oscillations in LVSV and ABP (Dornhorst et al., 1952b, Freyschuss and Melcher, 1976a, b). Thus, the independent influence of V_T upon the simultaneous behaviour of some cardiovascular parameters remains uncharacterised.

For example, little is known about the effects of SDB upon cardiac function, specifically LVSV and $\dot{Q}$; in particular, their within-breath behaviour in response to changes in f_R and/or V_T. Most of the current evidence regarding the respiratory modulation of cardiac function results from studies conducted in animal models (Brecher, 1952, Brecher and Hubay, 1955, Hoffman et al., 1965, Robotham et al., 1978, Robotham and Mintzner, 1979, Robotham et al., 1979, Scharf et al., 1979, Olsen et al., 1985, Robotham et al., 1988), or human cardiac patients (Ruskin, Bache, Rembert et al., 1973, Guz, Innes and Murphy, 1987). The other studies have been performed during quiet breathing (Karam et al., 1984, Guz et al., 1987, Innes, De Cort, Kox et al., 1993, Toska and Eriksen, 1993, Peirce, Panerai and Potter, 2001, Elstad, 2012, Claessen, Claus, Delcroix et al., 2014), or during exercise (Claessen et al., 2014), which also lack relevance to the contributions of f_R and/or V_T to cardiac function during SDB. The two studies that have explored paced breathing have done so at frequencies that were above 10 breaths·min^{-1} (Boutellier and Farhi, 1986, Peirce et al., 2001) and therefore also cannot be considered as

representative of the cardiac haemodynamic response during SDB. This gap in the literature is likely determined by the invasiveness of some of the interventions, as well as by the respiratory-imposed limitations inherent in more recent, non-invasive methods, such as Doppler ultrasound imaging (Armstrong and Ryan, 2012). However, the development of valid and reliable, non-invasive photoplethysmographic methods (Sugawara, Tanabe, Miyachi et al., 2003, van Lieshout, Toska, van Lieshout et al., 2003, Bogert and van Lieshout, 2005) currently allows for an easy and continuous evaluation of the cardiac response to respiratory challenges; albeit indirect. Despite not being in the context of SDB, one example is the study of Elstad (2012), which characterised the simultaneous variations in pulmonary and systemic blood flow in healthy human beings by combining Doppler ultrasound assessment of the right ventricular function and *Modelflow*, a mathematical model used to estimate LVSV and $\dot{Q}$, from the ABP waveform obtained by photoplethysmography. The understanding of volume and/or frequency-dependent left ventricular function responses is fundamental to the understanding of the acute response to SDB, and thus to the understanding of the mechanisms that might lead to any longer-term impact upon ABP.

Similarly, to better comprehend the relationships that are established between the different biological rhythms during SDB, it is important to clarify its impact upon the phase relationships between respiration, *fc* and ABP, as these are known to be altered by f_R. Using frequency domain techniques, Angelone and Coulter (1964) reported that at approximately 10 breaths·min^{-1}, respiration and *fc* tend to show an 180º phase relationship (opposite phase), which changed progressively with a reduction of f_R to 0º phase ≤ 4 breaths·min^{-1}. Others have suggested that respiration and *fc* are in phase (0º phase relation) at frequencies closer to 6 breaths·min^{-1} (Vaschillo et al., 2002). The same authors have argued that at 6 breaths·min^{-1}, the phase relation between respiration and ABP (in this case SBP) is 180º. This is consistent with a phase relationship between SBP and *fc* close to 180º at the frequencies close to 6 breaths·min^{-1}. However, experimental data from healthy individuals seems to contradict this idea, by showing a relatively unchanged SBP to *fc* phase relationship at around 90º, even for f_R below 6 breaths·min^{-1} (TenVoorde et al., 1995). Recent developments in time-domain analysis techniques have confirmed f_R-dependent alterations in the relationship between respiration, *fc* and SBP (Sin et al., 2010) that could not be extracted from classical frequency domain spectral methods. This new approach highlighted the presence of nonlinearities in

the respiratory-cardiovascular interactions in human beings (Sin et al., 2010). Thus, the influence of changes in f_R upon these phase relationships remains poorly understood. Furthermore, to the best of the author's knowledge no study has yet investigated whether the phase relationships between respiration, *fc* and SBP are in any way affected, or confounded, by variations in V_T.

Another suggested acute effect of SDB is to increase cardiac baroreflex sensitivity (BRS) (Bernardi et al., 2001, Lehrer et al., 2003). The determination and interpretation of BRS are known to be affected by posture (TenVoorde et al., 1995, O'Leary, Kimmerly, Cechetto et al., 2003), experimental protocol (Bowers and Murray, 2004a) and quantification method (Tzeng, Sin, Lucas et al., 2009). This probably explains why some studies have noted no relevant change in cardiac BRS with SDB (Shantsila et al., 2014a, Shantsila et al., 2014b), resulting in a lack of clarity in relation to the BRS response to SDB. Nonetheless, apart from TenVoorde's study, I am not aware of any study that has attempted to characterise cardiac BRS over a range of frequencies and volumes that are consistent with SDB.

To the author, the lack of consistent and comprehensive data that clearly characterises the independent acute cardiovascular impact of f_R and V_T justifies further research. Such understanding seems necessary to fully comprehend and identify the acute mechanisms behind the perturbing stimulus delivered to cardiovascular control system during SDB.

2-5.2 Effects of intra-thoracic pressure

Two recent studies have observed more profound and sustained effects of SDB training over the course of weeks, when used in combination with inspiratory resistances (Jones et al., 2010, Sangthong et al., 2016). These findings suggest that the acute and chronic influence of SDB might be enhanced by the addition of small respiratory resistances. Whilst I am not aware of any research that has examined specifically the acute cardiovascular response to resisted SDB, several studies have tested the effects of small inspiratory resistances; these data suggest that magnifying intra-thoracic pressure oscillations might modify the acute cardiovascular effects of breathing, due to the effect on venous return.

In healthy supine human beings, the use of a small inspiratory resistance reportedly results in increased ABP, with no change in cardiac BRS (Convertino, Ratliff, Ryan, Cooke et al., 2004), as well as increases in LVSV and $\dot{Q}$ (Convertino, Ratliff, Ryan, Doerr et al., 2004). In common with SDB (Lucas et al., 2013), application of small inspiratory resistances increases cerebral blood flow (CBF) and improves tolerance to orthostatic challenges and hypovolemia (Cooke, Lurie, Rohrer et al., 2006, Rickards, Ryan, Cooke et al., 2007, Rickards, Cohen, Bergeron et al., 2008, Rickards, Ryan, Cooke et al., 2011). One study in particular, explored the independent cardiovascular effect of various magnitudes of respiratory resistance (simultaneously during inspiratory and expiratory phases) during paced breathing at 12 breaths·min^{-1} with fixed V_T. The study demonstrated an unaltered phase relationship between surrogate measures of LVSV, as well as ABP and *fc*. The use of a range of respiratory resistances supported the existence of an important functional mechanical link between respiratory-related intra-thoracic pressure oscillations and changes in LVSV, ABP, and RSA. Collectively, these data suggest that studying of the combined influences of SDB and inspiratory loading may aid understanding of the contribution from intrathoracic pressure to changes in venous return and the associated cardiac haemodynamic responses. Furthermore, the characterisation of the acute effect of the combined use of the two interventions may contribute to identification of the factors underpinning a potential long-term benefit of inspiratory resisted SDB.

Finally, in line with the dearth of information relating to combining SDB with inspiratory resistances, no studies appear to have explored the cardiovascular impact of spontaneous breathing against small expiratory resistances, either in isolation or in conjunction with SDB. Similarly, little is known regarding the cardiovascular effects of the simultaneous use of inspiratory and expiratory resistances.

While this might seem to the reader to be a departure from the study of the cardiovascular responses to SDB, the use of inspiratory, expiratory and dual resistive breathing in conjunction with SDB is an integral part of yogic breathing techniques. These techniques require practitioners to breathe through a single-nostril, thus increasing the resistance to airflow and magnifying respiratory-induced intra-thoracic pressure oscillations. Indeed, single-nostril breathing has been suggested to have distinct beneficial effects upon ABP (Raghuraj and Telles, 2008,

Pal, Agarwal, Karthik et al., 2014), HRV (Pal et al., 2014) and autonomic activity (Telles, Nagarathna and Nagendra, 1994), with effects claimed to differ between right and left-nostril breathing. The confirmation of said differences between airway route (mouth, nostrils), and the understanding of the respiratory-phase dependent, within-breath cardiovascular responses to expiratory or dually resisted SDB, might contribute to a better understanding of the mechanisms underlying the acute response to SDB, as well as to enhancing the long-term efficacy of SDB interventions.

2-6 Overall summary

Respiration induces fluctuations in *fc*, ABP and MSNA that occur with a periodicity similar to the respiratory frequency. These oscillations are the result of the cyclic stimulation of mechanoreceptors in the lungs, heart and main vessels by increased inflation, and variations in blood volume. Similarly, cardiovascular rhythmicity has also been identified with a cadence of approximately six cycles per minute (0.1 Hz), in human beings.

Slowing breathing down to approximately 6 breaths·min^{-1} entrains the respiratory and cardiovascular rhythms and potentiates within-breath oscillations in *fc* (RSA) and ABP (BPV). This amplification phenomenon is thought to reflect resonance phenomena present in the baroreflex control mechanism of ABP. It could be that such a resonant effect creates a state of heightened systemic and autonomic stimulation that underpins the claimed positive, prospective clinical benefits of SDB.

However, no study has yet investigated the independent cardiovascular perturbation that might be delivered by independently manipulating f_R (within the range of slow breathing) and V_T, Similarly, no study to date has looked into whether different systemic haemodynamic variables tend to respond similarly, or differently to such manipulations, i.e. if there is a generalised tendency that favours breathing at an individualised resonant frequency.

Furthermore, secondary to deeper breathing, increased venous return is expected because of larger and more sustained (longer respiratory cycle duration) negative intrathoracic pressures throughout inspiration; this may also contribute to the amplitude of *fc* and ABP fluctuations during SDB. These respiratory-driven

oscillations in venous return and ABP can be amplified by breathing against small respiratory resistances, which might provide an adjunctive stimulus to the cardiovascular control mechanisms. Manipulation of this factor may also help to further the understanding of the mechanisms involved in the acute response to SDB, as well as how the effectiveness of such respiratory training regimes might be improved.

2-7 Aims of the present research and research hypotheses

The general aim of this thesis is to increase understanding of the acute responses to SDB, with an emphasis on the mechanical factors contributing to cardiovascular-respiratory interactions. In doing so, it is intended to facilitate the identification of a putative mechanistic link(s) between the acute response to SDB and its claimed chronic antihypertensive effects, with the purpose of optimising the factors involved.

The preceding review has summarised the existing literature regarding the interaction between respiration and cardiovascular regulation, as well as the known acute cardiovascular responses to SDB. Apart from the lack of understanding of the factors that translate the repeated practice of SDB into positive health benefits, the characterisation of within-breath acute impact of SDB upon key cardiac and haemodynamic variables is still poorly described. Furthermore, the independent effects of f_R, V_T, P_aCO_2 and intrathoracic pressure, and how these can be manipulated to deliver a more potent antihypertensive stimulus from SDB deserve deeper, systematic exploration.

To achieve the general aim, a series of systematic steps were taken; a summary of these follows and precedes a definition of the principal aims of this thesis, which guided the ensuing experimental chapters. The therapeutic SDB techniques currently in use might not be the most efficient in maximising acute cardiovascular perturbations, or promoting a maximal cardiovascular adaptive response; in particular:

1) Several existing techniques fail to recognise the existence of an individualised, respiratory frequency-dependent cardiovascular response to SDB;

2) The independent effect of V_T upon HRV and heart-lung interactions is not thoroughly understood and normally not accounted for;
3) Most studies examining the acute cardiovascular impact of SDB have failed to investigate it over a comprehensive range of f_Rs and V_Ts;
4) There is a general lack of data regarding the acute effects of SDB upon venous return, and hence cardiac haemodynamic responses to SDB.

Thus, there is the need for a systematic and comprehensive re-examination of the acute cardiovascular impact of SDB, which addresses the gaps in the literature articulated above.

In summary, the main aims and associated hypotheses of each chapter within this thesis are:

Chapter 4

Aims:

1) To elucidate the independent effect of f_R and V_T upon the magnitude of RSA and other cardiovascular fluctuations occurring with SDB;
2) To determine whether there is an optimal, individual breathing pattern that generates maximal acute cardiovascular responses;
3) To assess the contribution of respiratory synchronous fluctuations in LVSV, $\dot{Q}$ and ABP to the cardiac chronotropic response (RSA) to SDB.

Research hypotheses:

1) Reducing f_R will amplify RSA and ABP oscillations independently of V_T, with the maximum amplitude of fluctuations being attained at different, individualised, frequencies;
2) Increasing the magnitude of the change in lung volume (V_T) will increase the within-breath amplitude of LVSV and $\dot{Q}$, leading to increased ABP oscillations and amplified RSA

Chapter 5

Aims:

1) To determine if the simultaneous use of inspiratory resistance amplifies the acute cardiovascular responses to SDB;
2) To test whether the influence of inspiratory loads occurs via an effect upon in venous return;

Research hypotheses:

1) The addition of an inspiratory resistance will magnify the cardiovascular responses induced by SDB at similar f_R and V_T;
2) The cardiovascular response to resisted SDB will be coupled closely to the magnified within-breath fluctuations in intrathoracic pressure;
3) Reducing lower limb blood volume (by compression of the legs) will attenuate any potentiating effects of inspiratory resisted SDB;

Chapter 6

Aims:

1) To identify if the phase of breathing during which respiratory resistance is applied impacts the cardiovascular response to SDB.
2) To compare the influences of different respiratory loading modalities upon the cardiovascular response to SDB.

Research hypotheses:

1) Inspiratory, expiratory and dual resisted SDB will result in distinct cardiovascular responses to SDB;
2) Dual resisted SDB will amplify the cardiovascular response to SDB to a larger extent than the isolated application of inspiratory or expiratory resistances;
3) The scale of the cardiovascular response to resisted SDB will be determined by the magnitude of the load, and not by the type of load, or airway (mouth *vs.* nostril);
4) The extent of the cardiovascular response to single nostril SDB will not differ between left nostril *vs.* right nostrils.

CHAPTER 3 – GENERAL METHODS

3-1 Introduction

A detailed explanation of the general methods applied to the studies that form this thesis is outlined in this chapter. Specific methods that are unique to individual studies are described in the chapters relating to those studies. In some instances, due to their complexity, some specific procedures that are not common to all studies are outlined in the present chapter; in those cases, a clear rationale is provided.

3-2 Pre-test procedures

The ensuing section describes procedures carried out prior to experimental testing.

3-2.1 Ethical approval

Before commencing each of the studies, ethics committee approval was obtained from the Brunel University London - Department of Life Sciences Research Ethics Committee (Appendix III). All studies were performed in compliance with the latest version of the Declaration of Helsinki (World Medical Association, 2013).

3-2.2 Participants

Recreationally active, healthy, male participants between 18 and 35 years of age, were recruited for each study. The sample selection was based on the understanding that some variables of interest, particularly those related to heart rate variability (HRV), are affected by gender, age and health status. For example, total HRV power is known to decrease steadily with age (Zhang, 2007), whilst healthy young women appear to exhibit menstrual cycle fluctuations in HRV (Sato, Miyake, Akatsu et al., 1995, Yildirir, Kabakci, Akgul et al., 2001, Sato and Miyake, 2004).

Each participant received a detailed participant information sheet that described the testing procedures (Appendix IV). Written informed consent (Appendix V) and completion of a health screening questionnaire (Appendix VI) were required prior to testing. Participants who reported any contra-indicated health issues were excluded from the study. All participants were familiarised thoroughly with the testing procedures, on a separate occasion, before any data collection took place.

Participants were instructed to avoid drinking alcohol the day before the test and to refrain from caffeinated beverages during the 12 h prior to testing. Participants were

also advised to limit food ingestion in the 2 h leading up to testing. Acute alcohol ingestion has been showed to reduce HRV (Gonzalez Gonzalez, Mendez Llorens, Mendez Novoa et al., 1992, Bau, Moraes, Bau et al., 2011, Shi, Chen, Guo et al., 2014) and TPR (Kupari, 1983) while increasing *fc*, $\dot{Q}$, SBP and circulating catecholamines (Kupari, 1983, Ireland, Vandongen, Davidson et al., 1984). Similarly, substantial evidence supports the existence caffeine-induced acute cardiovascular effects, including effects on blood pressure, plasma catecholamine levels and arterial stiffness (Smits, Thien and Van 't Laar, 1985, Mahmud and Feely, 2001). The consumption of food and ensuing digestive process have been shown to increase both blood flow to the splanchnic organs (Moneta, Taylor, Helton et al., 1988, Waaler, Eriksen and Janbu, 1990, Sidery, Macdonald, Cowley et al., 1991) and $\dot{Q}$ (Kelbaek, Munck, Christensen et al., 1989), and to alter post-prandial HRV spectral indices (Lu, Zou, Orr et al., 1999, Chang, Ko, Lien et al., 2010), with these effects lasting up to 2 h (Waaler, Eriksen and Toska, 1991). Furthermore, the aforementioned pre-test dietary restrictions are aligned with the current recommendations regarding lung function testing (Miller, Crapo, Hankinson et al., 2005).

3-2.3 Anthropometry

Participant's date of birth was recorded and then converted to decimal age. Standing stature was measured to the nearest 1 cm using a stadiometer (SECA 798, Germany). Participants stood barefoot and straight, with their back, buttocks and both heels touching the stadiometer. Participants were instructed to orientate their head in the Frankfort horizontal plane (i.e. the inferior margin of the orbit and upper margins of the ear opening, aligned in a horizontal line) and to inspire fully; stature was recorded as the maximum distance from the floor to the vertex of the head (Stewart, Marfell-Jones and International Society for Advancement of, 2011). Body mass in minimal clothing was recorded to the nearest 0.1 kg using calibrated electronic scales (SECA 798, Germany).

3-2.4 Pulmonary function

Measurements of pulmonary function were carried out according to standardised procedures (Miller, Hankinson, Brusasco et al., 2005). Maximal flow-volume manoeuvres were performed using a hand-held spirometer (Micro Loop Mk8, MicroMedical, UK). The spirometer incorporated a digital turbine flow transducer,

which is claimed to exhibit excellent stability of measurement, even with changes in ambient temperature, altitude and humidity (Dirksen, Madsen, Pedersen et al., 1996).

All measurements were performed with participants in a seated upright position. Throughout the respiratory manoeuvres, all participants wore a nose clip and breathed through a disposable inline filter connected to the spirometer. For the forced vital capacity (FVC) manoeuvres, the participants were instructed to inhale rapidly and fully from functional residual capacity (FRC, i.e. starting from the end of a passive expiration) and to begin a forced exhalation with minimal pause or hesitation (<1 s) from total lung capacity (TLC) (Miller, Hankinson, et al., 2005). The participants were instructed not to “blow” the air, but to “blast” the air from their lungs until the end of the forced exhalation. The FVC, forced expiratory volume in the first second (FEV_1), as well as the ratio of FEV_1 to FVC, were derived from the flow-volume profiles.

In agreement with the American Thoracic Society/European Respiratory Society task force guidelines (Miller, Hankinson, et al., 2005) all FVC manoeuvres were performed to specific criteria, which included:

1) at least three reproducible measurements were made (i.e. the difference between the largest and the next largest FVC is ≤ 0.150 L, and the difference between the largest and next largest FEV_1 is ≤ 0.150 L);
2) an expiratory duration in the maximal expiratory effort of at least 6 s or, the existence of a plateau in the volume-time curve (i.e. volume kept unchanged over the last 2 s of exhalation);
3) extrapolated volume was < 5% of FVC or 0.150 L (criteria for the acceptable start of expiration).

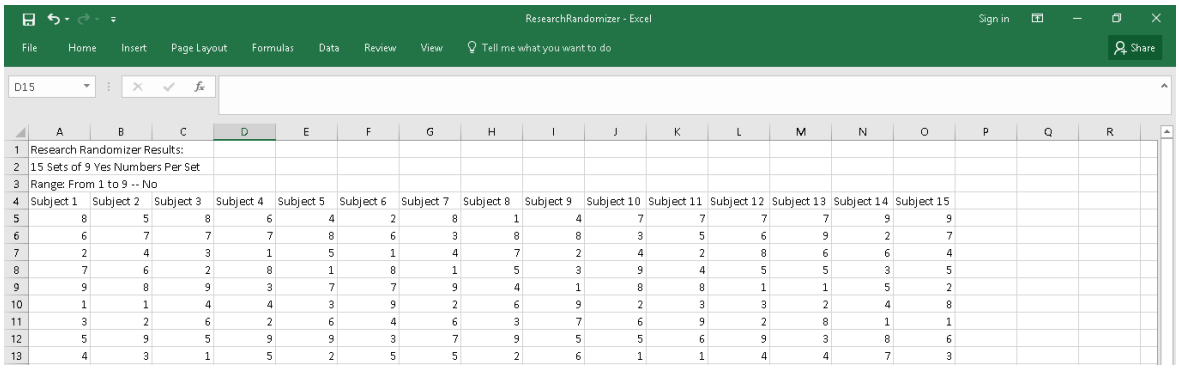
The largest FVC and FEV_1 were recorded after examination of data from all usable curves, even when not derived from the same curve.

3-2.5 Randomisation procedures

The conditions tested in all studies were randomised. Randomisation procedures involved the use of a freely available random number generator (www.randomizer.org). This software is best described as a ‘pseudo-random

number generator' as the numbers are generated by way of a complex algorithm that uses the computer's clock to generate quasi-random series of numbers.

For the first two studies (Chapters 4 and 5 respectively) the sets involving the smallest minute ventilation ($\dot{V}_E$) were always performed first, immediately following baseline measurements. This approach permitted identification of the $P_{ET}CO_2$ associated with the lowest $\dot{V}_E$, allowing for clamping of $P_{ET}CO_2$ at equivalent levels in succeeding, randomised sets. In Chapter 6, as all sets were performed at the same $\dot{V}_E$, full randomization of the intervention was undertaken (Figure 3-1).


ResearchRandomizer - Excel

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Research Randomizer Results:														
2	15 Sets of 9 Yes Numbers Per Set														
3	Range: From 1 to 9 -- No														
4	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12	Subject 13	Subject 14	Subject 15
5	8	5	8	6	4	2	8	1	4	7	7	7	7	9	9
6	6	7	7	7	8	6	3	8	8	3	5	6	9	2	7
7	2	4	3	1	5	1	4	7	2	4	2	8	6	6	4
8	7	6	2	8	1	8	1	5	3	9	4	5	5	3	5
9	9	8	9	3	7	7	9	4	1	8	8	1	1	5	2
10	1	1	4	4	3	9	2	6	9	2	3	3	2	4	8
11	3	2	6	2	6	4	6	3	7	6	9	2	8	1	1
12	5	9	5	9	9	3	7	9	5	5	6	9	3	8	6
13	4	3	1	5	2	5	5	2	6	1	1	4	4	7	3

Figure 3-1 – Example of .CSV export file from www.randomizer.com with randomised intervention order for study 3 (Chapter 6).

3-3 Apparatus and procedures

The following sections describe the different apparatus and procedures used in the studies. Information regarding other specialised apparatus or techniques used in individual studies is contained within the appropriate chapters.

3-3.1 Data acquisition and display

All respiratory and cardiovascular data were sampled at 250Hz via an analogue to digital converter (NI USB-6212 BNC, National Instruments Inc.) and analysed by a bespoke analysis system built using Labview acquisition and analysis software (LabView 13.0, National Instruments Inc.). The software was written by Professor Alison McConnell (primary supervisor). Respiratory airflow, instantaneous lung volume, respiratory pressure, arterial blood pressure (ABP), ECG, LVSV and end-tidal CO_2 ($P_{ET}CO_2$) signals were processed using both built-in and bespoke coded sub-routines that computed, mean, peak, nadir and amplitude of variations in relevant cardiovascular variables. Respiratory phase-dependent changes

(inhalation vs. exhalation) in the aforementioned and other variables were also calculated. In situations where a given cardiac cycle overlapped both respiratory phases, a criterion was established to include it in either inspiration or expiration based on the location of the R wave peak.

After each trial, a raw data file containing all analogue signals was created immediately, as well as a summary data file containing breath-by-breath values for all respiratory and the majority of cardiovascular variables of interest.

3-3.2 Device guided breathing and respiratory measurements

As well as recording data, a bespoke device guided breathing (DGB) system was incorporated within the Labview data acquisition software. The DGB displayed both visual guidance of the desired breathing pattern, as well as real time biofeedback of the person's actual respiratory pattern.

Device guided breathing

The front panel of the system displayed two similar bars (see figure 3-2, below). The right-hand side bar represented the desired instantaneous lung volume, while the left bar depicted the subject's measured volume, allowing the participants to match closely the two. The height of the bars represented the V_T for every breath while the rate of change of the height of the bar corresponded to respired flow rate. In so doing, it was possible to control both rate and volume simultaneously and independently.

The DGB system delivered three different breathing modes:

1) Semi-spontaneous breathing mode where only f_R was controlled. In this mode participants could breathe freely at any V_T and duty cycle, with the only restriction being the timing of each new breathing cycle, which was signalled by illumination of a green light on the screen ("Pacer" on the front panel in figure 3-6);
2) Semi-spontaneous breathing mode where only V_T was controlled, and all other dimensions of the breathing pattern were spontaneous. For this mode, the participants were required to inhale the defined V_T using a fixed predefined, volume target;

3) Fully controlled mode, where the target bar was set to deliver a specified respiratory duty cycle of 0.5 (inspiratory time = expiratory time), V_T (the height of the target bar) and flow rate corresponding to the desired f_R (the speed of the target bar movement).

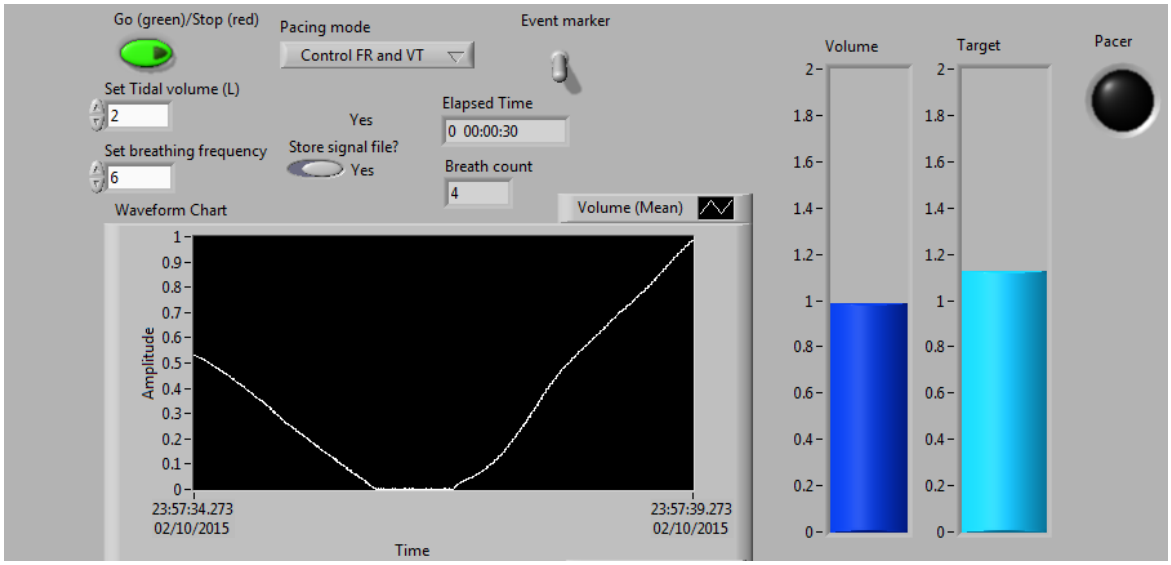


Figure 3-2 – Screenshot of the device guided breathing software's interface for a participant breathing at 6 breaths·min^{-1} and with prescribed tidal volume of 2 l. Dark blue bar accurately represents the actual breathing pattern of the participant, while the light blue bar provides indication of the desired breathing pattern.

Respiratory measurements

Respired flow rate (V') was measured continuously using a linear, Lilly type, bi-directional, heated pneumotachograph (Figure 3-3; Chapter 4 - Hans Rudolph 3813, Hans Rudolph Inc.; Chapters 5 and 6 - Hans Rudolph 3700, Hans Rudolph Inc.), controlled by a flow measurement system (Hans Rudolph RSS 100HR, Hans Rudolph Inc., Shawnee, KS, USA). The pneumotachographs interfaced with the participants through a mask that covered both mouth and nose. For the study in Chapter 4 we employed an Oro Nasal 7450 V2 Mask (Hans Rudolph Inc., Shawnee, KS, USA), while for the experiments in Chapters 5 and 6 an Intersurgical Quadralite Mask (Intersurgical Ltd., Wokingham Berkshire, UK) was utilised. According to the manufacturer, the pneumotachographs used in the studies comprised in this thesis tend to achieve laminar flow over an airflow range between 0 and 13.3 l·s^{-1} (for the Hans Rudolph 3813) and between 0 and 2.7 l·s^{-1} (for the Hans Rudolph 3700), both covered the airflow rates generated throughout the studies.

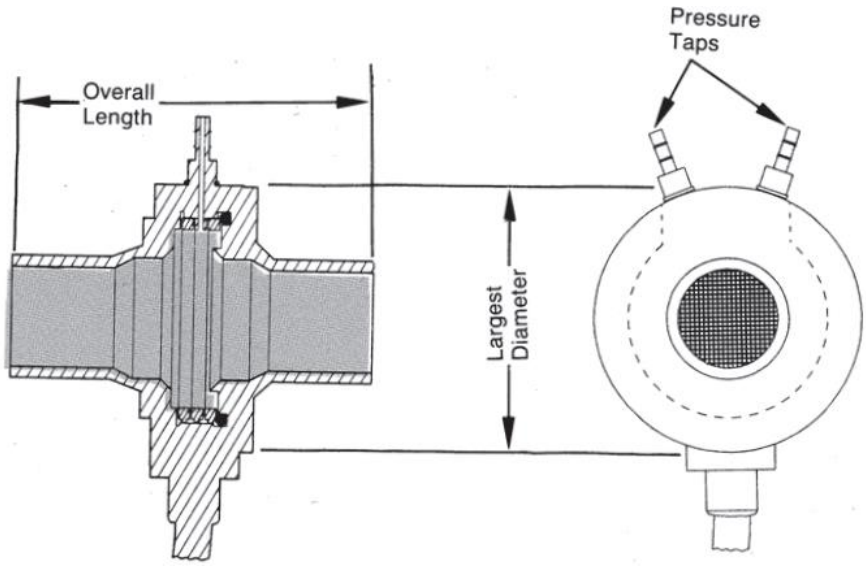


Figure 3-3 – Illustration of dead space volume (dark grey) and exterior configuration of the Hans Rudolph heated pneumotachographs. (Reproduced from Hans Rudolph pneumotachographs user's manual).

With the Lilly type pneumotachographs, airflow is derived from the pressure differential over a fine metal mesh. The pressure changes (P_1-P_2) across the mesh are proportional to V' when this is laminar, i.e. at relatively low flows. Respired volume is then computed by integrating V' over time (Figure 3-4), which in turn allows calculation of V_T as well as the inspiratory (T_I), expiratory (T_E) and total (T_{TOT}) durations for each breath, and f_R. The Labview software calculated all derived variables.

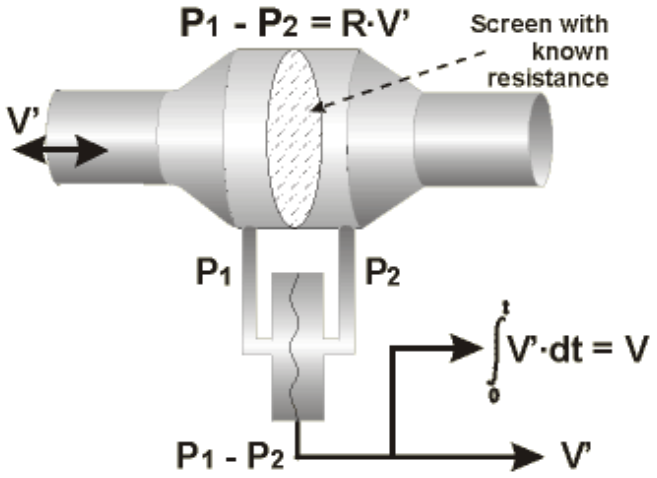


Figure 3-4 – Example of Lilly type pneumotachograph functioning. The pressure difference across the screen (mesh) is equal to the known resistance of the screen multiplied by airflow (V'). The integral of airflow over time allows to calculate air volume (V) going through the pneumotachograph. Reproduced from http://www.spirxpert.com/technical3.htm.

Before starting each study, a large servo motor driven piston pump (Hans Rudolph 1120 Flow/Volume simulator, Hans Rudolph Inc., Shawnee, KS, USA) was connected in series with the pneumotachographs and followed a built-in linearisation and calibration routine over which a predetermined range of continuous flow rates was delivered.

Prior to each laboratory test, the pneumotachograph was calibrated for volume. A calibration routine incorporated in the flow measurement system (Hans Rudolph RSS 100HR, Hans Rudolph Inc., Shawnee, KS, USA) consisted of repeatedly passing a known volume of gas (3 l) through the pneumotachograph. The necessary volume of air was delivered by way of a calibration syringe (Hans Rudolph 5530 Hans Rudolph Inc., Shawnee, KS, USA), which was stroked at a frequency that allowed to generate air-flow rates that were roughly half of the sensor's flow range. For a 160 L pneumotachograph sensor like the Hans Rudolph 3700, this equated to stroking the 3 L syringe in or out for 2.25 s. The calibration process was repeated by continuously stroking the syringe until the values for inspiratory and expiratory volumes recorded by the sensor differed < 5 mL from the volume of the calibration syringe. The flow measurement system automatically compared the measured gas volume with the syringe volume and calculated inspiratory and expiratory calibration factors, which were stored in the built-in non-volatile memory of the flow measuring system.

Respired gases were sampled continuously at the mouth via a fine catheter, and analysed for carbon dioxide (CO_2) concentrations using an analyser with laser diode absorption and infrared technologies, respectively (GA-200 gas analyser, iWorx Systems Inc., NH). End-tidal partial pressures of CO_2 ($P_{ET}CO_2$) were calculated from the analogue signals by the Labview software. Before each test, the gas analyser was switched on at least 30 min prior to testing to eliminate electrical drift. Pre-test procedures also included calibration across the normal physiological range using a standardised 15% O_2, 5% CO_2 and N_2 balance gas mixture (BOC Gases, Guilford, UK).

3-3.3 Rebreathing system

Both reductions (hypocapnia) and increases (hypercapnia) in P_aCO_2 beyond physiological values, are known to impact cardiovascular regulation (Price, 1960, Marshall, 1994, Henry, Lu, Beightol et al., 1998, Laffey and Kavanagh, 2002, Kara, Narkiewicz and Somers, 2003, Steinback, Salzer, Medeiros et al., 2009). To avoid the confounding effect of altered P_aCO_2 upon the cardiovascular and autonomic response to SDB, we clamped P_aCO_2 at a fixed, predetermined individual value. A variable volume re-breathing system was created to maintain a comparable P_aCO_2 across the range of fully controlled breathing patterns, which consisted of a series of different volume dead spaces. This system was based on the principle that P_aCO_2 levels could be kept constant even in situations of increased $\dot{V}_E$ by artificially increasing physiological dead space (therefore increasing $\dot{V}_D$). The following principles were applied in order to determine the appropriate volume for $\dot{V}_D$.

The minute ventilation ($\dot{V}_E$) represents the total volume of expired air in each minute, resulting from the product of tidal volume (V_T) and respiratory frequency (f_R):

$$\dot{V}_E = V_T \times f_R$$

Nonetheless, not all the mobilised air reaches the alveoli, where the gas exchange occurs (alveolar ventilation – $\dot{V}_A$). Part of the air remains in the conducting airways, therefore not entering in the so called respiratory zone (dead space ventilation – $\dot{V}_D$). The anatomical dead space is difficult to measure, but in healthy individuals it equates to roughly 150 mL per breath. This value tends to increase with large inspiratory efforts, as the bronchi are expanded due to the traction exerted by the adjacent lung parenchyma (West and Luks, 2016). For the sake of simplicity, throughout this thesis we considered anatomical dead space to be exactly 150 mL for every participant, and included instrument dead space (tubes, connectors, mask and pneumotachograph) for each study to provide the total dead space.

The $\dot{V}_E$ is the sum of $\dot{V}_A$ and $\dot{V}_D$:

$$\dot{V}_E = \dot{V}_A + \dot{V}_D$$

As anatomical and instrument dead space were constant, altering the volume of each inspiration (changing V_T) changes only $\dot{V}_A$. This is of fundamental importance

when considering the relation between $\dot{V}_A$ and CO_2 elimination. The alveolar ventilation equation depicts this relationship:

$$\dot{V}_A = (VCO_2 / P_{ET}CO_2) \times K$$

Where VCO_2 is carbon dioxide production, and K is a constant representing the difference between barometric pressure and water-vapour pressure. As all volumes measured by the heated pneumotachograph were at body temperature, pressure saturated with water vapour (designated BTPS), K related solely to the barometric pressure. Also, in normal, healthy, individuals at rest, $P_{ET}CO_2$ and the arterial partial pressure of CO_2 (P_aCO_2) are virtually identical (West and Luks, 2016) and therefore used interchangeably in this thesis. Throughout the studies there was no physical exertion and no alteration in metabolic activity. Thus VCO_2 remained unchanged. Therefore, any changes in V_A were inversely proportional to variations in P_aCO_2.

The added dead spaces consisted of adjustable pieces of tubing attached to one end of the respiratory circuit (see Figure 3-5). The target P_aCO_2, and thus the length of the tube (manipulation of $\dot{V}_D$), was estimated for each participant using the alveolar air equation. The target P_aCO_2 was defined by that observed during the condition with the lowest $\dot{V}_E$, which was always performed first. Throughout the subsequent breathing sets, $P_{ET}CO_2$ was inspected visually, and the length of tubing was adjusted manually (by extending/reducing) to achieve the target value.

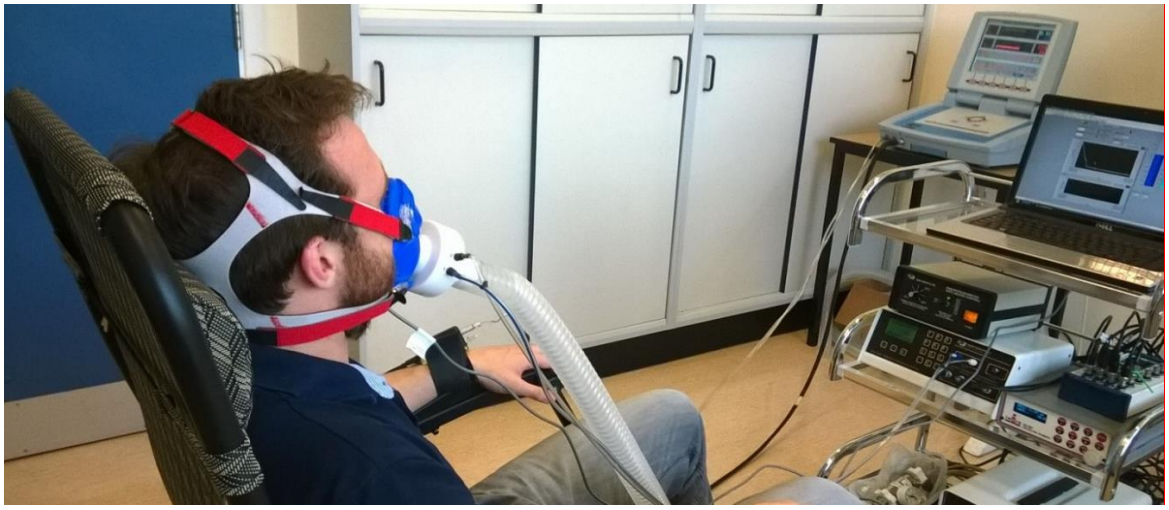


Figure 3-5 – Participant undergoing device guided slow and deep breathing exhibiting a piece of tubing attached to the pneumotachograph, in order to maintain a pre-determined arterial partial pressure of CO_2.

3-3.4 Loaded breathing

In this thesis, the imposition of respiratory loads was achieved through a bespoke flow resistive breathing circuit (Figure 3-6), and using proprietary pressure threshold loading devices (Philips Threshold IMT and Philips Threshold PEP; Philips Respironics, Murrysville, PA, USA). Loaded breathing was used in two studies to investigate the effects of changes in intrathoracic pressure upon the cardiovascular and autonomic response to SDB. The pressure threshold loading devices were only used in one of the studies comprised in this thesis (Chapter 6) and are described in detail in section 6-3.

The flow resistor inside the circuit comprised a number of nylon washers that reduced the internal diameter of the airway, creating resistance to flow according to Poiseuille's Law.

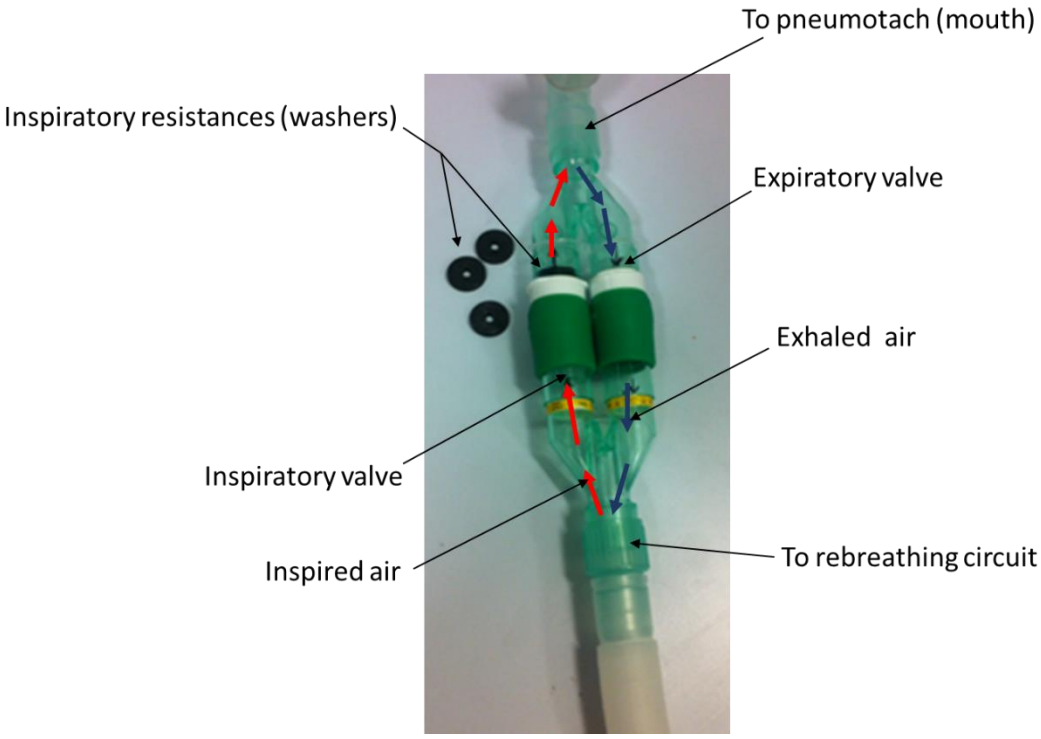


Figure 3-6 – Flow-dependent inspiratory resistance breathing device. Red arrows indicate the direction of the air entering in the circuit during inspiration, while blue arrows provide directionality of expired air.

According to Poiseuille's Law, if the flow is laminar, the resistance to airflow varies as a function of the airway diameter. In fluid dynamics, flow is considered to be laminar when the fluid flows in parallel, with no apparent disruption between the different layers (Batchelor, 2000), which tends to happen at slow fluid speeds or flow

rates. Poiseuille's Law is defined by the following equation, where *R* is the resistance to flow within the airways, *η* is the viscosity of the fluid flowing within the airways, *l* is the length of the airway and *r* represents the radius of the airways:

$$R = 8\eta l \,/\, \pi r^4$$

As the radius is raised to the power of four, a reduction of *r* to half means a 16-fold increase in the resistance that is generated for the same tube (airway) length (Hasan, 2010). The resistive load in centimetres of water produced by an airway of any given radius and length is also dependent upon the airflow rate and can be estimated by multiplying *R* by the mean inspiratory flow (MIF).

$$Respiratory\ load = R \times MIF$$

In the context of the present research, both the viscosity and MIF were assumed to be constant; the latter was achieved by maintaining the same f_R and V_T throughout all breathing sets that included resisted breathing, via the previously mentioned biofeedback system.

Alterations in the magnitude of inspiratory resistances were accomplished by changing the number of washers, thereby extending or reducing the length of the breathing resistor. Washers with different diameters were also used to guarantee that the desired load was administered. While it is unlikely that the conditions for laminar flow were met by the breathing circuit due to the interference of the unidirectional valves and the y-shaped connectors, it was guaranteed that the pressure generated by a given respiratory airflow was the intended by empirically adjusting the number of washers being used. Simultaneously, the y-shape of the breathing resistance circuit, and the inclusion of unidirectional inspiratory and expiratory valves ensured that the resistance could be applied selectively to a specific phase of the respiratory cycle, while also allowing the rebreathing of the exhaled air for the control of P_aCO_2 (see section 3-3.3).

3-3.5 Cardiovascular measurements

Beat-by-beat arterial blood pressure measurements

Arterial blood pressure (ABP) was estimated noninvasively using a Finometer (Finometer® Pro, Finapress Medical Systems, The Netherlands). This non-invasive

finger ABP measuring system is used widely in a multitude of settings and is considered to be within the Association for the Advancement of Medical Instrumentation (AAMI) the validation criteria (Guelen, Westerhof, van der Sar et al., 2008).

The Finometer ® Pro utilises the 'volume-clamp' method (Penaz, 1973, Boehmer, 1987, Imholz, van Montfrans, Settels et al., 1988), which involves the use of an inflatable finger cuff with inbuilt photo-electric plethysmography to detect finger pulse pressure waveforms (Figure 3-7). This technique, pioneered by Jan Penaz (1973), relies on the equalisation of the external (inflatable cuff) pressure with intraluminal pressure and subsequent clamping of said null transmural pressure at a constant level by continuous automatic adjustments. This zero transmural pressure condition also allows the measurement of the artery diameter by an infrared sensing technique. Any variations in intraluminal pressure associated with systole or diastole are proportional to a change in arterial diameter and detected by sensors within the cuff, which is rapidly inflated or deflated to maintain the zero transmural pressure 'set-point'. The external pressure adjustments delivered by the cuff to maintain vessel diameter are analogous to that being generated within the finger arteries themselves, and as such, allow continuous, accurate noninvasive determination of arterial pressure waveform (Wesseling, Jansen, Settels et al., 1993).

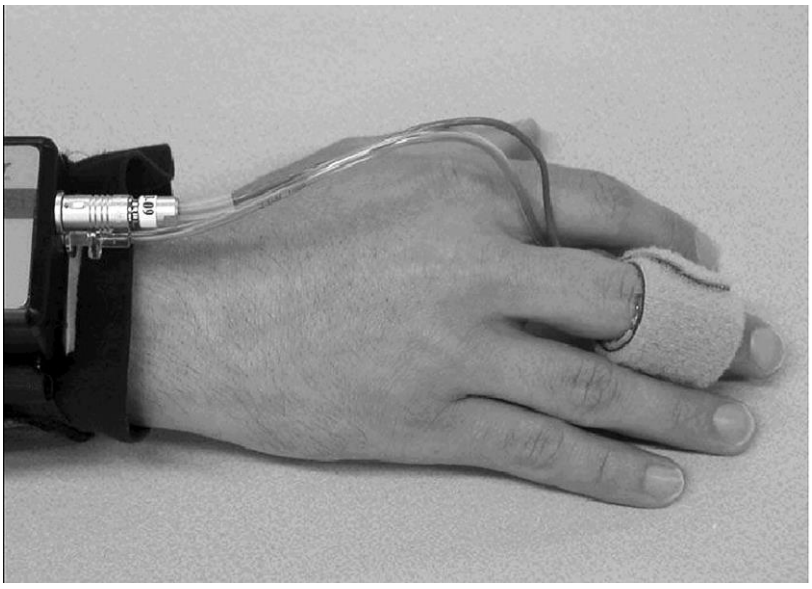


Figure 3-7 – Example of the fitting of a photoplethysmographic finger cuff for continuous non-invasive arterial blood pressure measurement. Reproduced from (Bogert and van Lieshout, 2005).

The ABP readings at the finger are inherently different to those at the brachial artery due to changes in pressure gradients and wave distortion along the artery. To counter such effect, the Finometer ® Pro incorporates a series of corrections, allowing the collection of valid and reliable estimates of brachial artery pulse pressure and derived variables. A good agreement between ABP values obtained by this method and direct recordings from the radial artery under an array of experimental conditions has been previously demonstrated (Imholz et al., 1988, Parati, Casadei, Groppelli et al., 1989, Imholz, Dambrink, Karemaker et al., 1990, Imholz, Settels, van der Meiracker et al., 1990).

The Finometer ® Pro also corrects for the hydrostatic height of the finger with respect to the heart by means of a height sensor. The system also corrects for the distortion of the pressure waveform along the arm, by measuring the brachial pressure in a traditional way (using an arm cuff). The latter, known as 'return-to-flow' correction (RTF), involves a protocol of stepwise arm cuff occlusion to supra-systolic levels, followed by gradual deflation. The first pulsation measured at the finger during cuff deflation is then compared to the arm cuff pressure, allowing for the RTF correction, and thus a reconstructed brachial artery pressure pulse wave to be constructed. The system uses a continuous built-in calibration, consisting of repeated zeroing of the finger pulse measurements at fixed intervals ranging from 10 to 60 s. For the purposes of the present studies, the RTF calibration was performed before the start of data collection and repeated throughout the testing session at roughly 30 min intervals. The auto-calibration function was turned off after the initial calibration, and re-started during each of the rest periods, allowing for uninterrupted sets of data, while maintaining proper calibration. Beat-to-beat reconstructed ABP waveforms, and LVSV were recorded by the bespoke data acquisition system (LabView, National Instruments Inc.) and averaged for each breath. A raw data file was also produced for offline analysis.

Heart rate, stroke volume and cardiac output measurements

The *fc* was monitored continuously using a 3-lead ECG (PysioControl VSM® 3, PhysioControl Inc., Redmond, WA, USA). An estimate of LVSV was calculated by the Finometer ® Pro software using the 'Modelflow method' by reconstructing raw brachial artery pressure waveforms. This approach utilises Wesseling's three-

element model (Wesseling et al., 1993), which calculates aortic flow using three known haemodynamic properties of the arterial system:

1) nonlinear pressure dependent aortic compliance (how effective the aorta and arterial system can store the elastic energy derived from the left ventricle upon contraction);
2) the characteristic impedance of the aorta (the extent to which the aorta impedes pulsatile flow);
3) time-dependent systemic vascular resistance (sum of the total resistance of all vascular beds).

By assuming these qualities, the Modelflow method calculates aortic flow over time (thus estimating LVSV). The LVSV measurements obtained through this approach have been shown to agree strongly with LVSV measured by Doppler ultrasound (van Lieshout et al., 2003). Cardiac output ($\dot{Q}$) was estimated by Labview by multiplying *fc* (ECG) by LVSV (Finometer).

3-3.6 Echocardiography

Echocardiography (or cardiac ultrasonography) was employed to determine changes in left ventricular volumes in response to specific interventions, more particularly, to different levels of lower body positive pressure (*see* Study 2, Chapter 5). Currently, echocardiography is the single most used tool to assess cardiac function in both the clinical and research settings; virtue of its easy and safe application, allied with its relatively low cost (Lang, Bierig, Devereux et al., 2005).

Principles of echocardiography

Echocardiography uses ultrasound to determine the structure and function of the heart. When these waves are discharged by a transducer, propagating through the body, the particles in the bodily tissues oscillate in parallel to the line of propagation, creating longitudinal waves (Armstrong and Ryan, 2012). The reflected ultrasound waves from the tissues interact with the piezoelectric crystals in the transducer and produce an electric signal that is converted into a digital grey-scale image (Armstrong and Ryan, 2012). The resolution of the created ultrasound imageis the result of interaction between the transmitted waves and the tissue properties. As

sound waves encounter materials with a different density (acoustical impedance), part of these waves is echoed back to the probe. The greater the difference between acoustic impedance, the larger the echo is, therefore translating into different shades of grey in the digital image, allowing the sonographer to differentiate different layers of tissue.

Procedures for image acquisition

In this thesis, echocardiography was used to assess systolic and diastolic left ventricular (LV) function as part of one experimental study examining the impact of progressive lower body positive pressure (LBPP) upon the cardiovascular response to deep and slow breathing (Chapter 5). Echocardiographic image acquisition and analysis were performed by an experienced sonographer (Dr Eurico Wilhelm) according to current guidelines for cardiac chamber assessment (Lang et al., 2005). Several published studies have investigated intra-observer reproducibility in either clinical populations or healthy cohorts. These have mostly reported low intra-observer variability in the evaluation of left ventricular function (Himelman, Cassidy, Landzberg et al., 1988, Pearlman, Triulzi, King et al., 1988, Nidorf, Picard, Triulzi et al., 1992, Gottdiener, Livengood, Meyer et al., 1995, Otterstad, Froeland, St John Sutton et al., 1997, Gottdiener, Bednarz, Devereux et al., 2004, Frikha, Girerd, Huttin et al., 2015).

Two-dimensional ultrasound imaging was recorded on a Vivid 7 (GE Medical, Horton, Norway) ultrasound machine, using an M4S 2–5 MHz probe (Figure 3-8) with the frequency set at 1.7 MHz on transmit and 3.6 MHz on receive. A built-in 3-lead electrocardiogram (ECG) recorded f_C with each image. Off-line analysis of LV function (including calculation of LV systolic and diastolic dimensions and ejection fraction) was performed and averaged on 2-3 consecutive cardiac cycles, using manufacturer provided software (EchoPAC, GE Medical, Horton, Norway, Version 7.0.0).

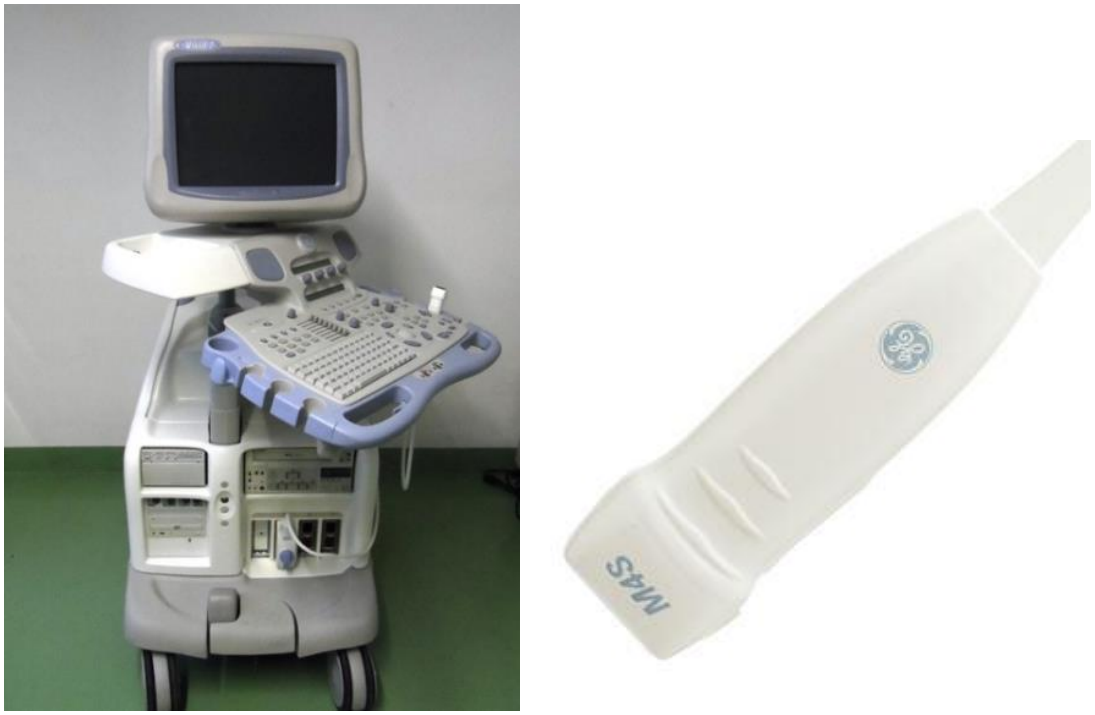


Figure 3-8 – Ultrasound system and probe. Images show (left panel) Vivid 7 ultrasound and (right panel) 2-D phased-array M4S transducer (both GE Medical, Horton, Norway) used in study 2 (Chapter 5).

Left ventricular volumes and ejection fraction

Two-dimensional images were recorded using the four-chamber and two-chamber apical views (Figure 3-9). Measurements for LV internal dimensions were taking by tracking the interior of the LV, between the endocardial border of the septum and the endocardial border of the posterior wall, both in systole and diastole (Figure 3-10). Manufacturer specific software (EchoPAC, GE Medical, Horton, Norway, Version 7.0.0) then calculated left ventricular end-diastolic volumes (EDV), end-systolic volumes (ESV), stroke volume (LVSV) and ejection fraction (EF) according to the modified Simpson's biplane rule (Figures 3-10 and 3-11), currently regarded by the American Society of Echocardiography as the most common and preferable method for volume measurements (Lang et al., 2005). With the Simpson's biplane method, total left ventricular volume is calculated from the summation of a stack of elliptical disks, with each disk representing a fraction of the left ventricle's long axis taken from the 2- and 4-chamber apical views (Lang et al., 2005, Evangelista, Flachskampf, Lancellotti et al., 2008). Linear echocardiographic measurements from 2D images have proven to be reproducible with low intra-observer and inter-observer variability (Ihlen, Endresen, Myreng et al., 1987, Pearlman et al., 1988, Nidorf et al., 1992, Palmieri, Dahlof, DeQuattro et al., 1999).

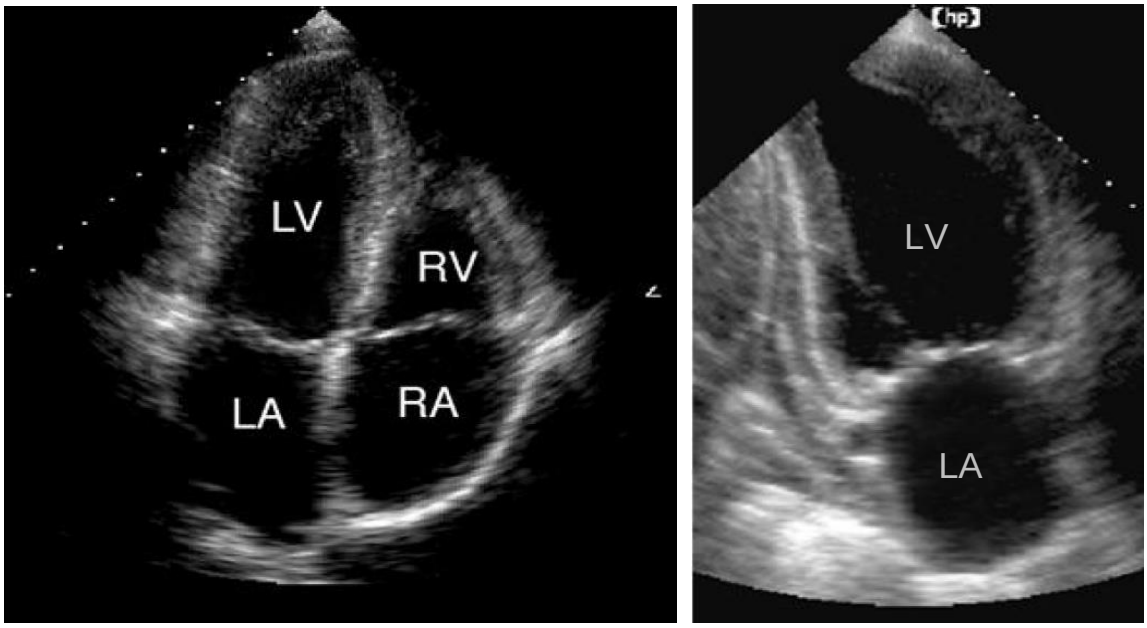


Figure 3-9 – Utilised Ultrasonographic views for left ventricular function assessment. Left: apical four chamber view. In this view the left (LV) and right ventricles (RV) as well as the left (LA) and right atria (RA) are visible. Right: apical two chamber view exhibiting left ventricle (LV) and left atria (LA). Adapted from Armstrong and Ryan (2012).

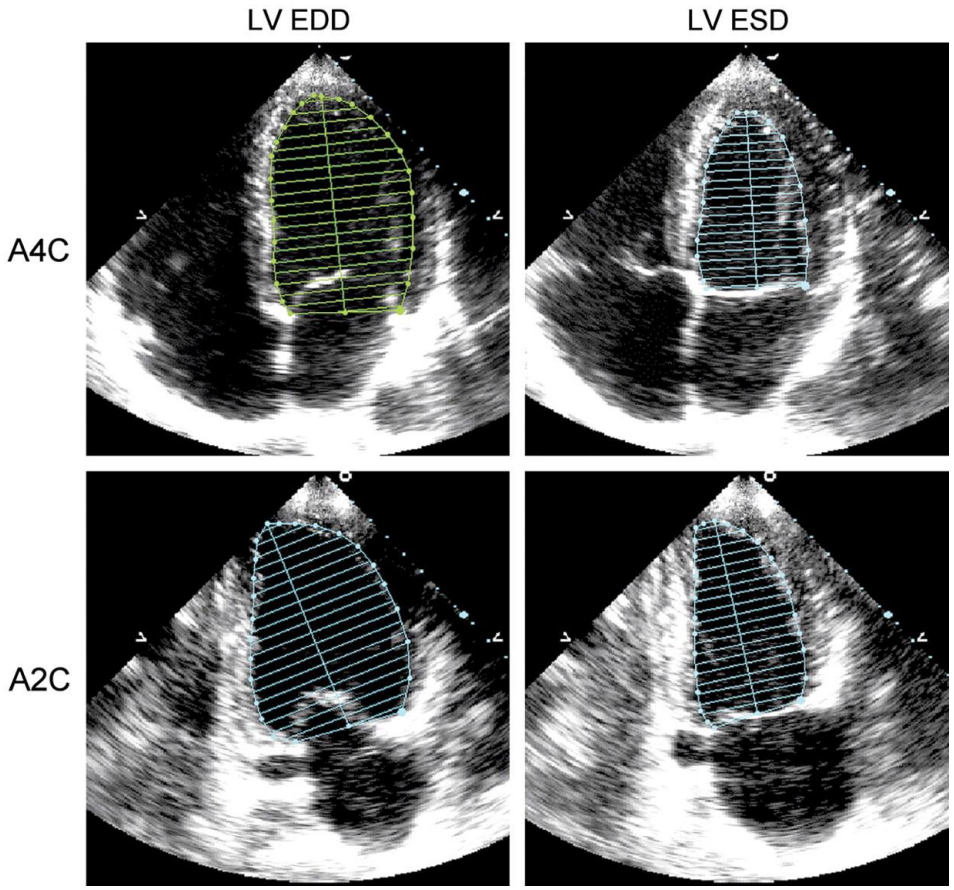


Figure 3-10 – Measurements for volume calculations using the biplane method of discs (modified Simpson's rule), in the apical four-chamber (A4C) and apical two-chamber (A2C) views at end diastole (EDV) and end-systole (ESV). In all panels, the tracking of the internal wall of the left ventricle is evident and shows a clear difference between end-systole and end-diastole. Reproduced from Lang et al. (2005).

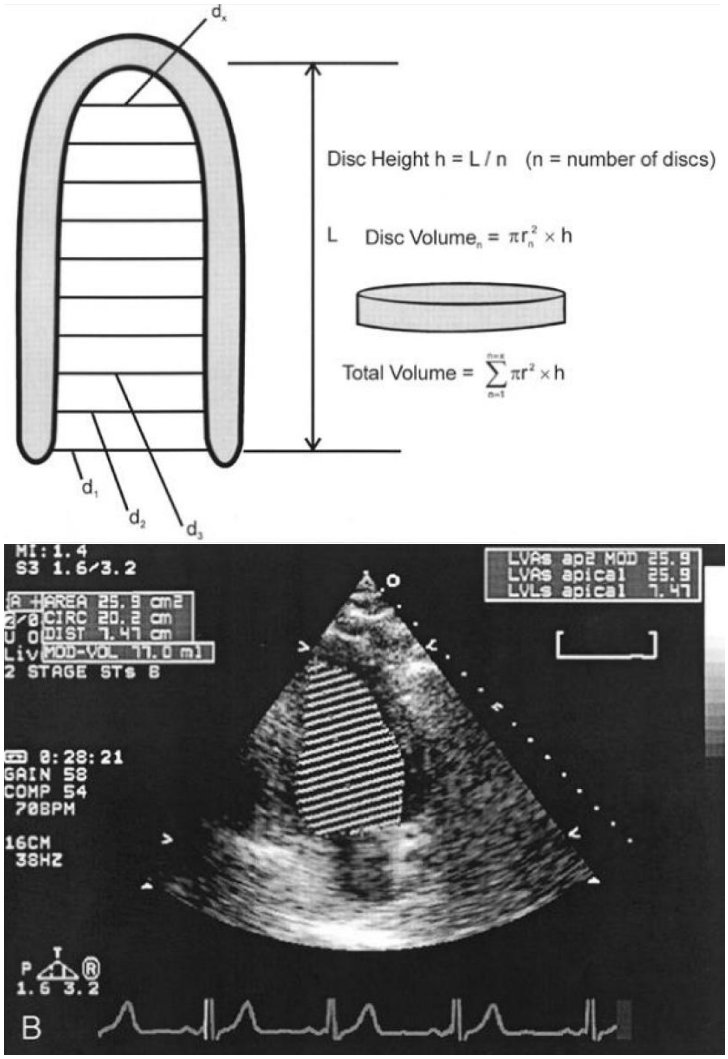


Figure 3-11 – Method for determining the left ventricular volume using the Simpson's biplane method. Top: The left ventricular cavity is arbitrarily divided into 'n' segments of equal height (d1, d2, d3… dn). The height of each cylinder corresponds to the overall length divided by the number of disks (h = L/n). Individual disk volume is calculated as noted, with the ventricular volume being the sum of the individual disk volumes. Bottom: Example of calculation of the left ventricular volume using an apical two-chamber view. Reproduced from Armstrong and Ryan (2012).

3-3.7 Heart rate variability

In this thesis, heart rate variability (HRV) analysis was performed on the raw ECG time series with the goal of obtaining indirect insight into the influence and control of the cardiovascular system by the autonomic nervous system. Throughout the thesis, two different tools were used to process and analyse HRV data. For the first experimental chapter (Chapter 4) the proprietary software Kubios 2.1 (University of Eastern Finland, Kuopio, Finland) was utilised, while for studies 2 and 3 (Chapters 5 and 6) all HRV analysis were performed using the MATLAB (MathWorks, Inc., Natick, MA, USA) based software suite 'Autonomic Nervous System Laboratory' (ANSLAB Professional, University of Salzburg, Salzburg, Austria).

Signal pre-processing

The last 3 min of each 5 min data collection period was used for HRV analysis. The ECG data were initially converted into RR times series. This is typically accomplished by evaluating the time difference between R-spikes from the QRS complexes. The preprocessing of the QRS usually involves bandpass filtering to reduce several sources of noise followed by a decision rule (Tarvainen and Niskanen, 2006). With Kubios this decision rule was included in the built-in QRS detection algorithm and comprised adaptively adjusted amplitude thresholds and expected times between adjacent R-waves (Tarvainen, Niskanen, Lipponen et al., 2014). Furthermore, to improve the time resolution, R-waves were interpolated at 2000 Hz, prior to R-wave extraction (Tarvainen et al., 2014).

When ANSLAB was utilised, RR time series were initially created by R-spike detection (within each individual QRS complex) using default decision criteria of minimum 375 ms length for inter-beat intervals and a minimal rise time to 0.53 mV of 38 ms for valid R-wave detection (Blechert, Peyk, Liedlgruber et al., 2016). Visual inspection of the detected R-peaks before the generation of the RR time series allowed for manual detection and correction of artefacts. Finally, with both software packages, cubic spline interpolation at a default interpolation sampling rate of 4 Hz was used to convert the RR time series into an equidistantly sampled series.

Artefact screening is fundamental in HRV computation as a single error within a relatively small RR time series is sufficient to impart substantial added variance in all frequency bands (Berntson and Stowell, 1998). With the Kubios software, visual inspection of the RR time series was performed initially and when the last 3 min of the data collection periods present artefacts, the closest artefact free section was selected. When that was not possible, the software's built in artefact removal option was utilised to make the necessary adjustments by way of cubic spline interpolation (Tarvainen et al., 2014).

Time domain analysis

The standard deviation of NN interval (SDNN) and the square root of the mean squared differences of successive NN intervals (RMSSD) were used. Both variables are calculated using the intervals between adjacent QRS complexes resulting from sinus node depolarizations, i.e. the normal-to-normal (NN) RR intervals (Task Force,

1996). The SDNN is the square root of the total variance. Since variance is mathematically equivalent to the total PSD, SDNN represents the entirety of cyclic variations in *fc* within the analysed recording period, therefore reflecting the overall HRV (Task Force, 1996). The RMSSD, on the other hand, is considered to be an estimate of the short-term, high-frequency RR interval fluctuations (Ewing, Neilson and Travis, 1984, Kleiger, Stein, Bosner et al., 1992, Task Force, 1996, Malik, 1997). The RMSSD is thought to correlate well with respiratory related high-frequency HRV fluctuations and has been demonstrated to be sensitive to cardiac vagal control, as suggested by pharmacological vagal blockade (Penttilä, Helminen, Jartti et al., 2001). Notwithstanding, at least one study has demonstrated that RMSSD also encompasses low-frequency fluctuations, suggesting that RMSSD can also reflect sympathetic influences, despite the high association between RMSSD and high-frequency spectral variability (Berntson, Lozano and Chen, 2005).

Frequency domain analysis

Analysis frequency bands were set according to the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task Force, 1996) - high frequency (HF) band: 0.15-0.4 Hz; low frequency (LF) band: 0.04-0.15 Hz and, very low frequency (VLF) band: 0-0.04 Hz.

Using Kubios, spectral analysis was performed through a Fast Fourier Transform (FFT) based Welch's periodogram. In this method, the RR time series is divided into overlapping segments using default window and window overlap of 265 s (240 s with ANSLAB) and 50%, respectively, which decreases the leakage effect (Tarvainen et al., 2014). The spectrum estimate was then obtained by averaging FFT spectra of the analysed segments. Before spectral analysis with ANSLAB a low pass filter set at 0.5 Hz was used for detrending, while the Kubios software applied no detrending method.

In this thesis, spectral results for the VLF band are not reported because the analysed segments were of very short duration (< 5 min). According to the Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, VLF assessed from short-term recordings should be avoided in the interpretation of power spectral density (PSD) of ECGs, as it seems to be deeply

affected by algorithms of baseline or trend removal (Task Force, 1996). The analysed variables were, therefore, the PSD for the HF (HRV_{HF}) and LF (HRV_{LF}) spectral bands, as well as the combined PSD (HRV_{TOT}), measured in milliseconds squared (ms^2).

Nonlinear analysis: Poincaré plot

The Poincaré plot of the RR time series was analysed as part of the built-in analysis options provided by both Kubios and ANSLAB software. The Poincaré plot is a graphical presentation of the correlation between consecutive RR intervals, i.e. a plot of RR_{i+1} as a function of RR_i (Figure 3-12). The basic descriptors of the Poincaré plot are:

- SD1 – the standard deviation of the dispersion of successive RR intervals perpendicular to the identity line of the plot (line where RR_n equals RR_{n+1});
- SD2 – the standard deviation of the points along the line of identity (Brennan, Palaniswami and Kamen, 2001) and,
- SD2/SD1 – the ratio of SD2 to SD1, which is thought to reflect the balance between long- and short-term HRV (Guzik, Piskorski, Krauze et al., 2007).

While SD1 is considered to describe short-term variability and relates strongly with time-domain RMSSD (Guzik et al., 2007), SD2 is commonly associated with measures of long-term variability, particularly SDNN (Brennan et al., 2001, Guzik et al., 2007).

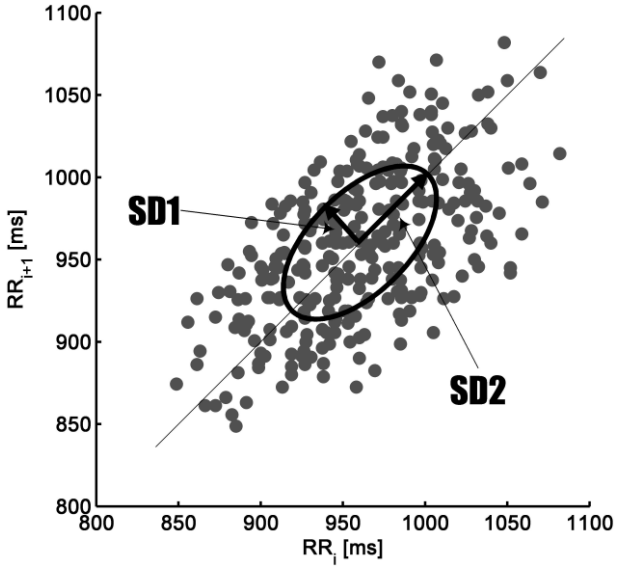


Figure 3-12 – Example of a Poincaré plot of a 5-min ECG recording. SD1 measures the dispersion of points across the identity line, and SD2 measures the dispersion of points along the identity line of the Poincaré plot. Both SD1 and SD2 are axes of an imaginary ellipse whose shape is visible in the diagram. Reproduced from Guzik et al. (2007).

3-3.8 Blood pressure variability

The last 3 min of each 5-min data collection period was considered for blood pressure variability (BPV) analysis. The analysis was performed using ANSLAB software and included an initial preprocessing of the raw ABP signal, which entailed visual inspection and manual removal of outliers. A built-in analysis routine then allowed for the determination of systolic (SBP) and diastolic blood pressure (DBP) time series.

For spectral analysis of ABP, only the SBP time series was considered. Analysis frequency bands were the same as those used for HRV - high frequency (HF) band: 0.15-0.4 Hz; low frequency (LF) band: 0.04-0.15 Hz and, very low frequency (VLF) band: 0-0.04 Hz. As for HRV, the VLF band results are not reported due to the short length of the epochs.

Detrending was performed ahead of the spectral analysis by using a low-pass filter set at 0.5 Hz. The same spectral analysis procedures used for HRV with ANSLAB were applied to the SBP time series, i.e. FFT based Welch's periodogram with a window size of 240 s and 50% window overlap. Results were presented in square millimetres of mercury ($mmHg^2$).

3-3.9 'Peak-Valley' methods applied to cardiovascular data

One commonly used method to quantify the amplitude of oscillating *fc* rhythms is the 'peak-valley' method (Katona and Jih, 1975, Hirsch and Bishop, 1981, Fouad, Tarazi, Ferrario et al., 1984, Grossman and Svebak, 1987, Schechtman, Kluge and Harper, 1988, Grossman, van Beek and Wientjes, 1990, Grossman and Kollai, 1993). This method has been traditionally used to quantify respiratory sinus arrhythmia (RSA), either by measuring the difference between the maximum and minimum instantaneous *fc* or RR interval associated with a specific respiratory phase or simply by determining the difference between the absolute peak and through, irrespective of the breathing phase in which it occurred. In the experimental chapters of this thesis both versions of RSA are calculated; a 'phase locked' version of RSA, which considered the minimum RR interval during inspiration and the longest interval accompanying expiration (Katona and Jih, 1975, Grossman et al., 1990), and one that considered the absolute minimum RR interval and ensuing maximum after the onset of inspiration (Hirsch and Bishop, 1981).

In the thesis, the difference between the absolute peak and through, irrespective of the breathing cycle was also calculated for other cardiovascular parameters to provide a time domain index of their variation, e.g. LVSV, SBP, DBP and PP. Despite limited evidence validating the use of this approach to cardiovascular indices other than RSA, we consider that this method might prove helpful in characterising the phasic cardiovascular variations induced by slow and deep breathing (SDB) interventions.

Regardless of the potential benefit of using this quantitative approach to characterise the cardiovascular response to SDB, this method is not without issues. These have been highlighted previously in the particular case of the utilisation of 'peak-valley' methods for the quantification of RSA (Porges and Byrne, 1992, Byrne and Porges, 1993). These involve the existence of complex trends and other periodic oscillations in cardiovascular parameters that are not perfect sine waves (e.g. baroreceptor influence upon *fc*), resulting in an underlying non-stationarity that may compress or stretch the amplitude of the analysed waveform. Simultaneously, the existence of non-sinusoidal slow periodic processes results in different durations in which the 'peak-valley' calculation will be increased or reduced. Thus, these trends can, in theory, lead to a variability in the 'peak-valley' estimates, even in the absence of changes to the respiratory pattern.

Finally, some 'peak-valley' techniques assume that any peak to trough difference must occur within a clearly defined respiratory cycle. This implies that phase relationships between breathing and the analysed cardiovascular signals remain constant, therefore disregarding the well-known impact that breath holding or respiratory manoeuvres (Valsalva and Mueller) have upon the phase relationships between respiration, *fc* and LVSV (Condos, Latham, Hoadley et al., 1987, Bernardi et al., 1989, Looga, 2001, 2002, 2005).

Accordingly, it has been suggested that 'peak-valley' methods can be used as a good approximation of instantaneous, breath-by-breath, phase relationships. When applied correctly, the 'peak-valley' methods can be advantageous, particularly if the vulnerabilities herein described result in a minor impact of the underlying trends, compared to the peak to through amplitude contribution that stems from a given intervention. In the particular case of RSA, 'peak-valley' estimates have been shown to correlate strongly with other signal processing methods in short-duration epochs

of 5 min (Grossman et al., 1990).The usefulness of 'peak-valley' methods is therefore dependent on the consideration of its inherent limitations, proper pre-processing of the data and removal of trends and careful interpretation of the findings, based on the characteristics of the applied "stress" mechanism. A summary of respiratory phase-related and 'peak-valley' variables used in this thesis can be found in Appendix II.

3-3.10 Baroreflex sensitivity

Baroreflex sensitivity (BRS) was calculated both by the sequence method (Bertinieri, di Rienzo, Cavallazzi et al., 1985, Parati, Di Rienzo, Bertinieri et al., 1988) and from the cross-spectral transfer function gain (BRS_{Freq}) (Robbe, Mulder, Ruddel et al., 1987). The sequence method is a time-domain technique that relies on the identification of a beat-to-beat series of SBP in which a monotonic change (either decrease or increase) of SBP is followed by a directionally opposite series of heart beats (sequence).

In this thesis, sequence BRS values were calculated via a built-in Labview subroutine (LabView 13.0, National Instruments Inc.). For a sequence to be considered valid a reference delay between the changes in SBP and the subsequent *fc* response of one beat, and a threshold change of 1 mmHg and 4 ms had to be met, for a minimum of three consecutive beats. The linear correlation between RR and SBP was computed for each sequence; if $r > 0.8$, the software calculated the slope of the regression line, which is considered to be an estimate of BRS and is expressed in $ms \cdot mmHg^{-1}$. Overall BRS (BRS_{Seq}) was calculated as the average between the sequences where the increases in SBP are paralleled by increases in RR interval (BRS_{up}) and the sequences representing decreases in SBP accompanied by concomitant heart period reductions (BRS_{down}; Figure 3-13).

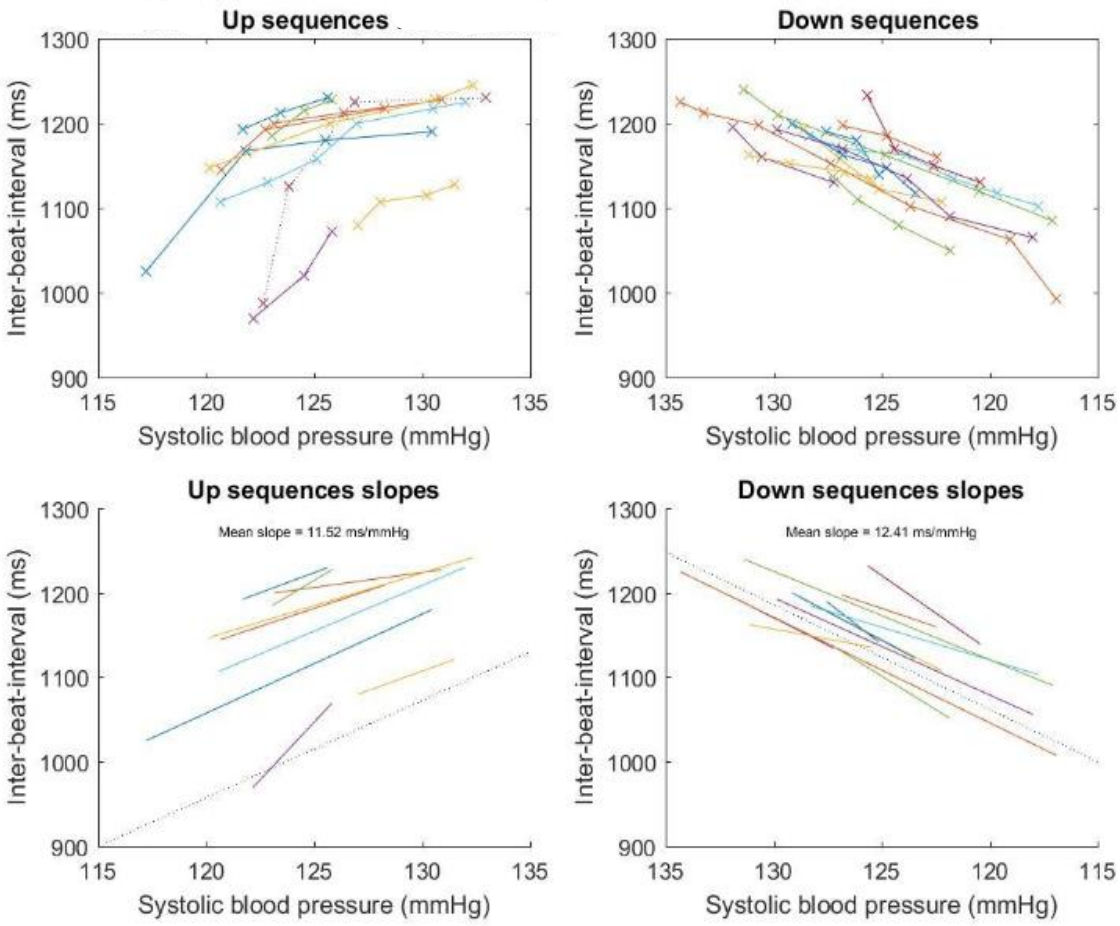


Figure 3-13 – Representation of the calculation of baroreflex sensitivity by the sequence method for one individual. The mean slope of all valid sequences is utilised as a measure of 'up' and 'down' baroreflex sensitivity. Overall baroreflex sensitivity is given by the average of the values (in this particular case,11.97 ms).

The frequency domain methods used in the calculation of the BRS_{Freq} represent an entirely distinct approach to the calculation of BRS. These methods assume that spontaneous SBP fluctuations and *fc* are linked due to the influence of a closed-loop baroreflex cardiovascular control (Robbe et al., 1987, Pagani, Somers, Furlan et al., 1988).

While there are several distinct spectral methods to quantify BRS, including the alpha method, the transfer function method and the trigonometric method (Parati, Saul and Castiglioni, 2004), only the transfer function method is explored and utilised in this thesis. This approach to BRS quantification was first proposed by Robbe and colleagues (1987) and is based on the premise that the SBP-RR interval relation is described by a linear system in which SBP is the input and the heart period is the noisy output.

The BRS estimates are computed by dividing the modulus of the SBP to RR interval cross-spectrum by the SBP spectrum (Figure 3-14). The transfer function is usually evaluated separately in both the LF and HF frequency bands. However, in this thesis, I only considered the transfer function calculation at the respiratory frequency, which means that in some instances (baseline epochs or sets at f_Rs > 8 breaths·min^{-1}) the HF transfer function was utilised as an estimate of BRS_{Freq}, instead of the LF transfer function value. Power spectrum density calculations and the transfer function between signals was performed by the ANSLAB software using an FFT based Welch's periodogram with a window size of 240 s and 50% window overlap.

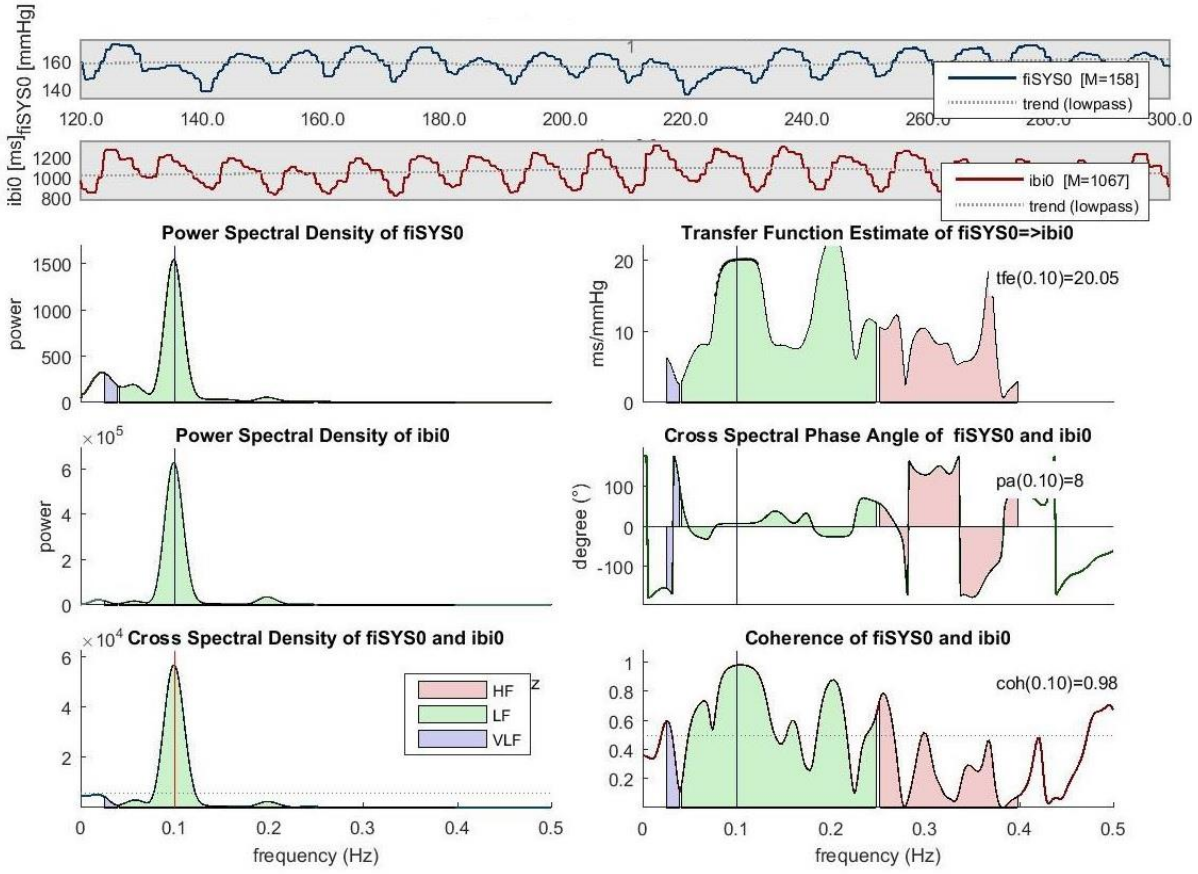


Figure 3-14 - Example of cross spectral analysis performed by ANSlab between heart rate period (ibi) and systolic blood pressure (SYS) for one individual at 6 breaths·min^{-1}. The analysis is performed at the frequency that shows highest cross spectral density within the spectral band of interest (left). Transfer function estimate (cross-spectral baroreflex gain), phase angle and coherence between signals are then calculated for that particular frequency (right).

3-3.11 Phase and time shift relationships

The use of the cross-spectral analysis also provided information on the phase relationship between respiratory and cardiovascular waveforms at the peak respiratory frequency. As high linear association between the two analysed waveforms is one of the premises of significant cross-spectral relationships, a squared coherence cut-off at 0.5 was deemed necessary for the phase values to be considered valid (Baselli, Cerutti, Civardi et al., 1986). Elstad and colleagues (2001) have previously suggested calculating average phase angles of cross-spectral data by weighing the phase angles with their squared coherence. Notwithstanding, as I encountered squared coherence values > 0.95 for the vast majority of individuals and SDB conditions, I opted to calculate the un-weighted circular mean ($\bar{\alpha}$) for phase angles for all relationships of interest, as suggested by Mardia and Jupp (2009). After converting the angles into their Cartesian coordinates, the following equation was used to calculate $\bar{\alpha}$:

$$\bar{\alpha} = arctan\left(\sum_{j=1}^{n} \cos \alpha_j \, , \sum_{j=1}^{n} \sin \alpha_j\right)$$

Circular standard deviation (v) was calculated by:

$v = \sqrt{-2 \ln(\bar{\alpha})}$,

while the circular standard error ($\hat{\sigma}$)was given by:

$$\hat{\sigma} = 1 \, / \sqrt{n\bar{\alpha}\hat{k}}$$

, where $\hat{k}$ represents the concentration parameter (Fisher, 1995).

The blood pressure waveforms (both SBP and DBP) phase data relative to the instantaneous lung volume (RESP) were advanced by 180 degrees so that the physiological phase and time differences between the increases in RESP and concurrent decreases in ABP could be more easily observed. Similarly, RR interval phase relationships were converted to *fc* by adding 180 degrees (Figure 3-15). The time delay (shift) between any two signals was calculated by dividing the cross-spectral phase angle by the peak respiratory period (i.e. 10 s for an f_R of 0.1 Hz or 6 breaths·min^{-1}) multiplied by 360 (Figure 3-15).

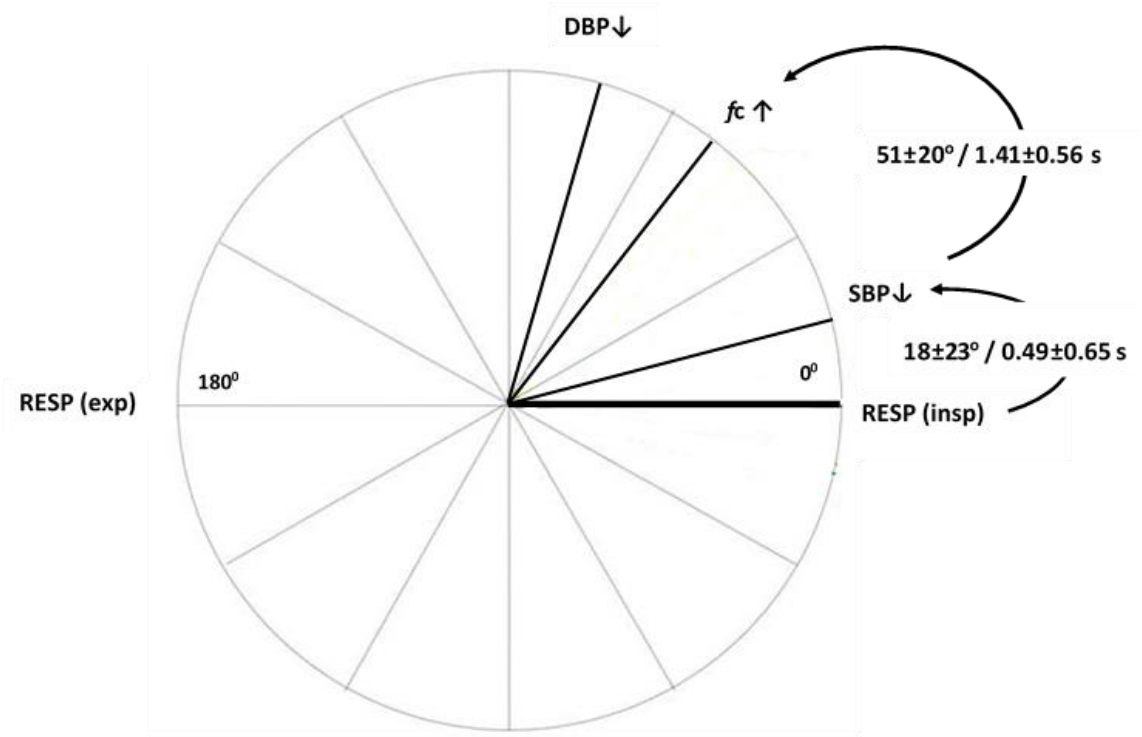


Figure 3-15 - Graphical representation of the relation between systolic blood pressure (SBP), heart rate (HR) and diastolic blood pressure (DBP) at 6 breaths·min^{-1} for one individual. Values are presented as phase shift and time delay between events. The diagram suggests that at 6 breaths·min^{-1} that SBP lags behind the respiratory fluctuations by approximately 1.41 s.

3-3.12 Statistical analysis and data presentation

Detailed descriptions of statistical methods can be found in each experimental chapter. All graphs were created using a commercially available graphing software (Prism 7, GraphPad Software, La Jolla, CA, USA). Circular data were analysed using a commercial statistical software package (Oriana 4, Kovach Computing Services, Pentraeth, Isle of Anglesey, UK), while all remaining data was processed using IBM® SPSS version 21.0 statistical software (IBM Corp., Armonk, NY, USA). All data are expressed as mean ± SEM and significance is set at a level of $P < 0.05$, unless stated otherwise.

3-4 Contribution

Within this thesis, I contributed to the definition of study design and establishment of the research hypothesis for each study. I undertook identification and recruitment of participants, their screening, consent, examination and data acquisition (except for cardiac ultrasonography, which was performed by an experienced sonographer – Dr Eurico Wilhelm). Additionally, I performed all data analysis, data interpretation and wrote all chapters of the thesis.

CHAPTER 4 – THE INDEPENDENT INFLUENCE OF BREATHING FREQUENCY AND TIDAL VOLUME UPON THE ACUTE CARDIOVASCULAR RESPONSES TO SLOW AND DEEP BREATHING

[Note: parts of this chapter were presented and accepted for publication in Proceedings of the 9th Annual Physiological Society Meeting – Physiology 2014; and in the Proceedings of the 2015 International Society for the Advancement of Psychophysiology Annual Conference.]

4-1 Abstract

Introduction and objective: Controlled, slower than normal, breathing frequencies have substantial effects on the cardiovascular and autonomic nervous systems (ANS). The exact mechanisms underlying the observed impact of slow and deep breathing (SDB) upon cardiac sympathovagal balance and other cardiovascular regulatory mechanisms are still not fully understood. Similarly, the independent influences of alterations in tidal volume (V_T), breathing frequency (f_R), and arterial partial pressure of carbon dioxide (P_aCO_2), during SDB remain unclear. Furthermore, the influence of SDB upon cardiac haemodynamics has not been explored systematically; in particular, there has been little investigation of the within breath changes (inhalation *vs.* exhalation). The purpose of this study was to clarify these issues; specifically, to explore how the well-established within-breath changes in heart rate (respiratory sinus arrhythmia; RSA) relate to other cardiovascular changes during SDB.

Materials and methods: Fourteen healthy males (28±3 y; 179±6 cm; 76.9±9.6 kg) undertook SDB in a seated upright position. In part 1 of the study the independent effect of f_R was assessed, whilst maintaining a constant V_T of 30% of forced vital capacity (FVC), and individual f_R optima identified (i.e. the individual f_R that generated the highest RSA amplitude). In part 2, the optimal f_R identified in part 1, was implemented across a range of V_Ts. Parts 1 and 2 included a semi-spontaneous condition in which only V_T or f_R were controlled (SSV_T and SSf_R, respectively). Conditions were randomised and a fixed magnitude of mild hypercapnia was maintained under all conditions, except for SSV_T and SSf_R.

Results and conclusion: Decrease in f_R and increase in V_T were both independently associated with higher amplitudes of RSA, as well as within-breath fluctuations of left ventricle stroke volume (ΔSV) and cardiac output ($\Delta\dot{Q}$), but no significant changes in MAP, $\dot{Q}$ and TPR (P<0.003). The RSA amplitude also increased linearly with both the decrease of f_R and increase V_T, but plateaued at 4 and 6 breaths·min^{-1}. Semi-spontaneous breathing resulted in no significant differences when compared to breathing with clamped f_R, V_T and P_aCO_2. Blood pressure variability (BPV_{TOT}) was smallest at the lowest f_Rs (≤ 6 breaths·min^{-1}), while heart rate variability (HRV_{TOT}) maximised at those same frequencies. Collectively, the data are consistent with the involvement of baroreflex-mediated mechanisms in minimising BPV and maximising RSA during SDB. The data also suggest the presence of respiratory induced modulation of right atrial filling, perhaps mediated

by differences in the magnitude of f_R- and/or VT-induced fluctuations in intra-thoracic pressure.

4-2 Introduction

There is a growing body of evidence that slow and deep breathing (SDB) may exert chronic antihypertensive effects when practiced daily, by people with hypertension, for several weeks (Grossman, Wilhelm, Kawachi et al., 2001, Schein et al., 2001, Viskoper et al., 2003, Elliott et al., 2004, Meles et al., 2004, Mourya et al., 2009, Singh, Gaurav and Parkash, 2011, Lin et al., 2012). In the acute context, SDB influences: i) RSA (Lehrer, Generelli and Hochron, 1997, Lehrer et al., 1999, Cysarz and Bussing, 2005), ii) baroreflex sensitivity (BRS) (Bernardi et al., 2001, Bernardi et al., 2002, Radaelli et al., 2004, Joseph et al., 2005), iii) spectral heart rate variability (HRV) indices (Cysarz and Bussing, 2005, Tharion et al., 2012), and iv) muscle sympathetic nerve activity (Raupach et al., 2008, Hering et al., 2013, Mozer, Fadel, Johnson et al., 2014).

The precise mechanisms underlying any antihypertensive effect of SDB remain unknown. A number of putative pathways have been suggested, including, 'strengthening' of baroreceptor homeostasis via neuroplasticity of the baroreflex (Vaschillo et al., 2002, Lehrer et al., 2003, Vaschillo et al., 2006) and changes in acid-base balance and renal sodium regulation induced by changes in P_aCO_2 (Anderson et al., 2009). However, the key to understanding the mechanism(s) underlying any chronic adaptation to physiological perturbation(s) is first to comprehend the mechanisms underpinning the acute adjustments. The exact mechanisms underlying the acute changes induced by SDB also remain unclear; in particular, whether the input variables (V_T and f_R) exert independent effects upon the cardiovascular system.

One of the most robust responses to SDB is its magnifying effect upon within breath fluctuations in heart rate, i.e. respiratory sinus arrhythmia (RSA). The functional significance of RSA has been the object of ample debate over the last century, and while some advances towards understanding its physiological purpose and underlying mechanisms have been made, disagreement still abounds (Eckberg, 2009b, Julien et al., 2009, Karemaker, 2009c, a). Fundamentally, however, RSA is a manifestation of the differing conditions that are experienced by the cardiovascular system during inhalation and exhalation. The RSA amplitude is known to maximise at f_Rs around 0.1Hz (Angelone and Coulter, 1964, Hirsch and Bishop, 1981, Song and Lehrer, 2003) and to increase with progressively higher V_Ts (Freyschuss and

Melcher, 1976c, Hirsch and Bishop, 1981). The reasons for this were explored in detail in section 2-4.3 of this thesis. In short, these include: i) the involvement of central mechanisms gating the responsiveness of cardiac vagal motoneurone throughout the respiratory cycle (a comprehensive review of this topic can be found in Eckberg [2003]); ii) the presence of baroreflex-mediated mechanisms (De Boer et al., 1987, TenVoorde et al., 1995, Karemaker, 2009a, c); iii) the mechano-electric and/or reflex response to atrial stretch, brought about by increased venous return to the right atria during inspiration (Bainbridge, 1915, Donald and Shepherd, 1978, Bolter and Wilson, 1999, Wilson and Bolter, 2002, Quinn and Kohl, 2012); iv) the contribution of lung stretch receptors (Anrep et al., 1936a, Seals et al., 1990).

Most of the mechanisms above either result from or contribute to, the generation of important within-breath cardiovascular changes induced by breathing. To further the understanding of the impact of SDB upon RSA and cardiovascular regulation, it is necessary to characterise the within-breath cardiovascular responses within the range of f_Rs and V_Ts typically implemented in SDB interventions. While this has been accomplished to some extent for RSA and other indices of HRV (Angelone and Coulter, 1964, Freyschuss and Melcher, 1976c, Hirsch and Bishop, 1981, Berger, Saul and Cohen, 1989a, Cooke et al., 1998), the characterisation of frequency and volume dependencies has yet to be extended to the within-breath haemodynamic and cardiac responses to SDB.

There is a general assumption that for f_Rs and V_Ts consistent with spontaneous breathing, LVSV during inhalation ($LVSV_I$) decreases at a time when heart rate (*fc*) increases (Dornhorst et al., 1952b, Robotham and Mintzner, 1979, Saul et al., 1989), leaving cardiac output ($\dot{Q}$) relatively unaffected by breathing (Elstad, 2012), minimising fluctuations in arterial blood pressure (ABP) induced by breathing (Elstad, Walløe, Holme et al., 2015). However, some of the handful of studies that have sought to characterise the frequency dependent respiratory modulation of ABP, and thus blood pressure variability (BPV), hint at an increased modulatory influence of breathing upon BPV at lower f_Rs (TenVoorde et al., 1995, Radaelli et al., 2004, Chang et al., 2013), supporting the argument that some of the within-breath cardiovascular responses observed during spontaneous breathing might be altered during SDB, particularly those involving LVSV and $\dot{Q}$.

The present study intends to shed light on how changes in f_R and V_T independently influence the within-breath cardiovascular response to SDB. It is hoped that the description of these response patterns can contribute to a better understanding of the mechanisms underpinning the generation and amplification of RSA by SDB, and reveal potential contributors to the reported antihypertensive effects of SDB programs

Thus, the specific objectives of this study are, 1) to elucidate the independent effect of f_R and V_T upon the within-breath cardiovascular response to SDB; 2) to identify whether there is an optimal breathing pattern (f_R and V_T) that maximises within-breath cardiovascular fluctuations; 3) to assess the contribution of respiratory-synchronous fluctuations of LVSV, $\dot{Q}$ and ABP, to the generation of RSA; and 4) to characterise the interrelationship of breathing, *fc* and ABP across a range f_Rs and V_Ts, using spectral analysis techniques.

4-3 Specific Methods

4-3.1 Overview

The study consisted of two experimental protocols, in which the independent influences of f_R (Protocol 1) and V_T (Protocol 2) upon the cardiovascular and autonomic response to SDB were assessed, with 'clamped' $P_{ET}CO_2$.

4-3.2 Participants

The study sample comprised fourteen healthy, recreationally-active men (28 ± 3 y). All participants were nonsmokers with no previous history of cardiovascular or respiratory disease, and self-reported to be free from medication usage. Descriptive characteristics of the participants are provided in Table 4-1.

Table 4-1 Descriptive characteristics of the participants.

	Mean ± SD	%Predicted
Anthropometry		
Age (y)	28 ± 2.5	-
Stature (cm)	179 ± 6	-
Body mass (kg)	76.9 ± 9.6	-
SBP(mmHg)	133 ± 11	-
DBP (mmHg)	74 ± 10	-
Resting pulmonary function		
FEV_1 (L)	4.7 ± 0.5	110 ± 10
FVC (L)	6.2 ± 0.8	121 ± 13
FEV_1/FVC	76.8 ± 5.5	93 ± 7

Values are mean ± SD for *n* = 14. Systolic blood pressure (SBP); diastolic blood pressure (DBP); forced expiratory in 1s (FEV_1); forced vital capacity (FVC). Predicted values for lung function were determined from the equations derived from Stanojevic, Wade, Stocks et al. (2008).

4-3.3 General Design

A within-subject design was utilised whereby participants underwent two experimental trials to access the independent effects of f_R and V_T upon cardiovascular and autonomic response. All participants undertook one familiarisation session and the two experimental trials (Protocols 1 and 2).

During their first visit, participants performed a baseline pulmonary function assessment. Those participants with a forced vital capacity (FVC) under 4.5 litres

(L), as well as individuals showing signs of asthma or other lung disease were excluded from the study. The exclusion of participants with FVCs below 4.5 L was deemed necessary after pilot testing demonstrated that these individuals would be unable to maintain stable and tolerable P_aCO_2 levels at the lowest breathing frequencies due to inadequate alveolar volume. The severe hypercapnia that would result from this situation would have led to unphysiological levels of P_aCO_2, leading to the activation of chemoreflex feedback mechanisms, precluding some participants from completing the experimental procedures, and compromising the study design. Also, a familiarisation test was undertaken using a bespoke device guided breathing (DGB) biofeedback system to assess participants' ability to follow an imposed breathing pattern accurately (Labview, National Instruments Inc.; please refer to Chapter 3, for a detailed description of pulmonary function assessment and familiarisation session protocols). Participants who were unable to follow the required pattern were excluded (only one participant out of 15 individuals tested did not meet the necessary inclusion criteria). Figure 4-1 depicts the study design.

During the second and third visits, participants undertook the two experimental protocols of DGB. Protocol 1 manipulated f_R and began with 5 min of quiet baseline breathing, followed by four 5 min sets at 4, 6, 8 and 10 breaths·min^{-1} and at a constant V_T corresponding to 30% of each participant's FVC. A condition where participants were allowed to breathe freely at the previously fixed V_T of 30% FVC was also included, henceforth referred to as 'semi-spontaneous breathing frequency' (SSf_R). Five minute rest periods separated each set. To enable the 'clamped' target level of $P_{ET}CO2$ to be identified, the 4 breaths·min^{-1} condition was always performed first, with full randomisation of all remaining sets (a detailed description of the randomisation procedures is presented in section 3-2.5). Protocol 2 manipulated V_T. Following an initial quiet breathing baseline 5 min set, participants performed 5 guided breathing sets at 20, 25, 30, 35 and 40% FVC at a constant f_R corresponding to the individual f_R that elicited the highest amplitude of peak-to-valley RSA (RSA for each participant). The RSA was defined as the difference between the maximum RR interval during expiration minus the minimum RR interval during inspiration, in ms (Grossman et al., 1990). The RSA was chosen as the variable on which to select the f_R for the evaluation of V_T manipulations because of its known association with cardiovascular system resonance and BRS (Vaschillo et al., 2002). A condition where participants were asked to breathe freely at the previously

designated f_R was also included; henceforth referred to as 'semi-spontaneous tidal volume' (SSV_T). Similar to the first experimental visit, sets were performed in fully randomised order, with the exception of the condition that would deliver the smallest $\dot{V}_E$ and consequently the highest $P_{ET}CO_2$ levels.

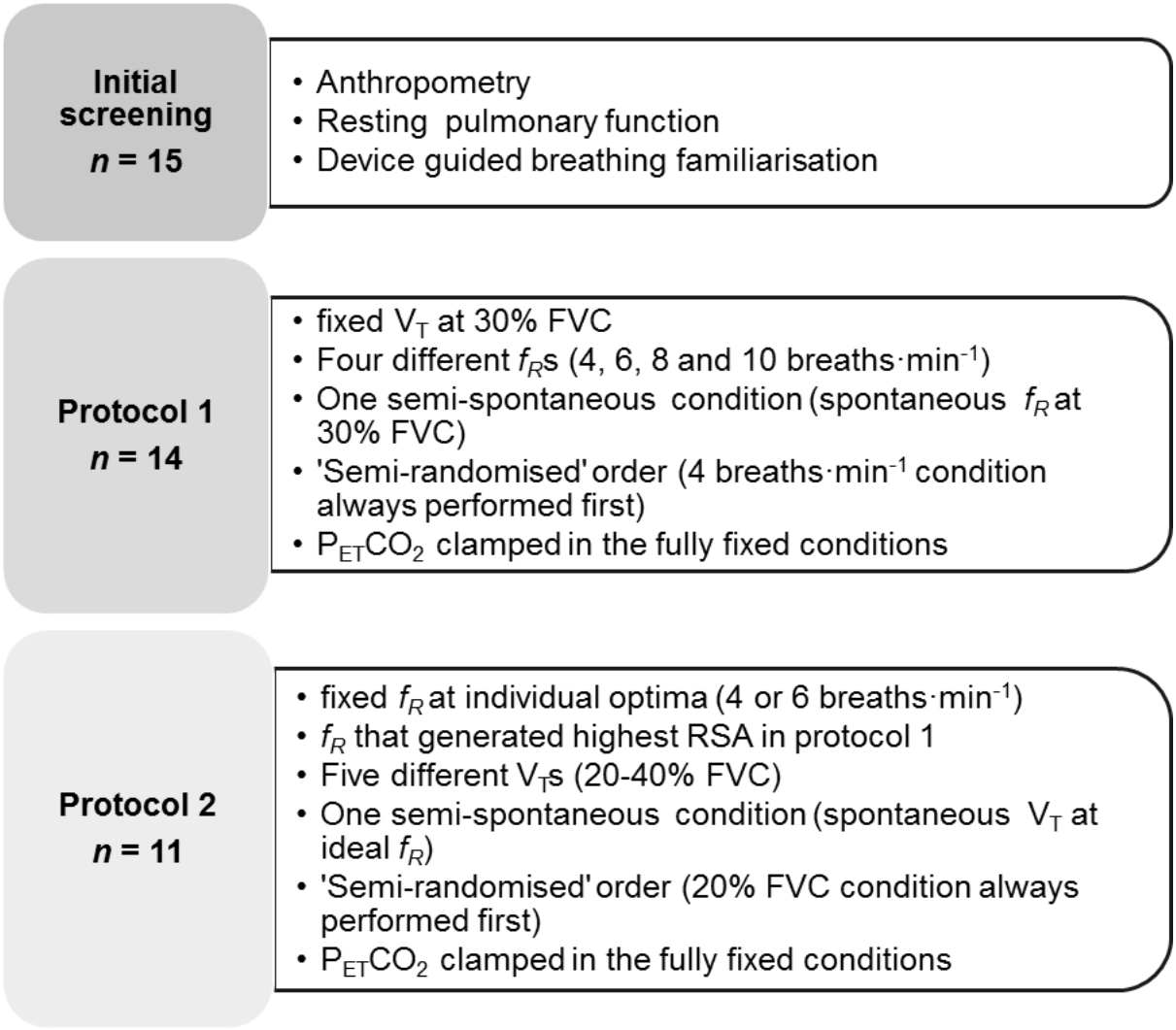


Figure 4-1 General study design. Tidal volume (V_T), forced vital capacity (FVC), breathing frequency (f_R), end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$), respiratory sinus arrhythmia (RSA).

4-3.4 Procedure

Ethics

Written informed consent was obtained from all participants prior to the start of the study. The study was approved by a local sub-panel of the Brunel University London Research Ethics Committee and conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013).

Participant preparation

Participants were asked to refrain from alcohol, caffeine and strenuous physical activity for at least 12 h before testing and to avoid consuming food during the two hours leading up to testing. All sessions were conducted at the same time of the day and were separated by at least 48 h but no more than one week. Throughout all sessions, participants assumed an upright-reclined position by sitting on a reclining lounge chair, set at an approximate angle of 60^{o}. They also breathed through a mask that allowed for normal mouth and/or nasal breathing (Oro Nasal 7450 V2, Hans Rudolph Inc., Shawnee, KS, USA). Room temperature was 22-25^{o}C and barometric pressure was recorded for each of the trial sessions. A detailed description of participant preparation guidelines as well exclusion criteria can be encountered in Chapter 3 (section 3-2.3).

Device guided breathing

Specified respiratory flow rates and a respiratory duty cycle of 0.5 (inspiratory duration = expiratory duration) were delivered using the aforementioned bespoke guided breathing biofeedback system (Labview, National Instruments Inc.) and measured using a heated pneumotachograph (Hans Rudolph 3813, Hans Rudolph Inc.). The $P_{ET}CO_2$ (GA-200 gas analyser, iWorx Systems Inc.) was maintained at a slightly hypercapnic level (defined by the lowest f_R and V_T conditions; see above) under all fully controlled conditions via a re-breathing system consisting of added dead spaces. Apparatus and procedural details are contained in Chapter 3 (section 3-3).

Cardiovascular measurements

Heart rate was monitored continuously using a 3-lead ECG (PhysioControl VSM® 3, PhysioControl Inc.). Non-invasive beat-to-beat arterial blood pressure was obtained using finger photoplethysmography (Finometer® PRO, Finapres Medical Systems, Amsterdam, The Netherlands). Total peripheral resistance (TPR) was calculated by dividing $\dot{Q}$ by the mean arterial pressure (MAP). Baroreflex sensitivity (BRS) was calculated by the sequence method (Bertinieri et al., 1985, Parati et al., 1988), as well as from the cross-spectral transfer function gain (Robbe et al., 1987). Phase angles and coherence between respiratory and cardiovascular (*fc*, SBP and DBP) waveforms were obtained from the cross-spectra at the peak respiratory frequency.

A more detailed description of these outcome variables can be found in the Chapter 3 (section 3-3).

4-3.5 Statistical analysis

Values are expressed as means ± standard error of the mean (SEM), unless stated otherwise. Repeated measures ANOVA with *post hoc* pairwise comparisons using Bonferroni correction were used to test differences between conditions after normality was confirmed via the Shapiro-Wilk test. Circular data (phase relationships) were analysed using multi-sample Watson-Williams F-test following confirmation of a Von Mises circular distribution (Fisher, 1995, Mardia and Jupp, 2009). Bonferroni corrections were applied to circular data for *post hoc* pairwise comparisons. A justification regarding the use of these statistical tests in instances where the requirements for the use of parametric statistics were not met can be found in Chapter 3 (section 3-3.12). Finally, the associations amongst f_R, V_T, and cardiovascular indices were accessed using linear correlation coefficients (Pearson's *r*) and respective coefficients of determination (R^2). For all analyses, *P* was set at 0.05.

4-4 Results

4-4.1 Respiratory responses elicited by each condition

A summary of the respiratory data associated with each experimental condition can be found in Tables 4-2 and 4-3. As intended, duty cycle (~0.5) remained constant throughout all conditions, while $P_{ET}CO_2$ was stable for all fully clamped conditions, in both the fixed V_T (40-41 mmHg) and fixed f_R protocols (41-42 mmHg). Both V_T (~30% FVC) and f_R (~5.4 breaths·min^{-1}) remained constant throughout Protocol 1 and 2, respectively (Tables 4-2 and 4-3). The semi-spontaneous conditions showed significantly lower $P_{ET}CO_2$ than the fully clamped conditions (SSV_T: ~36 mmHg *vs.* fully clamped: ~41 mmHg, Table 4-2; and SSf_R: ~35mmHg *vs.* fully clamped: ~42 mmHg, Table 4-3).

Table 4-2 Respiratory parameters for the fixed V_T protocol.

	Baseline	**4 breaths·min^{-1}**	**6 breaths·min^{-1}**	**8 breaths·min^{-1}**	**10 breaths·min^{-1}**	**Semi-Spontaneous (SSV_T)**
f_R	12.2±0.8	4.1±0.1*†	6.1±0.1*§¥ǂ	8.1±0.1*§ǂ†	10.1±0.1*§†	5.2±0.4*
V_T	12.3±0.7	29.9±0.4*	29.7±0.3*	29.7±0.4*	29.6±0.3*	29.9±0.4*
T_I/T_{TOT}	0.53±0.02	0.51±0.01	0.52±0.01	0.51±0.01	0.51±0.01	0.52±0.01
$P_{ET}CO_2$	38.2±1.4	41.3±0.8†	41.0±0.7†	40.9±0.7†	40.6±0.7†	35.5±1.3

Data represent mean ± SEM for 14 subjects. Breathing frequency (f_R) in breaths·min^{-1}, tidal volume (V_T) in % of forced vital capacity, duty cycle (Ti/T_{TOT}), partial pressure of end tidal carbon dioxide ($P_{ET}CO_2$) in mmHg. * different from baseline; † different from semi-spontaneous; § different from 4 breaths·min^{-1}; ¥ different from 8 breaths·min^{-1}; ǂ different from 10 breaths·min^{-1}. Signalled differences between conditions are significant at $P < 0.003$.

Table 4-3 Respiratory parameters for the fixed f_R protocol.

	Baseline	**20%FVC**	**25%FVC**	**30%FVC**	**35%FVC**	**40%FVC**	**Semi-Spontaneous (SSf_R)**
f_R	11.5±0.5	5.4±0.3*	5.5±0.3*	5.3±0.3*	5.4±0.3*	5.3±0.3*	5.3±0.3*
V_T	11.6±0.5	19.8±0.4*†	25.5±0.3*§†	30.2±0.5*§¥	34.9±0.6*§¥ǂ	40.2±0.5*§¥ǂ#†	31.4±2.5
T_I/T_{TOT}	0.54±0.01	0.51±0.02	0.52±0.01	0.52±0.01	0.52±0.01	0.52±0.01	0.52±0.03
$P_{ET}CO_2$	39.9±1†	42.1±1.6†	42.0±1.6†	42.1±1.6†	41.8±1.6†	41.7±1.7†	34.7±1.4

Data represent mean ± SEM for 11 subjects. Breathing frequency (f_R) in breaths·min^{-1}, tidal volume (V_T) in % of forced vital capacity, duty cycle (Ti/T_{TOT}), partial pressure of end tidal carbon dioxide ($P_{ET}CO_2$) in mmHg. * different from baseline; § different from 20% FVC; ¥ different from 25% FVC; ǂ different from 30% FVC; # different from 35% FVC; † different from semi-spontaneous. Signalled differences between conditions are significant at $P < 0.002$.

4-4.2 Cardiovascular response to SDB

Respiratory sinus arrhythmia and heart rate

The amplitude of RSA increased progressively with both the decrease of f_R and the increase of V_T, but exhibited a plateau for breathing frequencies ≤ 6 breaths·min^{-1} (P<0.001*;* Table 4-4; Figure 4-2A). The average gain of the change in RSA relative to the change in f_R was *circa* -20 ms·breath^{-1}·min^{-1}. The increased amplitude of the RSA fluctuations resulted from significant frequency dependent changes in *fc* during both inspiration (fc_I) and expiration (fc_E) (Table 4-4). Despite the tendency for fc_I to increase (R^2=0.89) and fc_E to decrease (R^2=0.70) with a reduction in f_R, the regression coefficient *b* was not significantly different from zero (P>0.05 for both; Figure 4-2A), indicating a small effect size of f_R upon fc_I and fc_E. Mean *fc* was only slightly, but significantly, affected by the changes in f_R (Table 4-4). The increments in V_T during the fixed f_R protocol (Protocol 2) also resulted in a linear increase in RSA (R^2=0.96, P=0.003, average gain: ~103 ms·L^{-1}; Figure 4-2E) accompanied by a linear decay in fc_E (R^2=0.90, P=0.015, Table 4-5; Figure 4-3D).

Stroke volume and cardiac output

The decrease in f_R resulted in increments in LVSV during expiration ($LVSV_E$, Table 4-4; Figure 4-3B). Despite the existence of a significant main effect of f_R upon $LVSV_I$, *post-hoc* analysis revealed no statistically significant differences between different f_Rs. Lower f_R*s* were associated with an inversion of the within-breath pattern of LVSV (ΔSV), i.e. $LVSV_E$ was higher than $LVSV_I$ for frequencies close to, or below, 6 breaths·min^{-1}, whilst the reverse was true for the higher f_Rs (Table 4-4, Figures 4-2B and 4-3B). Breathing at progressively higher V_Ts did not alter either $LVSV_I$ or $LVSV_E$, but a significant main effect of V_T was identified for ΔSV *(*Table 4-5*).* The *post-hoc* analysis only revealed significant differences relative to baseline, and not between the different V_Ts (Table 4-5*).*

No significant main effect of either f_R or V_T was detected for $\dot{Q}$ or the breath phase-specific variables ($\dot{Q}_I$ and $\dot{Q}_E$) (Tables 4-4 and 4-5). Nonetheless, while f_R changes did not significantly impact $\Delta\dot{Q}$ (Table 4-4, Figure 4-2C), increasing V_T at a fixed f_R resulted in a linear increase in $\Delta\dot{Q}$ (Figure 4-2G) resulting from a trend for $\dot{Q}_E$ to reduce with progressively higher V_Ts (Figure 4-3F).

Arterial blood pressure

Mean arterial pressure (MAP), pulse pressure (PP), SBP and DBP all increased across the range from 6 and 10 breaths·min^{-1}, with a significant main effect of f_R (P<0.001, Table 4-4). At 4 breaths·min^{-1}, MAP, PP, SBP and DBP returned to near baseline values. Breathing at a fixed individual optimal frequency (4 or 6 breaths·min^{-1}) resulted in no significant main effects of V_T upon MAP, PP, SBP or DBP (Table 4-5). Nevertheless, at its maximum (30% FVC), the difference in SBP compared to baseline averaged 10 mmHg (137±13 *vs.* 127±9 mmHg, 30%FVC *vs.* baseline, respectively).

Baroreflex sensitivity

A main effect of f_R was observed for all BRS indices, but *post-hoc* analysis only revealed significant differences between 6 and 10 breaths·min^{-1} for BRS_{down}, and between 6 breaths·min^{-1} and baseline using the cross-spectral index of BRS (BRS_{Freq}; Table 4-4). No significant main effect of V_T was observed for any of the analysed BRS variables (Table 4-5).

Semi-spontaneous breathing

Breathing with fixed V_T, but without clamped f_R or $P_{ET}CO_2$ (SSV_T) resulted in no significant differences from breathing under fully controlled conditions with similar f_Rs (i.e. 4 and 6 breaths·min^{-1}), for any of the analysed cardiovascular variables, except for SBP, DBP and MAP. For these variables, SSV_T exhibited slightly smaller, but significantly different values than those observed for f_Rs ≥ 6 breaths·min^{-1}. In contrast, breathing with fixed f_R, but without controlled V_T or $P_{ET}CO_2$ (SSf_R), did not result in any significant difference from any of the fully controlled conditions for any of the analysed cardiovascular variables.

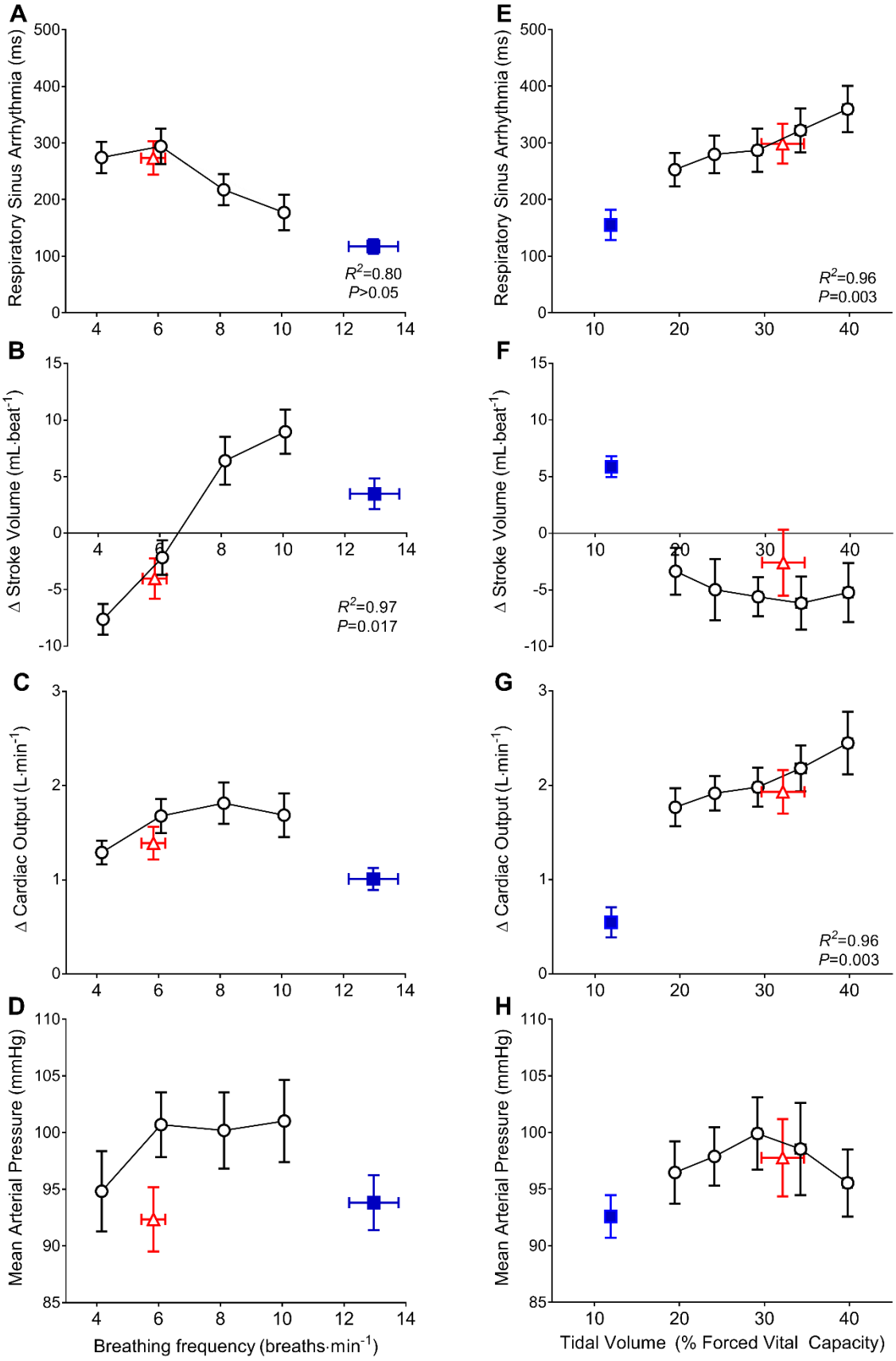


Figure 4-2 Cardiovascular response to SDB at different breathing frequencies (A, B, C, D) and tidal volumes (E, F, G, H). Respiratory sinus arrhythmia (A, E), amplitude of inspiratory to expiratory (Δ) stroke volume variation (B, F), amplitude of inspiratory to expiratory (Δ) cardiac output variation (C, G) and mean arterial pressure (D, H) Values are means ± SEM for fully fixed (○ black), semi-spontaneous (△red) and baseline (■ blue) breathing pattern. Presented R^2 represent Pearson's coefficient of determination. P < 0.05 indicate regression coefficients (b) that are significantly different from zero.

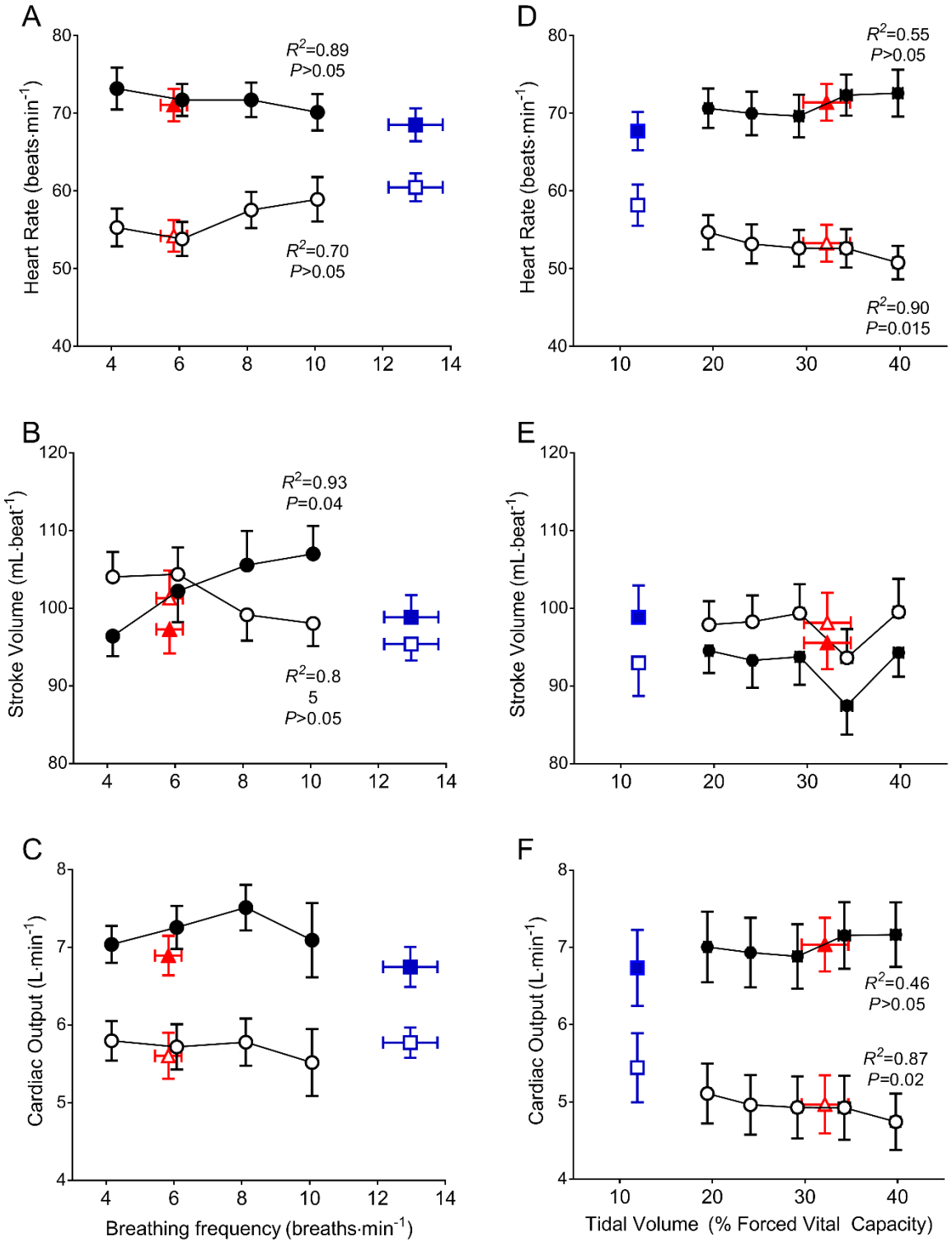


Figure 4-3 Within-breath cardiovascular response to SDB at different breathing frequencies (A, B, C) and tidal volumes (D, E, F). Inspiratory (solid shapes) and expiratory (open shapes) mean values are presented for heart rate (A, D), stroke volume (B, E) and cardiac output (C, F), Values are means ± SEM for fully fixed (○, ●, black), semi-spontaneous (△, ▲, red) and baseline (□,■, blue) breathing patterns. Presented R^2 represent Pearson's coefficient of determination. $P < 0.05$ indicate regression coefficients (*b*) that are significantly different from zero.

Table 4-4 Cardiovascular responses to SDB at fixed V_T (30% FVC).

	Baseline	4 breaths·min^{-1}	6 breaths·min^{-1}	8 breaths·min^{-1}	10 breaths·min^{-1a}	Semi-Spontaneous (SSV_T)
RSA (ms)	111±13	265±27*‡	274±35*‡¥	205±28*‡	166±31	259±31*‡
f_C (beats·min^{-1})	65±2	64±2	62±2‡	65±2	65±2	63±2
fc_I (beats·min^{-1}	68±2	72±3*	71±2	71±2	69±2	70±2
fc_E (beats·min^{-1})	60±2	55±2*‡	54±2*‡¥	58±2	59±3	54±2*‡¥
LVSV (mL·beat^{-1})	99±2	103±3	105±3	104±3	104±3	102±3
$LVSV_I$ (mL·beat^{-1})	99±3	98±3	103±3	106±3	107±5	98±3
$LVSV_E$ (mL·beat^{-1})	96±2	105±3*	105±4*‡	100±4	98±6	102±3
ΔSV (mL·beat^{-1})	4±1	-7±1*‡¥	-3±1‡¥	6±2	9±2	-4±2*‡¥
$\dot{Q}$ (L·min^{-1})	6.4±0.2	6.5±0.2	6.6±0.3	6.7±0.3	6.7±0.3	6.4±0.3
$\dot{Q}_I$ (L·min^{-1})	6.7±0.3	7.0±0.2	7.3±0.3	7.5±0.3	7.5±0.3	6.9±0.3
$\dot{Q}_E$ (L·min^{-1})	5.8±0.2	5.8±0.3	5.7±0.3	5.8±0.3	5.8±0.4	5.6±0.3
$\Delta\dot{Q}$ (L·min^{-1})	1.0±0.1	1.2±0.1*	1.5±0.2*	1.7±0.2*	1.7±0.2*	1.3±0.2*
SBP (mmHg)	133±3	135±4	144±4*†	143±4†	145±4*†	132±4
DBP (mmHg)	74±3	75±3	79±3*†	79±3	79±3	72±3
MAP (mmHg)	94±2	95±4	101±3*†	100±3†	101±3*†	92±3
PP (mmHg)	58±3	60±3	65±3*	65±3*	65±3*	60±3
TPR (mmHg·min·L^{-1})	15±1	15±1	16±1	16±1	16±1	15±1
BRS_{up} (ms·mmHg^{-1})	13±2	19±3	20±2	18±3	14±3	22±3
BRS_{down} (ms·mmHg^{-1})	11±1	14±1	14±1¥	11±1	11±2	14±2¥
BRS_{Seq} (ms·mmHg^{-1})	12±2	17±2	17±2	15±2	13±2	18±2¥
BRS_{Freq} (ms·mmHg^{-1})	7±2	17±2*	17±2¥	13±2	12±2	16±2

Values are Mean ± SEM for *n*=14. Respiratory sinus arrhythmia (RSA), heart rate (f_C), heart rate during inspiration (fc_I), heart rate during expiration (fc_E), left ventricle stroke volume (LVSV), stroke volume during inspiration ($LVSV_I$), stroke volume during expiration ($LVSV_E$), within-breath variation in stroke volume (ΔSV), cardiac output ($\dot{Q}$), cardiac output during inspiration ($\dot{Q}_I$), cardiac output during expiration ($\dot{Q}_E$), within-breath variation in cardiac output ($\Delta\dot{Q}$), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP), total peripheral resistance (TPR), sequence baroreflex sensitivity positive sequence gain (BRS_{up}), sequence baroreflex sensitivity negative sequence gain (BRS_{down}), sequence baroreflex sensitivity average gain (BRS_{Seq}); cross-spectral baroreflex sensitivity gain (BRS_{Freq}). * Different from baseline; † different from semi-spontaneous; § different from 4 breaths·min^{-1}; ¥ different from 8 breaths·min^{-1}; ǂ different from 10 breaths·min^{-1}. Signalled differences between conditions are significant at $P < 0.003$. Note: [a] a non-physiological outlier affecting the LVSV values was detected for one of the participants at 10 bpm, and was removed from the statistical analysis

Table 4-5 Cardiovascular responses to SDB at fixed individual ideal f_R (4 or 6 breaths·min^{-1}).

	Baseline	20% FVC	25% FVC	30% FVC	35% FVC	40% FVC	Semi-Spontaneous (SSf_R)
RSA (ms)	155±27	253±30*	280±33*	287±38*	322±39*†	360±41*†‡	298±35*
f_C (beats·min^{-1})	63±3	63±2	62±3	62±2	63±2	61±2	62±3
fc_I (beats·min^{-1})	68±3	71±2	70±3	70±2	72±2	73±2	71±2
fc_E (beats·min^{-1})	58±2	55±3	53±3	53±3*	53±3*	51±3*†‡	53±2*
LVSV (mL·beat^{-1})	95±4	96±3	95±3	96±4	91±3	97±3	97±3
LVSV$_I$ (mL·beat^{-1})	99±4	95±3	93±3	94±4	87±4	94±4	96±4
LVSV$_E$ (mL·beat^{-1})	93±4	98±3	98±4	99±4	94±4	100±3	98±3
ΔSV (mL·beat^{-1})	6±1	-3±2	-5±3	-6±2*	-6±2*	-5±3	-3±3
$\dot{Q}$ (L·min^{-1})	6.1±0.5	6.0±0.3	5.8±0.2	6.0±0.3	5.7±0.4	5.9±0.3	6.0±0.3
$\dot{Q}_I$ (L·min^{-1})	6.7±0.5	6.7±0.3	6.5±0.3	6.5±0.4	6.3±0.4	6.8±0.3	6.8±0.2
$\dot{Q}_E$ (L·min^{-1})	5.4±0.4	5.4±0.3	5.2±0.2	5.2±0.3	5.0±0.4	5.0±0.3	5.2±0.3
Δ$\dot{Q}$ (L·min^{-1})	1.3±0.1	1.3±0.2	1.3±0.3	1.3±0.3	1.4±0.3	1.8±0.3	1.5±0.3
SBP (mmHg)	127±3	133±4*	135±3*	137±4*	133±5	132±4	136±5
DBP (mmHg)	75±2	78±2	79±2	81±3	81±4	77±3	79±3
MAP (mmHg)	93±2	96±3	98±3	100±3	99±4	96±3	98±3
PP (mmHg)	52±2	54±2	56±2	56±3	52±3	55±2	57±3
TPR (mmHg·min·L^{-1})	16±1	17±1	17±1	18±1	18±1	17±1	17±1
BRS$_{up}$ (ms·mmHg^{-1})	19±4	21±4	20±3	28±5	23±4	27±5	25±5
BRS$_{down}$ (ms·mmHg^{-1})	17±2	15±1	18±2	15±2	14±1	16±2	16±2
BRS$_{Seq}$ (ms·mmHg^{-1})	18±3	18±2	19±3	21±3	18±3	21±3	21±3
BRS$_{Freq}$ (ms·mmHg^{-1})	18±3	18±2	20±3	20±2	21±4	21±2	20±3

Values are Mean ± SEM for n=11. Respiratory sinus arrhythmia (RSA), heart rate (f_C), heart rate during inspiration (fc_I), heart rate during expiration (fc_E), left ventricular stroke volume (LVSV), left ventricular stroke volume during inspiration (LVSV$_I$),left ventricular stroke volume during expiration (LVSV$_E$), within-breath variation in left ventricular stroke volume (ΔSV), cardiac output ($\dot{Q}$), cardiac output during inspiration ($\dot{Q}_I$), cardiac output during expiration ($\dot{Q}_E$), within-breath variation in cardiac output (Δ$\dot{Q}$), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP), total peripheral resistance (TPR), sequence baroreflex sensitivity positive sequence gain (BRS$_{up}$), sequence baroreflex sensitivity negative sequence gain (BRS$_{down}$), sequence baroreflex sensitivity average gain (BRS$_{Seq}$); cross-spectral baroreflex sensitivity gain (BRS$_{Freq}$). * different from baseline; ‡ different from 25% FVC; †different from semi-spontaneous. Signalled differences between conditions are significant at $P < 0.002$.

4-4.3 Heart rate and blood pressure variability responses to SDB

Heart rate variability

In common with RSA, other heart rate variability (HRV) parameters showed significant frequency-dependent responses with SDB (Table 4-6). The time domain variables SDNN and RMSSD, exhibited a significant main effect of f_R, increasing steadily from baseline and reaching maximal average values at 6 breaths·min^{-1} (Table 4-6). When breathing at a fixed, optimal f_R there was a significant main effect of V_T for SDNN, but not for RMSSD, with the peak SDNN values attained at the highest V_T (40%FVC, Table 4-7).

In the frequency domain, HRV_{LF} and HRV_{TOT} followed a similar trend, increasing with the reduction in f_R and maximising at 6 breaths·min^{-1} (Table 4-6), where a 6-fold increase in HRV_{LF} and 3-fold increase in HRV_{TOT} were observed. In contrast, HRV_{HF} peaked at 10 breaths·min^{-1}, with lower f_Rs resulting in significantly lower values (Table 4-6*),* as f_R moved into the low-frequency spectral band (<0.15 Hz).

The analysis of the two components of the nonlinear Poincaré plot method (SD1 and SD2) showed close similarities to the patterns observed in RMSSD and SDNN, respectively (Table 4-6). Both SD1 and SD2 increased with a reduction in f_R, peaking at 6 breaths·min^{-1}, with SD1 exhibiting a further marked decrease at 4 breaths·min^{-1} (P<0.001). The ratio between SD1 and SD2 reached a nadir at 4 breaths·min^{-1} (P<0.001; Table 4-6).

All frequency domain variables were also affected significantly by changes in V_T with both HRV_{LF} and HRV_{TOT} peaking at 40%FVC (P<0.001; Table 4-7) For HRV_{HF} *post-hoc* analysis revealed no statistically significant changes between the different V_Ts (P>0.05). Importantly, the f_Rs used in Protocol 2 were outside of the high-frequency spectral band, leading to HRV_{HF} values during the SDB sets that were always roughly one fifth of those recorded at baseline (e.g. 40%FVC = 538±545 *vs.* 2324±2300 ms^2 at baseline, Table 4-7*).*

No significant differences for SD1 and SD2 (P>0.05) were encountered when breathing at progressively higher V_Ts and fixed f_R, despite SD2 tracking the increments in V_T (Table 4-7). SD1/SD2 ratio showed a strong main effect of V_T

(P<0.001) but *post-hoc* analysis failed to demonstrate significant differences between conditions linear association with the changes in V_T (P>0.002; Table 4-7).

Breathing semi-spontaneously at a fixed V_T (SSV_T) did not result in any significant difference from breathing at 6 breaths·min^{-1} (P>0.05), nor from any of the different V_Ts (SSf_R). However, it was noticeable that the SSf_R condition elicited slightly higher (but not significantly different) average values across all analysed HRV indices, than those recorded at similar V_Ts, more precisely, the 30 and 35% FVC conditions (Table 4-7). Overall, SDNN, HRV_{LF} and HRV_{TOT} demonstrated a response pattern to changes in f_R and V_T that was similar to that described previously for RSA.

Table 4-6 Heart rate variability response to slow, deep breathing at fixed V_T (30% FVC).

	Baseline	4 breaths·min^{-1}	6 breaths·min^{-1}	8 breaths·min^{-1}	10 breaths·min^{-1}	Semi-Spontaneous (SSV_T)
SDNN (ms)	61±7	86±7*	98±9*‡	78±7*	68±8	94±8*‡
RMSSD (ms)	45±6	41±4	60±6*§	56±7§	54±8	53±5§
HRV_{LF} (ms^2)	1308±222	6901±952*‡¥	8099±1192*‡¥	4912±889*‡	1015±307	7517±973*‡
HRV_{HF} (ms^2)	1388±328	271±120*‡	493±103‡	331±68*‡	2951±749	561±142
HRV_{TOT} (ms^2)	2695±489	7172±991*‡	8592±1255*‡¥	5242±952*	3966±848	8078±1062*‡
SD1 (ms)	32±4	29±3	42±4*§	40±5§	38±5	37±4§
SD2 (ms)	79±9	118±9*‡	131±12*‡¥	102±9	88±10	127±10*‡
SD1/SD2	0.41±0.02	0.24±0.01*	0.33±0.01*§‡	0.38±0.02§	0.43±0.03§	0.29±0.01*‡¥

Values are Mean ± SEM for *n*=14. Standard deviation of normal-to-normal intervals (SDNN), root mean square of successive differences (RMSSD), low-frequency power (HRV_{LF}), high-frequency power (HRV_{HF}), total power (HRV_{TOT}), poincaré plot SD1 (SD1), poincaré plot SD2 (SD2). * different from baseline; † different from semi-spontaneous; § different from 4 breaths·min^{-1}; ¥ different from 8 breaths·min^{-1}; ‡ different from 10 breaths·min^{-1}. $P < 0.003$.

Table 4-7 Heart rate variability response to slow, deep breathing at fixed individual ideal f_R (4 or 6 breaths·min^{-1}).

	Baseline	20% FVC	25% FVC	30% FVC	35% FVC	40%FVC	Semi-Spontaneous (SSf_R)
SDNN (ms)	71±8	91±9	96±10*	99±9*	104±8*	113±10*	117±11*
RMSSD (ms)	58±10	54±7	56±8	57±7	60±7	65±8	70±8
HRV_{LF} (ms^2)	1612±423	7675±1270*	8029±1643*	8637±1406*	9150±1103*	12126±1877*	10863±2153*
HRV_{HF} (ms^2)	2324±693	476±105	471±119	513±103	557±161	538±164	689±171
HRV_{TOT} (ms^2)	3936±986	8152±1348*	8499±1743	9150±1483*	9706±1185*	12664±1997*	11552±2168
SD1 (ms)	41±7	38±5	40±6	41±5	42±5	46±6	50±5
SD2 (ms)	90±9	124±11	129±13	133±12	141±11	153±13	158±14
SD1/SD2	0.43±0.04	0.3±0.02	0.3±0.02	0.3±0.02	0.29±0.02	0.29±0.02	0.32±0.02

Values are Mean ± SEM for *n*=11. Standard deviation of normal-to-normal intervals (SDNN), root mean square of successive differences (RMSSD), low-frequency power (HRV_{LF}), high-frequency power (HRV_{HF}), total power (HRV_{TOT}), Poincaré plot SD1 (SD1), Poincaré plot SD2 (SD2). * different from baseline. $P < 0.002$.

Blood pressure variability

The blood pressure variability (BPV) also exhibited significant main effects of f_R for all indices analysed. The BPV_{LF} exhibited a pattern similar to that described previously for $LVSV_E$, demonstrating higher values at the highest f_Rs and peaking at 8 breaths·min^{-1}, followed by a steep decrease, with the lowest values at 4 breaths·min^{-1} (42.19 ± 28.46 *vs.* 580.94 ± 300.42 mmHg^2 at 4 breaths·min^{-1} and 8 breaths·min^{-1}, respectively). Similarly, both BPV_{HF} and BPV_{TOT} peaked at 10 breaths·min^{-1} and reached their lowest values at 4 breaths·min^{-1} (Table 4-8).

There was a significant main effect of V_T upon BPV_{LF} and BPV_{HF} (but not for BPV_{TOT}). However, *post-hoc* Bonferroni correction for multiple comparisons did not reveal any significant differences between conditions for BPV_{LF} (P>0.002, Table 4-9), while for BPV_{HF} these were only observed when comparing against baseline (P<0.002; Table 4-9).

Table 4-8 Blood pressure variability response to slow, deep breathing at fixed V_T (30% FVC).

	Baseline	**4 breaths·min^{-1}**	**6 breaths·min^{-1}**	**8 breaths·min^{-1}**	**10 breaths·min^{-1}**	**Semi-Spontaneous (SSV_T)**
BPV_{LF} (mmHg^2)	94.66±22.19	42.19±7.61	175.1±21.17§ǂ¥	580.94±80.29*†§	287.64±43.99*§	127.278±32.98
BPV_{HF} (mmHg^2)	96.56±24.03	8.34±3.04*	18.73±3.23ǂ	24.53±11.43	454.34±71.22*†§¥	11.63±3.18
BPV_{TOT} (mmHg^2)	191.23 ±39.13	50.53±7.88*	193.84±22.66§ǂ¥	605.57±77.33*†§	742.04±115.19*†§	138.92±33.24

Values are Mean ± SEM for *n*=14. Low-frequency power (BPV_{LF}), high-frequency power (BPV_{HF}), total power (BPV_{TOT}). * different from baseline; † different from semi-spontaneous; § different from 4 breaths·min^{-1}; ¥ different from 8 breaths·min^{-1}; ǂ different from 10 breaths·min^{-1}. $P < 0.003$.

Table 4-9 Blood pressure variability response to slow, deep breathing at fixed individual ideal f_R (4 or 6 breaths·min^{-1}).

	Baseline	**20% FVC**	**25% FVC**	**30% FVC**	**35% FVC**	**40%FVC**	**Semi-Spontaneous (SSf_R)**
BPV_{LF} (mmHg^2)	68.19±16.40	92.33±17.16	104.87±23.86	119.87±27.22	153.33±40.20	159.67±36.66	128.58±22.70
BPV_{HF} (mmHg^2)	81.58±13.80	10.48±3.29*	17.02±5.98*	16.75±4.18*	25.52±6.24	21.76±6.65*	41.75±21.65
BPV_{TOT} (mmHg^2)	149.78±19.59	102.82±18.98	121.89±27.75	136.63±29.32	178.85±40.80	181.44±39.23	170.34±36.23

Values are Mean ± SEM for *n*=11. Low-frequency power (BPV_{LF}), high-frequency power (BPV_{HF}), total power (BPV_{TOT}). * different from baseline. $P < 0.002$.

4-4.4 Relationships between cardiovascular parameters, heart rate and blood pressure variabilities

An analysis of the relationships between cardiovascular parameters, HRV and BPV is depicted in Tables 4-10 and 4-11. As expected, this confirmed the existence of a strong and significant correlation between RSA and HRV_{TOT} (r = 0.98 and r = 0.95 in the fixed V_T (Protocol 1) and fixed f_R (Protocol 2) protocols, respectively; Tables 4-10 and 4-11, both P < 0.01). Similarly, mean *fc* also correlated positively with HRV_{TOT} (r = 0.95, P < 0.01, for the fixed V_T protocol; and r = 0.77, P < 0.05 for the fixed f_R protocol), and negatively with RSA (r = -0.88 and r = -0.79, respectively; P < 0.05, Tables 4-10 and 4-11). Interestingly, *fc* correlated negatively with BRS_{Seq} in both protocols (r = -0.92, P < 0.01), while both RSA and HRV_{TOT} showed significant correlations with the cross-spectral baroreflex sensitivity (BRS_{Freq}), also in both protocols (RSA: r = 0.97, P < 0.01, and r = 0.88, P < 0.05; HRV_{TOT}: r = 0.92 and 0.72, P < 0.05, for fixed VT and fixed f_R, respectively; Tables 4-10 and 4-11). In contrast, only in the fixed V_T protocol were RSA and HRV_{TOT} correlated with BRS_{Seq} (RSA: r = 0.95; HRV_{TOT}: r = 0.98, P < 0.01; Table 4-10).

The within-breath variations in left ventricular stroke volume (∆SV) where moderately/strongly inversely correlated with RSA and HRV_{TOT}, despite only being significant for Protocol 2 (r = - 0.71 and r = -0.77, P > 0.05, in Protocol 1 and; r = -0.88 and r = -0.76, P < 0.05, in Protocol 2 for RSA and BPV_{TOT}, respectively). In contrast, only in the Protocol 1 was there a strong correlation between ∆SV and BPV_{TOT} (r = 0.90, P < 0.05). The ∆SV also related strongly and negatively with SBP, MAP and TPR in response to changes in V_T at a fixed, optimal f_R, but did not translate into similarly significant correlations when breathing at different f_Rs. An opposite pattern was encountered for the within-breath amplitude of cardiac output fluctuations (∆$\dot{Q}$), which correlated strongly with SBP (r = 0.89, P < 0.05), MAP (r = 0.89, P < 0.05) and PP (r = 0.95, P < 0.05) during Protocol 1, but not with Protocol 2. The mean $\dot{Q}$ also correlated positively and strongly with SBP (r = 0.87, P < 0.05), MAP (r = 0.87, P < 0.05) and PP (r = 0.86, P < 0.05), as well as BPV_{TOT} (r = 0.88, P < 0.05) in the fixed V_T protocol (Protocol 1), but showed no significant correlation with the same variables in the second part of the study (Protocol 2 - fixed f_R conditions). Perhaps surprisingly, HRV_{TOT} and BPV_{TOT} were not significantly correlated in any of the protocols (r = -0.50 and -0.48, P > 0.05). Finally, while both baroreflex sensitivity indices (BRS_{Seq} and BRS_{Freq}) exhibited a significant correlation

during Protocol 1 (r = 0.88, P < 0.05), the same did not occur during Protocol 2 (r = 0.69, P > 0.05), raising questions regarding the interchangeability of these methods in assessing baroreflex sensitivity.

Table 4-10 Correlations between cardiovascular parameters, HRV and BPV indices for slow, deep breathing at 4 to 10 breaths.min^{-1} with fixed V_T (30% FVC).

	RSA	***fc***	**LVSV**	**ΔSV**	**$\dot{Q}$**	**Δ$\dot{Q}$**	**SBP**	**MAP**	**PP**	**TPR**	**HRV_{TOT}**	**BPV_{TOT}**	**BRS_{Seq}**	**BRS_{Freq}**
RSA	–	-0.88*	0.70	-0.71	-0.04	0.37	0.11	0.04	0.26	0.20	0.98**	-0.41	0.95**	0.97**
fc		–	-0.51	0.70	0.32	-0.18	-0.01	0.06	-0.13	-0.26	0.95**	0.55	-0.92*	-0.75
LVSV			–	-0.04	0.64	-0.89*	0.77	0.72	0.86*	0.73	0.61	0.32	0.50	0.74
ΔSV				–	0.64	0.38	0.54	0.57	0.45	0.40	-0.75	0.90*	-0.78	-0.65
$\dot{Q}$					–	0.84*	0.87*	0.87*	0.85*	0.62	-0.19	0.88*	-0.28	0.11
Δ$\dot{Q}$						–	0.89*	0.84*	0.95**	0.79	0.27	0.67	0.19	0.42
SBP							–	0.99**	0.98**	0.92**	0.19	0.71	-0.12	0.16
MAP								–	0.96**	0.91*	-0.06	0.72	-0.21	0.08
PP									–	0.92*	0.17	0.67	0.04	0.29
TPR										–	0.17	0.50	0.02	0.15
HRV_{TOT}											–	-0.50	0.98**	0.92*
BPV_{TOT}												–	-0.55	0.28
BRS_{Seq}													–	0.88*
BRS_{Freq}														–

The values represent *r* for mean group values for *n*=14. Respiratory sinus arrhythmia (RSA), heart rate (*fc*), left ventricular stroke volume (LVSV), within-breath variation of left ventricular stroke volume (ΔSV), cardiac output ($\dot{Q}$), within-breath variation of cardiac output (Δ$\dot{Q}$), systolic blood pressure (SBP), mean arterial pressure (MAP), pulse pressure (PP), total peripheral resistance (TPR), heart rate variability total power (HRV_{TOT}), blood pressure variability total power (BPV_{TOT}), sequence baroreflex sensitivity (BRS_{Seq}) and cross-spectral baroreflex sensitivity (BRS_{Freq}). *$P < 0.05$, **$P < 0.01$.

Table 4-11 Correlations between cardiovascular parameters, HRV and BPV indices for slow, deep breathing at 20 to 40% FVC with fixed f_R (4 or 6 breaths·min^{-1}).

	RSA	***fc***	**LVSV**	**ΔSV**	**$\dot{Q}$**	**Δ$\dot{Q}$**	**SBP**	**MAP**	**PP**	**TPR**	**HRV_{TOT}**	**BPV_{TOT}**	**BRS_{Seq}**	**BRS_{Freq}**
RSA	–	-0.79*	-0.05	-0.88*	-0.62	0.66	0.61	0.60	0.44	0.67	0.95**	0.46	0.62	0.88**
fc		–	-0.36	0.68	0.30	-0.58	-0.62	-0.48	-0.68	-0.43	0.77*	-0.30	-0.92**	-0.70
LVSV			–	0.19	0.77*	0.32	0.15	-0.17	0.67	-0.57	0.19	-0.16	0.55	-0.31
ΔSV				–	0.71	-0.25	-0.79*	-0.83*	-0.47	-0.81*	-0.76*	-0.07	-0.46	-0.72
$\dot{Q}$					–	-0.07	-0.32	-0.53	0.16	-0.87*	-0.37	-0.31	-0.03	-0.77*
Δ$\dot{Q}$						–	0.00	-0.13	0.23	-0.01	0.77*	0.71	0.58	0.60
SBP							–	0.94**	0.79*	0.65	0.61	-0.10	0.61	0.41
MAP								–	0.54	0.83*	0.51	-0.05	0.43	0.49
PP									–	0.11	0.58	-0.16	0.75	0.15
TPR										–	0.47	0.29	0.30	0.77
HRV_{TOT}											–	0.48	0.69	0.76*
BPV_{TOT}												–	0.39	0.71
BRS_{Seq}													–	0.56
BRS_{Freq}														–

The values represent *r* for mean group values for *n*=11. Respiratory sinus arrhythmia (RSA), heart rate (*fc*), left ventricular stroke volume (LVSV), within-breath variation of left ventricular stroke volume (ΔSV), cardiac output ($\dot{Q}$), within-breath variation of cardiac output (Δ$\dot{Q}$), systolic blood pressure (SBP), mean arterial pressure (MAP), pulse pressure (PP), total peripheral resistance (TPR), heart rate variability total power (HRV_{TOT}), blood pressure variability total power (BPV_{TOT}), sequence baroreflex sensitivity (BRS_{Seq}) and cross-spectral baroreflex sensitivity (BRS_{Freq}). *$P < 0.05$, **$P < 0.01$.

4-4.5 Analysis of parameters contributing to respiratory sinus arrhythmia

A three-predictor model that included $\Delta\dot{Q}$, *fc* and ΔSV provided a significant regression equation (F (3, 69) =121.675, $P < 0.001$), with an $R^2 = 0.834$.

Predicted RSA = 594 + 1.62 ($\Delta\dot{Q}$) – 8.976 (*fc*) – 12.766 (ΔSV)

Where $\Delta\dot{Q}$ is measured in L·min-1, *fc* in beats·min^{-1} and ΔSV in mL·beat^{-1}.

Thus, RSA varied by around 1.62 ms for each L·min^{-1} of within-breath variation of $\dot{Q}$ ($\Delta\dot{Q}$), varied by 8.97 ms for each beat.min^{-1} if *fc* and varied by 12.76 ms for each mL·beat^{-1} change in within-breath LVSV (ΔSV). All three variables were significantly correlated with RSA.

4-4.6 Cardiorespiratory time shift and phase angle

The cross-spectral analysis of the relevant cardiovascular and respiratory signals revealed high squared coherence (> 0.5) at the respiratory frequency for all different combinations of f_R and V_T, including for the baseline conditions. Tables 4-12 and 4-13 show mean values ± SEM of the results of cross-spectral analysis for phase angle (in degrees) between the respiratory airflow (RESP) and SBP, DBP and *fc*. Phase angle differences between SBP and DBP and *fc* are also presented. Figure 4-4 depicts the time shifts graphically (in seconds) corresponding to the abovementioned phase angle differences between signals.

Phase varied significantly between all variables except between SBP and DBP; in this case, the angular relation between the SBP and DBP waveforms remained relatively stable across different f_Rs (Table 4-12). Notwithstanding, the increase in breath duration resulting from the reduction in f_R accounted for noticeable increases in time delay at the lower f_Rs (~2.6 s at 4 breaths·min^{-1} *vs*. ~1 s at the remaining analysed f_Rs; Figure 4-4E).

Heart rate, SBP and DBP all lagged behind RESP, with both the phase angle and time delay increasing with a decrease in f_R, consistent with frequency dependent behaviour. On the other hand, *fc* tended to lag SBP, but both phase angle and the

temporal difference between the two variables were shortened by the decrease in f_R, tending to be completely in phase at 4 breaths·min^{-1} (Table 4-12, Figure 4-4D).

Significant main effects of V_T upon phase angle were observed for all analysed relations, except between SBP and DBP. However, *post-hoc* statistical tests revealed no significant differences in phase angle (P>0.002) between different V_Ts at the same fixed f_R for any analysed relationships. Time shift values were largely unaffected by the changes in V_T. Notwithstanding, the temporal lag between DBP and RESP exhibited a significant linear relationship with an increase in V_T (R^2=0.93, P=0.008; Figure 4-4H).

Finally, none of the semi-spontaneous conditions (SSV_T and SSf_R) differed from the previously described frequency and volume-dependent relationships. Phase angle and time shift values for SSV_T were situated between those observed for 4 and 6 breaths·min^{-1} as illustrated by Figure 4-4 (panels A through E) and Table 4-12. Breathing at a fixed f_R but without regulating V_T or $P_{ET}CO_2$ levels (SSf_R) resulted in no significant differences in either phase angle or time shift when comparing to fully controlled breathing sets (Figure 4-4[F-J]; Table 4-13).

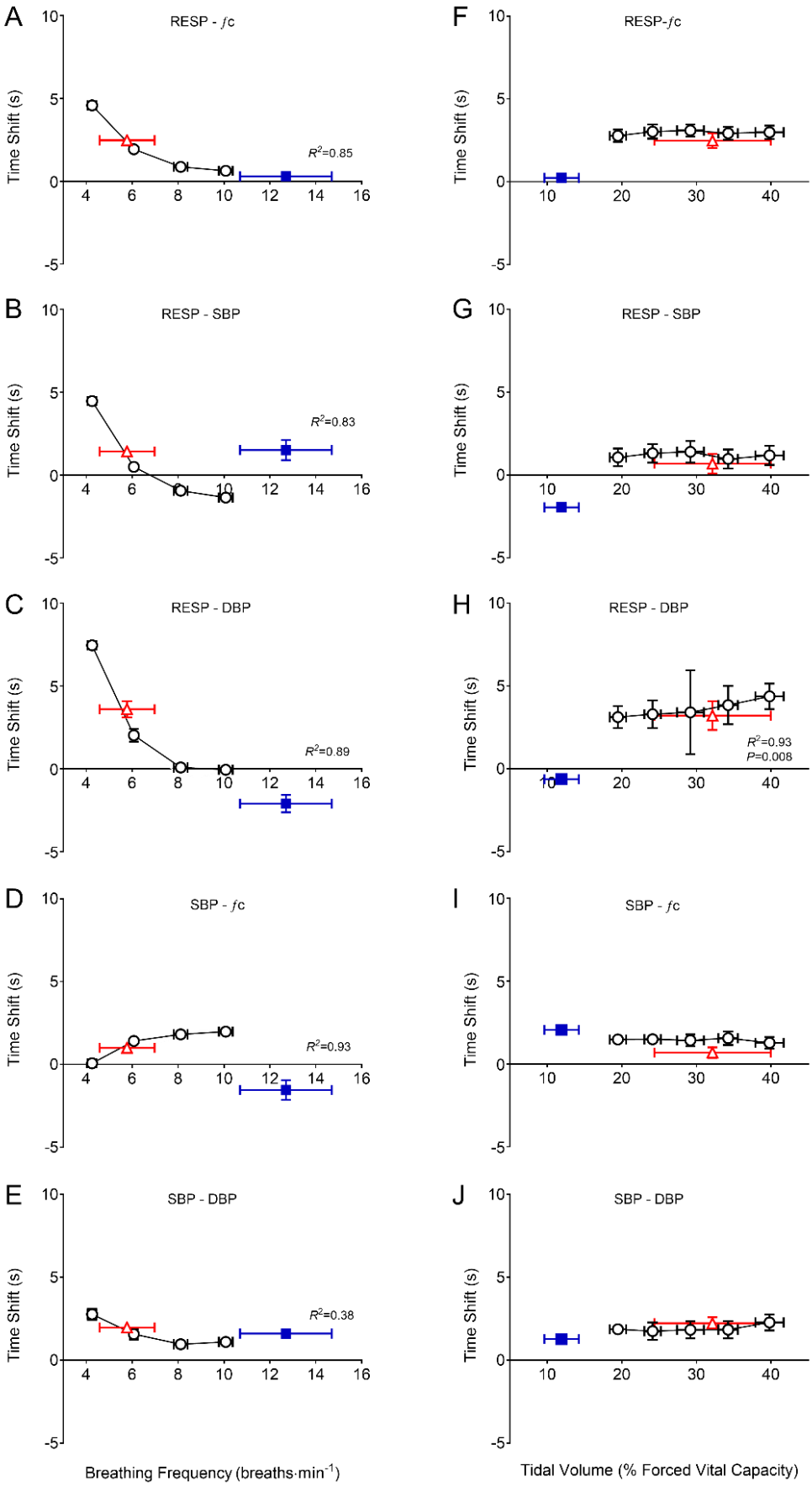


Figure 4-4 Time shift responses between respiration (RESP), systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (*fc*) to variations in breathing frequency (A-E) and tidal volume (F-J). Values are means ± SEM for fully fixed (○ black), semi-spontaneous (△red) and baseline (■ blue) breathing pattern. Presented R^2 represent Pearson's coefficient of determination.

Table 4-12 Phase angle responses to SDB at fixed V_T (30% FVC).

	Baseline	4 breaths·min^{-1}	6 breaths·min^{-1}	8 breaths·min^{-1}	10 breaths·min^{-1}	Semi-Spontaneous (SSV_T)
RESP – *fc*	22±6	109±5*†§¥‡	70±8*¥‡	42±7*†	38±6†	81±9*
RESP – SBP	111±168	106±6†§¥‡	18±7*¥‡	-45±4*†	-81±5*†	47±13
RESP – DBP	-156±38	177±7†§¥‡	73±14*¥‡	4±12*†	-4±13*†	118±16
SBP – *fc*	-113±163	2±7§¥‡	51±6*¥‡	87±7*†‡	119±7*†	33±8*
SBP – DBP	117±20	65±8*	57±12*	46±12*	66±14*	65±8

For the determination of phase angle, the initiation of the respiratory cycle and *fc* and/or SBP fluctuations were considered. Values are presented as mean ± SEM. Signalled significant differences are of *post-hoc* Bonferroni corrected multiple comparisons after the application Watson-Williams F-Test. * different from baseline; † different from semi-spontaneous; § different from 6 breaths·min^{-1}; ¥ different from 8 breaths·min^{-1}; ‡ different from 10 breaths·min^{-1}. Signalled differences between conditions are significant at $P < 0.003$.

Table 4-13 Phase angle responses to SDB at a fixed individual ideal f_R (4 or 6 breaths·min^{-1}).

	Baseline	20%FVC	25%FVC	30%FVC	35%FVC	40%FVC	Semi-Spontaneous (SSf_R)
RESP – *fc*	16±7	89±12*	96±14*	98±11*	93±12*	94±13*	79±14*
RESP – SBP	-133±11	34±17*	42±18*	44±20*	31±18*	38±18*	22±19*
RESP – DBP	-42±16	99±21*	104±27*	108±80*	122±37*	138±24*	101±27*
SBP – *fc*	141±7	47±9*	47±9*	45±11*	49±13*	40±11*	22±10*
SBP – DBP	87±17	59±9	56±17	58±16	58±16	72±15	71±12

For the determination of phase angle, the initiation of the respiratory cycle and *fc* and/or ABP fluctuations were considered. Values are presented as mean ± SEM. Signalled significant differences are of *post-hoc* Bonferroni corrected multiple comparisons after the application Watson-Williams F-Test. * different from baseline. Signalled differences between conditions are significant at $P < 0.002$.

4-5 Discussion

The objectives of this study were to systematically characterise the cardiovascular response to SDB, across a range of f_Rs and V_Ts, with particular emphasis on the respiratory-synchronous fluctuations in LVSV, $\dot{Q}$, *fc* and ABP. Simultaneously, we sought to clarify any contribution of haemodynamic mechanisms to the generation and frequency modulation of RSA. The data from the current study showed no change in MAP, $\dot{Q}$ and TPR when increasing FVC from 20% to 40% or altering f_R from 4-10 breaths·min^{-1}. However, significant within-breath responses were observed for LVSV (ΔSV), $\dot{Q}$ ($\Delta\dot{Q}$) and *fc* (RSA). The data suggest that across the range of f_Rs and V_Ts studied, f_R exerts a more potent within-breath influence upon the cardiovascular system than V_T, whilst RSA appears to attenuate respiratory-related fluctuations, thereby stabilising $\dot{Q}$ and $\Delta\dot{Q}$. A simple, three component mathematical model including $\Delta\dot{Q}$, ΔSV and *fc* seems able to account for most of the variability of the RSA response to SDB. The total variability of the systolic blood pressure (BPV_{TOT}) was highest between 8 and 10 breaths·min^{-1}, but fluctuations appeared to be dampened at frequencies ≤ 6 breaths·min^{-1}. In contrast, heart rate variability (HRV_{TOT}) maximised at the lowest f_Rs, but surprisingly, HRV_{TOT} and BPV_{TOT} were not correlated. Altering V_T while breathing at a fixed f_R did not seem to influence BPV, while HRV changes paralleled those in V_T. Furthermore, changing f_R contributes to important alterations of the phase and time relationships between breathing, *fc* and SBP, whilst altering V_T has seemingly no influence. Breathing semi-spontaneously at either a controlled low f_R or high V_T did not result in any significant cardiovascular differences, compared to breathing with a fully controlled breathing pattern. Overall, while not providing conclusive evidence regarding which mechanisms are involved in the regulation of the acute cardiovascular response to SDB, data from this study hint at a relevant contribution from the enhancement of within-breath fluctuations in *fc* and LVSV with SDB. They also suggest that the cardiac baroreflex is insufficient to explain the pattern of responses observed, and that other putative mechanisms are a work during SDB.

4-5.1 Cardiovascular responses to SDB

Impact upon fc and RSA

The frequency and respiratory volume dependency of RSA has been thoroughly described over the past decades (Angelone and Coulter, 1964, Freyschuss and

Melcher, 1976c, Hirsch and Bishop, 1981, Song and Lehrer, 2003) and was confirmed by the present study, which showed a twofold increase in RSA from baseline when breathing at 6 breaths·min^{-1} at a fixed V_T of 30% FVC. The RSA increased linearly with increments in V_T, with maximal values occurring at the highest V_T (40% FVC); this effect was entirely independent of f_R, which was fixed. Lower f_Rs produced no further increases in average RSA values, despite approximately half of the participants showing maximal RSA at 4 breaths·min^{-1}. While it is accepted that RSA tends to maximise at frequencies between 6 and 5.5 breaths·min^{-1} (Hirsch and Bishop, 1981, Vaschillo et al., 2002), several reports have shown the existence of higher magnitude respiratory-driven fluctuations of *fc* at even lower f_Rs (Song and Lehrer, 2003). Previous studies have also shown gender and height dependency (Lehrer, 2007), suggesting an individualised f_R at which RSA can be maximised.

In the present study, the changes in RSA, in the presence of a largely constant mean *fc,* except for a small (~3 beats·min^{-1}), but significant reduction in *fc* at 6 breaths·min^{-1}. Nonetheless, the 2-3 beats·min^{-1} average reduction from baseline is unlikely to be physiologically relevant. These findings confirm those of others (Hayano, Mukai, Sakakibara et al., 1994, Cooke et al., 1998, Pitzalis, Mastropasqua, Massari et al., 1998, Song and Lehrer, 2003), who found no significant changes in mean *fc* during SDB interventions. The increase in RSA with V_T had been demonstrated previously and is associated with both an increased fc_I and decreased fc_E (Freyschuss and Melcher, 1976c). Both fc_I and fc_E responded linearly to the changes in f_R and V_T; increases in fc_I followed reductions in f_R and/or increases in V_T, while fc_E exhibited the opposite pattern (Figure 4-3[A, D]). However, the variation in fc_E was slightly more pronounced than that observed for fc_I, confirming early observations (Eckberg, 1983, Song and Lehrer, 2003). These authors hypothesised that sino-atrial node activity is inhibited during expiration due to the effects of the bolus of acetylcholine (ACh) released throughout expiration (Eckberg, 2003) A longer expiration at lower f_Rs would allow for a larger bolus of ACh to be released therefore contributing to a greater decrease in fc_E. A longer respiratory interval also allows for a more complete hydrolisation of ACh by acetylcholinesterase, thus resulting in a more pronounced increase in fc_I. Importantly, these respiration-related fluctuations of cholinergic inhibition are asymmetrical, such that expiratory inhibition of the sinoatrial node is more abrupt than its decay throughout inspiration (Eckberg, 1983, 2003). Potential

contributions from haemodynamic respiratory-related changes to RSA are discussed below.

Impact upon stroke volume and cardiac output

The observation of a lower left ventricular expiratory stroke volume ($LVSV_E$) during spontaneous breathing contradicts previous reports (Kim, Ishida, Tsuneoka et al., 1987, Elstad, 2012), and was also present for other f_Rs above 6 breaths·min^{-1} (Figure 4-3B). A novel finding of the present study was the observation that for f_Rs close to (or below) 6 breaths·min^{-1}, $LVSV_E$ exceeded $LVSV_I$ (Figure 4-3C). These findings suggest the existence of a complex, breathing frequency-dependent response. Respiratory-induced fluctuations in LVSV are thought to be related to a complex interplay of several different mechanisms, which include according to Hamzaoui et al. (2013): 1) the passive transmission of the decreased intrathoracic pressure to the cardiac structures; 2) the effects of the variations of intra-abdominal pressure upon cardiac preload and afterload; 3) the mechanical effects of lung expansion upon the surrounding structures; 4) the existence of interventricular dependence phenomena; and 5) the existence of a delay between RVSV and LVSV interposed by the pulmonary circulation (pulmonary transit time - PulTT). This is the first time that such a pattern of LVSV response has been demonstrated during SDB, and we believe that it might be underpinned by the capacitance of the pulmonary circulation, i.e. to the effect of PulTT. However, the confounding effect of postural and breathing pattern dissimilarities (not only with regards to lung excursion but also diaphragmatic *vs.* thoracic breathing) between studies should not be overlooked.

The theory underpinning the influence of transit time upon LVSV has previously been proposed (Dornhorst et al., 1952b, Hamzaoui et al., 2013), but to the best of the author's knowledge, never demonstrated experimentally. The PulTT introduces a phase lag between RVSV and LVSV, such that during breathing at any given frequency, inspiratory-induced increases in venous return (Brecher and Hubay, 1955) and RVSV are delayed, and not translated immediately to LVSV. Inter-individual variation in pulmonary transit time ranges from 5 to 10 s (Zavorsky et al., 2003), and when combined with different f_Rs, induces different effects upon LVSV. The complexity of these interrelationships is further exacerbated by the fact that PulTT is influenced by $\dot{Q}$ (Lund, Flø, Rasmussen et al., 1997, Zavorsky et al., 2003), which not only differs between f_Rs, but also between inspiration and expiration

(Tables 4-4 and 4-5; Figure 4-3[C,F]). Lower f_Rs allow more time for an increased RVSV to transit the pulmonary circulation within the same respiratory cycle; thus, as illustrated by this chapter's data, allowing an inspiratory-generated increase in LVSV to occur during the immediately ensuing expiration, rather than during the inspiration of the subsequent breath. This supposition is supported by the fact that there was no statistically significant difference in mean LVSV between f_Rs, suggesting that the mechanism responsible modulates, but does not impair or enhance, mean LVSV during SDB.

Recently, it was demonstrated that the amplitude of the respiratory-related, LVSV variations is similar to that in the right ventricle (Elstad, 2012). Assuming the respiratory-driven variations in venous return are not buffered by the right side of the heart and pulmonary circulation, enhanced venous return during inspiration is the most likely cause of the observed increase in $LVSV_E$ at 4 and 6 breaths·min^{-1}. Increments in V_T are known to affect both preload and afterload, mainly by impacting right-atrial filling pressure and aortic transmural pressure (Freyschuss and Melcher, 1976a, Scharf et al., 1979, Karam et al., 1984). This is due to the increase in intraabdominal pressure stemming from the descent of the diaphragm during inspiration and concurrent decrease in intrathoracic pressure with the increase in lung volume, as well as the gradient that is established between the two (Scharf et al., 1979, Takata, Wise and Robotham, 1990, Takata and Robotham, 1992, Scharf, 1995). This gradient can be affected by breathing mechanics, i.e. abdominal *vs.* thoracic breathing (Kimura et al., 2011, Osada, Nagata, Murase et al., 2011). The clamping of V_T throughout Protocol 1 allows to exclude the potential influence of changes in V_T at lower f_Rs upon the frequency dependent pattern of within-breath variations in LVSV (Figure 4-3B). However, while we cannot completely discard the existence of slight changes in breathing pattern, from abdominal to thoracic breathing pattern (or vice-versa) between sets, it is reasonable to assume that diaphragmatic excursion and the amplitude of variations of intrathoracic and abdominal pressures were significantly altered throughout Protocol 1.

If one accepts the premise that SDB influences venous return to the right atrium during inspiration, it would be expected that larger amplitude swings in intrathoracic pressure would result in larger LVSV and SBP oscillations (Dornhorst et al., 1952b, Freyschuss and Melcher, 1976a). Accordingly, increasing V_T might, therefore, be predicted to magnify the LVSV-related changes induced by SDB. However, a

relatively modest influence of V_T upon LVSV was observed, with no significant alteration of mean $\dot{Q}$ (Table 4-5). Mean LVSV, $LVSV_I$ and $LVSV_E$ exhibited no significant response to changes in V_T, with the important changes in $\Delta\dot{Q}$ (Figure 4-3G) being attributable mainly to the fluctuations in *fc*, particularly during expiration. Previous findings that suggest increases in mean $\dot{Q}$ following an increase of V_T at fixed breathing rates (Boutellier and Farhi, 1986) were not confirmed in the present study. This is most likely explained by the fact that the f_Rs utilised by Boutellier and Farhi ranged between 20 and 40 breaths·min^{-1}. These data also tend to lend support to the aforementioned modulatory role for pulmonary transit time, rather than the amplitude of intra-thoracic pressure swing *per se*, in the intra-breath haemodynamic fluctuations that were observed in the present study. Another way in which enhanced inspiratory venous return might influence $\dot{Q}$ is via a cardio-acceleratory atrial stretch mechanism, whereby the mechanical distention of the atrial sinus by expansion of the right atria with increased venous return produces a chronotropic response. This cardio-acceleration has been suggested to have either a reflex origin (Bainbridge, 1915, 1920, Pawelczyk and Levine, 1995), or to arise from mechanical factors, i.e. a stretch-responsive intracardiac 'myogenic' mechanism (Cooper and Kohl, 2003, Quinn and Kohl, 2012), and might therefore contribute either to the amplification (if $LVSV_I$ also increases) or maintenance (if $LVSV_I$ decreases) of $\dot{Q}_I$. The role of intrathoracic pressure and venous return will be explored in more detail in subsequent studies of this thesis.

Impact upon blood pressure and blood pressure variability

Blood pressure related variables (SBP, DBP, MAP and PP) were higher at f_Rs between 6 and 10 breaths·min^{-1} when compared to baseline and semi-spontaneous breathing (SSV_T). This increase in ABP (~11 mmHg for SBP, ~5 mmHg for DBP and ~7 mmHg for both MAP and PP) is not consistent with previous studies in healthy people during SDB, which have reported reduced (Radaelli et al., 2004, Pramanik, Sharma, Mishra et al., 2009, Pramanik, Pudasaini and Prajapati, 2010, Mason, Vandoni, Debarbieri et al., 2013, Zhang, Wang, Wu et al., 2016) or unchanged ABP when breathing at 6 breaths·min^{-1} (Bernardi et al., 2002, Limberg et al., 2013, Paprika, Gingl, Rudas et al., 2014). Similarly, a relatively large number of studies in hypertensive individuals have observed significant, acute reductions in ABP during SDB (Joseph et al., 2005, Kaushik, Kaushik, Mahajan et al., 2006, Oneda et al., 2010, Chang et al., 2015). The increases in ABP in the present study at f_Rs between

6 and 10 breaths·min^{-1} were not present at 4 breaths·min^{-1}, nor during semi-spontaneous breathing at the same fixed V_T. While in the latter the lower P_aCO_2 ($P_{ET}CO_2$: 6 breaths·min^{-1} = 41±1 mmHg *vs.* SSV_T = 36±1 mmHg) could support an argument for the potential contribution of a chemoreflex driven reduction in ABP, the reported values are within the normal physiological range. Furthermore, this is backed by the lack of any significant differences between conditions with regards to TPR. Instead, the higher ABP during SDB observed in the present study are interpreted to be indicative of strong contribution from small changes in $\dot{Q}$ and $\Delta\dot{Q}$. The lowest values for both $\dot{Q}$ and $\Delta\dot{Q}$ were observed during the baseline, SSV_T and 4 breaths·min^{-1} conditions, and $\Delta\dot{Q}$ explained 79-90% of the total variation in the aforementioned ABP variables; this made $\Delta\dot{Q}$ the variable that best described the general behaviour in ABP in response to changes in f_R[2]. Similarly, $\dot{Q}$ accounted for ~74% of the variation of SBP, MAP and PP.

Only SBP showed significant differences with baseline values for V_Ts of 20, 25 and 30%, where it reached its maximal values (baseline: 127±3 mmHg *vs.* 30%FVC: 137±3 mmHg, $P < 0.002$). All analysed ABP variables exhibited a pattern of initial increase between 20 and 30% FVC (with SBP and MAP increasing ~4 mmHg between 20 and 30% FVC, while DBP mean values increased by ~3 mmHg) followed by a fall at higher percentages of FVC. This pattern was likely associated to changes in the LVSV within-breath amplitude as suggested by the strong, negative correlations between ΔSV and SBP ($r = -0.79$, $P < 0.05$) and MAP ($r = -0.83$, $P < 0.05$). Despite showing no change with the increase in V_T, TPR was also significantly correlated with MAP ($r = 0.83$, $P < 0.05$), which questions its relative contribution to the observed ABP behaviour.

As no changes in mean TPR in response to variations in f_R or V_T were observed in the present study, the significant increase in all MAP related variables (SBP, DBP and PP) between 6 and 10 breaths·min^{-1} likely reflect a slight, non-significant, respiratory-induced increase in mean $\dot{Q}$. Unfortunately, the within-breath responses for TPR and SBP were not calculated in the present study, precluding the impact of SDB upon afterload to be judged, as well as the influence of the increased

[2] As TPR was calculated based on MAP and $\dot{Q}$ values according to the formula TPR ≈ MAP ÷ $\dot{Q}$ instead of a direct output by the finometer I did not want to include it in the discussion of the ABP data, despite an indication that TPR could explain ~84% of the total variability found in SBP, MAP and PP (Table 4-10). Such approach is supported by a recent study showing that estimations of TPR from MAP and $\dot{Q}$ underestimates the TPR results calculated by the Finometer's ModelFlow method (Hill et al., 2013).

modulation of LVSV upon SBP fluctuations. However, this is addressed in later studies within this thesis.

The response of BPV did not show the anticipated f_R-dependent pattern of an increase in BPV_{TOT} and BPV_{LF} at the lowest f_Rs (Radaelli et al., 2004, Chang et al., 2013), due to increased respiratory modulation of SBP (TenVoorde et al., 1995). Previously, it has been proposed that the teleological role of RSA is limiting the impact of increased BPV by effectively counteracting unwanted excessive BPV with HRV (De Boer et al., 1987, Karemaker and Wesseling, 2008). Data from the fixed V_T protocol supports this concept, with a marked reduction of BPV at the lowest f_Rs, where the recorded HRV_{TOT} was highest. In addition, the fixed f_R protocol highlighted that at those same low frequencies, BPV is only slightly affected (Table 4-9) by changes in V_T (slight non-significance trend to increase with the progressively higher V_Ts), while HRV_{TOT} tended to linearly follow the increase in V_T. The small number of studies exploring the frequency-dependency of BPV have neglected to consider the effect of the f_R-related changes in V_T induced by SDB (TenVoorde et al., 1995, Radaelli et al., 2004, Chang et al., 2013), which necessarily raises a question in relation to the interpretation of the resulting data.

The HRV and BPV data of the present study is interpreted as a possible indication of the effectiveness of the baroreflex control of blood pressure at frequencies around 0.1Hz. This observation seems to be aligned with the previous reports in sino-atrial denervated cats, suggesting that baroreflex regulation of ABP (and BPV) is ineffective at high breathing frequencies (Mancia et al., 1999). Studies employing mathematical models also suggest that the effectiveness of baroreflex regulation for frequencies close to or below 0.1Hz implies that most BPV is controlled and minimised (Wesseling and Settels, 1993). At the same time, the moderate correlation coefficients between HRV_{TOT} and BPV_{TOT} (r = -0.50 and r = 0.48 between HRV_{TOT} and BPV_{TOT}, for Protocols 1 and 2, respectively) argue against the idea of a uniquely (or predominantly) baroreflex-determined contribution to the magnitude of HRV (and RSA), suggesting that mechanisms such as direct stretch-driven stimulation of the sinoatrial node by venous-return may be more important than previously thought (Bernardi et al., 1989, Saul et al., 1991).

The present data highlight the complexity of the phenomena being studied. Nonetheless a common feature seems to lie within the modulatory effect that

breathing exerts upon LVSV, ABP and *fc*. These appear to be inherently linked to the way breathing impacts venous return to the heart, and therefore a more careful evaluation of the effects of changing venous return and intrathoracic pressure may help to further the understanding of the mechanisms involved in the cardiovascular and autonomic response to SDB. Future studies within this thesis will explore these themes.

Impact on baroreflex sensitivity

We analysed cardiac BRS using both the sequence-method and the cross-spectral transfer function between SBP and *fc* (at a frequency corresponding to f_R). Protocol 1 showed that the highest BRS values for both methods (BRS_{Freq} and BRS_{Seq}) were encountered at the lowest f_Rs and in the SSV_T condition (Table 4-4). Nonetheless, statistical significance was only obtained for a small number of conditions, which highlights not only the strictness of the *post-hoc* analysis criterion (number of analysed comparisons) but also the variability within the BRS data. No indication of any significant alteration of BRS was found in Protocol 2, suggesting that changing V_T at a pre-determined fixed f_R fails to influence the sensitivity of the cardiac-vagal baroreflex (Table 4-5). Furthermore, while the results obtained in Protocol 2 show a strong and significant correlation between the BRS estimated using the two methods (r = 0.88, P < 0.05; Table 4-10), the data arising from the fixed V_T intervention (Protocol 1) shows only a moderate association between methods (r = 0.56, Table 4-11).

We believe these data are indicative of the limitations of these methods of assessing spontaneous baroreflex-mediated control of ABP during SDB interventions, and the interpretation and use of BRS should be object of carefull consideration. This interpretation stems from a number of factors, which include:

- baroreflex control is not limited to a single cardiac vagal efferent limb but relies instead on distinct vagal and sympathetic pathways to the heart, and sympathetic pathways to the peripheral vasculature. In fact, several studies suggest that the blood pressure buffering capacity of the arterial baroreflex is mainly determined by the sympathetic vascular baroreflex (van de Vooren et al., 2007, Di Rienzo et al., 2009), while BRS, as traditionally measured, translates almost exclusively the vagal efferent gain to the sino-atrial node

(Pomeranz, Macaulay, Caudill et al., 1985, Abrahamsson, Ahlund, Nordlander et al., 2003, van de Vooren et al., 2007);

- baroreflex buffering seems only to be effective at frequencies below the ABP resonant frequency, i.e. <0.1Hz (Di Rienzo et al., 2009);
- the presence of a resonant effect on the ABP and *fc* behaviour at 0.1Hz, which might preclude a correct BRS evaluation during SDB;
- the existence of other sources of variability in RR interval and ABP that are unrelated to the cardiac baroreflex pathway, including:
- feedforward mechanisms by which ABP variations are produced/limited by Starling and *Windkessel* effects driven by *fc* changes (Porta, Baselli, Rimoldi et al., 2000);
- the presence of reflex driven changes in *fc* resultant from stimulation of pulmonary (McMahon et al., 2000, Moore et al., 2004a, b, 2011) or low-pressure atrial baroreceptors (Bainbridge, 1915, 1920, Pawelczyk and Levine, 1995);
- the presence of respiratory modulation of the responsiveness of vagal–cardiac motoneurones influencing *fc* phasically (Eckberg, 2003).

In summary, the usefulness of both time- and frequency-domain mathematical based techniques for the evaluation of spontaneous cardiac baroreflex responses to respiratory challenges is, in my view, questionable. Some limitations of these techniques have been emphasised previously by others and involve the criteria used to determine valid baroreflex sequences, the number of sequences used in the calculations, the poor agreement with modified Oxford method (gold standard method to access arterial baroreflex function), and the inability to distinguish between baroreflex and non-baroreflex respiratory-synchronous fluctuations in *fc* (Di Rienzo, Castiglioni, Mancia et al., 2001, Di Rienzo, Parati, Castiglioni et al., 2001, Rothlisberger et al., 2003, Laude, Elghozi, Girard et al., 2004, Reyes del Paso, Cea, Gonzalez-Pinto et al., 2006, Reyes del Paso, Hernandez and Gonzalez, 2006, Tzeng et al., 2009, Hart, Joyner, Wallin et al., 2010), particularly at f_Rs close to, or multiples, of 0.1 Hz (Bothova, Honzikova, Fiser et al., 2010). Furthermore, ABP buffering and BRS are unrelated unless the contribution of all baroreflex limbs is assumed (van de Vooren et al., 2007).

4-5.2 Insights from transfer function analysis

Some valuable insights regarding the interplay between respiratory and cardiovascular changes can be garnered from transfer function analysis. In the present study, variations in V_T resulted in no apparent phase or temporal changes between RESP, SBP and *fc,* except for a non-significant increase of the phase angle between RESP and DBP (Figure 4-4H); increases in V_T resulted in an average difference of 40^0 (1.2 s) between the lowest and highest analysed V_T conditions. This might be attributable to an increase in diastolic 'run-off' period, following the decrease in *fc* during exhalation, i.e. as *fc* decreases the length of the diastolic relaxation period increases (while left ventricular ejection period remains unaltered) leading to a greater delay of DBP change relative to RESP. Simultaneously, the greater diastolic 'run-off' can promote a DBP decay due to the *Windkessel* properties of the aorta (De Boer, Karemaker and Wieling, 1985, De Boer, Karemaker and Strackee, 1985b). In contrast, the influence of changes in f_R upon phase angle and time delays was more pronounced, with both phase angle and the time shift increasing with decreases in f_R for RESP-*fc*, RESP-SBP and RESP-DBP. In contrast, a reduction in both phase and time shift was observed for SBP-*fc*, and no significant changes were detected for the SBP-DBP phase relation.

These findings contradict previous studies, using both cross-spectral (De Boer, Karemaker, et al., 1985b, De Boer, Karemaker and Van Montfrans, 1986, Pitzalis et al., 1998, Sin et al., 2010) and time-domain techniques (Sin et al., 2010), which found an increase in both phase and time shift between SBP and the ensuing RR interval changes, with the decrease in f_R. Notably, seemingly contradictory results between different studies regarding the SBP to *fc* phase relation have been widely reported in the literature. In common with the present study, some authors have found that SBP changes lead *fc* fluctuations (De Boer et al., 1986, De Boer et al., 1987, Pagani et al., 1988, Saul et al., 1991, Pitzalis et al., 1998), while others have argued that the RR fluctuations lead, rather than follow, the beat-to-beat SBP changes (Weise, London, Guerin et al., 1995, Zhao, Yamamoto, Munakata et al., 1999).

The reasons for these inconsistencies include (but may not be limited to):

- Individual differences in resonant frequency[3] in the low-frequency band (Vaschillo et al., 2002);
- Postural differences leading to a baroreflex-driven or sympathetically mediated phase shift at the respiratory frequency (Berger, Saul and Cohen, 1989b, Berger et al., 1989a, Saul et al., 1989, TenVoorde et al., 1995, Taylor and Eckberg, 1996, Cooke et al., 1999, Gilad, Swenne, Davrath et al., 2005);
- Differences in breathing patterns, particularly duty cycle (Mason et al., 2013) *et al.*, 2013), but also P_aCO_2 levels (Tzeng, Larsen and Galletly, 2007), and the presence/absence of V_T control (Laude, Goldman, Escourrou et al., 1993);
- Differing methods used to calculate phase/time relations and distinct methods/strategies to define the peaks and troughs for both ABP and *fc* fluctuations (Bowers and Murray, 2004b). This issue is particularly relevant to the present study, as the SBP and DBP signals were inverted by 180^0 to better reflect the expected physiological response to respiratory fluctuations; by doing so, the phase angle values reflect the known reduction in ABP that occurs during inhalation. Thus, the transfer function analysis quantified the relationship between the onset of inhalation (a positive change) and the start of a reduction in ABP (a negative change). Importantly, it was confirmed that duty cycle (T_I/T_{TOT}) was 0.5 and that the SBP and DBP waveforms closely resembled sinusoid waveforms. More details regarding this approach can be found in General Methods section (Chapter 3).

While it has been suggested that at a particular 'resonant' frequency, usually close to 0.1 Hz (Vaschillo et al., 2002), the SBP-*fc* function is ~0^0 in-phase and RESP-SBP function is ~180^0 out of phase, the present study only observed such relationships for SBP-*fc* at even lower f_Rs (4 breaths·min^{-1}). Simultaneously, RESP-SBP was ~180^0 out of phase at 6 breaths·min^{-1} (0.1 Hz). The former may reflect lower resonant frequencies in our participants. Importantly, the time shift between SBP and *fc* observed at 6 breaths·min^{-1} was consistent with the known delays in the cardiac baroreflex reflex arc (Baskerville, Eckberg and Thompson, 1979).

[3] Resonance refers to the physical phenomenon in which an oscillatory system oscillates with increased amplitude at a specific natural (resonant) frequency, by action of a small periodic stimulus of similar frequency as the resonant frequency of the system. In the particular case of SDB this is accomplished, by breathing at an f_R of similar periodicity to the natural low-frequency oscillations in ABP and *fc,* close to 0.1Hz.

Unlike Vaschillo and colleagues, data from the present study showed phase increases in RESP-*fc* at the lowest f_Rs, with values of 109±5^o at the lowest breathing frequencies, compared with 38±6^o at the highest (Table 4-12). In situations of strong cardio-ventilatory coupling, like the one we expect to encounter at a common resonant frequency, the shortest RR intervals tend to occur during late inspiration, while maximal RR intervals typically appear just before inspiratory onset (Galletly and Larsen, 1998). If this construct is applied to the present findings (with equal inspiratory and expiratory durations), the smallest RR interval is expected to occur a quarter of a respiratory cycle after the start of inhalation or approximately 90^o. This pattern, which is consistent with the present data and those of others (Bowers and Murray, 2004c), supports the presence of strong cardio-ventilatory coupling at frequencies between 4 and 6 breaths·min^{-1}.

Importantly, the consistent increase in the RESP-SBP phase and time relation with the decrease in f_R might be interpreted as evidence of the presence of a relatively constant lag in the respiratory increase in systemic venous return during SDB, and the translation of this lag into variations in SBP. As mentioned earlier, such a lag is generated mainly by the long PulTT (5 to 10 sec). At f_Rs above 6 breaths·min^{-1}, the SBP increases take place mainly during exhalation as can be inferred by the negative time lag (SBP precedes RESP). However, at 6 breaths·min^{-1} this lag becomes positive (SBP lags RESP) and both time and phase shifts continue to increase when f_R is reduced further to 4 breaths·min^{-1}. Therefore the zero-crossing (x-axis) of the RESP-SBP transfer function (Figure 4-4B) likely reflects the average cardiopulmonary transit time and closely matches the likely f_R at which $LVSV_E$ equals $LVSV_I$ (Figure 4-3B).

Collectively these findings highlight the presence of nonlinearities that cannot be explained simply by the presence of the baroreflex mechanisms. An argument can be made regarding the contribution of non-reflex mechanisms, particularly those directly related to the respiratory modification of LVSV during SDB, which underpin alterations of the relationship between ABP and *fc*. Overall, the cross-spectral transfer function analysis showed a substantial impact of f_R upon the phase relations between respiration, *fc* and ABP, possibly reflecting alterations in the relative contribution of central, reflex and mechanical factors; in line with the other findings from this study. This tends to support a pivotal role for respiratory driven-fluctuations

in LVSV in the cardiovascular response to SDB, particularly by affecting the timing of the respiratory modulation of ABP.

4-5.3 Impact of stringent control of breathing pattern vs. semi-spontaneous breathing

When SDB is implemented in trials of its anti-hypertensive effects, only f_R is controlled, leading to the question of whether any effects are due to f_R *per se*, the increase in V_T *per se* or the combined influence of both f_R and V_T. The present study sought to evaluate these possibilities in two ways; firstly, by assessing the independent effects of f_R *and* V_T, and secondly, by including a semi-spontaneous condition in which only f_R was controlled. For completeness, we also included a semi-spontaneous condition in which only V_T was controlled. Neither of the semi-spontaneous conditions resulted in any changes to minute ventilation ($\dot{V}_E$). Thus, any differences between the semi-spontaneous and the fully clamped conditions, were most likely due to the only other factor that differed, which was P_aCO_2 (Tables 4-2 and 4-3).

A small number of studies have compared the effects of different f_Rs and V_Ts upon RSA (Angelone and Coulter, 1964, Freyschuss and Melcher, 1976a, c, Hirsch and Bishop, 1981, Berger et al., 1989a, Cooke et al., 1998), but none has done so in a way that has standardised P_aCO_2, or maintained CO_2 at physiological levels. Instead, participants typically breathed freely, varying their V_T in order to maintain a prescribed f_R or $\dot{V}_E$ (Hayano et al., 1994, Patwardhan, Evans, Bruce et al., 1995, Bernardi, Wdowczyk-Szulc, Valenti et al., 2000, Stark, Schienle, Walter et al., 2000, Wilhelm, Grossman and Coyle, 2004). Breathing at a fixed f_R and V_T potentially generates a $\dot{V}_E$ that falls above or below that required to clear metabolic CO_2 production, eventually leading to either hypocapnia or hypercapnia. The P_aCO_2 has a powerful influence on both the cardiovascular and autonomic systems (Marshall, 1994, Sasano et al., 2002, Cooper et al., 2005, Simmons et al., 2007, Tzeng et al., 2007); acute hypercapnia can lead to a sympathetically mediated pressor response (Shepard, 1990, Serebrovskaya, 1992), while hypocapnia has the opposite effect (Rothe, Flanagan and Maass-Moreno, 1990). Thus, failure to control this influence creates uncertainties in relation to differentiation between frequency- and volume-driven responses to SDB. Although we clamped $P_{ET}CO_2$ at slightly hypercapnic levels (*circa.* 41 mmHg), this exceeded the $P_{ET}CO_2$ during spontaneous breathing

by only around 2 mmHg. A significant pressor effect would require $P_{ET}CO_2$ levels far beyond those encountered in the present study (Serebrovskaya, 1992). Accordingly, we are confident that the clamping of $P_{ET}CO_2$ did not influence any of the findings.

The stringent control of the breathing pattern with 'clamped' $P_{ET}CO_2$, in both protocols, resulted in no discernible differences in RSA compared to semi-spontaneous breathing at either fixed f_R or V_T, despite significantly different $P_{ET}CO_2$ levels, but similar $\dot{V}_E$. My results are contrary to those of others (Yasuma and Hayano, 2001, Sasano et al., 2002, Tzeng et al., 2007) who found a dose-response effect of P_aCO_2 upon RSA. Such dissimilarities between studies are likely attributable to important methodological differences, which include species differences, the use of different methods to quantify RSA, the application of f_Rs that are not consistent with SDB, or the establishment of hypercapnia at consistently higher $P_{ET}CO_2$ values than those in the present study, which leads me to believe that in my study the degree of hypercapnia was insufficient to stimulate a chemoreflex response.

The increased blood pressure response that was seen in the 'fully clamped' conditions at f_Rs ≥ 6 breaths.min^{-1}, during Protocol 1, is consistent with the findings of others (Cooper et al., 2005). In contrast, during Protocol 2, there were no significant differences between 'semi-spontaneous' breathing and any of the 'fully clamped' breathing patterns for any variables. At the same time, the absence of changes, in TPR argues against the potential role of sympathetic involvement in the changes in ABP. In our study, MAP was significantly lower for SSV_T, compared to other tested f_Rs. Notwithstanding, the MAP was similar during both SSV_T and the 'fully-clamped' 4 breaths·min^{-1}, suggesting that any potential pressor effect was unlikely to be related to hypercapnia *per se*. Inspection of the LVSV and $\dot{Q}$ responses indicate that these exhibit a similar pattern to that of MAP (despite not achieving significance) suggesting that any observed differences in MAP are likely attributable to the same mechanisms that determine the LVSV response to SDB. Overall, the present study did not reveal any advantage of simultaneously clamping f_R, V_T and P_aCO_2, over controlling just f_R or V_T. A significant pressor response was observed during Protocol 1 in the fully clamped breathing conditions, which was likely associated with a respiratory-driven increase in LVSV and $\dot{Q}$, and not with a chemoreflex response.

4-5.4 Implications for future research on SDB interventions

The use of RSA as a control variable for numerous SDB interventions, particularly those using HRV biofeedback, relies on the amplification of *fc* fluctuations (Lehrer, 2013, Lehrer and Gevirtz, 2014). While HRV exhibits a somewhat 'chaotic' structure involving various superimposed oscillatory rhythms, related nonlinearly to each other (Ivanov, Amaral, Goldberger et al., 1999), the exact mechanisms by which SDB increases RSA and HRV are not entirely understood. A proposed theory involves the confluence of a series of reflex processes, operating as negative feedback loops, leading to increased amplitude of *fc* oscillations (Lehrer, 2013, Lehrer and Eddie, 2013). These include, but might not be limited to:

1) phase relationships between heart rate oscillations and breathing at specific frequencies;
2) phase relationships between heart rate and blood pressure oscillations at specific frequencies;
3) the activity of the baroreflex;
4) resonance characteristics of the cardiovascular system (Lehrer and Gevirtz, 2014).

The present study demonstrated no significant differences for the RESP-*fc* and SBP-*fc* phase relationships with the changes in $f_{R,}$ thus counteracting points 1 and 2. Instead, the data suggest that these *fc* fluctuations are, to some extent, secondary to variations in RVSV brought about by increased venous return during SDB. While the findings do not eliminate a contribution from the baroreflex modulation of *fc* and the amplification of LVSV and ABP fluctuations by way of resonances in the cardiovascular system, they support a contribution from a mechanical stretch of the sinoatrial node. The data suggest that the contribution of this mechanism to the amplitude of acute respiratory-driven ABP and *fc* swings during SDB, may be larger than previously suggested (Saul et al., 1991). Studies using inspiratory resisted breathing have showed increased *fc* fluctuations (RSA) secondary to mechanically driven swings in LVSV and ABP (Freyschuss and Melcher, 1976c, Blaber and Hughson, 1996). Breathing against modest inspiratory resistances (< 10 cmH_2O) results in increased LVSV, $\dot{Q}$ and SBP, secondary to respiratory-driven increased venous return (Convertino, Ratliff, Ryan, Doerr, et al., 2004, Ryan, Cooke, Rickards et al., 2008, Convertino, Ryan, Rickards et al., 2011). Therefore, future research on the effectiveness of SDB should focus on understanding the effect of interventions

that maximise venous return and LVSV, as well as how these factors affect both haemodynamic and autonomic responses to SDB. These issues will be explored further in subsequent studies of this thesis.

4-6 Methodological considerations

Several methodological aspects should be taken into account when interpreting the results of the present study. Firstly, Protocol 2 was performed at the theoretical optimal individual f_R for RSA based on the results of Protocol 1. By taking this approach, it was recognised that some of the participants would be unable to complete Protocol 2 (due to hypercapnia), leading to the exclusion of 3 participants for the second part of the study. These participants all exhibited maximal RSA at 4 breaths·min^{-1}, which combined with a V_T of only 20% FVC resulted in insufficient $\dot{V}_E$ to prevent progressive hypercapnia. Overall, the reduction of *n* for Protocol 2 contributed to a decrease in statistical power, which may have been reflected on the borderline non-significant results seen in some of the study's outcome variables. Secondly, by asking participants to breathe at a specific f_R (4 or 6 breaths·min^{-1}) throughout Protocol 2, the full expression of volume-dependent cardiovascular responses to paced breathing might have been limited. These 'optimised' f_Rs had a strong impact on the cardiovascular response, independently of V_T during Protocol 1, which may have attenuated the effects resulting from the manipulation of V_T during Protocol 2. Thirdly, in the semi-spontaneous breathing conditions, the re-breathing method for controlling $P_{ET}CO_2$ prevented the maintenance of $P_{ET}CO_2$ at the same levels present in the fully controlled sets. While this might limit the ability to interpret differences between the semi-spontaneous conditions and the other conditions, it did allow comparison of the effectiveness of a natural semi-spontaneous breathing *versus* a more rigorously controlled breathing pattern. Fourthly, the use of cross-spectral methods assumes that respiratory and cardiovascular fluctuations occur in parallel, and are bounded by fixed phase relations (Sin et al., 2010), i.e. that the signals are stable and truly sinusoidal. By making some adjustments to the pre-processing of the cardiovascular signals (see footnote 2), advancing one of the signals by half a cycle will have changed the reference point for calculation of phase differences, which considering the underlying waveform deviations from a true sinus shape, might have contributed to either increased or decreased error in the phase and time shift estimates. Fifthly, the f_Rs used during the first part of the study were primarily aimed at providing a

general overview of the behaviour of the different dependent variables that were tested, over a broad range of slow frequencies. Thus, any assumptions on the effectiveness of the different frequencies must be seen at the light of this limitation. The use of smaller intervals between different f_Rs (one or half a breath·min^{-1}) would have allowed for further detail, but would have increased the length of the testing sessions dramatically, imposing unreasonable demands upon the participants. Finally, the existence of a possible 'hangover' effect of undertaking a large number of SDB interventions in a single session, with only a short break between each condition, cannot be ignored. Pilot-testing conducted prior to the trials demonstrated that 5 min rest periods appeared to be sufficient to restore mean cardiovascular (LVSV, $\dot{Q}$ and SBP) and respiratory ($P_{ET}CO_2$) variables to baseline levels, following each 5 min SDB intervention. A recent study has shown that the recovery kinetics for HRV following SDB might not follow a similar pattern (Dick, Mims, Hsieh et al., 2014). Notwithstanding, Dick's study involved a much longer (20 min) SDB epoch than the ones employed in the present study, and the randomisation of the SDB conditions certainly limited any potential impact of a systematic 'hangover' order effect, upon the study's results.

4-7 Conclusion

In summary, the independent effects of f_R and VT upon the cardiovascular response to SDB have been assessed, and the findings confirmed the established frequency and volume-dependent response of RSA, which is maximised at breathing frequencies between 4 and 6 breaths·min^{-1}. Also, the data support existing evidence that RSA contributes to the attenuation of the respiratory modulatory effect of SDB upon LVSV and ABP, and that ABP fluctuations are mostly determined by within-breath variations of LVSV. Furthermore, the data provided new evidence that the impact of SDB on the cardiovascular response is mainly determined by the changes in f_R. At 4 and 6 breaths·min^{-1}, a previously undescribed influence upon LVSV was observed, whereby $LVSV_E$ exceeded $LVSV_I$, and the f_R at which this occurs is likely related to the individual length of pulmonary transit time. Finally, while being consistent with stimulation and involvement of baroreflex regulation during SDB, the presented data hint at a relevant involvement of other sources of heart rate variability during SDB, likely arising from respiratory induced modulation of right atrial filling.

CHAPTER 5 – THE INFLUENCE OF INTRATHORACIC PRESSURE UPON RESPIRATORY MODULATION OF CARDIOVASCULAR CONTROL DURING SLOW, DEEP BREATHING

5-1 Abstract

Introduction and objective: Slow and deep breathing (SDB)has substantial effects on the cardiovascular and autonomic nervous systems. The use of inspiratory resistances has been shown to increase left ventricular stroke volume (LVSV) in normo- and hypovolemic individuals. Loading also magnifies the respiratory sinus arrhythmia (RSA) during paced, high frequency, breathing. The present study tested the hypothesis that magnifying inspiratory pressure (P_I) swings via inspiratory loading amplifies the cardiovascular response to SDB and that the effect is mediated primarily by mechanical mechanisms that modulate systemic venous return.

Materials and methods: Eleven healthy males (25±4 years; 179±5 cm; 76.5±10.5 kg) were tested in a seated upright-reclined position. The protocol consisted of eight 5 min bouts of SDB at a breathing frequency (f_R) of 6 breaths·min^{-1} and tidal volumes (V_T) of 25% and 40% of forced vital capacity (FVC). The SDB was combined systematically with two different inspiratory loads of -9.2±0.8 (IL1) and -22.9±1.1 (IL2) cmH_2O and two different lower body positive pressures of 10 (LBPP1) and 20 (LBPP2) mmHg. All interventions were randomised and interleaved with 5 min of unrestricted, spontaneous breathing.

Results: The response to SDB was proportional to IL magnitude for respiratory sinus arrhythmia (RSA) (40%FVC = 347±33; IL1 = 432±44; IL2 = 459±41 ms, $P < 0.002$), cardiac output ($\dot{Q}$) (6.2±0.3, 6.5±0.3, 6.8±0.4 L·min-1, $P < 0.002$), within-breath stroke volume (ΔSV) (2±2, -6±2, -11±2 mL·beat^{-1}, $P < 0.002$), heart rate variability (HRV) (HRV_{TOT}: 16396±2733, 23282±4042, 26621±4138 ms^2·Hz^{-1}, $P < 0.002$) and blood pressure variability (BPV) (BPV_{TOT}: 149±24, 452±57, 722±117 mmHg^2·Hz^{-1}, $P < 0.002$). Both within-breath cardiac output ($\Delta\dot{Q}$) and ΔSV were moderately correlated with RSA (R^2 = 0.75 and 0.52, respectively, $P < 0.001$), while time-shifts between instantaneous lung volume, systolic blood pressure and heart rate waveforms correlated strongly with RSA, ΔSV, HRV and BPV ($P < 0.05$). Isolated LBPP did not alter left ventricular function as revealed by Doppler ultrasound. However, when combined with IL, a significant main effect of LBPP was detected for $\dot{Q}$, with LBPP counteracting the effects of IL upon $\dot{Q}$ ($P < 0.05$).

Conclusion We conclude that the imposition of an IL during to SDB amplifies the effects of SDB alone. The data support the notion that respiratory-related intrathoracic pressure swings are the predominant breathing-related stimulus underlying the acute cardiovascular response to SDB. While the exact contribution of baroreflex mediated mechanisms to the amplification of RSA, HRV and BPV by

IL is unclear. The data suggest that amplification of these variables necessarily involves the augmentation of within-breath fluctuations of venous return and LVSV.

5-2 Introduction

Study 1 (Chapter 4) identified an important acute impact of specific f_Rs upon within-breath fluctuations of LVSV and $\dot{Q}$. Other variables, commonly associated with heightened parasympathetic activity (Berntson, Bigger, Eckberg et al., 1997), such as RSA, were also greatly affected by f_R. These results hinted that acute and chronic responses to SDB might be mediated via stimulation of cardiac, aortic and/or carotid baroreceptors. Such stimulation is most likely underpinned by respiratory-driven fluctuations in venous return, maximising at f_Rs around 6 breaths.min^{-1}.

Breathing against an inspiratory load (IL) exaggerates cardiovascular responses to spontaneous breathing (Dornhorst et al., 1952b, Kilburn and Sieker, 1960, Wise, Robotham and Summer, 1981, Coast, Jensen, Cassidy et al., 1988, Convertino, Ratliff, Ryan, Doerr, et al., 2004), and potentially also that to SDB. The underlying stimulus from IL is most likely a more negative pleural pressure during inspiration, which increases transmural pressure across the heart chambers and intrathoracic vessels, impacting both preload and afterload (Alian and Shelley, 2012). Recently, a study with healthy people reported increases in LVSV, $\dot{Q}$ and MAP during SDB (Lucas et al., 2013). This pattern is similar to that observed with small inspiratory loads (Convertino, Ratliff, Ryan, Cooke, et al., 2004, Convertino, Ratliff, Ryan, Doerr, et al., 2004), suggesting the involvement of similar underlying mechanisms. Data from this thesis (Chapter 4) also indicates that SDB modulates the within breath oscillations in LVSV and $\dot{Q}$. The use of respiratory loading under constant f_R and V_T conditions has also been used previously to test the link between mechanically coupled variations in LVSV and the activation of baroreflex mediated *fc* responses (Blaber and Hughson, 1996). This study revealed no differences in SBP and LVSV, while RSA and the transfer function magnitude between SBP and RR interval both increased, with the increasing respiratory (inspiratory and expiratory) load magnitude, supporting the hypothesis that mechanisms other than the arterial baroreflex are involved in the RSA response to SDB with respiratory loads. However, it is unclear if the same response occurs when only the inspiratory phase of breathing is loaded.

Importantly, the application of IL is believed to contribute to a marked reduction in right atrial pressure, which in turn contributes to augmentation of the pressure gradient for central venous return, driving an increased quantity of blood to the right atrium during inspiration (Robotham et al., 1978, Convertino, Cooke and Lurie,

2005). Increases in venous return can also be accomplished by altering body position or by compression of the extremities by a positive pressure. The compression at the lower limbs with lower body positive pressure (LBPP) produces a displacement of blood into the abdominal and thoracic compartments, effectively increasing central blood volume; increasing central venous return has been shown to promote rises in LVSV and ABP (Shi, Crandall and Raven, 1993). We reasoned that if inspiratory 'suction' during inhalation increased venous return and LVSV, that effect could be attenuated by squeezing blood from the lower legs into the abdominal compartment. Such a shift would make it easier for breathing to enhance venous return, thereby lessening the benefit of magnifying the 'suction' effect using an IL.

For this study it was hypothesised that 1) the addition of an IL would magnify the cardiovascular and autonomic responses induced by SDB at similar f_R and V_T; 2) this response would be closely coupled to heightened within-breath fluctuations in intrathoracic pressure, and 3) the addition of LBPP would attenuate the combined effects of SDB and IL.

5-3 Specific Methods

5-3.1 Participants

Eleven healthy, recreationally-active men participated (mean ± SD; age 25±4 years; 179±5 cm; 76.5±10.5 kg). All participants were nonsmokers with no previous history of cardiovascular or respiratory disease and reported not taking any medication.

5-3.2 Ethical approval

Fully informed, written consent was obtained from the participants prior to the study. All procedures were approved by the Brunel University Research Ethics Committee (RE17-14) and conformed to the guidelines of the Declaration of Helsinki (World Medical Association, 2013).

5-3.3 Protocol

Participants visited the laboratory on two different occasions. On the first session, the participants were introduced to the experimental set-up and familiarised with the

methodology. A baseline pulmonary function assessment was performed with those participants showing signs of asthma or other lung disease being excluded from the study. Additionally, a familiarisation trial using a bespoke device guided breathing biofeedback system was performed (Labview, National Instruments Inc.) (please refer to the General Methods section – Chapter 3 - for a detailed description of the pulmonary function assessment and familiarisation session protocols). Finally, participants also underwent an ultrasonography cardiac function analysis to evaluate their individualised response to graded LBPP (at compression levels corresponding to 0, 10, 20 and 30 mmHg; see *General Methods* – Chapter 3 - for details of LBPP). Cardiac ultrasound measurements (echocardiography) were performed by a trained researcher to estimate the change in left ventricular blood volume at different levels of LBPP. Procedures followed the recommendations of the American Society of Echocardiography (Lang et al., 2005). Participants were tested in an upright-reclined position at an angle similar to that used during the second visit. Four consecutive beats were taken in both the apical two and four-chamber views and used to estimate end-diastolic and end-systolic left ventricular volumes, as well as LVSV and $\dot{Q}$ through the Simpson-biplane method (Lang et al., 2005).

During the second visit, participants undertook device guided SDB. The trial session began with 5 min of quiet baseline spontaneous breathing, followed by eight 5 min sets of device guided SDB. All interventions were performed at an f_R of 6 breaths·min^{-1}. Two sets included SDB at a constant V_T corresponding to 25% and 40% of each participants' FVC, respectively. The remaining conditions were all performed at a fixed V_T of 40% FVC and included a combination of SDB against two different inspiratory resistances (IL) and with two different levels of LBPP. Five minute rest periods separated each condition (Figure 5-1). The 6 breaths·min^{-1} at 25% FVC condition was always performed first as this condition delivered the smallest V_E and consequently highest $P_{ET}CO_2$ levels. All remaining conditions were conducted in a fully randomised order (a detailed description of the randomisation procedures can be found in the General Methods section - Chapter 3).

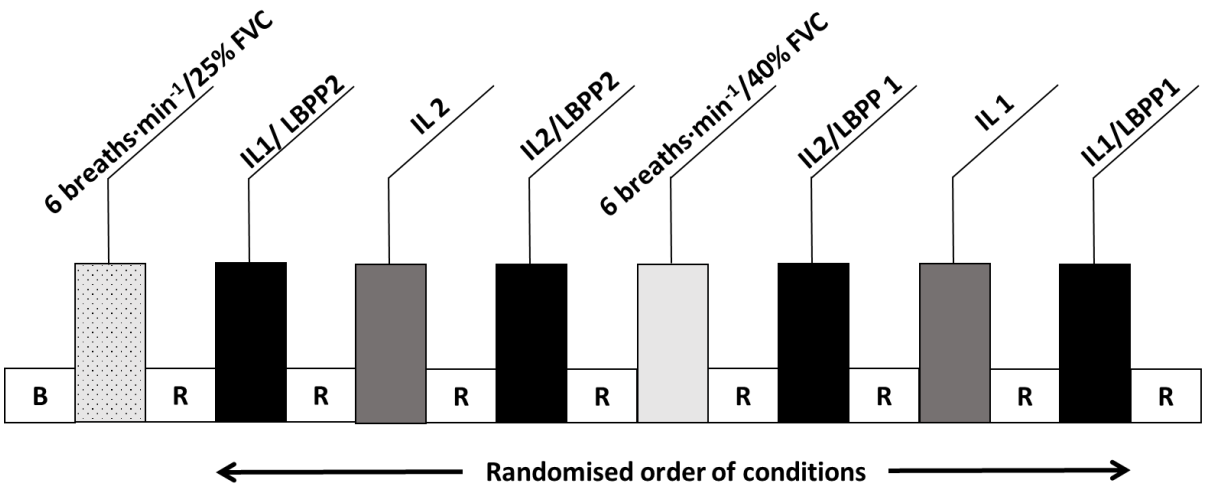


Figure 5-1 - Sequence of the experimental protocol. Participants were exposed to 5 min of spontaneous baseline (B) breathing followed by device guided slow and deep breathing at 6 breaths·min^{-1} and 25% FVC (dotted light grey bar). Subsequent, randomly assigned, device guided slow and deep breathing sets included unloaded slow and deep breathing (light grey), inspiratory loaded slow and deep breathing (dark grey) and combined inspiratory loaded slow and deep breathing conditions with lower body positive pressure (dark). Interventions lasted for 5 min and were interleaved by 5 min of spontaneous, unrestricted breathing (R).

5-3.4 Procedures and instrumentation of participants

Participants were asked to refrain from alcohol, caffeine and strenuous physical activity for at least 12 h before testing and to avoid consuming food during the 2 h leading up to testing. All sessions were conducted at the same time of the day, generally in consecutive days, but no more than one week apart. Throughout the entirety of the trials, participants assumed an upright-reclined position by sitting on a reclining lounge chair, set at an approximate 60^{o} angle, and breathed through a mask that allowed for normal mouth and/or nasal breathing (Intersurgical Quadralite Mask; Intersurgical Ltd., Wokingham Berkshire, UK). Room temperature remained at a comfortable 22-25^{o}C and barometric pressure was recorded for each of the trial sessions.

Specified respiratory flow rates and a respiratory duty cycle of 0.5 (inspiratory time = expiratory time) were delivered using the bespoke guided breathing biofeedback system described in the General Methods section (Labview, National Instruments Inc.) and measured using a heated pneumotachograph (Hans Rudolph 3700, Hans Rudolph Inc.). $P_{ET}CO_2$ was maintained at a slightly hypercapnic level (GA-200 gas analyser, iWorx Systems Inc.) under all conditions via a re-breathing system consisting of added dead spaces. A summary of the experimental respiratory parameters can be found in the Results section of this chapter (Table 5-2).

The inspiratory loads were imposed by a bespoke breathing circuit containing a flow resistor that comprised a number of nylon washers that reduced the internal diameter of the airway, thus creating resistance to flow and generating a precisely calibrated inspiratory load. As flow was kept constant throughout each step, via the aforementioned DGB system, the addition and removal of washers allowed the length of the breathing resistor to be adjusted, thereby altering resistance to flow according to the Hagen–Poiseuille equation. The y-shape and separate inspiratory and expiratory valves ensured that the load only acted upon the inspiratory portion of the respiratory cycle, whilst the joining of the inspiratory and expiratory limbs allowed for the aforementioned rebreathing of exhaled air to maintain isocapnia. A detailed description of this flow resistive breathing circuit can be encountered in Chapter 3 – General Methods (section 3-3.4).

The increase in central blood volume was attempted through application of LBPP (Figure 5-2), delivered by an anti-G suit (Royal Air Force MK2A Anti-G trousers) inflated at two different pressures of 10 and 20 mmHg and permanently controlled through a pressure meter (Comark C9553, Comark Instruments, Norwich, Norfolk, UK).

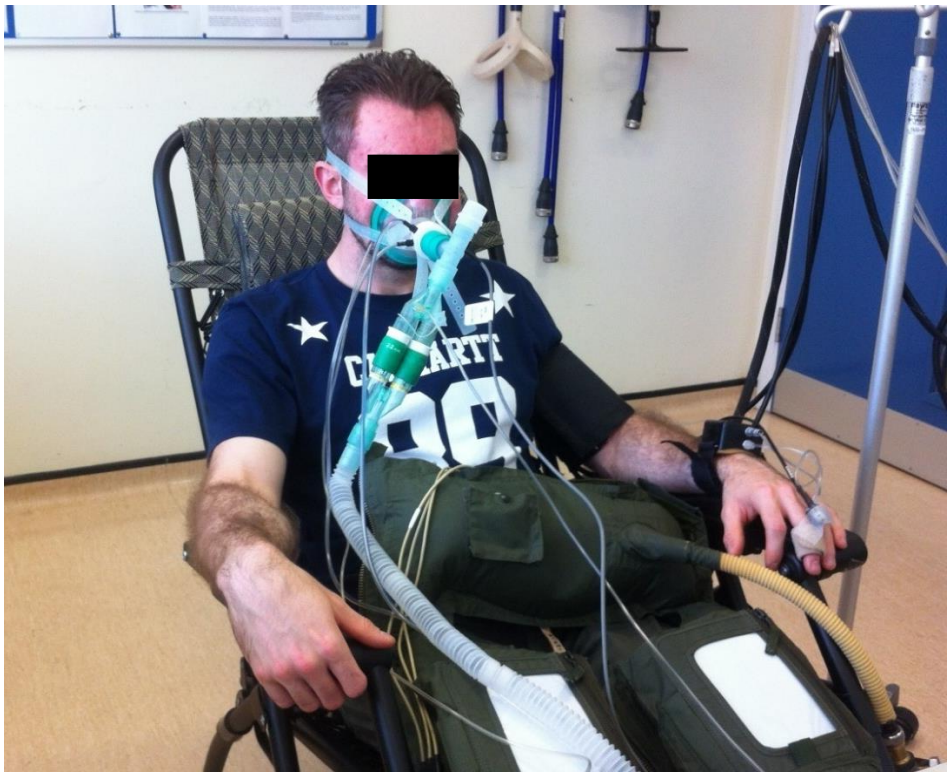


Figure 5-2 - Experimental set-up showing participant sitting in a semi-reclined chair while breathing against an inspiratory resistance and legs being compressed with anti-G trousers. The abdominal portion of the suit was kept loose to avoid compression of the abdomen.

Non-invasive beat-to-beat arterial blood pressure was obtained using finger photoplethysmography (Finometer® PRO, Finapres Medical Systems, Amsterdam, The Netherlands), whilst heart rate was monitored using a 3-lead ECG (PysioControl VSM® 3, PhysioControl Inc.). Total peripheral resistance (TPR) was calculated by dividing $\dot{Q}$ by the mean arterial pressure (MAP). Baroreflex sensitivity (BRS) was calculated by the sequence method (Bertinieri et al., 1985, Bertinieri, Di Rienzo, Cavallazzi et al., 1988, Parati et al., 1988) as well as from the cross-spectral transfer function gain (Robbe et al., 1987). Phase angles and coherence between respiration and cardiovascular (heart rate, SBP and DBP) waveforms were obtained from the cross-spectra transfer function at the peak respiratory frequency. Average phase angles were calculated as the un-weighted circular mean ($\bar{\alpha}$) for phase angles for all relationships of interest (Mardia and Jupp (2009). A more detailed description of the BRS and cross-spectral calculations can be encountered in Chapter 3 (section 3-3).

5-3.5 Statistical analysis

Data were analysed using IBM® SPSS version 21.0 statistical software (IBM Corp.). Values are expressed as means ± SEM unless otherwise stated. Repeated measures ANOVA with *post hoc* pairwise comparisons using Bonferroni correction were used initially to test differences between conditions after normality was confirmed via the Shapiro-Wilk test. Two-way repeated measures ANOVA with similar *post-hoc* testing was conducted to test the independent effect of IL and LBPP,on selected variables. Circular (cross-spectral) data were analysed using multi-sample Watson-Williams F-test following confirmation of the existence of a Von Mises circular distribution (Fisher, 1995, Mardia and Jupp, 2009). Bonferroni corrections were applied for *post-hoc* paired sample comparisons.

Despite some violations of requirements for the multisample ANOVA and Watson-Williams F-test, involving deviations from the required distributions that could not be corrected using data transformation, a decision was made to use the parametric options rather than the nonparametric alternatives. The use of continuous data, the robustness of the repeated measures ANOVA against moderate deviations in normality (Glass, Peckham and Sanders, 1972), and the similarity of results encountered when analysed the data using the nonparametric alternatives (Friedman and Mardia-Watson-Wheeler tests), provide assurance that the findings

drawn from the data were not affected by false positives deriving from the author's approach. Finally, for the analysis of the relationship between cardiovascular, HRV and BPV indices, multiple regression for within-subject repeated measures was applied (Bland and Altman, 1995), while simple, linear regression analysis was used to evaluate the relationship between cross-spectral time-shift and the aforementioned variables. For all analyses, *P* was set at 0.05.

5-4 Results

5-4.1 Cardiac function response to lower body positive pressure

The ultrasonographic analysis of the impact of progressive increases in LBPP upon left ventricular cardiac function revealed no changes in average steady-state values for end diastolic (EDV), end systolic (ESV) volumes, LVSV, *fc*, $\dot{Q}$ or MAP (Table 5-1).

Table 5-1 – Left ventricular function in response to progressive LBPP

	Rest	**10 mmHg (LBPP1)**	**20 mmHg (LBPP2)**	**30 mmHg**
EDV (mL)	137±3	136±3	137±4	137±3
ESV (mL)	40±3	38±2	38±3	38±3
LVSV (mL)	97±2	99±1	98±1	99±2
***fc* (beats·min^{-1})**	58±2	57±2	56±2	57±2
$\dot{Q}$ (L·min^{-1})	5.6±0.2	5.6±0.2	5.6±0.2	5.6±0.2
MAP (mmHg)	88±3	90±3	91±3	92±3

Data represent mean ± SEM for 11 subjects. End diastolic volume (EDV) in mL, end systolic volume (ESV) in mL, left ventricular stroke volume (LVSV) in mL, heart rate (*fc*) in beats·min^{-1}, cardiac output ($\dot{Q}$) in L·min^{-1} and mean arterial pressure (MAP) in mmHg. All variables were acquired by Doppler ultrasonography while MAP was acquired using infrared photoplethysmography. $P < 0.05$.

5-4.2 Respiratory changes with each condition

A summary of the respiratory data each experimental condition can be found in Table 2. As intended, duty cycle (~0.5), $P_{ET}CO_2$ (41-43 mmHg) and f_R (~6 breaths·min^{-1}) remained constant throughout all interventions (Table 2). Mean P_I was ~10 cmH_2O for the IL1 conditions, while for the IL2 it was set at ~23 cmH_2O (Table 2). The V_T in the IL2 conditions was slightly, but non-significantly, higher than that during the IL1 conditions (< 0.15 L·breath^{-1}; Table 5-2).

Table 5-2 - Experimental respiratory parameters.

	Baseline	25%FVC	40%FVC	IL 1	IL1/LBPP1	IL1/LBPP2	IL 2	IL2/LBPP1	IL2/LBPP2
f_R (breaths·min^{-1})	13.4±1.1	6.3±0.1*	6.1±0.1*	6.1±0.1*	6.0±0.1*	6.0±0.1*	6.1±0.1*	6.0±0.1*	6.1±0.1*
V_T (L·breath^{-1})	0.64±0.05	1.49±0.11*	2.30±0.12*†	2.39±0.12*†	2.34±0.13*†	2.32±0.12*†	2.48±0.14*†	2.49±0.12*†	2.45±0.12*†
T_I/T_{ToT}	0.55±0.02	0.50±0.02	0.51±0.01	0.49±0.01	0.49±0.02	0.50±0.01	0.48±0.01	0.48±0.02	0.48±0.02
$P_{ET}CO_2$ (mmHg)	40.9±2.8	41.9±1.4	41.3±1.4	41.0±1.4	42.1±1.4	42.5±1.5	42.1±1.3	42.9±1.1	42.7±1.6
P_I (cmH_2O)	-0.5±0.1	-0.5±0.1	-0.5±0.1	-9.2±0.8*	-10.0±1.5*	-10.0±1.4*	-22.9±1.1*ǂ	-23.8±1.0*ǂ	-23.7±0.8*ǂ

Data represent mean ± SEM for 11 subjects. Breathing frequency (f_R) in breaths·min^{-1}, tidal volume (V_T) in % of forced vital capacity, duty cycle (Ti/T_{TOT}), partial pressure of end tidal carbon dioxide ($P_{ET}CO_2$) in mmHg, inspiratory pressure (P_I) in cmH_2O. * different from baseline, † different from 25%FVC, ǂ different from IL1. $P < 0.002$.

5-4.3 Cardiovascular response to slow and deep breathing, inspiratory loading and lower body positive pressure

The use of inspiratory loads (IL1 and IL2) resulted in significant increases in RSA when compared to unloaded SDB at the same V_T. This was accompanied by unaltered *fc* and fc_E. The fc_I followed a pattern similar to RSA, with the effect being more noticeable during IL2 and IL2/LBPP1. The use of IL2 also increased $LVSV_E$, ΔSV, $\dot{Q}$, $\dot{Q}_I$ and $\Delta\dot{Q}$ significantly, particularly when compared to baseline and the unloaded SDB conditions (Table 5-3).

When compared to unloaded breathing at 40% FVC a significant main effect of inspiratory loading was detected for $LVSV_E$, ΔSV, $\dot{Q}$, $\dot{Q}_I$ and $\dot{Q}_E$, while a significant main effect of LBPP was only present for $\dot{Q}$ (Figure 5-3, $P < 0.05$).

The MAP, SBP, PP and TPR responses to both unloaded and inspiratory loaded breathing did not reach statistical significance, while DBP exhibited significantly higher values in the IL1/LBPP2 condition when compared to baseline (Table 5-3).

The response of the different BRS indices revealed that both the sequence method and BRS_{Freq} were largely unaffected by the interventions; however, BRS_{up} was significantly higher than baseline with SDB in the 40% FVC condition (Table 5-3).

Table 5-3 – Systemic haemodynamic responses to slow and deep breathing with different grades of inspiratory loading and lower body positive pressure.

	Baseline	25%FVC	40%FVC	IL 1	IL1/LBPP1	IL1/LBPP2	IL 2	IL2/LBPP1	IL2/LBPP2
RSA (ms)	170±21	268±25*	347±33*	432±44*	432±44*†	424±44*	459±41*†	479±36*†‡	426±36*†
f_C (beats·min^{-1})	61±2	62±2	61±2	61±2	60±2	60±2	62±1	61±2	63±1
fc_I (beats·min^{-1})	64±2	69±3	71±3	74±3	77±5	72±3	80±4	90±11	79±3*¥
fc_E (beats·min^{-1})	56±2	53±2	50±2	50±2	49±2	49±2	50±2	49±2	50±2
LVSV (mL·beat^{-1})	100±5	102±7	103±5	108±7	105±6	105±6	111±6	106±6	103±6
$LVSV_I$ (mL·beat^{-1})	100±5	103±6	102±6	105±7	101±5	101±6	104±5	100±5	97±5
$LVSV_E$ (mL·beat^{-1})	98±4	102±6	99±6	110±7	107±6	107±6	115±7*‡	113±7	110±7
ΔSV (mL·beat^{-1})	2±2	1±2	2±2	-6±2†‡	-7±2†	-6±2†	-11±2†‡	-13±3*†‡	-12±3*†‡
$\dot{Q}$ (L·min^{-1})	6.1±0.3	6.2±0.4	6.2±0.3	6.5±0.3	6.2±0.4	6.2±0.3	6.8±0.4*‡	6.5±0.4	6.4±0.4
$\dot{Q}_I$ (L·min^{-1})	6.4±0.4	7.1±0.5	7.2±0.4	7.7±0.5	7.6±0.5	7.3±0.5	8.3±0.5	8.8±0.9	7.7±0.5
$\dot{Q}_E$ (L·min^{-1})	5.4±0.3	5.4±0.3	5.0±0.3	5.4±0.3	5.2±0.4	5.2±0.3	5.7±0.3	5.5±0.3	5.5±0.3
Δ$\dot{Q}$ (L·min^{-1})	0.9±0.3	1.7±0.2	2.3±0.2*	2.3±0.4	2.4±0.4	2.1±0.3	2.6±0.4	3.3±0.8	2.3±0.2*
SBP (mmHg)	123±3	123±5	124±4	130±4	136±4	136±4	131±4	131±3	129±3
DBP (mmHg)	72±2	72±2	74±1	77±2	79±2	79±2*	76±2	76±2	76±2
MAP (mmHg)	89±2	89±3	91±2	94±2	98±3	98±2	94±2	95±2	94±2
PP (mmHg)	51±2	51±3	50±3	54±3	57±3	57±3	56±3	55±3	53±3
TPR (mmHg·min·L^{-1})	15±1	15±1	15±1	16±1	17±1	17±1	15±1	16±1	16±1
BRS_{up} (ms·mmHg^{-1})	19±4	30±6	41±6*	40±10	33±5	33±7	26±4	28±5	25±3
BRS_{down} (ms·mmHg^{-1})	22±6	17±2	20±3	18±3	18±3	21±5	18±3	12±1	16±2
BRS_{Seq}(ms·mmHg^{-1})	20±4	23±4	30±4	29±6	26±4	27±6	22±3	20±3	20±2
BRS_{Freq} (ms·mmHg^{-1})	21±5	20±2	33±6	16±1	18±2	17±2	17±2	15±1	16±2

Data represent mean ± SEM for 11 subjects. Respiratory sinus arrhythmia (RSA), heart rate (f_C), heart rate during inspiration (fc_I), heart rate during expiration (fc_E), left ventricle stroke volume (LVSV), stroke volume during inspiration ($LVSV_I$), stroke volume during expiration ($LVSV_E$), within-breath variation in stroke volume (ΔSV), cardiac output ($\dot{Q}$), cardiac output during inspiration ($\dot{Q}_I$), cardiac output during expiration ($\dot{Q}_E$), within-breath variation in cardiac output (Δ$\dot{Q}$), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP), total peripheral resistance (TPR), sequence baroreflex sensitivity positive sequence gain (BRS_{up}), sequence baroreflex sensitivity negative sequence gain (BRS_{down}), sequence baroreflex sensitivity average gain (BRS_{Seq}); cross-spectral baroreflex sensitivity gain (BRS_{Freq}).* different from baseline, † different from 25%FVC, ‡ different from 40%FVC, ¥ different from IL1/LBPP2. $P < 0.002$.

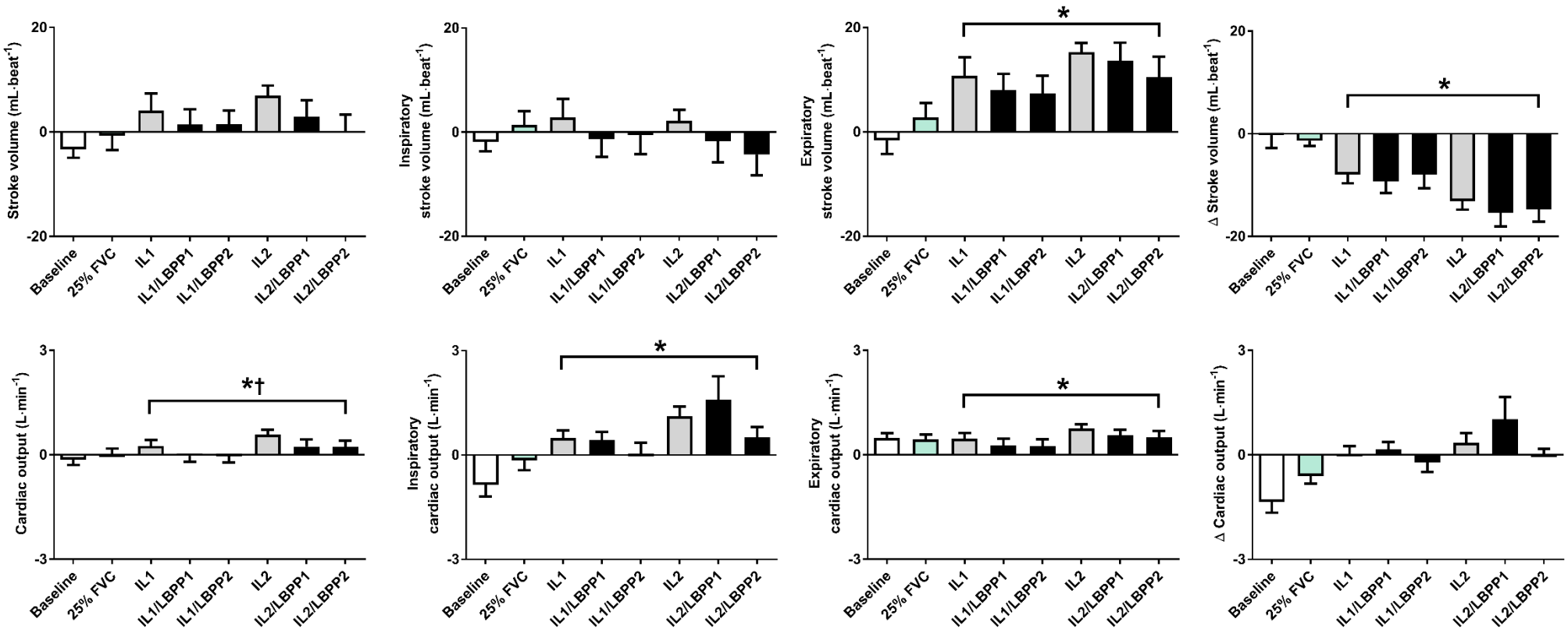


Figure 5-3 – Difference relative to slow and deep breathing at 6 breaths·min^{-1} and 40% of forced vital capacity. Data are mean ± SEM for 11 participants. Differences for baseline (white), unloaded breathing at 25% of forced vital capacity (light blue), combined slow and deep breathing with inspiratory resistances (grey) and combined inspiratory resisted slow and deep breathing with lower body positive pressure (black) are presented. Two-way repeated measures ANOVA was conducted to test the existence of *Significant main effect for inspiratory loading, † significant main effect for lower body positive pressure. Significant main effects for the interaction between inspiratory loading and lower body positive pressure were not detected. $P < 0.05$.

5-4.4 Relationship between cardiac haemodynamics and respiratory sinus arrhythmia

The RSA displayed a moderate linear correlation with both $LVSV_E$ and ΔSV (R^2 = 0.19 and 0.27, respectively; $P < 0.001$). Stronger correlations with RSA were observed for $\dot{Q}_I$ (R^2 = 0.41; $P < 0.001$) and $\Delta\dot{Q}$ (R^2 = 0.56; $P < 0.001$), while significant correlations were absent for LVSV, $LVSV_I$, $\dot{Q}$ and $\dot{Q}_E$ (Figure 5-4).

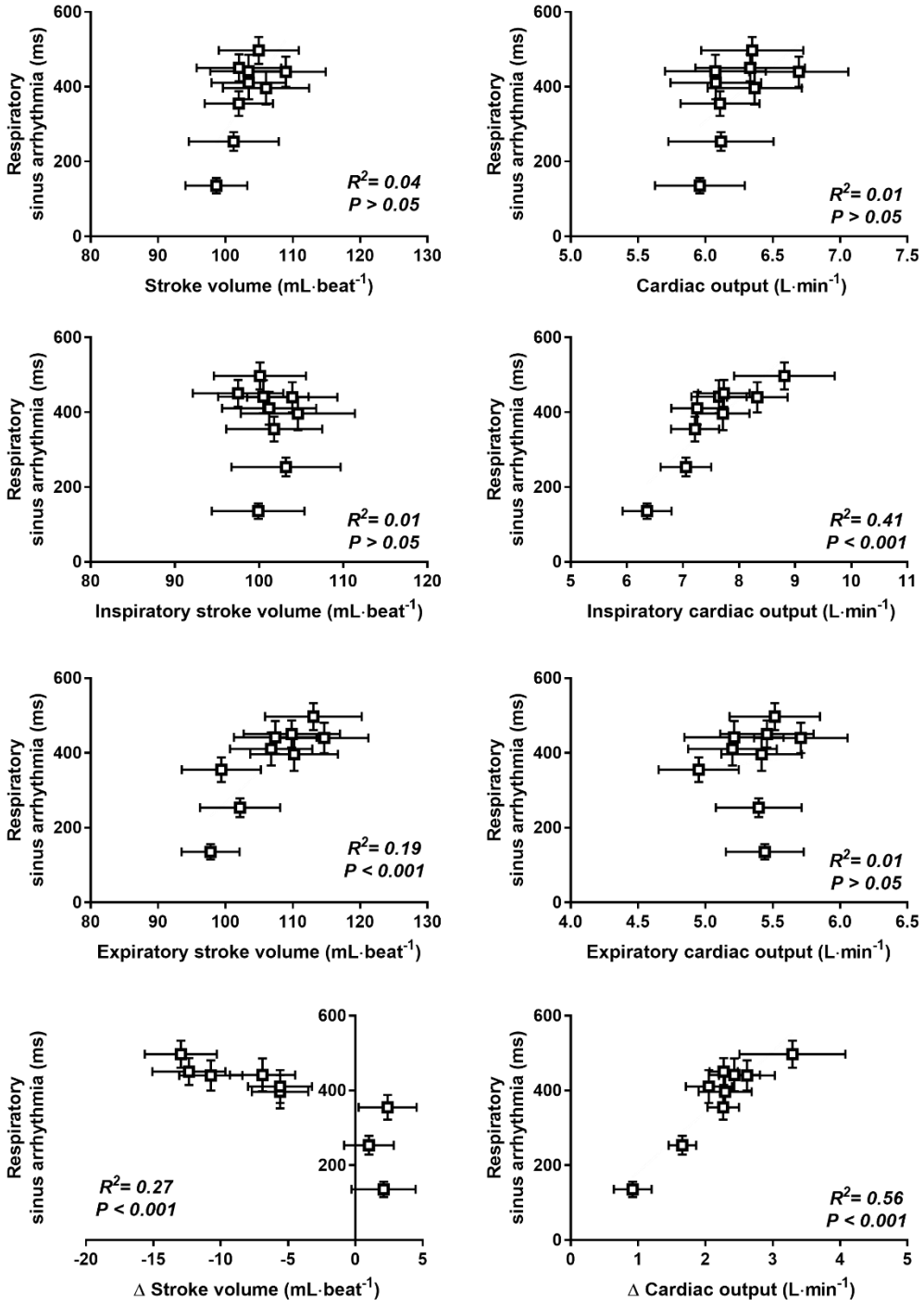


Figure 5-4 – Relationship between stroke volume (left panel), cardiac output (right panel) and respiratory sinus arrhythmia. Data are mean ± SEM for 11 participants. Corresponding R^2 values are calculated using previously described multiple regression analysis.

Strongest influences upon respiratory sinus arrhythmia to slow and deep breathing inspiratory loading and lower body positive pressure

An estimation of RSA using the previously applied three predictor model rendered similar results to the ones described in Chapter 4. When applying said three predictor model including ΔSV, *fc* and $\Delta\dot{Q}$, a significant regression equation (F (3, 95) =55.188, $P < 0.001$) with an $R^2 = 0.635$, was obtained.

Predicted RSA = 241 – 12 (ΔSV) – 7 (*fc*) + 52 ($\Delta\dot{Q}$), where ΔSV is measured in $mL \cdot beat^{-1}$, *fc* in $beats \cdot min^{-1}$ and $\Delta\dot{Q}$ in $L \cdot min^{-1}$.

Participants' RSA decreased 12 ms by each $mL \cdot beat^{-1}$ increase in within-breath variation in LVSV, reduced 7 ms by each $beat \cdot min^{-1}$ and increased 52 ms by each $L.min^{-1}$ of within-breath variation of $\dot{Q}$ ($\Delta\dot{Q}$). All three variables were significantly correlated with RSA.

5-4.5 Heart rate and blood pressure variabilities

All the analysed HRV and BPV indices exhibited statistically significant differences between some of the conditions, except in the case of the HRV_{HF} (Figure 5-5, Table 5-4). The HRV_{LF} and HRV_{TOT} indices exhibited significantly smaller values at baseline when compared to all SDB conditions (Figure 5-5, Table 5-4). These differences are especially evident in all loaded conditions, with the highest absolute values for both HRV_{LF} and HRV_{TOT} occurring with IL2, which also exhibited statistically higher values than those observed during the unloaded SDB conditions (Figure 5-5, Table 5-4). Similar trends to those found for HRV_{LF} and HRV_{TOT} were also present in the time domain variables SDNN and RMSSD and both Poincaré plot indices (SD1 and SD2; Table 5-4).

The pattern across condition observed for most BPV variables was similar to that observed for HRV spectral indices, with the highest values for BPV_{LF}, BPV_{HF} and BPV_{TOT} being observed in the IL2 sets (Figure 5-5, Table 5-4). The highest values for these three variables were recorded when IL2 was combined with a small compression at the legs (IL2/LBPP1). In this particular condition, the values were statistically higher than those of all of the unloaded breathing and IL1 breathing conditions, as well as baseline (Figure 5-5, Table 5-4).

Importantly, BPV_{TOT} correlated significantly with ΔSV (Figure 5-6, R^2 = 0.98, P < 0.001 with group mean values and R^2 = 0.30, P < 0.001 with multiple regression analysis).

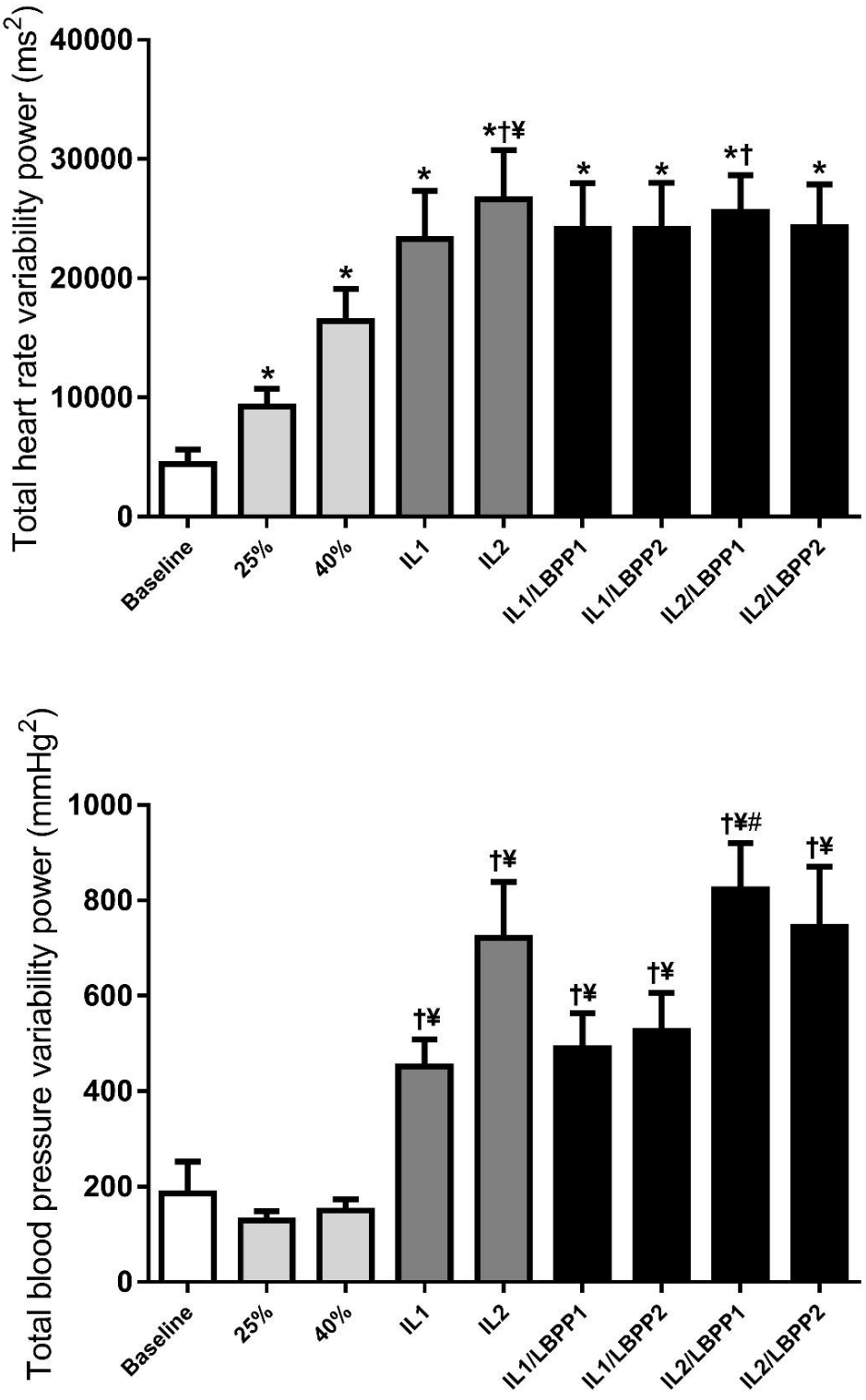


Figure 5-5 – Heart rate and blood pressure variabilities total power responses to combined slow and deep breathing, inspiratory loading and lower body positive pressure. Data are mean ± SEM for 11 participants. * Significantly different from baseline. † Significantly different from breathing at 25%FVC. ¥ Significantly different from breathing at 40%FVC, # Significantly different from all light inspiratory loaded conditions (IL1, IL1/LBPP1 and IL2/LBPP2). P < 0.05.

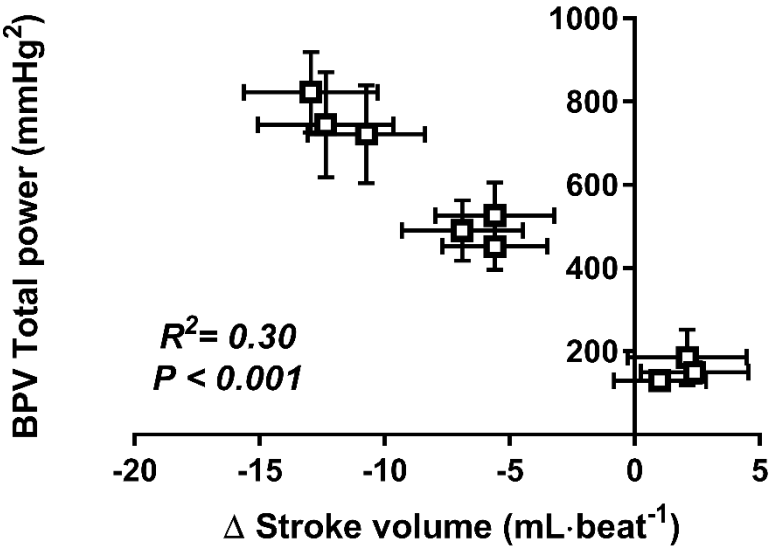


Figure 5-6 – Relationship between the within-breath variation in stroke volume and blood pressure variability total power. Data are mean ± SEM for 11 participants. Corresponding R^2 values are calculated using previously described multiple regression analysis.

Table 5-4 – Heart rate and blood pressure variabilities response to slow and deep breathing with different grades of inspiratory loading and lower body positive pressure.

	Baseline	**25%FVC**	**40%FVC**	**IL 1**	**IL1/LBPP1**	**IL1/LBPP2**	**IL 2**	**IL2/LBPP1**	**IL2/LBPP2**
SDNN (ms)	79±9	103±8*	131±11*†	157±14*†	158±13*†	158±15*†	167±14*†‡	167±9*†‡	161±12*†
RMSSD (ms)	62±8	73±8	103±13	121±16	129±16	130±17	132±15*†	139±12*†	129±14*†
HRV_{LF} (ms^2)	2849±1047	7863±1182*	13845±2143*	20080±3423*	20370±2999*†	19927±2927*†	22216±3218*†‡	20876±2278*†	20234±2910*
HRV_{HF} (ms^2)	1572±318	1402±532	2551±750	3202±832	3754±1131	4213±1250	4405±1059	4702±1007	4050±847
SD1 (ms)	44±6	51±6	73±9	86±11	91±12	92±12	93±11*†	99±9*†	91±10*†
SD2 (ms)	101±12	136±10	169±14*	203±18*†	203±16*†	204±18*†	216±17*†‡	213±11*†‡	208±15*†
SD1/SD2	0.45±0.06	0.37±0.02	0.42±0.03	0.41±0.03	0.43±0.03	0.44±0.03	0.42±0.02	0.46±0.03	0.43±0.02
BPV_{LF} ($mmHg^2$)	84±33	117±17	131±23	342±43†‡	354±56†‡	390±61†‡	472±70*†‡¥ŧ	485±53*†‡¥ŧ	446±70*†‡
BPV_{HF} ($mmHg^2$)	102±37	13±4	18±5	110±24†‡	136±23†‡	136±24†‡	250±59*†‡¥ŧ	338±54*†‡¥ ŧ#	299±65†‡

Data represent mean ± SEM for 11 subjects. * different from baseline, † different from 25%FVC, ‡ different from 40%FVC, ¥ different from IL1, ŧ different from IL1/LBPP1, # different from IL1/LBPP2, $P < 0.002$. Note: cf. Figure 5-5 for total variability power data.

5-4.6 Cross-spectral phase angle and time shift

There were significant differences in phase angle and time shift for all of the test conditions when compared to baseline for the RESP-*fc*, RESP-SBP and SBP-*fc* relations (Tables 5-5 and 5-6). At baseline, the decrease in SBP seemed to precede the inspiratory onset (cf. General Methods – Chapter 3; and Chapter 4; for a detailed explanation), while the opposite was observed during SDB (both loaded and unloaded). No significant differences between conditions were found for the RESP-DBP and SBP-DBP relations, despite the existence of the main effect of SDB for SBP-DBP (Tables 5-5 and 5-6).

Table 5-5 **–** Phase angle response to slow and deep breathing with different grades of inspiratory loading and lower body positive pressure for respiration, heart rate and blood pressure.

Relation	Baseline	25%FVC	40%FVC	IL 1	IL1/LBPP1	IL1/LBPP2	IL 2	IL2/LBPP1	IL2/LBPP2
RESP – *fc* (deg)	20±9	74±7*	74±9*	93±6*	93±4*	91±7*	100±5*	102±7*	104±6*
RESP – SBP (deg)	-64±14	48±11*	51±9*	62±8*	64±5*	62±5*	65±9*	70±5*	67±6*
RESP – DBP (deg)	69±20	101±15	116±20	104±16	127±14	129±14	113±13	123±10	117±11
SBP – *fc* (deg)	82±8	24±8*	22±11*	30±7*	29±7*	29±7*	35±6*	32±6*	37±7*
SBP – DBP (deg)	116±22	53±13	63±18	43±12	62±14	65±15	49±10	54±6	43±7

Data represent mean ± SEM for 11 subjects. * Different from baseline. $P < 0.002$.

Table 5-6 **–**Time shift responses to slow and deep breathing with different grades of inspiratory loading and lower body positive pressure for respiration, heart rate and blood pressure.

Relation	Baseline	25%FVC	40%FVC	IL 1	IL1/LBPP1	IL1/LBPP2	IL 2	IL2/LBPP1	IL2/LBPP2
RESP – *fc* (s)	0.27±0.12	2.05±0.21	2.05±0.25	2.58±0.18	2.59±0.11	2.52±0.19	2.79±0.15	2.85±0.19	2.90±0.16
RESP – SBP (s)	-0.87±0.19	1.35±0.30	1.43±0.26	1.72±0.21	1.77±0.13	1.71±0.14	1.81±0.24	1.94±0.15	1.86±0.18
RESP – DBP (s)	0.94±0.26	2.82±0.43	3.22±0.55	2.90±0.44	3.54±0.38	3.59±0.39	3.13±0.36	3.42±0.27	3.26±0.31
SBP – *fc* (s)	1.11±0.11	0.66±0.22	0.62±0.31	0.84±0.21	0.80±0.21	0.79±0.21	0.98±0.17	0.89±0.16	1.04±0.19
SBP – DBP (s)	1.57±0.30	1.48±0.37	1.76±0.51	1.18±0.32	1.73±0.38	1.80±0.42	1.35±0.28	1.49±0.17	1.20±0.19

Data represent mean ± SEM for 11 subjects.

5-4.7 Relationship between cardiovascular, heart rate variability, blood pressure variability and cross-spectral time shift indices.

The strongest linear correlations with the different time shift indices were observed for ΔSV (RESP-*fc*: R^2 =0.98; RESP-SBP: R^2 =0.93; SBP-*fc*: R^2 =0.87; $P < 0.001$; Figure 5-7) and BPV_{TOT} (RESP-*fc*: R^2 =0.96; RESP-SBP: R^2 =0.94; SBP-*fc*: R^2 =0.83; $P < 0.001$; Figure 5-8). In contrast, $\Delta\dot{Q}$ only exhibited a moderate relationship with RESP-SBP (R^2 =0.57; $P < 0.05$) while no association was found with RESP-*fc* and SBP-*fc* ($P > 0.05$; Figure 5-7).

The RSA and HRV_{TOT} displayed moderate to high correlations with RESP-*fc*, RESP-SBP and SBP-*fc* (RSA: R^2 = 0.79, 0.91 and 0.54 respectively, $P < 0.05$; HRV_{TOT}: R^2 = 0.79, 0.87 and 0.59 respectively, $P < 0.05$ Figure 5-8).

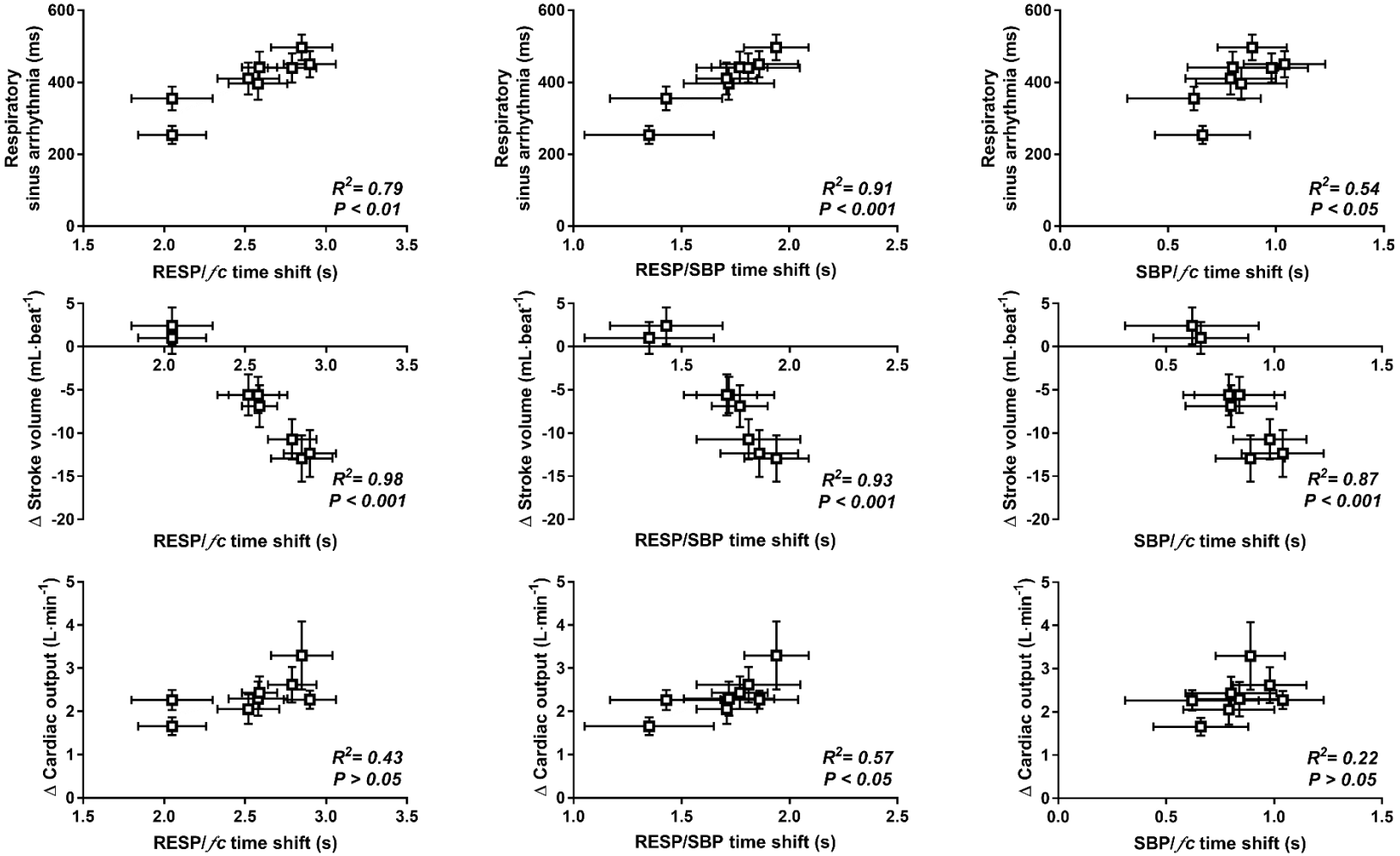


Figure 5-7 – Relationship between cardiovascular and cross-spectral time shift indices. Data are mean ± SEM for 11 participants. Corresponding R^2 values are calculated using group mean values.

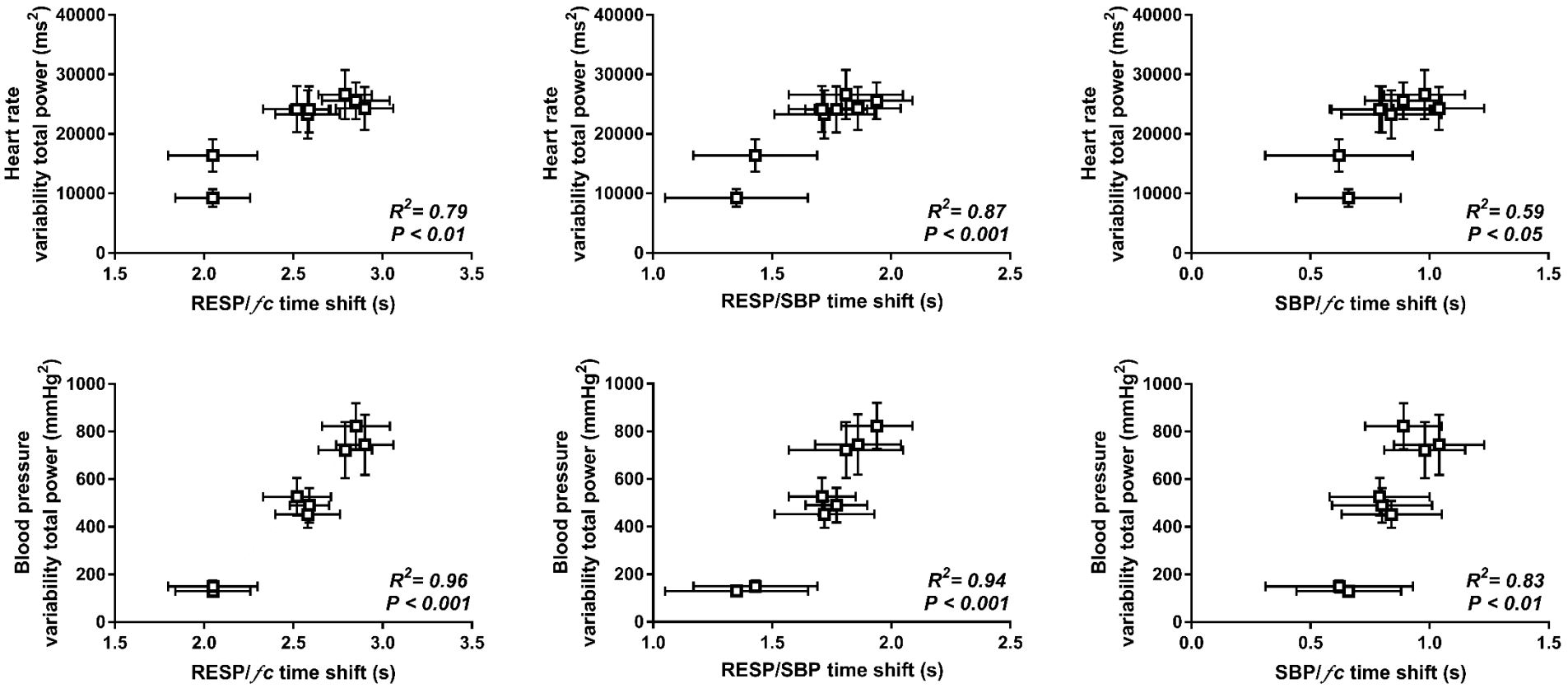


Figure 5-8 – Relationship between heart rate variability total power, blood pressure variability total power and cross-spectral time shift indices. Data are mean ± SEM for 11 participants. Corresponding R^2 values are calculated using group mean values.

5-5 Discussion

This study sought to clarify whether the addition of small inspiratory loads amplifies the known acute haemodynamic effects of SDB and whether mechanically-driven variations in systemic venous return underpin such cardiovascular responses. The study also attempted to shed light on whether the application of inspiratory loading could provide a further understanding of the acute control mechanisms relevant to the cardiovascular response to SDB. A major finding was that the combined application of SDB and IL promoted significant increases in $LVSV_E$ and ΔSV, as well as $\dot{Q}$, $\dot{Q}_I$, $\dot{Q}_E$ and RSA, when compared to unloaded SDB, while only $\dot{Q}$ was affected by LBPP. These changes occurred without any significant alteration of MAP during to both IL or LBPP. The application of LBPP attenuated the response of $\dot{Q}$ to IL, supporting the idea that IL operates via a mechanism that involves mechanical modulation of systemic venus return. The RSA correlated strongly with the amplitude of within-breath fluctuations of LVSV and $\dot{Q}$, providing support for an important functional link between the respiratory driven fluctuations in LVSV and RSA. Consistent with this notion, were the significant effects of IL upon spectral measures of HRV and BPV. Collectively, the data support the existence of an important contribution of intrathoracic pressure swings to the generation of within-breath fluctuations in LVSV, leading to *fc* and ABP variations, which are attributable to both mechanical and reflex mechanisms. The addition of small inspiratory loads to SDB seems to amplify the acute cardiovascular and autonomic responses to SDB.

5-5.1 Effects of loaded breathing upon systemic haemodynamic response

A first aim of the study was to examine the haemodynamic response to inspiratory resisted SDB. Significant main effects were detected for IL upon $\dot{Q}$, $\dot{Q}_I$ and $\dot{Q}_E$ (Table 5-2, Figure 5-3). Despite significant main effects of IL and/or LBPP being found for MAP, SBP, DBP and TPR, only DBP exhibited a significant difference between conditions after *post-hoc* analysis; more precisely, DBP differed between baseline and IL1/LBPP2 (72±2 vs 79±2 mmHg, respectively). Nonetheless, it is important to highlight that the magnitude of changes of SBP between baseline and SDB with IL1 (~7 mmHg increase with IL1, and ~13 mmHg increase if the combined IL1 and LBPP conditions) are comparable to previous studies, which have reported significant ABP increases with small inspiratory resistances (Convertino, Ratliff, Ryan, Cooke, et al., 2004, Convertino, Ratliff, Ryan, Doerr, et al., 2004, Cooke et al., 2006). Therefore,

the results reported herein are consistent with the known acute effects of breathing against small inspiratory resistances (between 6 and 12 cmH_2O), which include increases in LVSV, $\dot{Q}$ and MAP in both normovolemic supine individuals (Convertino, Ratliff, Ryan, Doerr, et al., 2004, Cooke et al., 2006) and in hypovolemic models (Convertino, Ratliff, Crissey et al., 2005, Ryan et al., 2008, Convertino et al., 2011). A salient finding of the present study was the difference in ΔSV, which became increasingly negative (bigger $LVSV_E$ relative to $LVSV_I$) with the increase in inspiratory load magnitude. It has been suggested that the left ventricular expression of respiratory related changes in systemic venous return is delayed by the resistance and capacitance of the pulmonary circulation in such a way that an inspiratory increase in right-atrial filling is expressed as an increase in $LVSV_E$ (Dornhorst et al., 1952b, Hamzaoui et al., 2013), which helps to explain the changes in $LVSV_E$ and ΔSV observed with our intervention. As discussed in the previous chapter (cf. 4-5.1), PulTT has a high inter-individual variability and is sensitive variations in $\dot{Q}$ (Lund et al., 1997, Zavorsky et al., 2003). Accordingly, our results might be limited to individuals with similar characteristics to our sample. In healthy individuals, a fall in intrathoracic pressure is accompanied by increase in transmural pressures for the left atria, pulmonary artery, right atria and aorta, as a result of pleural pressure falling more than cardiac chamber pressures (Alian and Shelley, 2012). Thus, a favourable pressure gradient is created throughout inspiration as a result of a progressively more negative right atrial pressure with the decrease in intrathoracic pressure, contributing to an inspiratory increase in systemic venous return (Brecher, 1952, Guyton et al., 1957). This effect and the impact it has upon LVSV is limited by a combination of factors that include: i) venae cava collapse (Guyton and Adkins, 1954, Kimura et al., 2011), ii) ventricular interdependent mechanisms (Brinker, Weiss, Lappe et al., 1980, Amoore and Santamore, 1989, Amoore, Santamore, Corin et al., 1992) and, iii) the interplay between intrathoracic and abdominal pressures (Miller, Pegelow, et al., 2005b). These factors impact both cardiac and aortic transmural pressures and therefore preload and afterload (Robotham and Mintzner, 1979, Scharf et al., 1979, Robotham et al., 1985, Takata et al., 1990, Scharf, 1995). The influence of these mechanisms helps to explain why human $\dot{Q}$ and MAP are relatively insensitive to fluctuations in venous return (Triedman and Saul, 1994).

Another salient finding from the current study is the significant magnification of RSA with IL, which was accompanied by unchanged mean *fc*, suggesting no changes in

cardiac vagal tone with the application of IL. The increase in RSA was accompanied by a trend for the inspiratory *fc* to increase (fc_I: 64±2 vs. 71±3 vs. 74±3 vs. 80±4 beats·min^{-1} for baseline, unloaded SDB at 40%FVC, IL1 and IL2, respectively), confirming previous observations with inspiratory (St. Croix et al., 1999) and dually resisted breathing (Blaber and Hughson, 1996, Calabrese, Dinh, Eberhard et al., 1998), which demonstrated the existence of a positive association between RSA and the magnitude of respiratory loading.

The study findings support an additive effect of IL to the within-breath responses of $\dot{Q}$ and LVSV to SDB, via a respiratory driven increase in venous return. However, it is not clear exactly which mechanisms are responsible for the observed increase in RSA. Some possibilities include, but are not limited to, a) reflex (Bainbridge, 1915, Bernardi et al., 1989, Pawelczyk and Levine, 1995, Barbieri et al., 2002) or direct mechanical stimulation of the sinus by atrial distention (Bolter and Wilson, 1999, Wilson and Bolter, 2002, Cooper and Kohl, 2003, Quinn and Kohl, 2012), b) carotid and aortic baroreflex response to variations in left ventricular output (Triedman and Saul, 1994, Karemaker, 2009a), and/or c) stimulation of lung stretch receptors (Anrep et al., 1936a, Gandevia et al., 1978, Taha et al., 1995). Furthermore, the fact that the role of *fc* oscillations (with emphasis on RSA) upon the amplification or buffering of the arterial ABP oscillations is surrounded by controversy (Buchner, Żebrowski and Gielerak, 2010) makes the identification of the underlying physiological mechanisms, particularly difficult. Fundamentally, the contradictions between studies seem to be due primarily to postural differences. For example, Elstad and colleagues (2001) reported opposite effects of RSA upon SBP and MAP in supine, but not in upright humans, while drastic reductions in RSA due to vagal blockade (Taylor, Carr, Myers et al., 1998) and fixed rate atrial pacing (Taylor and Eckberg, 1996) are accompanied by reduced SBP oscillations in the supine, but not in the upright position.

5-5.2 Potential influences upon baroreceptor stimulation

Cardiac BRS indices were analysed based on both the sequence-method and the cross-spectral transfer function between SBP and *fc* at a frequency corresponding to f_R. Interpretation of these data carries the same caveats as were articulated in the previous chapter (cf. 4-5.1 – 'Impact on baroreflex sensitivity'). No indication of any significant alteration of BRS in the presence of SDB with IL or IL combined with

LBPP was found. The only exception was the difference observed between unloaded breathing at 6 breaths·min^{-1} at 40% FVC and baseline for BRS_{up} (41±6 and 19±4 ms·mmHg^{-1}, respectively; P <0.05; Table 5-1), which is in line with the results from Study 1 (Chapter 4). Mean *fc* remained unchanged throughout the intervention and no statistical differences between conditions were found for mean MAP, SBP and TPR (despite a significant main effect of the combined application of IL and LBPP being detected by the repeated measures one-way ANOVA). Nonetheless, it is important to mention that the magnitude of the trend of SBP and MAP between baseline and IL1 (SBP: ~7 mmHg with IL1, and reaching ~13 mmHg if the combined IL1 and LBPP conditions are considered; MAP: ~5 mmHg with IL1) is comparable to other studies that reported significant ABP increases with small inspiratory resistances (Convertino, Ratliff, Ryan, Cooke, et al., 2004, Convertino, Ratliff, Ryan, Doerr, et al., 2004, Cooke et al., 2006). The application of small inspiratory impedances (~10 cmH_2O) has been proposed previously to induce a 'resetting' of the cardiac vagal baroreflex response, as suggested by unaltered *fc* and BRS in the presence of significant MAP increase (Convertino, Ratliff, Ryan, Cooke, et al., 2004). Hypothetically, this change in the operating point of the baroreflex provides a physiological advantage by allowing increases in f_C even with high MAP, thus improving $\dot{Q}$. While the author might be inclined to interpret the presented data as a confirmation of Convertino's (2004) construct, there are methodological concerns that must be emphasised. Firstly, the usefulness of both time- and frequency-domain mathematical based techniques for the evaluation of cardiac baroreflex response to respiratory challenges is questionable. Some limitations of these techniques have been highlighted previously in Chapter 4 (cf. 4-5.1). Furthermore, the analysis of the cardiac baroreflex response may yield different outcomes depending on the ABP variable considered: SBP or MAP (Elstad et al., 2001, Buchner et al., 2010). Arguably, the use of MAP, and not SBP, provides a better representation of the stimulus being sensed by the baroreceptors, particularly as the respiratory-driven fluctuations in MAP are less dependent on the position of the body (Buchner et al., 2010). Moreover, body position seems to play a relevant role on the ability of the cardiac baroreflex responses to counteract increased BPV. In the supine position, the magnitude of ABP fluctuations seems to be amplified by HRV itself, as *fc* seems to lead SBP fluctuations, while the opposite occurs with upright postures at the respiratory frequency (Taylor and Eckberg, 1996, Cooke et al., 1999, Buchner et al., 2010), adding another confounding factor to the interpretation of both time-domain and spectral analysis of the cardiac baroreflex

control loop. These caveats make the interpretation BRS data problematic and highlight the confounding nature that posture might have on the evaluation of cardiovascular response to SDB challenges. Thus, it is not possible to discern whether inspiratory loaded SDB acts acutely to alter the gain of the vagal cardiac baroreflex response.

The addition of IL magnifies the negative intrathoracic pressure at any given V_T (Seals et al., 1993). This amplifies the known, V_T-dependent, fluctuations in intrathoracic, transmural and intravascular pressures that occur with normal, un-resisted breathing (Dornhorst et al., 1952b, Wise et al., 1981). Intravascular pressures primarily influence the carotid baroreceptors, while aortic baroreceptors are mostly influenced by aortic transmural pressure (Angell James, 1971, Fitzgerald, Robotham and Anand, 1981). This becomes particularly relevant with resisted inspiratory efforts as aortic transmural pressure is increased due to a more pronounced decrease in intrathoracic pressure than the observed reduction in systemic intravascular pressure (Robotham and Mintzner, 1979, Karam et al., 1984, Peters, Fraser, Stuart et al., 1989). The increase in aortic transmural pressure can alter the relative stimulation of cardiopulmonary, aortic and carotid baroreceptors (Wallin and Fagius, 1988) when compared to spontaneous, un-resisted, breathing. In other words, during inhalation, the intravascular pressure is decreased, which 'unloads' the carotid baroreceptors, while aortic transmural pressure is increased, which 'loads' the aortic baroreceptors. Contrarily, during expiration, as LVSV increases, both aortic and carotid baroreceptors might be stimulated simultaneously due to increased intravascular pressure. The net baroreflex response is thus a composite of the signals from aortic and carotid baroreceptors.

As mentioned previously in this chapter, inspiratory loaded breathing is believed to augment systemic venous return to the right atrium and enhance right atrial filling. Right atrial blood volume increases are known to elicit tachycardic responses in human beings (Pawelczyk and Levine, 1995, Barbieri et al., 2002); commonly known as Bainbridge reflex. This chronotropic effect can also be accomplished by direct mechanical stretch of the sinus node, without the involvement of neuronal pathways (Bolter and Wilson, 1999, Wilson and Bolter, 2002, Cooper and Kohl, 2003, Quinn and Kohl, 2012), and has been previously been suggested to occur in healthy human beings with inspiratory loaded breathing (Cooke et al., 2006). Despite not assessing RVSV in the current study, the significant increase in ΔSV

and $LVSV_E$ (ΔSV: 40% FVC = 2±2, IL1 = -6±2, IL2 = -11±2 mL·beat^{-1}; $LVSV_E$: 40% FVC = 99±6, IL1 = 110±7, IL2 = 115±7 mL·beat^{-1}) is most likely due to respiratory fluctuations in systemic venous return, as demonstrated recently in resting human beings in the left lateral decubitus position (Elstad, 2012).

Simultaneously, it is assumed that amplified LVSV oscillations result in phasic variations of ABP throughout the respiratory cycle; this assumption is supported by the observed increase in BPV_{TOT} with increasingly negative intrathoracic pressures (BPV_{TOT}: unloaded 40% FVC < IL1 < IL2; Figure 5-5, $P < 0.05$) and by significant relationship between the BPV_{TOT} and ΔSV (Figure 5-6, $R^2 = 0.98$, $P < 0.001$). Based on the previous reasoning, if the cardiac chronotropic response to inspiratory resisted SDB was determined mainly by baroreflex mechanisms, a decrease in *fc* would be expected during the expiratory phase; furthermore, the conflicting effects of IL upon aortic and carotid baroreceptors throughout inspiration would be expected to limit the net cardiac-baroreflex response. No differences were observed in either fc_E and fc_I, despite the existence of a slight, non-significant, trend for fc_I to increase with the increments in IL (40% FVC = 71±3, IL1 = 74±3, IL2 = 80±4; Table 5-1). The author's findings support a potential involvement of atrial stretch mechanisms to the magnification of fc_I and RSA, thereby questioning the contribution of baroreflex mediation of respiratory-driven *fc* fluctuations in inspiratory resisted SDB.

5-5.3 Effects of loaded breathing upon HRV and BPV

Heart rate variability

The response of HRV to different breathing patterns was systematically evaluated in chapter 4 of this thesis and has also received attention from other researchers (Cooke et al., 1998, Lehrer et al., 1999, Stark et al., 2000, Song and Lehrer, 2003, Chang et al., 2013). In contrast, the effect of breathing with an inspiratory load upon HRV has been largely unexplored, and to the best of the author's knowledge, no studies have been conducted in human beings exploring the acute, combined effects of SDB and inspiratory loading.

A main finding of this study is the existence of significant increments in HRV in the time domain (SDNN and RMSSD), frequency domain (HRV_{LF} and HRV_{TOT}) and Poincaré indices (SD1 and SD2) with increments in IL magnitude during SDB. The consensus is that for both time and frequency domain HRV parameters, there is a

positive linear correlation with V_T, whilst the correlation is negative for f_R (Cooke et al., 1998, Song and Lehrer, 2003, Larsen et al., 2010). By fixing f_R, V_T and P_aCO_2, my study design eliminated the confounding effect of changes in breathing pattern between conditions and permits the conclusion that increases in HRV with IL stem from the increasingly negative intrathoracic pressures. These findings contradict, in part, those of Calabrese and colleagues (2000), who studied the impact of progressive resisted breathing of both breath phases, simultaneously, upon HRV and RSA. In common with the present study, they observed a significant increase in HRV and RSA that paralleled the magnitude of the respiratory resistance. However, because Calabrese and colleagues' study was conducted in spontaneously breathing individuals, the increments in load were accompanied by a systematic reduction in f_R and increase in V_T. When Calabrese and colleagues statistically controlled for the changes in breathing pattern, no significant differences between loaded and unloaded conditions were observed, leading the authors to conclude that HRV and RSA amplitude during loaded breathing was determined mainly by ventilatory compensatory mechanisms associated with loaded breathing (Calabrese et al., 2000). The presented data do not support this interpretation as the changes in HRV and RSA in response to IL occurred in the absence of variations in f_R and V_T (and therefore $\dot{V}_E$). Instead, I believe that the significant correlations between RSA and ΔSV, $\Delta\dot{Q}$ and $\dot{Q}_I$ (Figure 5-4), as well as the very strong correlations between BPV_{TOT} and ΔSV (Figure 5-6) support the influence of mechanical effects of breathing upon LVSV and the existence of a functional link with RSA.

The latter interpretation is supported by an earlier study (Blaber and Hughson, 1996), which used fixed-paced breathing at 12 breaths·min^{-1} with simultaneous inspiratory and expiratory loading, and suggested that the increases in LVSV associated with resisted breathing were intimately related to the increase in HRV at the respiratory frequency. My study has important methodological differences to both of these previous studies (Blaber and Hughson, 1996, Calabrese et al., 2000); in particular, the fact that the present study loaded only inhalation. The discrepancies between studies raise the question of whether different modalities of loaded breathing have distinct effects upon the cardiovascular response to SDB, and if this translates to concomitant dissimilarities in HRV. This uncertainty will be addressed in the final study of this thesis (Chapter 6).

The potentially important role of right atrial filling in the chronotropic response (Bainbridge reflex) to inspiratory resisted SDB was discussed above. This mechanism most likely impacts HRV at the respiratory frequency, but the contribution of vagal feedback from lung stretch receptors also merits consideration as a potential contributor to HRV, as suggested by a much reduced of RSA in lung denervated patients breathing at a fixed f_R and high V_T and/or IL, when compared with healthy individuals (Taha et al., 1995). Furthermore, evidence from spontaneously breathing animal models indicates increased activation of slowly adapting pulmonary stretch receptors (Widdicombe, 1961a, Davenport et al., 1981, Barrière, Delpierre, Del Volgo et al., 1993) with resisted breathing. These data support the case for a contribution from respiratory vagal afferent feedback to increases in HRV and RSA with inspiratory loading.

Blood pressure variability

The assessment of combined SDB and IL also yielded novel findings in relation to BPV. A noteworthy finding was a significant main effect of IL for BPV_{LF}, BPV_{HF} and BPV_{TOT}. Compared to unloaded SDB, BPV_{LF} was more than doubled with IL1 and quadrupled with IL2. Similarly, BPV_{HF} increased progressively with the increase in inspiratory load (18±5 vs. 110±24 vs. 250±59 $mmHg^2$ for SDB alone, IL1 and IL2, respectively). The frequency-dependent response of BPV during paced breathing was established recently (Chang et al., 2013), but the present study is the first to describe the response during IL. The BPV_{LF} is believed to arise from changes in vascular tone and peripheral resistance and to reflect the sympathetic modulation of vascular regulation, while BPV_{HF} has been ascribed primarily to the respiratory modulation of ABP (Pagani, Lombardi, Guzzetti et al., 1986, Di Rienzo et al., 1991, Parati, Saul, Di Rienzo et al., 1995). Both f_R and V_T seem to have important modulatory effects upon muscle sympathetic nerve activity (MSNA). This is most likely associated with vagally mediated lung inflation feedback (Seals et al., 1990, Seals et al., 1993, St. Croix et al., 1999, Limberg et al., 2013), since increasing respiratory motor output with IL does not seem to affect sympathetic nerve activity discharge (Seals et al., 1993, St. Croix et al., 1999). In the present study, f_R and V_T were identical in all conditions (except for unloaded SDB at 25%FVC), and f_R remained in the LF band. Therefore, in the present study, the BPV_{LF} represents SBP variations due to respiratory modulation of LVSV, *fc* and $\dot{Q}$, entrained with naturally occurring fluctuations in ABP at 0.1Hz. These are unlikely to be associated to the

respiratory modulation of MSNA, but instead to be related to: 1) resonant oscillations in the sympathetic component of the baroreflex loop (De Boer et al., 1987, Malpas et al., 2001, Julien, 2006); 2) the intrinsic regulation of vascular regulation by endothelial nitric oxide release following cyclic variations in ABP (Malpas, 2002), or 3) the manifestation of the myogenic activity of the smooth muscle cells (Stefanovska and Bracic, 1999a). While it is impossible to quantify the contribution and interplay of these mechanisms to the observed changes in BPV_{LF}, it is reasonable to infer that the results represent the direct impact of the respiratory driven variations in left ventricular output, as well as cardiac and aortic transmural pressures, upon SBP fluctuations.

While the association between the respiratory modulation of LVSV and low-frequency BPV seems reasonable due to the low f_Rs employed in this study, the significant increase in BPV_{HF} as a result of the application of progressive IL is harder to explain, as it is unlikely any contribution from a high-frequency respiratory component. Earlier it has been suggested that vagally mediated changes in *fc* and $\dot{Q}$ might contribute to the BPV_{HF} (Saul et al., 1991). Saul's study reduced the respiratory impact of breathing upon spectral analysis by leading participants to breathe at irregularly spaced intervals corresponding to f_Rs from 4 to 60 breaths·min-1. Nonetheless, the existence of HF respiratory oscillations was likely sufficient to generate relevant HF HRV and BPV. One plausible explanation for the present results involves the presence of clear harmonics of the 0.1 Hz peak in both the HRV and BPV spectrum, for most participants (Figure 5-9). These harmonic components result from the RR and SBP waveforms not being true sine wave signals. Consequently, Fourier analysis applied to such data produces power spectra that consists of the fundamental frequency component (first harmonic), together with the second, third and even fourth order harmonics (Ramirez, 1985). It is not clear if harmonics in BPV reflect vagal activity, but a few [scarce] studies have reported an increase in HRV_{HF} with SDB (Strauss-Blasche, Moser, Voica et al., 2000, Van Diest, Verstappen, Aubert et al., 2014). Interestingly, in our study the power spectrum of both SBP and RR interval showed similar harmonics, which has been previously proposed to potentially signify the contribution of cardiac baroreflex regulation (Van Diest et al., 2014). The same authors attributed the presence of harmonics on HRV data to an imperfect sine wave respiratory signal and/or to deficient tracking of the imposed breathing pattern by the participants. Despite not reporting the power spectrum of the respiratory signal (RESP) in my study, the observation of the

individual tachograms allows to affirm that the observed harmonics are unlikely to be related to any respiratory component, as no HF peaks were identified, in any of the participants breathing at 6 breaths·min^{-1}. Further to the presence of the harmonics, the non-true sine wave characteristics of cardiovascular waveforms makes the application of spectral Fourier transformation result in the creation of new spectral peaks at a given repetitive frequency. This effect known as leakage is limited by the use of 'windowing' techniques, though its application to HRV and BPV data might not be sufficient to entirely eliminate its presence. The efficacy of such techniques in counteracting spectral leakage seems to be inherently related to the window size (Singh, Vinod, Saxena et al., 2004).

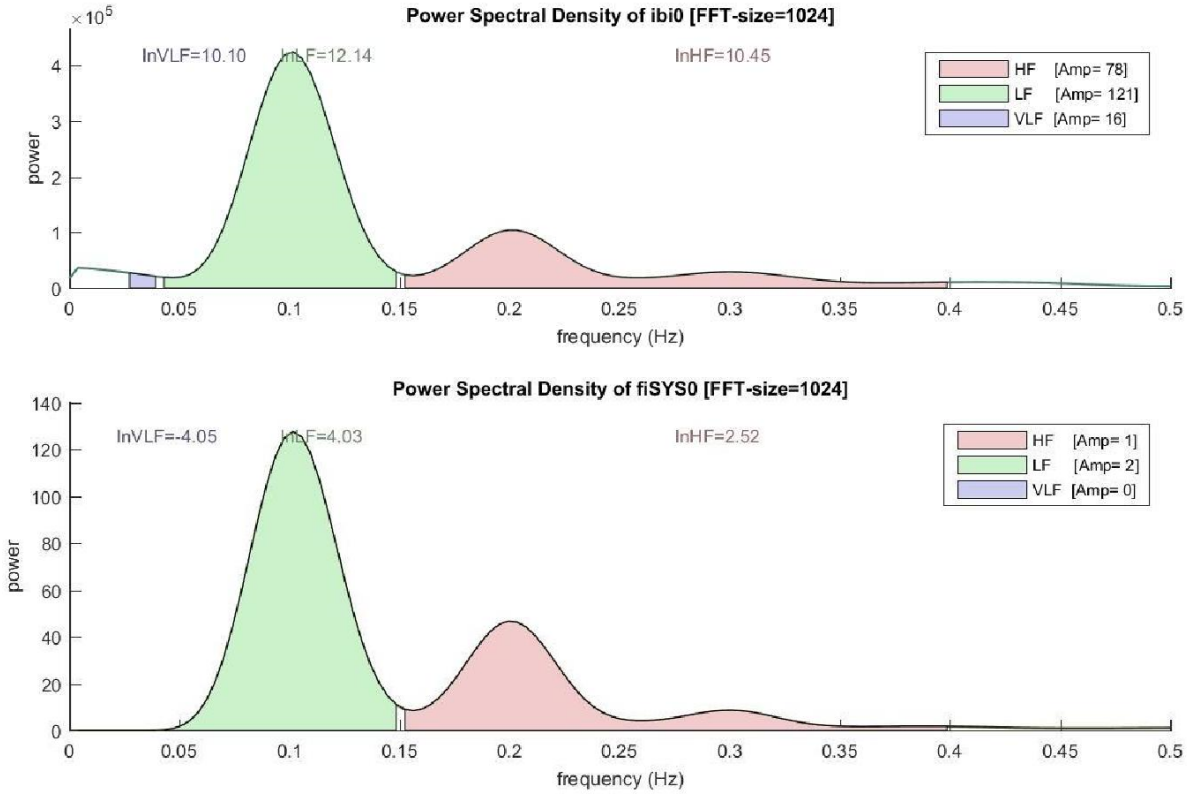


Figure 5-9 – Data from one individual breathing at 6 breaths·min^{-1} denoting the presence of high-frequency harmonic components in the HRV (top) and BPV (bottom) tachograms. Note how the dominant low-frequency peak is replicated with decay at 0.2, 0.3Hz despite the inexistence of high-frequency respiratory oscillations.

Whether the significant increases in BPV_{HF} encountered in the present study are a reflection of the presence of physiological mechanisms, or the expression of the limitations of the application of frequency domain methods to non-sinusoidal data, is unclear and merits further investigation.

In common with HRV, the low-frequency component of BPV contained the majority of the observed variability (Figure 5-5). The general assumption is that short term variations in ABP are dampened by the vagal cardiac-baroreflex, effectively trading high-frequency BPV for HRV (Karemaker and Wesseling, 2008). However, the significant increases in BPV_{LF} with the application of IL seemed not to be paralleled by similar-sized fluctuations in HRV_{LF} (Table 5-4). This suggests a finite ability of *fc* fluctuations to buffer increased BPV during IL. This interpretation of the present findings agrees with data from atrial pacing studies in healthy human beings (Taylor and Eckberg, 1996) showing increased BPV with the abolition of HRV. The unaltered BPV at the respiratory frequency with sino-atrial denervation in conscious cats (Mancia et al., 1999) provides further support to the argument of a limited buffering ability of BPV by HRV, through the action of a cardiac baroreflex mechanism. Additionally, Taylor and Eckberg (1996) demonstrated that the dampening properties of baroreflex modulation of *fc* act predominantly upon DBP, increasing diastolic runoff time (De Boer, Karemaker and Strackee, 1985a, De Boer et al., 1987), and that the effects upon SBP are only evident in situations of augmented sympathetic outflow, i.e. not observed in supine, resting individuals. While DBP variability was not measured in the present study, the moderate association between HRV and BPV total spectral powers suggests that in seated healthy individuals, undergoing inspiratory loaded SDB, HRV cannot be exclusively determined by baroreflex modulation, and/or that such a mechanism is inherently unable to buffer fluctuations in SBP under these conditions. Thus, other sources of variability, such as that resulting from sino-atrial node stretch and vagally mediated feedback arising from the lungs, should be considered when interpreting HRV data.

5-5.4 Effects of loaded breathing upon phase angle relationships

To the best of the author's knowledge, the temporal relationship between breathing, *fc* and ABP during (exclusively) inspiratory resisted SDB has not been described previously. In this study, a significant increase was observed in RESP-*fc*, decrease in SBP-*fc* and inversion of RESP-SBP phase angle (from SBP fluctuations 'preceding' respiratory swings to trailing them with SDB) from baseline to all SDB conditions. A small, non-significant trend for RESP-*fc* to increase from unloaded SDB at 6 breaths·min^{-1}, and during 40% FVC with the addition of both IL1 and IL2 was also observed (Table 5-5). These findings are interpreted as being indicative of the involvement of the same mechanisms in both light-loaded and unloaded SDB.

These results contrast those obtained previously in supine individuals, in which combined inspiratory and expiratory resistances led to reduced lag between the inhalation and the RR interval (RESP-*fc*) shortening, without affecting the SBP to RR interval (SBP-*fc*) phase relation (Blaber and Hughson, 1996). Contrarily, in a similar study to Blaber and Hughson's, no phase differences were observed between RESP and *fc* in seated, healthy human beings (Calabrese et al., 2000). Despite using similar resistances to those employed in the present study (0-16cmH_2O), both of the aforementioned studies applied flow resisted breathing to both phases of the respiratory cycle, which raises the question of how exactly the isolated use of inspiratory resisted breathing might have a distinct cardiovascular impact to that of combined inspiratory and expiratory resisted breathing, or even breathing against expiratory resistances alone. Furthermore, the conflicting results between studies strengthen previously stated concerns regarding the implications that posture might have in the relationship between ABP and *fc* (Taylor and Eckberg, 1996, Taylor et al., 1998, Cooke et al., 1999, Elstad et al., 2001, Buchner et al., 2010).

Interestingly, phase angle (and time shift) correlated strongly with other cardiovascular indices (Figures 5-7 and 5-8). Most importantly, the time lag between the onset of inhalation (RESP) and SBP correlated strongly and positively with RSA (R^2 = 0.91), HRV_{TOT} (R^2 = 0.87) and BPV_{TOT} (R^2 = 0.94), and negatively with ∆SV (R^2 = 0.93). Furthermore, the RESP-*fc* lag exhibited similar associations with the same variables (R^2: RSA = 0.79; HRV HRV_{TOT} = 0.79; BPV_{TOT} = 0.96 and; ∆SV = 0.98), while SBP-*fc* only correlated strongly with ∆SV (R^2 = 0.87) and BPV_{TOT} (R^2 = 0.83). These relationships are interpreted as being representative of a strong mechanical link between the respiratory fluctuations in intrathoracic pressure and subsequent LVSV swings, resulting in substantial ABP fluctuations. This is corroborated by the high correlation observed between ∆SV and BPV_{TOT} (R^2 = 0.98 for group mean values and; R^2 = 0.30, P < 0.001, with multiple regression analysis with individual fixed effects model), as well as by the moderate to low correlation of SBP-*fc* with RSA and HRV_{TOT} (R^2: RSA = 0.54; HRV_{TOT} = 0.59; P < 0.05). The latter suggests that factors other than a simple mechanical effect of IL upon LVSV underpin changes in RSA. This is further supported by the increase in RSA amplitude with the increase in IL (Table 5-2) and by the different behaviours of HRV and BPV in response to IL and LBPP; HRV_{TOT} was virtually unaltered by IL and LBPP, whereas BPV_{TOT} was influenced by IL and LBPP (Figure 5-5). This tends to

support the notion that changes in RSA are due to an ensemble contribution from reflex, neural and mechanical factors (Anrep et al., 1936a, b, Triedman and Saul, 1994, Blaber and Hughson, 1996, Eckberg, 2003, 2009b, Julien et al., 2009, Karemaker, 2009c, a). Also, it also highlights the importance of mechanical mechanisms in the *fc* response to loaded SDB. The observations also support previous findings indicating the inability of baroreflex modulation of *fc* to fully buffer ABP oscillations at the respiratory frequency (Mancia et al., 1999). Considering the likely increase in systemic venous return with IL during inspiration (Robotham et al., 1978, Convertino, Cooke, et al., 2005, Elstad, 2012) it would be expected that the resulting increased blood volume entering the pulmonary circulation could contribute to a transient increase in $\dot{Q}$. As mentioned previously in this thesis, increases in $\dot{Q}$ result in in reduced PulTT (Zavorsky et al., 2003), with a concomitant alteration of the RESP-SBP and potentially RESP-*fc* (if considered the presence of a carotid baroreflex mechanism) phase relations. However, an increase in the capacitance of the pulmonary circulation (blood pooling) in response to increased inspiratory venous return can offset this effect and maintain pulmonary transit time (Wilkins et al., 2001, *cit by* Zavorsky et al., 2003); the combined effect of both mechanisms could contribute to the stabilisation of the time delay in the expression of the mechanical effects of breathing upon LVSV. This mechanism can also explain why $LVSV_E$ tends to increase with the increase in IL, while $LVSV_I$ is apparently unaltered (as inspiratory increases in RVSV will only be expressed in left ventricular output during the ensuing expiratory phase due to the delay interposed by the pulmonary circulation).

Unfortunately, the ANSlab software was unable to calculate phase relationships for LVSV, and this is an interesting area for future study. Finally, the lack of any significant changes in either RESP-DBP or SBP-DBP, suggests the presence of a much smaller respiratory modulation of DBP, and smaller transfer of respiratory-driven changes in LVSV into DBP, most likely because of the *arterial Windkessel* effect (TenVoorde et al., 1995).

5-5.5 Haemodynamic impact of lower body positive pressure

The level of LBPP was altered systematically in a seated position and permitted evaluation of the impact of light lower limb compression upon the left ventricular cardiac function. No alteration was observed in steady-state EDV, ESV, LVSV, $\dot{Q}$

and MAP for compressions ranging from 10 to 30 mmHg (Table 1). Previous studies have shown significant steady-state cardiovascular changes with LBPP, and the discrepancy with the present findings is most likely due to posture and the method used to deliver the LBPP. Unlike most previous studies, which applied LBPP to supine individuals using a LBPP box (Shi, Crandall, et al., 1993, Shi, Potts, Foresman et al., 1993, Shi, Foresman and Raven, 1997), participants in the present study were upright-reclined, and LBPP was delivered using anti-gravitational compressive trousers. The observed rise in MAP, in the steady-state evaluation of LBPP (88±3 mmHg *vs* 92±3 mmHg; no compression *vs* LBPP at 30 mmHg, respectively; Table 5-1), is comparable to the 3-6 mmHg increase at 20-30 mmHg LBPP reported for supine human beings (Bevegard, Castenfors and Lindblad, 1977, Shi, Crandall, et al., 1993, Shi, Potts, et al., 1993, Shi et al., 1997). The existence of a posture-dependent cardiovascular response to graded LBPP has been shown previously, indicating ABP, but not LVSV and $\dot{Q}$, to be more responsive to LBPP in the supine position than in a seated upright position (Nishiyasu, Nagashima, Nadel et al., 1998). It is also reasonable to suggest that the increase in hydrostatic pressure associated with a more upright position in the current study, might have attenuated the expression of LBPP in the applied compression range. Moving from supine to an upright position results in an estimated 500-600 mL of blood shifting to the lower limbs (Sjöstrand, 1953; Gauer and Thron, 1965, *cit* by Nishiyasu et al, 1998). Previous studies reported changes in central venous pressure > 1mmHg with the application of LBPPs in the same range those used in the present study (Shi, Crandall, et al., 1993, Shi, Potts, et al., 1993). Since it is well documented that small variations in central venous pressure of just a few millimetres of mercury can significantly impact venous return (Guyton, 1955, Fessler, Brower, Wise et al., 1991, Gelman, 2008), it is possible that the changes in central venous pressure with LBPP were insufficient to counteract the hydrostatic pressure. Furthermore, given that LBPP was only applied to the lower limbs, and not to the abdominal compartment, it is conceivable that an important volume of blood has been shifted from the extremities and pooled in the abdominal compartment, particularly in the splanchnic and renal vasculature. The splanchnic circulation receives ~25% of $\dot{Q}$ and holds 20-25% of total blood volume (Rowell, 1990). Splanchnic veins are much more compliant than the veins from extremities (Hainsworth, 1986) and are known to play a fundamental role in the maintenance of $\dot{Q}$, even without the contribution of reflex mechanisms (Rothe, 1983, Rothe and Gaddis, 1990). More importantly, splanchnic venous return is greatly dependent on the pressure gradient between the inferior

vena cava and the hepatic vein (Guyton et al., 1955, Aliverti, Bovio, Fullin et al., 2009). Under resting conditions, when the effect of the abdominal pump (by increase of intraabdominal pressure) upon mean splanchnic vascular resistance is minute, an increase in the inferior vena cava's intraluminal pressure by compression of the lower limbs can limit venous flow from splanchnic circulation (Aliverti et al., 2009), thus not affecting venous return to the right atria (and LVSV and $\dot{Q}$). Furthermore, the existence of a respiratory alternation between systemic venous flow and splanchnic flow into the inferior vena cava has been demonstrated both in dogs and resting humans (Moreno, Burchell, Van der Woude et al., 1967, Moreno and Burchell, 1982), with systemic venous flow predominating through inspiration while splanchnic flow is increased during expiration. Under this construct, the increased inferior vena cava's intra luminal pressure with LBPP would make the oscillations in right atrial pressure with IL less relevant towards the creation of a favourable pressure gradient for venous return to the right atrium during inspiration. Notwithstanding, when combined with SDB and IL, a significant main effect for LBPP upon $\dot{Q}$ was found (Figure 5-3). This suggests that the application of LBPP attenuated the impact of IL upon $\dot{Q}$. This observation is consistent with the hypothesis that increasing abdominal blood volume lessens the importance of the 'suction' effect created by inhalation, thereby supporting the notion that the haemodynamic changes induced by SDB and IL are underpinned by fluctuations in intra-thoracic pressure.

Finally, it is pertinent to note that the magnitude of the applied LBPP was limited to a maximum of 20 mmHg during the main intervention, as previous reports suggested activation of intramuscular pressure-sensitive receptors above this level, which would, in theory, have counteracted the reflex response to cardiopulmonary baroreceptor loading (Shi, Crandall, et al., 1993).

5-5.6 Translational relevance

The amplification of the influence of intrathoracic pressure swings upon venous return has proven to be useful in an array of clinical conditions, particularly those where hypotension is involved (Convertino, Cooke, et al., 2005, Convertino, Ryan, Rickards et al., 2007, Rickards et al., 2007, Rickards et al., 2008, Ryan et al., 2008, Convertino et al., 2011, Rickards et al., 2011, Convertino, Parquette, Zeihr et al., 2012, Metzger, Rees, Segal et al., 2013, Segal, Yannopoulos, Truchot et al., 2013).

Furthermore, recent studies suggest improved antihypertensive efficacy of daily SDB practice with the addition of a small IL, when compared to SDB alone (Sangthong et al., 2016). Moreover, a study of respiratory muscle resistance training highlighted the importance of large intrathoracic pressure swings, but not large lung volumes, to the antihypertensive effect of such training in healthy human beings (Vranish and Bailey, 2015).The data presented herein suggest that inspiratory loading amplifies acute cardiovascular and autonomic responses to SDB, leading to the hypothesis that LVSV oscillations may underpin the anti-hypertensive effects of both SDB and respiratory loading. Despite the suggestion that the increased acute cardiovascular response suggests that, on balance, inspiratory resisted SDB is beneficial, additional studies are necessary to identify if other modalities of resisted SDB might also impact the cardiovascular and autonomic nervous system. The isolated or combined (with IL) use expiratory resistances, and the deployment of alternative loading devices (e.g., pressure threshold loading) are just two examples of future research paths.

5-6 Conclusions

This study provides evidence that the application of small ILs enhances the within-breath response (inhalation *vs.* exhalation) of LVSV, *fc* and $\dot{Q}$ to SDB, without affecting mean *fc* or MAP. This behaviour suggests the absence of significant tonic parasympathetic or sympathetic alterations during loaded SDB. Furthermore, the significant main effect of LBPP also supports the existence of a mechanical 'suction' mechanism, since the presence of LBPP attenuated the improvements created by IL.

Also, the responses of RSA, HRV and BPV to graded inspiratory loading during tightly-regulated SDB were characterised. The data suggest that IL magnifies inspiratory pressure-driven increases independently of changes in V_T and f_R. Furthermore, strong evidence is provided that the amplitude of within-breath fluctuations in both LVSV and ABP are highly correlated with small phase shifts between cyclic fluctuations in lung volume, SBP and *fc*. We interpreted these findings as evidence of the contribution of both mechanical and reflex factors to the generation and amplitude of RSA during loaded SDB. Further investigation of the role of these mechanisms in the acute and chronic response to SDB will help to improve the potential therapeutic use of SDB as an antihypertensive intervention.

CHAPTER 6 – THE INFLUENCE OF DIFFERENT MODALITIES OF AIRWAY RESISTANCE UPON THE ACUTE CARDIOVASCULAR RESPONSES TO SLOW AND DEEP BREATHING

[Note: parts of this chapter were accepted for publication in Proceedings of the 38th International Union of Physiological Sciences Congress]

6-1 Abstract

Introduction and objective: The brief daily bouts of breathing via inspiratory and/or expiratory resistances has recently been shown to exert antihypertensive effects in both healthy and hypertensive individuals. The present study tested the hypothesis that adding inspiratory and/or expiratory resistances during slow and deep breathing (SDB) enhances acute cardiovascular and autonomic responses, compared to SDB alone. The study also explored whether different loading methods (flow resisted breathing *vs.* threshold loaded breathing *vs.* single nostril breathing) affected the magnitude of the cardiovascular response to resisted SDB.

Materials and methods: Fifteen healthy males (25±4 years; 179±8 cm; 73.4±9.3 kg) were tested in a seated upright-reclined position. Participants completed nine 5 min bouts of SDB at a breathing frequency (f_R) of 6 breaths·min^{-1} and tidal volume (V_T) of 30% of forced vital capacity (FVC). One bout consisted of SDB alone, while six sets combined SDB with inspiratory and/or expiratory loads of approximately 10 cmH_2O delivered by a flow resistive or pressure threshold device. Two other sets consisted of single-nostril (right and left) SDB. All interventions were randomised.

Results and conclusion: The cardiovascular and HRV responses to resisted SDB did not differ between different loading devices, with one exception; with the threshold loaded breathing, heart rate (*fc*) and total peripheral resistance (TPR) during inspiration were significantly higher than other conditions. The addition of an expiratory resistance resulted in a significant pressor response when compared to inspiratory resisted SDB, or SDB alone. A small inspiratory resistance in conjunction to SDB increased RSA and within-breath LVSV swings without acutely increasing MAP. Finally, the use of single nostril breathing, which generated comparable respiratory pressures to the ones encountered with the resisted SDB, resulted in no significant differences from flow resisted breathing, while there was no suggestion of a differentiated cardiovascular or autonomic response when comparing right *vs.* left nostril breathing. The data support the notion that magnified respiratory-related intrathoracic pressure swings are the dominant breathing-related alteration underlying cardiorespiratory interactions. While it is not clear what is the exact contribution of baroreflex mediated mechanisms to the amplification of RSA, HRV and BPV with inspiratory loaded SDB, the data are consistent with the involvement of important mechanical respiratory effects, acting upon venous return and right atrial stretch.

6-2 Introduction

The previous chapter reported the acute cardiovascular response to the combination of fixed pace SDB with different inspiratory loads; a positive correlation was found between the magnitude of inspiratory resistances (more negative intrathoracic pressure) and changes in expiratory LVSV, inspiratory $\dot{Q}$ and the magnitude of within-breath LVSV fluctuations (ΔSV), without a concomitant increase in MAP. Similarly, RSA, HRV and BPV were significantly higher in the presence of inspiratory loading and related strongly with the resulting time shift variations between the changes in lung volume, SBP and *fc*. These results built upon previously reported data from healthy individuals (Convertino, Ratliff, Ryan, Cooke, et al., 2004, Convertino, Ratliff, Ryan, Doerr, et al., 2004, Cooke et al., 2006); hypovolemia/hypotension models (Convertino, Cooke, et al., 2005, Convertino et al., 2007, Rickards et al., 2007, Rickards et al., 2008, Ryan et al., 2008, Convertino et al., 2011, Rickards et al., 2011, Convertino et al., 2012), and fixed-paced resisted breathing (Blaber and Hughson, 1996). Collectively, these data suggest an important functional link between respiratory driven fluctuations in right atrial filling, left ventricular output and the acute cardiovascular and autonomic response to inspiratory resisted SDB.

Recently, it has been suggested that large intrathoracic pressure swings (either negative or positive) result in chronic reductions in SBP, DBP and MAP in normotensive individuals, following a 6-week training period, while more modest intrathoracic pressure variations led to no improvements, independent of the lung volume (Vranish and Bailey, 2015). This study highlights the importance of understanding the acute cardiovascular and autonomic responses to both inspiratory and expiratory loaded slow and deep breathing, in order to better comprehend its potential clinical relevance.

As mentioned previously in Chapters 2 and 5, breathing against an inspiratory resistance places significant mechanical strain upon the cardiovascular system and promotes important changes in systemic venous return, while contributings to enhanced right ventricular filling during inspiration Simultaneously, the more negative intrathoracic pressure also contributes to an increase in left ventricular afterload as a result of increased aortic transmural pressure (Robotham et al., 1978,

Karam et al., 1984), with the final impact upon the cardiac function being determined by the interaction among the changes in preload, contractility and afterload.

In contrast, breathing against an expiratory resistance mimics the increase in intrathoracic pressure associated with the Valsalva manoeuvre albeit with decreasing lung volume (Laciuga et al, 2012); an initial increase in LVSV and SBP is observed due to the direct mechanical effect of increased pleural pressure upon the heart and intrathoracic vessels, assisting blood ejection and contributing to the increase in aortic intravascular pressure (Eckberg, 1980, Looga, 2005). Similarly, expiration against a moderate expiratory resistance (24 cmH_2O) in healthy individuals results in an increase in LVSV during the first couple of heart beats following the start of the expiratory effort (Natarajan, Wise, Karam et al., 1987). The authors showed that this increment was due to an increase in left ventricular filling and not to an increase in left ventricular contractility or decrease in afterload. However, the scarcity of studies means the acute response to expiratory loading remains very poorly understood.

A handful of studies have characterised *fc* and ABP responses to expiratory resisted efforts, but most did not involve SDB, and/or imposed much larger expiratory loads (relative to maximal expiratory pressure - P_{Emax}). One particular study reported unchanged in *fc* in response to brief and repetitive exertions with swallowing manoeuvres in orthopaedic patients, while supraventricular tachycardia and premature atrial and ventricular contractions were encountered in stroke patients (Chaudhuri, Hildner, Brady et al., 2002). Conflicting findings have also arisen regarding the acute cardiovascular effects of applying large expiratory resistances (60-75% P_{Emax}) in healthy individuals, with reports of both unchanged (Laciuga, Davenport and Sapienza, 2012) and increased *fc* (10-15 $beats \cdot min^{-1}$) and SBP (~20 mmHg) following expiratory resisted breathing sessions (Derchak, Sheel, Morgan et al., 2002). These studies suggest that the application of expiratory resistances possibly has a meaningful acute cardiovascular impact in healthy individuals, but might be contraindicated in patients with a history of cardiovascular disease.

The application of resistance to breathing can be accomplished through two different methods. Firstly, flow resistive loading creates the resistance by having individuals breathing through an orifice, whereby, for a given flow, the resistance to breathing increases with the decrease in the size of the orifice. Secondly, pressure threshold

loading requires the production of sufficient airway pressure to overcome the threshold load on a valve, in order to initiate respiration (McConnell and Romer, 2004). Importantly, threshold loading provides an inertial load that results in a rapid pressure build-up, which precedes the production of airflow, thereby creating a different pattern of intrathoracic pressure changes, compared to the flow dependent resistance of flow resistive loading. While both methods have the ability to deliver the same absolute resistance to breathing, no study has yet compared the acute cardiovascular impact of these two distinct types of loading during resisted SDB. Furthermore, studies using single nostril breathing appear to have similar effects to the observations from Chapter 5 of this thesis, but with claims of different effects for right-nostril *vs.* left-nostril breathing (Raghuraj and Telles, 2008). It is likely that these effects are simply the result of the inherent flow resistance of the nasal airway (in single nostril breathing), but to the author's knowledge, this has not been tested experimentally, particularly under conditions in which the airflow resistance of the nostrils has been quantified.

Accordingly, the aims of this study were to compare: 1) the influence of adding inspiratory and/or expiratory resistances upon the responses to SDB alone; 2) the effects of inspiratory and expiratory resistances upon responses to SDB; 3) the cardiovascular responses to flow resistive loading and pressure threshold loading, and 4) compare the cardiovascular response to the different routes of respiratory airflow, specifically mouth, right nostril and left nostril.

6-3 Specific Methods

6-3.1 Participants

Fifteen healthy, recreationally-active men participated (mean ± SD; age 25±4 years; 179±8 cm; 73.4±9.3 kg). All participants were nonsmokers with no previous record of cardiovascular or respiratory disease and reported not taking any medication.

6-3.2 Ethical Approval

Fully informed, written consent was obtained from the participants before the study. All procedures were approved by the Brunel University Research Ethics Committee (RE49-14) and conformed to the guidelines of the Declaration of Helsinki (World Medical Association, 2013).

6-3.3 Protocol

Participants visited the laboratory on a single occasion. Initially, the participants were introduced to the experimental set-up and familiarised with the measurement methods. A baseline pulmonary function assessment was performed; any participants showing signs of asthma or other lung disease were excluded from the study. In addition, a familiarisation trial using a bespoke device guided breathing biofeedback system was performed (Labview, National Instruments Inc.) (refer to Chapter 3 - General Methods, for a detailed description of pulmonary function assessment and familiarisation session protocols).

After the initial screening, participants undertook a session of device guided SDB. The trial session began with 5 min of quiet baseline spontaneous breathing, followed by ten, randomised 5 min sets of device guided SDB.

These interventions were all performed at an f_R of 6 breaths·min^{-1} and a V_T corresponding to 30% each participants' forced vital capacity (FVC) and included:

- SDB with no added resistance (unloaded; UL);
- SDB combined with flow resistive inspiratory (IF), expiratory (EF) and dual (DF) loading;
- SDB coupled with pressure threshold inspiratory (IT), expiratory (ET) and dual (DT) loading, and,

- SDB through the right (RN) or left nostril (LN), with contralateral nostril occluded.

Five minute rest periods separated each 5 min set (Figure 6-1). All sets were conducted in a fully randomised order (a detailed description of the randomisation procedures can be found in the General Methods section).

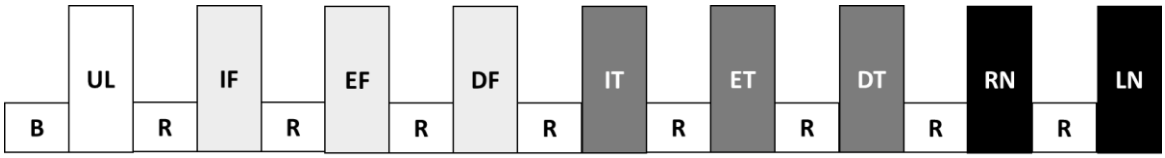


Figure 6-1 – Example sequence of the experimental protocol (order was randomised). Participants were exposed to 5 min of spontaneous baseline breathing (B) followed by randomly assigned device guided slow and deep breathing sets, with and without resistance. Unloaded slow and deep breathing (large white bar: UL), flow resistive slow and deep breathing (light grey: IF, EF and DF), pressure threshold slow and deep breathing (dark grey: IT, ET and DT) and single nostril breathing (black: RN and LN) 5 min interventions were interleaved by 5 min of spontaneous, unrestricted breathing (R).

6-3.4 Procedures and instrumentation of participants

Participants were asked to refrain from alcohol, caffeine and strenuous physical activity for at least 12 h and to avoid consuming food during the 2 h leading up to testing. Throughout the entirety of the trials, participants assumed an upright, reclined position by sitting on a reclining lounge chair, set at an approximate 60^{o} angle, and breathed through a mask that allowed for normal mouth and/or nasal breathing (Intersurgical Quadralite Mask; Intersurgical Ltd., Wokingham Berkshire, UK). Room temperature remained at a comfortable 22-25^{o}C and barometric pressure was recorded for each of the trial sessions. Specified respiratory flow rates and a respiratory duty cycle (T_i/T_{TOT}) of 0.5 (inspiratory time = expiratory time) were delivered using the aforementioned bespoke guided breathing biofeedback system (Labview, National Instruments Inc.). Respiratory flow rates were measured using a heated pneumotachograph (Hans Rudolph 3700, Hans Rudolph Inc.), whilst airway pressure was measured using a separate, auxiliary differential pressure transducer. A summary of the respiratory parameters can be found in Table 5-1.

Table 6-1 – Characterisation of respiratory parameters during all loaded breathing sets.

	Baseline	Unloaded	IF	EF	DF	IT	ET	DT	RN	LN
f_R (breaths·min^{-1})	11.5±1.0	5.9±0.2*	5.8±0.2	6.0±0.2	5.9±0.2	5.9±0.2	5.9±0.2	5.9±0.2	5.8±0.2	5.8±0.2
V_T (L·min^{-1})	0.82±0.08	1.58±0.07*	1.61±0.07	1.63±0.07	1.64±0.07	1.61±0.07	1.65±0.07	1.62±0.07	1.61±0.08	1.61±0.08
T_I/T_{ToT}	0.44±0.01	0.49±0.01*	0.50±0.01	0.53±0.01	0.52±0.01	0.47±0.01	0.51±0.02	0.49±0.01	0.48±0.01	0.48±0.01
$P_{ET}CO_2$ (mmHg)	42.0±1.0	40.2±1.1	40.3±1.2	42.2±1.3	41.6±1.2	40.2±1.0	42.7±1.1	40.4±1.0	40.0±1.1	40.7±1.1
P_I (cmH_2O)	-0.5±0.1	-0.5±0.1	-10.3±0.5‡	-3.5±0.1	-10.0±0.6‡	-10.3±0.3§	-3.2±0.1	-10.0±0.1§	-8.6±1.1	-8.7±1.4
P_E (cmH_2O)	0.5±0.1	0.5±0.1	2.7±0.1	9.8±0.3†	9.8±0.5†	1.8±0.1	12.3±0.3#	12.6±0.1#	8.9±1.9	9.0±2.2

Data represent mean ± SEM for 15 subjects. Slow and deep breathing conditions performed at 6 breaths·min^{-1} and 30% FVC. Breathing frequency (f_R) in breaths·min^{-1}, tidal volume (V_T) in % of forced vital capacity, duty cycle (T_I/T_{TOT}), partial pressure of end tidal carbon dioxide ($P_{ET}CO_2$) in mmHg, inspiratory pressure (P_I) in cmH_2O, expiratory pressure (P_E) in cmH_2O. IF – flow dependent inspiratory load, EF – flow dependent expiratory load, DF – flow dependent dual inspiratory/expiratory load, IT – threshold inspiratory load, ET – threshold expiratory load, DT – threshold dual inspiratory/expiratory load, RN – right nostril breathing, LN – left nostril breathing. * unloaded slow and deep breathing different from baseline, † different from IF, ‡ different from EF, # different from IT, § different from ET. $P < 0.004$.

Flow resistive loading

A bespoke breathing circuit imposed the flow resistive loads, as previously described in Chapter 3 (Figure 3-6). To allow for selective inspiratory, expiratory and dual loading, the flow resistors were placed in the inspiratory, expiratory or both paths of the device. The y-shape and separate inspiratory and expiratory valves ensured that the load only acted upon the desired portion of the respiratory cycle.

Threshold loaded breathing

Threshold loaded resisted breathing was performed by a bespoke breathing circuit that included a y-shape connector and inspiratory and expiratory valves, similar to the ones used in the aforementioned flow-dependent resistor. Inspiratory and/or expiratory spring-loaded threshold devices (inspiratory: Philips Threshold IMT; expiratory: Philips Threshold PEP; Philips Respironics, Murrysville, PA, USA) were attached to inspiratory and/or expiratory arm of the breathing circuit (Figure 6-2).

Figure 6-2 - Left panel: expiratory (Threshold PEP) and inspiratory (Threshold IMT) threshold loading devices. Right panel: Bespoke threshold loading circuit, including threshold loading devices, inspiratory and expiratory valves, pneumotachograph sensor and connection to pressure transducer.

Single-nostril breathing sets involved the use of a nasal probe for nostril pressure measurements (CareFusion UK 232 Ltd., Basingstoke, UK; Figure 6-3). Prior to the insertion of the nasal probe, participants performed a nasal wash with saline solution to remove accumulated nasal mucous.

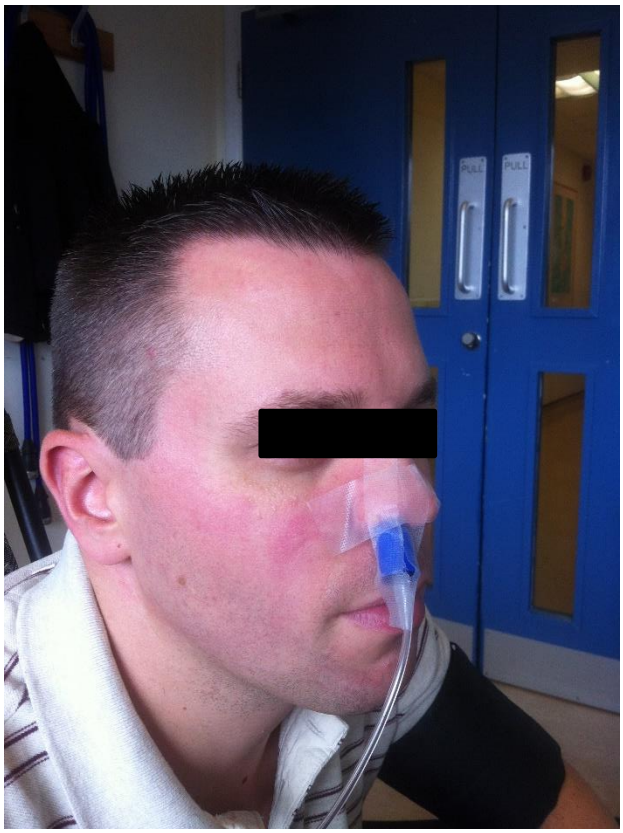


Figure 6-3 – Placement of nasal probe for single left nostril breathing. The nasal probe was attached to the pneumotachograph auxiliary pressure channel for continuous respiratory pressure measurement. The probe was taped tightly to avoid leaks, particularly during expiration.

Cardiovascular measurements are described in detail elsewhere (*General Methods* – Chapter 3), but briefly, non-invasive beat-to-beat arterial blood pressure was obtained using finger photoplethysmography (Finometer® PRO, Finapres Medical Systems, Amsterdam, The Netherlands), while heart rate was monitored using a 3-lead ECG (PysioControl VSM® 3, PhysioControl Inc.). Total peripheral resistance (TPR) was calculated by dividing cardiac output ($\dot{Q}$) by the mean arterial pressure (MAP). Baroreflex sensitivity (BRS) was calculated by the sequence method (Bertinieri et al., 1985, 1988, Parati et al., 1988), as well as from the cross-spectral transfer function gain (Robbe et al., 1987). Phase angles and coherence between respiration and cardiovascular (heart rate, SBP and DBP) waveforms were obtained from the cross-spectra transfer function at the peak respiratory frequency. Average phase angles were calculated as the un-weighted circular mean ($\bar{\alpha}$) for phase angles

for all relationships of interest (Mardia and Jupp, 2009). A more detailed description of the BRS and cross-spectral calculations can be found in section 3-3.

Statistical Analysis

Data were analysed using IBM® SPSS version 21.0 statistical software (IBM Corp.). Values are expressed as means ± SEM unless stated otherwise. After normality was confirmed via the Shapiro-Wilk test, repeated measures ANOVA with *post hoc* planned, pairwise comparisons (determined by study aims), using Bonferroni correction (see below) was used to assess differences between conditions. Circular (cross-spectral) data were analysed using multi-sample Watson-Williams F-test following confirmation of the existence of a Von Mises circular distribution (Fisher, 1995, Mardia and Jupp, 2009). Bonferroni adjusted alpha levels of 0.004 per test (0.05/13; Baseline *vs*. UL; IF *vs*. EF; IF *vs*. DF; IF *vs*. IT; EF *vs*. DF; EF *vs*. ET; DF *vs*. DT; IT *vs*. ET; IT *vs*. DT; ET *vs*. DT; DF *vs*. RN; DF *vs*. LN; RN *vs*. LN) were used in this study for both ANOVA and the Watson-Williams multiple sample *posthoc* analyses. Despite some violations of requirements for the multisample ANOVA and Watson-Williams F-test, involving deviations from the required distributions that could not be corrected using data transformation, a decision was made to use the parametric options rather than the nonparametric alternatives. This was justified because:

1) We were working with continuous rather than ordinal data;
2) repeated measures ANOVA is relatively robust against moderate deviations in normality (Glass et al., 1972);
3) similar results encountered when we analysed the data using the nonparametric Friedman and Mardia-Watson-Wheeler (for circular data) alternatives.

To assess the existence of a causal path between the use of inspiratory *vs.* expiratory resisted breathing upon the response of key cardiovascular variables, mechanism analysis as described by Hopkins (2003). With this method, we plotted the change in mean respiratory pressure between inspiratory (X-pre), and expiratory (X-post) loaded conditions against the corresponding change in the key dependent variables and visually analysed the directionality and consistency of the individualised responses. To complement the analysis of the relationship between mean respiratory pressure and the cardiovascular response, a multiple regression

for within-subject repeated measures was used (Bland and Altman, 1995). Finally, after confirmation of the homoscedasticity, multicollinearity and normality of residuals assumptions, stepwise multiple linear regression was employed to help determine how strongly selected cardiovascular variables contributed to changes in RSA (Field, 2013).

6-4 Results

6-4.1 Cardiovascular response

Effects of unloaded slow and deep breathing

The RSA response to SDB showed significantly higher values for the unloaded SDB condition than for baseline (Baseline: 189±29 *vs.* Unloaded SDB: 306±32, $P < 0.004$; Figure 6-4). Similarly, $LVSV_E$, $LVSV_{I/E}$, SBP_{Emax}, ΔSBP-DBP, PP, PP_I and PP_E all increased with Unloaded SDB ($P < 0.004$; Table 6-2). In contrast, fc_E decreased, while ΔSV became negative ($LVSV_I < LVSV_E$) with SDB ($P < 0.004$; Table 6-2). No changes in any other analysed cardiovascular variables were observed with unloaded SDB.

Comparison of flow resistive and pressure threshold loading

There were small, but significant differences between the two types of inspiratory load (IF *vs.* IT) for fc_I (71±2 *vs.* 74±2 beats·min^{-1}, respectively) and TPR_I (14±1 *vs.* 13±1 mmHg·min·l^{-1}, respectively; Table 6-2; $P < 0.004$). For the two types of expiratory load (EF *vs.* ET), there were no significant differences for any of the analysed variables. Similarly, dual inspiratory and expiratory loading did not differ between loading types (DF *vs.* DT), or between single nostril breathing and oral DF (RN and LN *vs.* DF).

Comparison of right vs. left single nostril breathing

No significant cardiovascular differences were observed between right *vs.* left single nostril breathing (Table 6-2, Figure 6-4, $P < 0.004$).

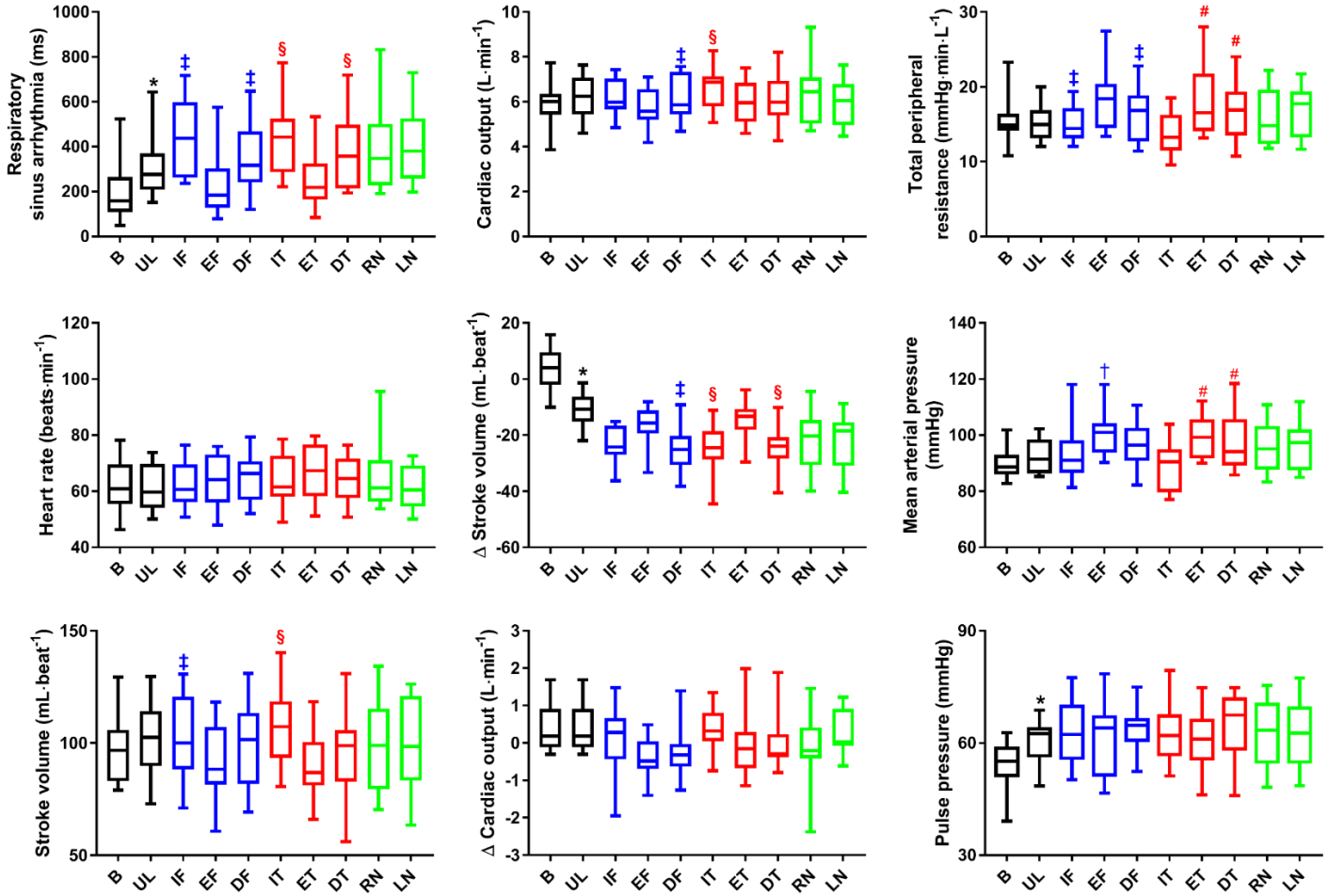


Figure 6-4 – Systemic haemodynamic responses to resisted slow and deep breathing. Black: B- Baseline, UL -Unloaded slow and deep breathing; Blue: IF – flow dependent inspiratory load, EF – flow dependent expiratory load, DF – flow dependent dual inspiratory/expiratory load; Red: IT – threshold inspiratory load, ET – threshold expiratory load, DT – threshold dual inspiratory/expiratory load; Green: RN – right nostril breathing, LN – left nostril breathing. Data are for 15 participants (*n*=14 for RN and LN due to issues with placement of the nasal probe in one participant). * different from baseline, ‡ different from EF, § different from ET. $P < 0.004$

Table 6-2 – Systemic haemodynamic responses.

	Baseline	UL	IF	EF	DF	IT	ET	DT	RN	LN
f_{C_I} (beats·min^{-1})	65±3	67±2	71±2	67±2	71±2	74±2†	72±3	73±2	72±2	70±2
f_{C_E} (beats·min^{-1})	60±2	56±2*	55±2	61±2	58±2	55±2	62±3	57±2	59±4	54±2
$LVSV_I$ (mL·beat^{-1})	100±5	97±4	91±4	84±4	86±4	93±4	84±4	85±5	88±5	89±5
$LVSV_E$ (mL·beat^{-1})	96±4	109±4*	114±5‡	100±5	112±5‡	118±5§	98±4	108±6	110±7	111±7
$LVSV_{I/E}$ (mL·beat^{-1})	14±1	19±1*	29±2	27±2	30±2	31±2	27±2	29±1	29±3	28±2
$\dot{Q}_I$ (L·min^{-1})	6.4±0.3	6.4±0.2	6.3±0.2‡	5.6±0.3	6.1±0.3‡	6.8±0.2§	6.0±0.3	6.1±0.3	6.2±0.3	6.1±0.3
$\dot{Q}_E$ (L·min^{-1})	5.8±0.3	6.0±0.2	6.2±0.2	6.0±0.2	6.4±0.3	6.4±0.3	6.0±0.3	6.1±0.2	6.4±0.4	5.8±0.3
SBP (mmHg)	120±2	126±1	125±3	132±3	129±2	121±3	129±2#	129±3#	128±2	127±2
SBP_{Imax} (mmHg)	121±2	127±2	128±3	133±3	130±2	126±3	129±2	130±3	129±2	129±3
SBP_{Emax} (mmHg)	126±2	135±1*	137±3	144±3	143±2	132±2	143±3#	146±3#	141±2	141±2
$SBP_{I/E}$ (mmHg)	16±1	20±1	29±1	21±2	29±2‡	29±2	23±1	34±2§	31±3	30±3
DBP (mmHg)	72±1	73±1	73±2‡	81±2	77±2	70±2	80±2#	78±2#	77±2	75±2
$DBP_{I/E}$ (mmHg)	9±1	9±1	11±1	19±2†	17±1†	11±1	21±1#	18±1#	14±2	14±2
MAP_I (mmHg)	88±1	90±2	89±2‡	95±2	90±2	85±2	93±2#	90±2	90±2	89±3
MAP_E (mmHg)	91±1	95±2	97±3‡	107±2	104±2	92±2	107±2#	104±3#	102±2	100±2
PP_I (mmHg)	52±2	58±1*	61±2	61±2	62±2	62±2	58±2	62±3	60±2	61±3
PP_E (mmHg)	55±2	63±2*	64±2	64±2	66±2	64±2	63±2	67±2	65±3	66±2
$PP_{I/E}$ (mmHg)	10±1	14±1	21±1‡	16±2	17±1	21±2§	15±1	20±2	19±2	19±2
TPR_I (mmHg·min·L^{-1})	14±1	14±1	14±1‡#	18±1	16±1‡	13±1	16±1#	16±1#	15±1	15±1
TPR_E (mmHg·min·L^{-1})	16±1	16±1	16±1‡	19±1	17±1‡	15±1	19±2	18±1#	17±1	18±1
BRS_{up} (ms·mmHg^{-1})	17±3	22±2	27±4	15±2	20±4	23±5	17±3	17±2	22±2	23±2
BRS_{down} (ms·mmHg^{-1})	15±2	15±1	14±2	11±1	11±2	12±1	13±2	10±1	12±2	16±3
BRS_{Seq}(ms·mmHg^{-1})	16±2	18±2	20±3‡	13±2	16±2	18±3	15±2	14±1	17±1	20±2
BRS_{Freq} (ms·mmHg^{-1})	19±4	17±2	15±1	16±4	12±2	17±2	10±1	11±1	12±1	15±2

Data represent mean ± SEM for 15 participants. Respiratory sinus arrhythmia (RSA), heart rate (f_C), heart rate during inspiration (f_{C_I}), heart rate during expiration (f_{C_E}), left ventricle stroke volume (LVSV), stroke volume during inspiration ($LVSV_I$), stroke volume during expiration ($LVSV_E$), within-breath variation in stroke volume (ΔSV), 'peak-valley' amplitude of LVSV ($LVSV_{I/E}$), cardiac output ($\dot{Q}$), cardiac output during inspiration ($\dot{Q}_I$), cardiac output during expiration ($\dot{Q}_E$), within-breath variation in cardiac output ($\Delta\dot{Q}$), systolic blood pressure (SBP), peak systolic blood pressure during inspiration (SBP_{Imax}), peak systolic blood pressure during expiration (SBP_{Emax}), 'peak-valley' amplitude of systolic blood pressure ($SBP_{I/E}$), diastolic blood pressure (DBP), 'peak-valley' diastolic blood pressure ($DBP_{I/E}$), mean arterial pressure during inspiration (MAP_I), mean arterial pressure during expiration (MAP_E), pulse pressure during inspiration (PP_I), pulse pressure during expiration (PP_E), 'peak-valley' pulse pressure ($PP_{I/E}$), total peripheral resistance during inspiration (TPR_I), total peripheral resistance during expiration (TPR_E), sequence baroreflex sensitivity positive sequence gain (BRS_{up}), sequence baroreflex sensitivity negative sequence gain (BRS_{down}), sequence baroreflex sensitivity average gain (BRS_{Seq}); cross-spectral baroreflex sensitivity gain (BRS_{Freq}). Slow and deep breathing conditions performed at 6 breaths·min^{-1} and 30% FVC. UL – unloaded slow and deep breathing, IF – flow dependent inspiratory load, EF – flow dependent expiratory load, DF – flow dependent dual inspiratory/expiratory load, IT – threshold inspiratory load, ET – threshold expiratory load, DT – threshold dual inspiratory/expiratory load, RN – right nostril breathing, LN – left nostril breathing. Data for RN and LN are for 14 participants, due to issues with placement of the nasal probe in one individual. * unloaded slow and deep breathing different from baseline, † different from IF, ‡ different from EF, # different from IT, § different from ET. $P < 0.004$.

6-4.2 Inspiratory vs. expiratory resistances during slow and deep breathing

The analysis of the pooled (combined data from flow resistive loading and pressure threshold loading), individualised, systemic cardiovascular response to the application of inspiratory and expiratory resistances (dual inspiratory and expiratory resisted breathing conditions not included) showed that in all individuals, except one, RSA was higher during inspiratory loading (Figure 6-5A). Similar patterns were found for LVSV (Figure 6-5C) and $\dot{Q}$ (Figure 6-5E), while the opposite was observed for ΔSV, TPR and MAP (Figure 6-5D, G-H), indicating lower values of these variables with inspiratory loading, compared expiratory loading (Figure 6-5). In contrast, no clear pattern emerged for *fc* and $\Delta\dot{Q}$ (Figures 6-5B and 6-5F) responses for either directionality, or magnitude of the change, which varied widely between participants.

A regression analysis of the individualised response of RSA to inspiratory loaded, expiratory loaded and unloaded SDB was consistent with the aforementioned response patterns, i.e., mean respiratory pressure (average pressure during the entire respiratory cycle) related strongly and negatively with RSA (R^2 = 0.64; P < 0.001; Figure 6-6). Similarly, moderate positive correlations were observed between mean respiratory pressures and ΔSV, TPR and MAP (R^2 = 0.53, 0.46 and 0.47, respectively; P < 0.001; Figure 6-6). Weaker, but significant, relationships emerged between mean respiratory pressure and $\dot{Q}$ (R^2 = 0.32; P < 0.001), LVSV (R^2 = 0.22; P < 0.001), $\Delta\dot{Q}$ (R^2 = 0.3; P < 0.01) and *fc* (R^2 = 0.08; P < 0.05; Figure 6-6).

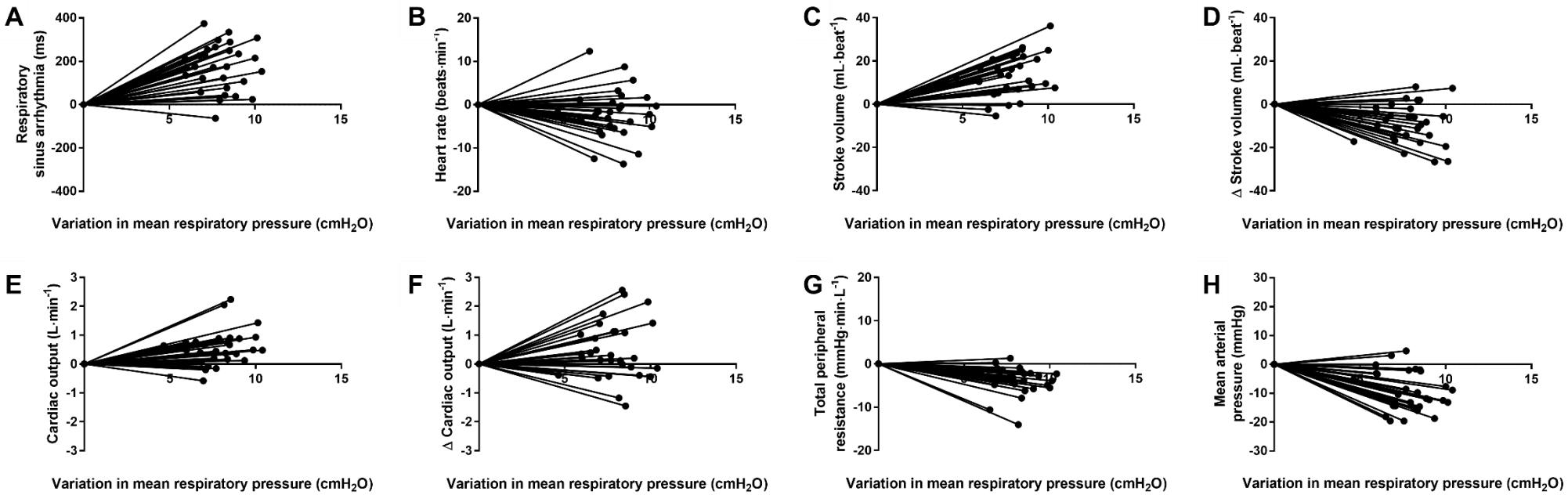


Figure 6-5 - Individual cardiovascular responses to inspiratory and expiratory resisted breathing. The 0 (zero) values in both axes refer to individual values for the expiratory loaded conditions, while the remaining dots represent the difference between the inspiratory and expiratory loaded conditions. Positive y-axis values indicate an individual increase in the variable of interest with the application of inspiratory resisted breathing vs.similar magnitude expiratory resisted breathing. The figure depicts data from 15 participants for both flow resisted breathing and threshold loaded breathing for a total of 30 data points.

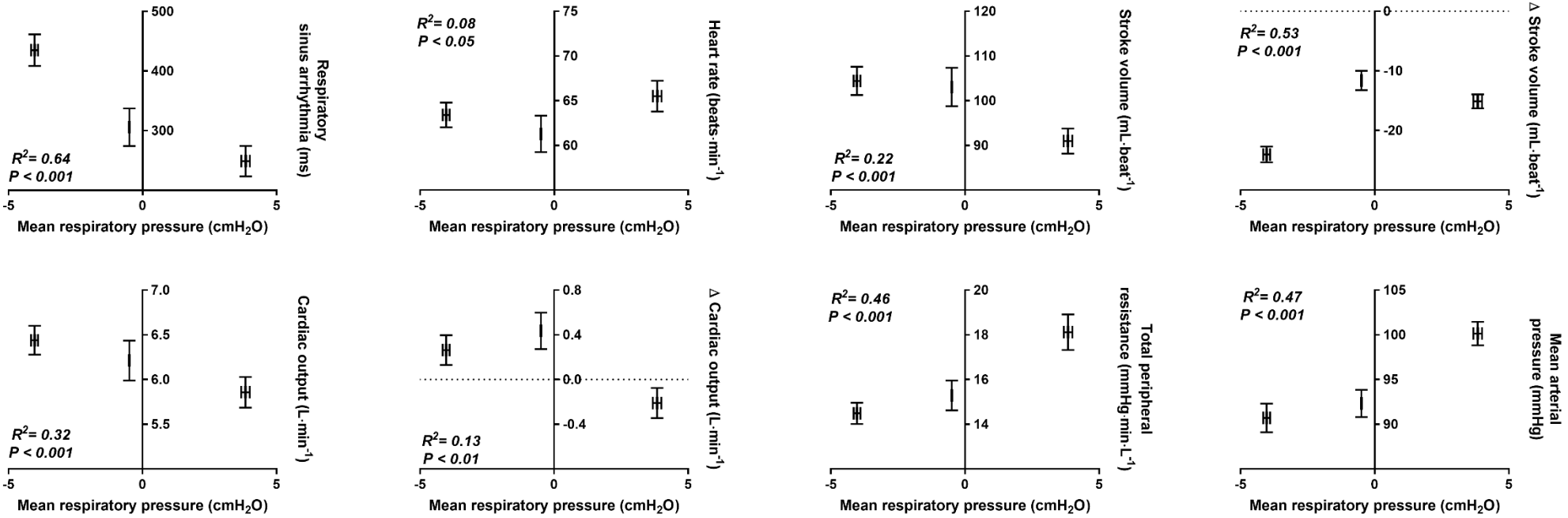


Figure 6-6 – Relationship between mean respiratory pressures (average pressure during the entire respiratory cycle) and the cardiovascular response to inspiratory loading, expiratory loading and unloaded SDB. Data are mean ± SEM for 15 participants. Corresponding R^2 values are calculated using previously described multiple regression analysis.

6-4.3 Strongest influences upon respiratory sinus arrhythmia during loaded breathing

A four-predictor model including $PP_{I/E}$, $\Delta\dot{Q}$, *fc* and ΔSV provided a significant regression equation (F (4, 143) = 92.473, $P < 0.001$), with an $R^2 = 0.721$.

Predicted RSA = 281.13 + 12.864 ($PP_{I/E}$) + 83.931 ($\Delta\dot{Q}$) – 3.794 (*fc*) – 3.736 (ΔSV), where $PP_{I/E}$ is measured in mmHg, $\Delta\dot{Q}$ in $L \cdot min^{-1}$, *fc* in $beats \cdot min^{-1}$ and ΔSV in $mL \cdot beat^{-1}$.

Thus, RSA increased by around 13 ms for each mmHg increase in $PP_{I/E}$ amplitude, 84 ms by each $L.min^{-1}$ of within-breath variation of $\dot{Q}$ ($\Delta\dot{Q}$), reduced 3.79 ms by each $beats \cdot min^{-1}$ increase and varied inversely to within-breath amplitude in LVSV by 3.74 ms by each $mL \cdot beat^{-1}$ change. All variables were significantly correlated with RSA.

This four-predictor model provided a better estimation of RSA than the previously applied (cf. Chapters 4 and 5). When applying said three predictor model including ΔSV, *fc* and $\Delta\dot{Q}$, a significant regression equation (F (3, 144) =68.487, $P < 0.001$), with an $R^2 = 0.588$.

Predicted $RSA_{(I/E)}$[4] = 201 - 9 (ΔSV) –5 (*fc*) +98 ($\Delta\dot{Q}$), where ΔSV is measured in $mL \cdot beat^{-1}$, *fc* in $beats \cdot min^{-1}$ and $\Delta\dot{Q}$ in $L \cdot min^{-1}$.

Participants' $RSA_{(I/E)}$ decreased 9 ms by each $mL \cdot beat^{-1}$ increase in within-breath variation in LVSV, reduced 5 ms by each $beats \cdot min^{-1}$ and increased 98 ms by each $L.min^{-1}$ of within-breath variation of $\dot{Q}$ ($\Delta\dot{Q}$). All three variables were significantly correlated with $RSA_{(I/E)}$.

[4] In the previous experimental chapters, RSA was calculated by the difference between the maximum RR interval during expiration minus the minimum RR interval during inspiration, as described by Grossman et al. (1990). Contrarily, in the current experimental chapter, RSA was calculated as the maximal difference between maximum and minimum RR interval within a breath cycle, independently of the respiratory phase in which they occurred (Hirsch and Bishop, 1981).

6-4.4 Heart rate and blood pressure variability response

Effects of unloaded slow and deep breathing

Unloaded SDB resulted in significant increments of SDNN (75±8 ms) and Poincaré plot's SD2 (158±15 ms) when compared to baseline (SDNN: 51±7 and SD2: 104±13 ms; Table 6-3, $P < 0.004$), with no impact in time domain RMSSD and spectral HRV indices. Similarly, BPV spectral components did not change significantly from baseline with the application of SDB alone (Table 6-3, $P > 0.004$).

Comparison of flow resistive and pressure threshold loading

The application of different methods of loading (flow resisted breathing *vs*. threshold loaded breathing) did not result in any significant differences in any of the analysed HRV and BPV variables. Similarly, no differences were observed for either HRV and BPV indices during single nostril breathing (right nostril *vs.* left nostril; Table 6-3, $P > 0.004$).

Comparison of right vs. left single nostril breathing

No significant differences were found between single nostril breathing and the dually (inspiratory and expiratory) flow resisted breathing condition (DF), for any of the HRV and BPV analysed indices. Similarly, the comparison between right and left nostril breathing revealed no dissimilarities between these two conditions (Figure 6-7, Table 6-3, $P<0.004$).

Inspiratory vs. expiratory resistances during slow and deep breathing

When comparing the effects of inspiratory, expiratory and dual loaded breathing, a consistent pattern emerged for both time domain and Poincaré plot HRV indices. The SDNN, RMSSD, SD1 and SD2 all showed systematically higher values during the inspiratory (IF and IT) and dual (DF and DT) loading conditions than during the expiratory (EF and ET) resisted breathing trials (Table 6-3, $P < 0.004$)

The HRV_{TOT} during the IF condition was significantly larger than that observed during the EF condition (Figure 6-7, Table 6-3, $P < 0.004$), but the same pattern was not observed during threshold loading (IT *vs*. ET). BPV was highest with the combined use of inspiratory and expiratory loads (DF and DT). The BPV_{TOT} for the

dual loading conditions was significantly greater than the expiratory resisted breathing conditions using the same type of loading device (DF *vs.* EF and DT *vs.* ET; Figure 6-7, Table 6-3, $P<0.004$).

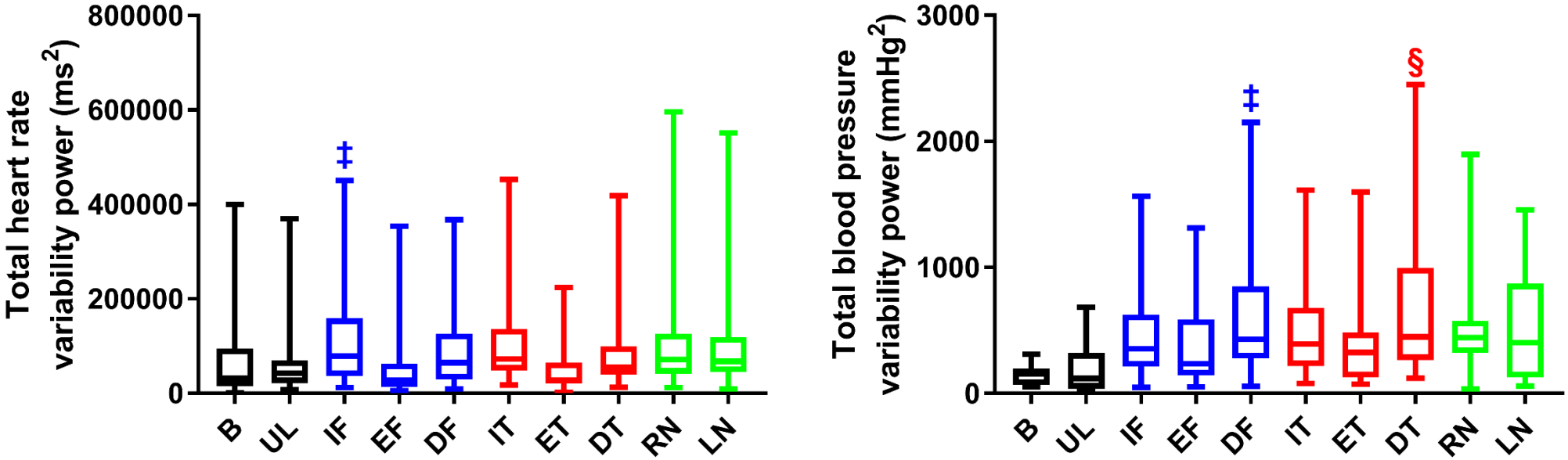


Figure 6-7 – Total heart rate variability and blood pressure variability power responses to resisted slow and deep breathing. Black: B- Baseline, UL -Unloaded slow and deep breathing; Blue: IF – flow dependent inspiratory load, EF – flow dependent expiratory load, DF – flow dependent dual inspiratory/expiratory load; Red: IT – threshold inspiratory load, ET – threshold expiratory load, DT – threshold dual inspiratory/expiratory load; Green: RN – right nostril breathing, LN – left nostril breathing. Data are for 15 participants (*n*=14 for RN and LN due to issues with placement of the nasal probe in one participant). ‡ different from EF, § different from ET. $P < 0.004$.

Table 6-3 – Heart rate and blood pressure variabilities

	Baseline	Unloaded	IF	EF	DF	IT	ET	DT	RN	LN
SDNN (ms)	51±7	75±8*	98±9	63±9†	80±9†	101±8§	66±8	86±9§	95±10	97±10
RMSSD (ms)	43±8	51±7	73±9	48±11†	60±9†	75±9§	47±10	61±8§	74±13	75±12
HRV_{LF} (ms^2)	49841±23801	50381±19485	78462±23811	34256±12899	55807±16262‡	91034±26488	23928±7168	61789±19389	58914±12837	67506±21236
HRV_{HF} (ms^2)	16560±5809	20580±5049	36782±9625	27664±11816	36150±9312	34818±9251	34127±14329	30953±8161	51211±27708	46402±17639
SD1 (ms)	49±9	58±8	86±10	57±12†	70±10†	86±10§	53±11	75±9§	83±12	85±11
SD2 (ms)	104±13	158±15*	211±18	132±17†	172±19†	213±16§	139±15	189±13§	203±23	205±21
SD1/SD2	0.47±0.05	0.36±0.02	0.40±0.02	0.40±0.04	0.40±0.02	0.40±0.02	0.35±0.03	0.40±0.05	0.41±0.04	0.42±0.05
BPV_{LF}($mmHg^2$)	100±24	194±52	408±108	297±89	564±148	437±123	320±98	677±173	410±98	446±110
BPV_{HF} ($mmHg^2$)	53±14	16±3	101±14	91±21	109±19	136±12	82±18	101±22	106±36	102±34

Data represent mean ± SEM for 15 subjects. Slow and deep breathing conditions performed at 6 breaths·min^{-1} and 30% FVC. IF – flow dependent inspiratory load, EF – flow dependent expiratory load, DF – flow dependent dual inspiratory/expiratory load, IT – threshold inspiratory load, ET – threshold expiratory load, DT – threshold dual inspiratory/expiratory load, RN – right nostril breathing, LN –left nostril breathing. Data for RN and LN are for 14 participants, due to issues with placement of the nasal probe in one individual. * unloaded slow and deep breathing different from baseline, † different from IF, ‡ different from EF, # different from IT, § different from ET. $P < 0.004$.

6-4.5 Phase angle and time shift

There were no differences in phase and time shift for the SBP-*fc* transfer function ($P > 0.004$ for all selected comparisons).

Effects of unloaded slow and deep breathing

Significant differences between baseline and unloaded SDB were found for the RESP-*fc* (baseline: 0.21 ± 0.09 s *vs.* UL: 1.91 ± 0.23 s; $P < 0.004$) and RESP-SBP relationships (baseline: -0.93 ± 0.32 s *vs.* UL: 1.48 ± 0.48 s; $P < 0.004$; Figure 6-8, Table 6-4) alone.

Comparison of flow resistive and pressure threshold loading

The use of different loading devices did not result in significant differences for any of the analysed phase relations ($P > 0.004$; Figure 6-8, Table 6-4).

Comparison of right vs. left single nostril breathing

Single nostril breathing conditions (RN and LN) showed similar behaviour for all analysed transfer functions, while also not differing significantly from dual loaded flow resisted breathing (DF; $P > 0.004$).

Inspiratory vs. expiratory resistances during slow and deep breathing

Both RESP-DBP and SBP-DBP functions exhibited larger phase angle and time shift values for the inspiratory loaded conditions (IF and IT) when compared to the expiratory and dual loaded breathing conditions using the same loading devices (IF > EF and DF; IT > ET and DT; $P < 0.004$; Figure 6-8, Table 6-4).

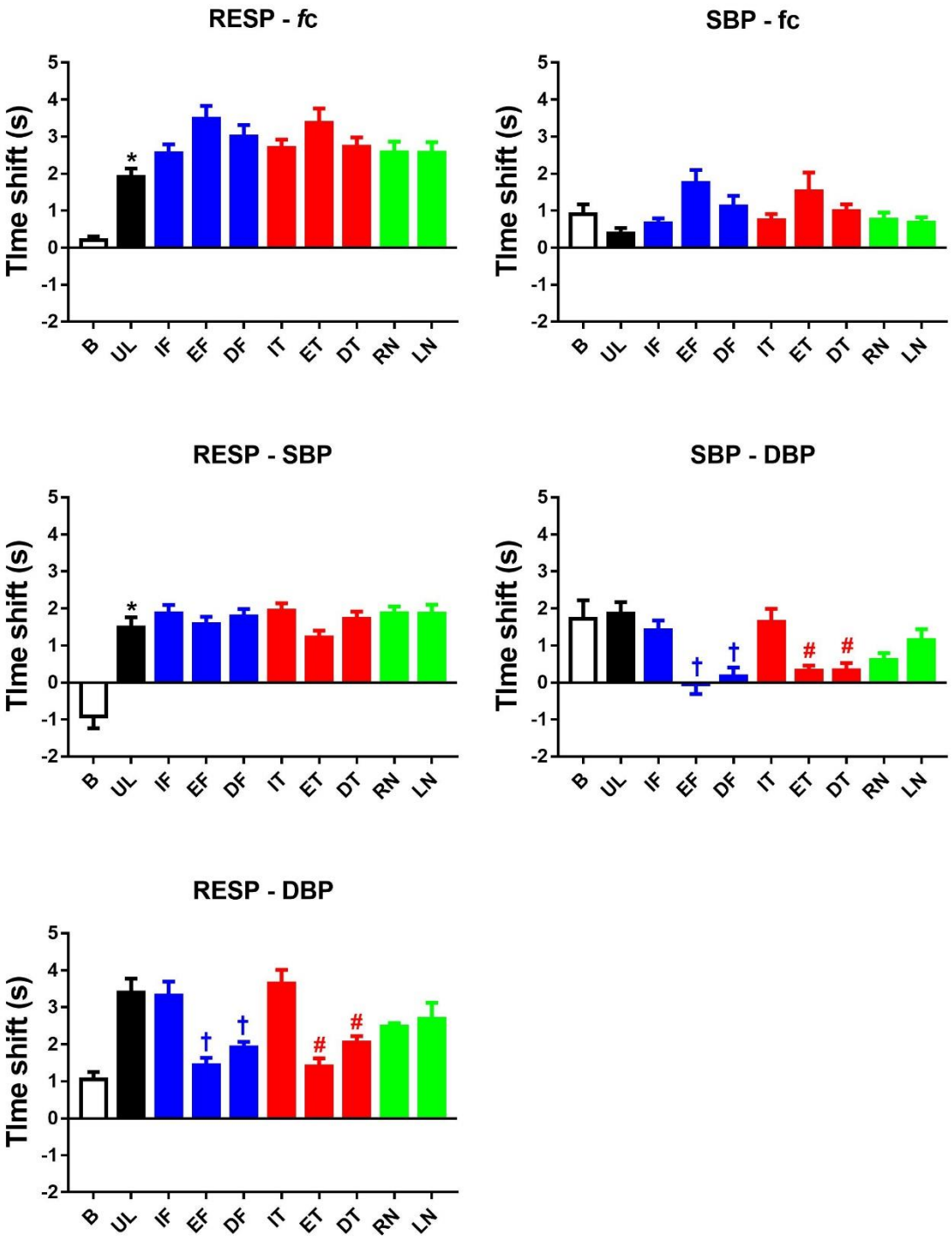


Figure 6-8 – Respiratory, heart rate and blood pressure time shift variations with resisted slow and deep breathing. Black: B- Baseline, UL -Unloaded slow and deep breathing; Blue: IF – flow dependent inspiratory load, EF – flow dependent expiratory load, DF – flow dependent dual inspiratory/expiratory load; Red: IT – threshold inspiratory load, ET – threshold expiratory load, DT – threshold dual inspiratory/expiratory load; Green: RN – right nostril breathing, LN – left nostril breathing. Data are mean ± SEM for 15 participants (n=14 for RN and LN due to issues with placement of the nasal probe in one participant). Signalled significant differences are for corresponding phase angles (Table 4). * UL different from B, † different from IF, # different from IT. $P < 0.004$.

Table 6-4 – Respiratory, blood pressure and heart rate phase angle response to loaded slow and deep breathing.

	Baseline	Unloaded	IF	EF	DF	IT	ET	DT	RN	LN
Resp-*fc* (deg)	14±6	66±8*	88±8	120±12	103±10	92±8	114±13	94±9	88±10	88±10
Resp-SBP (deg)	-60±21	51±10*	64±8	54±6	61±7	67±7	41±6	59±7	64±6	64±9
Res-DBP (deg)	68±13	116±13	114±13	49±6†	66±5†	125±13	47±7#	70±6#	85±4	92±15
SBP-*fc* (deg)	59±18	13±5	23±4	60±12	38±10	26±6	51±17	34±6	26±7	23±5
SBP-DBP (deg)	112±33	64±11	49±9	-2±9†	6±8†	56±12	11±4#	11±7#	21±6	39±10

Data represent mean ± SEM for 15 subjects. Slow and deep breathing conditions performed at 6 breaths·min^{-1} and 30% FVC. IF – flow dependent inspiratory load, EF – flow dependent expiratory load, DF – flow dependent dual inspiratory/expiratory load, IT – threshold inspiratory load, ET – threshold expiratory load, DT – threshold dual inspiratory/expiratory load, RN – right nostril breathing, LN –left nostril breathing. Data for RN and LN are for 14 participants, due to issues with placement of the nasal probe in one individual. * unloaded slow and deep breathing different from baseline, † different from IF, # different from IT. $P < 0.004$.

6-5 Discussion

This investigation sought to elucidate the acute cardiovascular and autonomic response to loaded SDB in healthy human beings by establishing: 1) the influence of adding inspiratory and/or expiratory resistances upon the efficacy of SDB alone; 2) the effects of inspiratory and expiratory resistances upon cardiovascular responses to SDB; 3) the cardiovascular responses to flow resistive loading and pressure threshold loading, and 4) the cardiovascular response to the different routes of respiratory airflow, specifically mouth, right nostril and left nostril.

A major finding was that the application of resistances that resulted in more negative intrathoracic pressures was correlated significantly with an increase in RSA and HRV indices. These responses were accompanied by increases in LVSV and the amplitude of ΔSV (such that $LVSV_E > LVSV_I$), while MAP, similarly to the previous studies, remained unchanged from unloaded SDB. In contrast, the application of expiratory loads promoted small, but significant 'pressor-like' response during SDB. Consistent with this notion, the use of expiratory resistances affected the phase relationships for DBP, with both the instantaneous variations in lung volume (RESP) and SBP showing a shortening of the delay in their relationships with DBP. Other important findings included the absence of any differences between right and left nostril breathing, and between these and dual flow resisted breathing (DF), at a similar respiratory pressure. When combined with the fact that no meaningful differences existed between the two types of loading, these data suggest that the acute cardiovascular response to loaded SDB is primarily driven by variations in mean respiratory pressure. Overall, the addition of a small inspiratory resistance seems to amplify of the acute within-breath LVSV and $\dot{Q}$ responses to SDB, as well as HRV, without a concomitant increase in blood pressures, whilst the use of expiratory resistances increases blood pressures without enhancing the amplitude of other cardiovascular and HRV responses observed with inspiratory resistances or unloaded SDB.

6-5.1 Effects of inspiratory vs. expiratory resisted breathing

Cardiovascular effects

Similar magnitude inspiratory and expiratory resistances were applied systematically using two different loading methods, as well as single nostril breathing. A major finding was the discovery of a tight inverse relation between changes in RSA magnitude and mean respiratory pressure (Figures 6-5 and 6-6). This pattern might be attributable mechanistically to increases in systemic venous return and LVSV with the application of inspiratory resistances, as previously suggested by others (Lurie, Mulligan, McKnite et al., 1998, Lurie, Zielinski, McKnite et al., 2000, Convertino, Ratliff, Ryan, Cooke, et al., 2004, Convertino, Ratliff, Ryan, Doerr, et al., 2004), and supported by data from Chapters 4 and 5 of this thesis. Several observations underpin this interpretation.

Firstly, in all but one participant, RSA was higher with the use of inspiratory resistances than with expiratory loaded breathing. Furthermore, similar individualised patterns were found for the LVSV and $\dot{Q}$, when comparing the application of inspiratory and expiratory resistances, while an opposite response (decrease with inspiratory resisted breathing *vs.* expiratory loaded breathing) was observed for TPR, MAP and ΔSV. The more negative values for ΔSV with the application of inspiratory resistances, under conditions where LVSV tends to have the opposite behaviour, suggests that any increases in LVSV with the inspiratory loaded breathing occur mainly during the expiratory portion of the respiratory cycle ($\uparrow LVSV_E$). This is consistent with findings from the two previous studies (Chapters 4 and 5) and likely reflects a delay between the changes in venous return to the right side of the heart and its expression in left ventricular output, interposed by relatively large pulmonary transit time, when compared to the normal length of the respiratory cycle, in spontaneously breathing individuals (5 to 10 s *vs.* 2.5 s, respectively) (Blumgart and Weiss, 1927b, Dornhorst et al., 1952b).

Secondly, multiple individual fixed effects regression analysis confirmed the close relationship between the increase (more positive) value of mean respiratory pressure and a decrease in RSA (R^2 = -0.80; $P < 0.001$), with weaker relationships observed for the within-breath LVSV amplitude (ΔSV: R^2 = 0.53; $P < 0.001$), MAP (R^2 = 0.47; $P < 0.001$), TPR (R^2 = 0.46; $P < 0.001$), $\dot{Q}$ (R^2 =0.32; $P < 0.001$) and LVSV (R^2 = 0.22; $P < 0.001$). Finally, stepwise multiple regression analysis

generated a four-predictor linear model that suggested RSA was most strongly influenced by $PP_{I/E}$, $\Delta\dot{Q}$, *fc* and ΔSV (R^2 = 0.721, P < 0.001). The presence of $PP_{I/E}$ (i.e. maximal amplitude in pulse pressure within the epoch), $\Delta\dot{Q}$ and ΔSV in this model, highlight the contribution of respiratory-driven fluctuations in left ventricular output to the generation and amplitude of RSA. However, it must be refered that, similarly to the previous experimental chapters, $\Delta\dot{Q}$ and *fc* are not independent from RSA, as RSA effectively represents the within-breath amplitude of *fc* and $\dot{Q}$ is given by the product of *fc* by LVSV. Since, participants breathed at 0.1 Hz, it is possible that the respiratory variations in $\dot{Q}$ enhanced RSA by entraining with naturally occurring low-frequency ABP oscillations at the same frequency (Elstad, Walloe, Chon et al., 2011). The complexity of the contributory mechanisms makes it extremely difficult to understand if $\Delta\dot{Q}$ is in fact enhancing RSA by its effect on ABP and subsequent stimulation of carotid baroreceptors, or whether RSA is in fact contributing to (or buffering) the phasic fluctuations in $\dot{Q}$ (Elstad et al., 2011).

Recently, results from a 6-week trial in healthy people of different loaded breathing patterns (at normal or slightly raised f_R) showed no difference in the magnitude of chronic reductions in blood pressure between expiratory and inspiratory loads, independent of V_T (Vranish and Bailey, 2015). The authors concluded that similar acute mechanisms should be involved for expiratory and inspiratory loading. However, the present data indicate that expiratory and inspiratory loads induce very different cardiovascular responses, which does not support Vranish and Bailey's interpretation. Two methodological differences are important to highlight before further consideration of the findings; firstly, the present study used much lower absolute respiratory loads and f_R, compared with Vranich and Bailey. A consistent pressor-like response was observed in the current study with the application of expiratory resistances, which was not present in the inspiratory resisted breathing conditions, suggesting there may be different aortic and carotid baroreceptor loading patterns, depending on the phase of breathing that is loaded. The elevations in MAP, SBP and DBP during expiratory loading are not consistent with earlier observations by Laciuga et al. (2012), who found no alterations in ABP with short (5 s) Valsalva manoeuvres and expiratory muscle training in healthy individuals. However, the magnitude of the expiratory load applied in Laciuga's study was much higher (75% of individual maximum expiratory pressure) and the length of the resisted breathing tasks lower (12 consecutive breaths, followed by another 13 breaths after measurement), compared to the present study. Nonetheless, the

present data also contradict previous reports of significant elevations in ABP following light inspiratory resisted breathing (6 to 12 cmH_2O) in human beings (Convertino, Ratliff, Ryan, Cooke, et al., 2004, Convertino, Ratliff, Ryan, Doerr, et al., 2004). Since BRS was unchanged in both Convertino's studies, the authors interpreted the increase ABP as evidence of 'resetting' of the operating point for SBP on the baroreflex stimulus-response continuum. In contrast, the present study found no changes in SBP, MAP or DBP during inspiratory loading, relative to baseline or unloaded SDB; furthermore, all BRS indices remained relatively unchanged throughout all interventions (except BRS_{Seq}, which was significantly higher for IF than for EF). For Convertino and colleagues, the changes in SBP were considered to be due to increased venous return, LVSV and $\dot{Q}$. However, the present study found no evidence to support the idea that the addition of a small inspiratory load during SDB could significantly increase LVSV and $\dot{Q}$ beyond what is generated by SDB alone. In contrast, when comparing the responses to inspiratory and expiratory loading, the present study found that a higher LVSV and $\dot{Q}$ was present during inspiratory loaded SDB, despite reductions in MAP. These observations are in agreement with previous observations by Seals and colleagues (1993), who reported an abrupt decrease in MAP during early inspiration with the application of inspiratory resistances of ~20 cmH_2O.

Within the constraints of the present study, it is reasonable to suggest that ABP fluctuations were mainly determined by the differing effects of loading inspiration and expiration, upon TPR and systemic venous return. This is consistent with echocardiographic evidence from studies of Valsalva and Mueller manoeuvres (as mentioned previously, breathing against an expiratory resistance can be likened to a partial Valsalva, with a similar logic being applied to inspiratory loaded breathing and the Mueller manoeuvre). Under this construct, the increase in MAP with expiratory resisted breathing is likely due to the combined effect of the following factors operating during expiration: 1) decreased ABP during the preceding unloaded inspiratory phase (Murgo, Westerhof, Giolma et al., 1980, Looga, 1997); 2) reflex response from arterial baroreceptors and lung vascular mechanoreceptors leading to a rise in *fc* and TPR in response to the above-mentioned decrease in ABP (Looga, 2005), 3) this reflex response is combined with the effects of an increase venous return during the preceding inspiratory phase upon LVSV, further contributing to an in increase in ABP (Looga, 2005), 4) the pneumatic effect of the enhanced expiratory effort compressing the heart and intrathoracic vessels, forcing

blood into periphery, despite an increased aortic intravascular pressure (Eckberg, 1980, de Burgh Daly, 1986). In contrast, inspiratory loading would resemble the cardiovascular response observed in the Mueller manoeuvre, with an initial increase in systemic venous return to the RV, accompanied by a rise in aortic transmural pressure (increased afterload), but followed rapidly by a reduction in preload as the difference between the negative intrathoracic pressure and positive intraabdominal pressure causes the large extrathoracic vessels to collapse at the thoracic inlets (Condos et al., 1987). Due to the length of PulTT (averaging ~6.5s at rest, according to Blumgart and Weiss [1927b]), during SDB the inspiratory increases in venous return to the RV translate into an expiratory rise in LVSV (Dornhorst et al., 1952b); similarly, the LVSV during inspiratory loading is determined by the preceding expiratory phase of breathing. Simultaneously, aortic transmural pressure decreases, contributing to a maintenance of MAP during inspiratory loaded SDB. Collectively, these observations suggest that the phase of the respiratory cycle where loading is applied, combined with inherent system lags (e.g. PulTT) determines the pattern of the cardiovascular response to loaded breathing, affecting MAP, but also left ventricular output, and consequently the magnitude of RSA.

Heart rate and blood pressure variability effects

Results from the present study show that loading the inspiratory phase of breathing results in significantly higher time domain (SDNN and RMSSD) and non-parametric HRV indices (SD1 and SD2) when compared to expiratory loaded SDB (Table 6-3). Loading simultaneously both phases of the respiratory cycle resulted in values between those observed for inspiratory and expiratory loaded SDB. In contrast to the time domain indices, in the frequency domain, only HRV_{TOT} seemed to be significantly increased by IF (but not IT) compared to a similar expiratory load delivered by the same method (IF: 115,243±29,563 *vs.* EF: 61,920±23,985 ms^2). Respiration is known to represent a significant portion of spectral HRV, profoundly altering HRV independent of changes in autonomic cardiac activity (Angelone and Coulter, 1964, Melcher, 1976, Hirsch and Bishop, 1981, Brown et al., 1993) making the quantification of spectral variability arising from different sources particularly challenging. The maintenance of an identical respiratory pattern across all conditions guaranteed that any significant differences in spectral HRV herein reported were either direct or indirect consequences of the different types and phases of loading. It must be emphasised that HRV measurements are not

analogous to direct measurements of either sympathetic nerve activity or cardiac parasympathetic outflow, providing a merely qualitative reflection of changes in cardiac autonomic regulation (Billman, 2011). As described in other sections of this thesis (see Chapter 2) there are several concurrent mechanisms interacting in a complex way that contribute to the variations in HRV, in such a way that any evaluation of cardiac autonomic activity based on the power spectral density in any of the HRV spectral bands is no more than an over-simplification of a nonlinear, multi-faceted phenomenon. The acute elevation of SDNN, RMSSD and HRV_{TOT} represents an increase in the overall cardiac short-term variability (Task Force, 1996) and is thought to positively contribute to an improved health status (Bigger, Fleiss, Steinman et al., 1995, Task Force, 1996, Bigger, 1997, Billman, 2011). Thus, the increases HRV in the present study likely reflect the action of mechanical factors contributing to changes in right atrial filling and stretch (Bainbridge, 1915, 1920, Bernardi et al., 1989) and respiratory regulated reflex responses involving aortic and carotid baroreceptor stimulation (Eckberg and Orshan, 1977, Eckberg, Rea, Andersson et al., 1988, Eckberg, 2003, Rothlisberger et al., 2003). These observations supplement those of Calabrese et al. (2000) who, using simultaneous inspiratory and expiratory resisted breathing, observed no changes in spectral HRV with an increase of load, after correcting for the differences in respiratory pattern across their different conditions. These authors argued that the applied load (0-12.5 cmH_2O) had either been insufficient to alter baroreceptor input to cardiac motoneurones or that the marked within-breath ABP fluctuations were not the determinant factor involved in the changes in RSA and HRV. There is some support for the latter interpretation in our data, as suggested by the larger BPV_{TOT} values in the dual loaded conditions. Furthermore, in Calabrese's study inspiratory and expiratory phases were loaded simultaneously (similar to DF and DT in our study), and our HRV results for dual loading sets are akin to those observed with unloaded SDB, and thus consistent with those of Calabrese, under similar loading conditions. However, during single breath phase loading, our study did show a distinct HRV patterns, which were directly related to the phase of breathing that was loaded. Collectively, these findings point to a dissociation between the amplitude of ABP fluctuations and HRV, strengthening the case for a significant contribution arising from mechanically driven atrial stretch.

Cardiorespiratory phase angle and time shift

The DBP transfer function phase angle and time shift with both the instantaneous lung variation (RESP-DBP) and SBP (SBP-DBP) decreased substantially when an expiratory load was applied (either isolated or combined with an inspiratory resistance) despite RESP-*fc*, RESP-SBP and SBP-*fc* remaining fairly unaltered across all conditions (Table 6-4; Figure 6-8). These findings expand on previous research (Blaber and Hughson, 1996) and show that loading the expiratory phase of breathing has an almost immediate, mechanically driven, impact upon peripheral resistance as DBP is determined by the TPR, the viscoelastic properties of the arteries, and by diastolic interval (*fc*). As mentioned in chapter 4 (section 4-5.2), increased TPR decreases systolic run-off while diastolic run-off is increased, resulting in an elevation of MAP (London and Guerin, 1999), which is consistent with the changes in TPR and MAP observed in the present study (Table 6-2). Blaber and Hughson (Blaber and Hughson (1996) implemented dual inspiratory and expiratory resisted breathing, founding no changes in the phase relationship between LVSV, SBP and RR interval with the increase in applied resistance, suggesting that these phase relationships were primarily mechanically-driven by respiration. Similarly, in the present study, the phase angle for SBP-*fc* remained relatively constant across conditions. Moreover, while the RESP-SBP was unaltered, irrespective of the breathing phase being loaded, the SBP-DBP phase angle reduced from ~50º (1.5 s) in the unloaded or inspiratory loaded conditions to 0-10º (0-0.3 s) when expiratory resistances were applied. As fluctuations in SBP and DBP are occurring almost simultaneously, and LVSV has a much more pronounced impact upon SBP than DBP, the SBP-DBP relation is likely a reflection of the respiratory mechanical effects of expiratory loading upon TPR. Due to insurmountable methodological limitations, within the time constraints of this work, we were unable to calculate LVSV phase relationships with RESP, *fc* and SBP.

6-5.2 Flow resisted breathing vs. threshold loaded breathing

The third aim of the present study was to examine if the application of different respiratory loading methods, at similar respiratory pressures, would influence the cardiovascular and autonomic response to the loaded SDB; flow resistive loading was compared with pressure threshold loading. To the best of the author's knowledge, this is the first such comparison. Thus, a novel finding emerging from

the study is the absence of any significant differences between the two loading methods for any cardiovascular variables, or HRV and BPV, irrespective of the respiratory phase in which the load was applied (inspiratory, expiratory or both). The only exception was a small, but significantly higher fc_I (IF: 71±2 *vs.* IT: 74±2 beats·min^{-1}) and slightly lower TPR_I (IF: 14±1 *vs.* IT: 13±1 mmHg·min·L^{-1}) with IT, when compared to IF. We associate these small differences to slightly different pressure variation and airflow profiles between the two methods, particularly when the respiratory loading is applied during the inspiratory phase. With inspiratory threshold loading, the airflow is obstructed until enough pressure is generated to open the inspiratory valve. In order to be able to generate airflow and follow the visual feedback corresponding to a sinusoidal flow profile in the IT condition, participants had to produce the necessary opening pressure very early in the inspiratory phase, leading to a very abrupt decrease in intrathoracic pressure. In contrast, during flow resisted breathing, the pressure changes developed more progressively, for most participants (Figure 6-9).

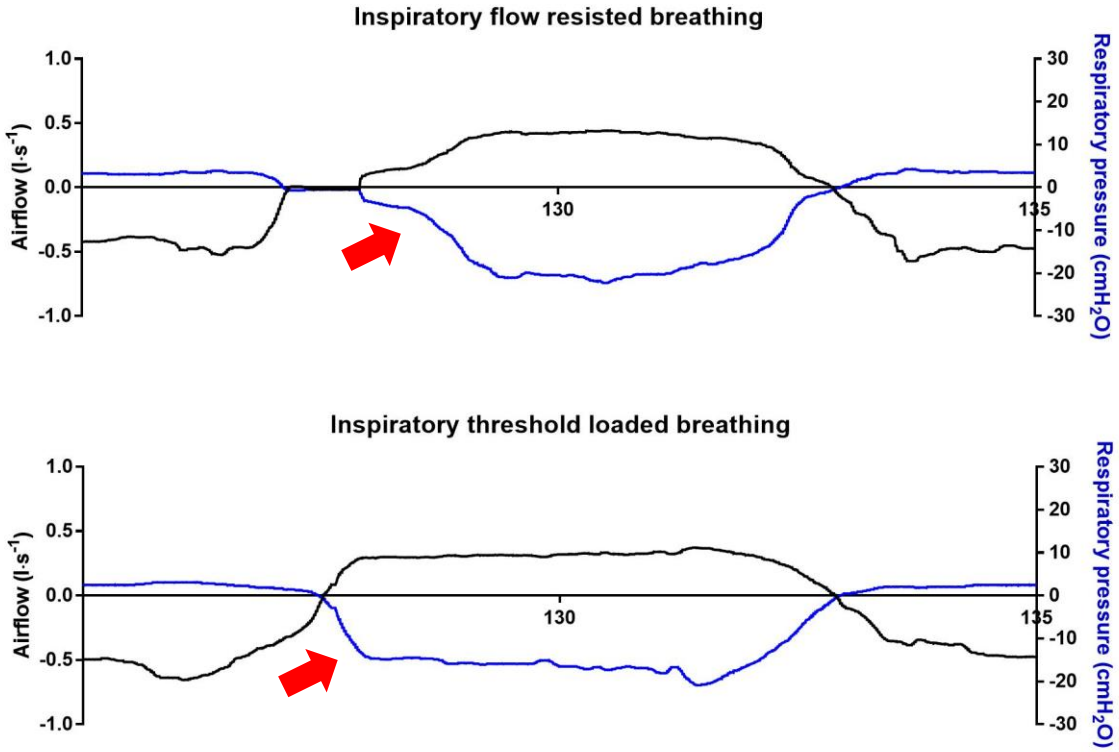


Figure 6-9 - Comparison of airflow and respiratory pressures for one individual using a flow resisted breathing device (upper panel) and a threshold loading device (lower panel). Red arrows highlight differences between the two methods during early inspiration.

One potential effect of a quicker decrease in intrathoracic pressure might be an earlier (and more pronounced) increase in systemic venous return to the right atrium, with a more potent activation of cardiac mechanical stretch receptors, leading to the observed small inspiratory increase in fc_I with IT. Notwithstanding this nuance, the findings reported in this chapter show for the first time, that the loading method does not affect the cardiovascular and HRV responses to loaded SDB, provided that the respiratory pressures and respiratory pattern are similar. Small differences in *fc* between methods might be due to dissimilarities in the rate of respiratory pressure change during early inspiration.

6-5.3 Single-nostril breathing

Over the last couple of decades, some evidence has arisen suggesting that unilateral nostril breathing can have distinct beneficial effects upon ABP (Raghuraj and Telles, 2008, Pal et al., 2014), HRV (Pal et al., 2014) and autonomic activity (Telles et al., 1994). Breathing through the right nostril alone has been shown to contribute to an acute increase in SBP, DBP and MAP, and heightened sympathetic activation, while the opposite has been observed for left nostril breathing (Telles et al., 1994, Raghuraj and Telles, 2008, Pal et al., 2014). An explanation offered by authors for these observations is that single nostril breathing differentially activates the brain hemispheres, impacting both cognition and emotional states (Block, Arnott, Quigley et al., 1989, Schiff and Rump, 1995).

The existence of a cyclic engorgement of left and right nasal mucosa has been documented to range from 25 to 200 min, leading to an alternation of the nostril through which airflow resistance is greatest (Schiff and Rump, 1995). This phenomenon has been related to known lateralised rhythmic activation patterns of the cerebral hemispheres (Werntz, Bickford, Bloom et al., 1983). This relationship between increased airflow and the(contralateral) hemisphere activation is also present with single nostril breathing, as suggested by increased EEG activity in the contralateral hemisphere to the open nostril (Werntz, Bickford and Shannahoff-Khalsa, 1987).

A novel finding of the present study was that, in contrast to previous research, we found no differences in the cardiovascular and HRV response to single nostril breathing. Further to this main finding, no differences in any of the analysed variables were observed between the single nostril breathing conditions and the dual flow resistive breathing condition (both inspiratory and expiratory phases loaded). Unlike any of the studies mentioned above, air flow rate was controlled closely, and respiratory pressure monitored so that respiratory flow rates were identical for DF, RN and LN, while both P_I and P_E were quantified. The latter were very similar, despite larger intra-individual differences between RN and LN (Table 6-1). As an additional precaution, excess nasal mucus was removed by nasal wash with saline prior to any of the single nostril breathing sets, guaranteeing that any differences between right and left nostril breathing respiratory pressure were determined by individual anatomical differences. Collectively, the present data suggest that any differences between nostrils during single nostril breathing

encountered in previous studies are likely due to different airflow resistances resulting from anatomical differences or mucus accumulation, leading to dissimilar inter-nostril resistances and associated pressures. Notwithstanding, the consistency across previous studies suggesting a sympathoexcitatory effect of right nostril breathing, with somewhat opposite effects associated with left nostril breathing, may warrant further investigation.

6-6 Limitations

In this study, evidence is provided for the existence of an important functional link between the within-breath fluctuations in LVSV and BPV. Further to this finding, it was speculated that there is a contribution of systemic venous return variations, in phase with respiration, to the generation of RSA. However, the absence of spectral and cross-spectral data relating to the LVSV waveforms is a limitation that leaves a gap in the characterisation and understanding of said mechanical link.

6-7 Conclusions

Inspiratory loaded SDB acutely heightens RSA, HRV, LVSV and the amplitude of within breath LVSV fluctuations, without concomitant increases in MAP. The addition of inspiratory loads amplifies the cardiovascular and HRV of SDB alone. Expiratory loaded breathing, on the other hand, failed to elicit the same cardiovascular and HRV response, while generating a significant pressor response (likely associated with mechanically-driven increases in TPR), and might therefore indicate that expiratory loads should be used with caution in certain clinical populations, especially as they seem less 'effective'. The data lent support to the previous construct (see Study 2 – Chapter 5) that RSA is a 'composite' index to both mechanical and reflex effects of SDB, with the existence of an important functional link between respiratory driven variation in LVSV and the amplitude of RSA. The application of multiple regression analysis to this study's data indicates that $PP_{I/E}$, $\Delta\dot{Q}$, *fc* and ΔSV are the main determinants of RSA. The use of different loading methods (flow resistive breathing *vs.* pressure threshold) seems to have no impact on the acute response to loaded SDB. Considering that the quantification of the resistance with flow resisted devices involves a precise control of the respiratory

flow rate, which normally can only be accomplished with the assistance of expensive equipment, the use of an inspiratory pressure threshold loading device may represent a more reliable and affordable alternative. Finally, the use of single nostril SDB proved to generate similar responses to that of dual flow resistive breathing, while no differences between left and right nostril breathing was identified. The observation of comparable outcomes with single nostril breathing, at similar airflow rates and respiratory pressures warrant further investigation, particularly how its application in combination with other respiratory techniques may provide potential therapeutic benefits in certain clinical populations, particularly in hypertensive individuals. Furthermore, whilst an argument has been made for a potential therapeutic effect of combining SDB with small inspiratory loads, future studies are required to test its clinical efficacy, particularly as an anti-hypertensive therapy. Results from this study have provided evidence of positive cardiovascular and HRV acute responses with inspiratory resisted breathing that have been previously shown to be related with an improved health status. Whether the magnitude of the observed acute changes is sufficient to cause long term cardiovascular and autonomic adaptation, and whether the same benefits are observed in hypertensive/elderly populations, in comparison to the healthy cohort herein reported, remains to be scrutinised.

CHAPTER 7 – GENERAL DISCUSSION

7-1 Introduction

The goal of this chapter is to appraise the major findings of this thesis and to discuss them in the context of the existing state-of-the-art. A mechanistic perspective is provided by integrating findings from this thesis and previous work described in the extant literature. The chapter concludes by outlining potential future research paths.

7-2 Overview of Objectives

The primary aim of this thesis was to characterise the acute cardiovascular and autonomic responses to SDB, in healthy individuals. To meet this goal, a number of objectives were addressed, which included:

1) determine the independent effects of f_R, V_T and P_aCO_2 to the acute cardiovascular and autonomic responses to SDB;
2) ascertain the contribution of intrathoracic pressure swings to the acute cardiovascular and autonomic responses to SDB;
3) compare the acute impact of different methods of creating intrathoracic pressure swings during SDB.

7-3 Main Findings

7-3.1 Effects of breathing frequency, tidal volume and P_aCO_2

The aims of Chapter 4 were to evaluate the independent effects of f_R, V_T and P_aCO_2 to the responses to SDB. The main findings of chapter 4 confirmed previous observations that heart rate variability (HRV) and RSA magnitudes tended to increase with the decrease in f_R, with average maximal values reached at frequencies close to 0.1 Hz (Angelone and Coulter, 1964, Hirsch and Bishop, 1981, Cooke et al., 1998, Song and Lehrer, 2003). Simultaneously, increments in V_T promoted linear increases HRV, independently of f_R. Furthermore, blood pressure variability (BPV) was significantly reduced at the lowest f_Rs, likely due to the involvement of a baroreflex mediated responses, while V_T did not impact BPV significantly. We speculate that RSA worked to buffer the impact of respiratory-driven fluctuations in venous return upon left ventricular cardiac output ($\dot{Q}$) and arterial blood pressure (ABP). A novel finding of Chapter 4 was that for f_Rs < 8 breaths·min^{-1}, SDB promoted an inversion of the normal within-breath (inhalation *vs.* exhalation) pattern of left ventricular stroke volume (LVSV) such that expiratory

LVSV exceeded inspiratory LVSV. We attribute this finding to the influence of a lag between enhanced right atrial filling and RVSV during inspiration, and its expression in LVSV. We believe this lag was created by the effect of the time required for blood to transit the pulmonary circulation. Finally, there appeared to be a limited influence of P_aCO_2 upon the cardiovascular and HRV response to SDB.

7-3.2 Effects of intrathoracic pressure variation

The data presented in Chapter 5 supported the important contribution of respiratory-driven increases in right-atrial filling during inspiration, and suggests a relevant contribution of previously underappreciated reflex, and/or 'myogenic', cardiac response mechanisms, to the amplification of RSA and HRV during SDB. Inspiratory loading during SDB (i.e. magnified negative intrathoracic pressure) increased inspiratory pressure-driven fluctuations in LVSV and *fc,* and enhanced $\dot{Q}$, independently of changes in V_T and f_R. When SDB was loaded, the within-breath (inhalation *vs.* exhalation) behaviour of LVSV, *fc* and $\dot{Q}$, combined with the non-existence of significant changes in MAP and TPR suggested the absence of meaningful, additional acute autonomic changes. Evidence was provided regarding a causative relationship between within-breath fluctuations of LVSV and BPV. Furthermore, respiratory-driven fluctuations of LVSV (ΔSV), SBP (BPV) and *fc* (RSA) were strongly, linearly related to phase shifts between cyclic fluctuations in lung volume, SBP and *fc*. Overall, Chapter 5 provided support for a contribution of both mechanical and reflex factors in the generation and amplitude of RSA during loaded SDB.

7-3.3 Effects of different methods of creating intrathoracic pressure variations

The aims of chapter 6 were to examine the effects of specific methods of increasing the magnitude of within-breath intrathoracic pressure fluctuations upon the responses to SDB. The findings in chapter 6 suggested that the method of loading did not affect the cardiovascular response to resisted SDB. In other words, flow resisted breathing and threshold loaded breathing resulted in similar patterns of acute response at the same level of resistance. Notwithstanding, the directionality of the resistance (i.e. inspiratory *vs.* expiratory) impacted the response pattern to SDB; the isolated use of inspiratory SDB amplified the effects observed with un-resisted SDB (reported in chapter 5), while the application of expiratory resistances

increased the ABP and total peripheral resistance (TPR) response to SDB, while limiting RSA, LVSV and $\dot{Q}$, when compared to inspiratory resisted SDB. The simultaneous application of inspiratory and expiratory resistances did not result in increased magnitude of acute cardiovascular responses, when compared to inspiratory resistances alone. Finally, single nostril breathing provided similar results to those observed with the simultaneous application of inspiratory and expiratory resistances of a similar magnitude, while no differences were found between the right and left nostril.

7-4 Interpretation of findings

The following section focuses on an interpretation of the main findings previously described in point 7-3. Figure 7-1 is a schematic representation of a potential model depicting the different factors that interplay to determine the acute cardiovascular response to SDB and frames the discussion that follows.

The primary aim of the current chapter is to interrogate the findings of the present thesis in the context of the extant literature. Considering the accumulated of data that currently exists, and to which we believed to have been able to contribute with this thesis, it seems reasonable to depict an integrated model to portray the mechanistic pathways involved in the acute cardiovascular response to SDB.

The proposed model aims to provide a possible exemplar from which a clearer understanding of how the manipulation of breathing pattern, as performed during the studies that comprise this thesis, impacts key cardiovascular variables acutely. The extreme complexity and multiplicity of systems that interplay and contribute towards this phenomenon mean that, to date, most studies exploring cardio-respiratory interactions have examined potential mechanisms in isolation. Thus, the vast majority of quantitative data examining the acute cardiovascular effects of SDB breathing have been derived from reductionist human models that have, in my view, failed to systematically address the potentially independent effects of f_R, VT, P_aCO_2 and the amplitude of intrathoracic pressure variation.

In contrast, the development of complex multifactorial mathematical models has allowed for the advancement of the state-of-the-art in this area of physiology, and

for the simulation of expected cardiovascular responses to a variety of perturbing stimuli. However, said models are far too complex to enable the identification of the key features that make the largest contribution(s) to cardiovascular system perturbation during SDB, in an integrated human model. While the proposed model draws from diverse sources of research, it mostly reflects the findings and interpretation of the data that emerged from the current thesis, and should, therefore, be interpreted within the constraints of this specific experimental paradigm.

The model is based upon a hierarchy of respiratory, cardiovascular and autonomic interactions, where for simplicity, many intermediary factors have been omitted. The model proposes that the manipulation of the respiratory pattern by SDB induces direct and indirect physiological responses to the key mediating inputs: intrathoracic pressure (ITP), tidal volume (V_T) and breathing frequency (f_R). These inputs also carry a degree of interdependence.

A general mechanistic description of the effects of SDB, based on the model presented in Figure 7-1, is underpinned by inspiratory increases in venous return associated with a more negative intrathoracic pressure throughout inspiration. The latter amplifies the pressure gradient between the right atrium and the systemic venous pressure, effectively 'sucking' blood from the major venous vessels into the right atrium (Brecher, 1952, Dornhorst et al., 1952b, Brecher and Hubay, 1955, Robotham et al., 1978, Robotham and Mintzner, 1979, Kim et al., 1987, Peters et al., 1989, Innes et al., 1993). Breathing at larger V_Ts amplifies the inspiratory decrease in intrathoracic pressure, and promotes increased intra-abdominal pressure, as the diaphragm obtrudes upon the abdominal cavity (Willeput et al., 1984, Boutellier and Farhi, 1986, Kimura et al., 2011, Mesquida, Kim and Pinsky, 2012), thereby contributing to the pressure gradient between the right atria and systemic venous pressure. This pressure gradient can be further amplified by the addition of an inspiratory resistance, thus promoting a more accentuated decrease in intrathoracic pressure throughout inspiration. Finally, f_R can also impact cardiovascular dynamics, not only via an effect upon ITP (as f_R decreases air flow rate is reduced for the same V_T, thus attenuating ITP swings; this effect is likely negligible during SDB), but more potently, by altering the duration of the periods of facilitated venous return and the relation between the expression of increased venous return to the right atrium and the expression of such variations into

augmented LVSV, due to the lag interposed by the pulmonary circulation. Furthermore, at specific f_Rs, entrainment occurs between respiratory, cardiac and systemic oscillations, which have been attributed to the resonant properties of the systemic baroreflex; this phenomenon is believed to greatly contribute to the amplification of ABP and *fc* oscillations at the predominant respiratory frequency (Vaschillo et al., 2002, Vaschillo et al., 2006).

Finally, while not included in the model, this section also includes an overview of the significance of the findings regarding the impact of P_aCO_2 changes upon the acute cardiovascular response to SDB.

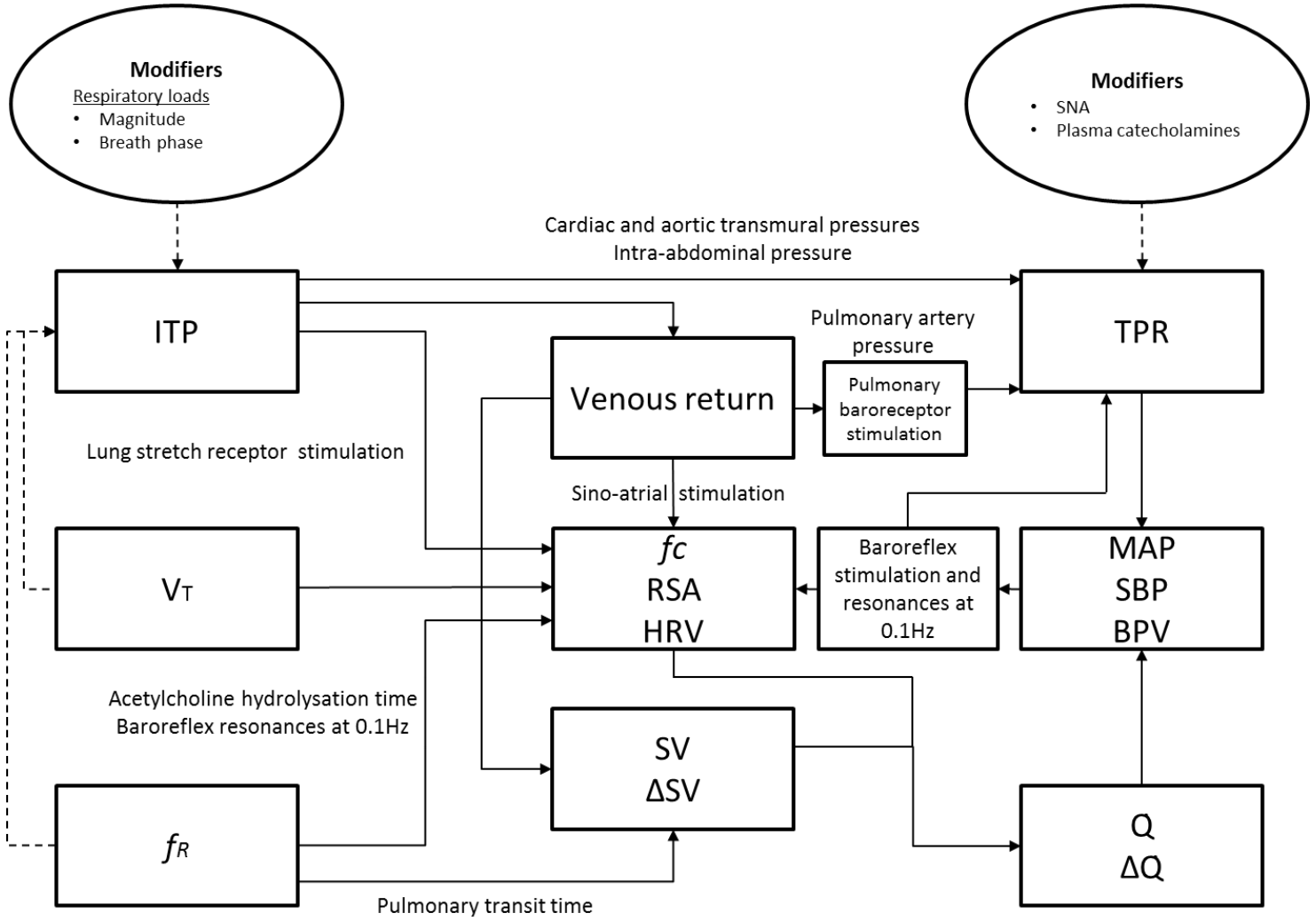


Figure 7-1 – A schematic of an integrated mechanistic model to describe the acute cardiovascular responses to slow and deep breathing

7-4.1 Effect of breathing frequency

Relation with pulmonary transit time

A novel finding of the present study was the frequency-dependent interrelationship of the within-breath (inspiration *vs.* expiration) changes in LVSV (Figure 4-3B). The apparently paradoxical decrease in LVSV during inhalation observed at some f_{Rs} is due to the delay between increases in venous return to the right atrium being expressed in the LVSV, likely induced by the pulmonary circulation (Dornhorst et al., 1952b, Harrison, Goldblatt, Braunwald et al., 1963, Hamzaoui et al., 2013). Thus, the inversion of inspiratory LVSV relative to expiratory LVSV below 6.6 breath·min^{-1} can be explained by the influence of pulmonary transit time (PulTT). During spontaneous breathing, increased right ventricular filling during inspiration and is translated into increased left ventricular filling and LVSV during the ensuing expiration (Figure 7-2).

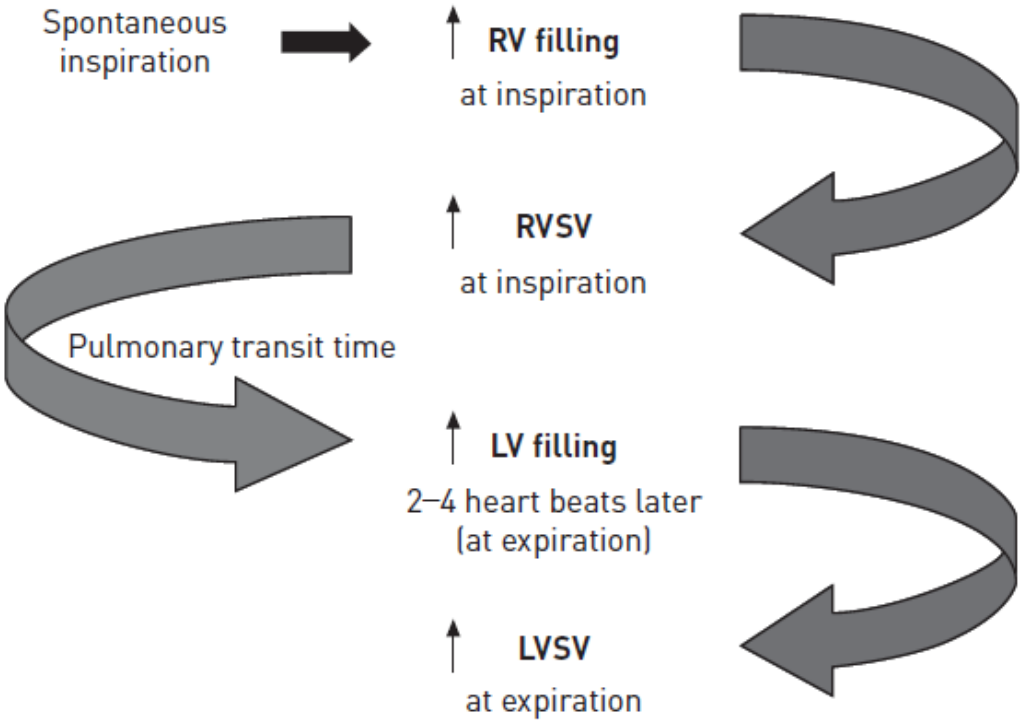


Figure 7-2 - Effect of pulmonary transit time upon the transmission of right to left ventricular filling changes, during spontaneous breathing. Inspiration results in increased right ventricular (RV) filling and stroke volume (SV). Pulmonary transit time interposes a phase lag between the right ventricle and the left ventricle (LV) so that LVSV is maximal during expiration. Adapted from Hamzaoui et al. (2013)

Cross-spectral analysis of the relationship between instantaneous lung volume changes and SBP further supports the interpretation of a significant role of PulTT in determining LVSV phasic changes during SDB. A variable lag was identified between the start of inspiration and the induced reduction in SBP. This lag was, on average, 0º (completely in phase) at an f_R of approximately 6.7 breaths·min^{-1}. A possible explanation is that, at this frequency, the expiratory LVSV (LVSVe), and the associated decrease in SBP, lag behind the inspiratory increase in venous return by half a respiratory cycle (breath); in other words, the onset of inspiration coincides with the arrival at the left ventricle of the [attenuated] RVSV generated by the preceding exhalation.

Previous reports suggest PulTT ranges from 5 to 17s (Blumgart and Weiss, 1927b), with an average PulTT of ~6.5s-7.9s in healthy human beings at rest (Blumgart and Weiss, 1927b, Levinson, Pacifico and Frank, 1966). A simple mathematical exercise based on the zero lag between the change in lung volume and the variation in SBP, at an f_R of 6.7 breaths·min^{-1}, leads to an estimation of a group mean PulTT of around 4.5s. Whilst this crude estimate is lower than the aforementioned reported PulTT, it worth pointing out the vast variation in methods and criteria that exist in relation to estimates of PulTT, perhaps the most important of which being, 1) health status of the participants; 2) the start and end points for the estimation, e.g. right atrium to left ventricle. Furthermore, if we consider that increases in $\dot{Q}$ can significantly impact PulTT, as demonstrated by the considerable reduction of PulTT when moving from rest to exercise (Zavorsky et al., 2003), it is plausible that small changes in $\dot{Q}$ and $\Delta\dot{Q}$ associated with SDB might have resulted in a small reduction in average PulTT in our healthy, young male participants, compared to a spontaneous breathing condition. Despite the small discrepancy, my crude calculations nonetheless support the potential influence of the pulmonary circulation upon determining the amplitude and directionality (inspiratory *vs.* expiratory) of the within-breath variations in LVSV. Future research using first pass radionuclide cardiography (Zavorsky et al., 2003) during SDB, might help to further clarify the role of PulTT in the within-breath cardiovascular patterns.

Spontaneously breathing individuals (paced breathing at the spontaneous f_R) normally demonstrate phase opposition between RVSV and LVSV (Gabe, Gault, Ross et al., 1969, Elstad, 2012). However, while other factors like interventricular dependence (Bove and Santamore, 1981, Amoore and Santamore, 1989, Peters et

al., 1989, Amoore et al., 1992) and the compliance of the right ventricle (Santamore and Amoore, 1994) can possibly play a role in determining such phase relations, most studies examining this phenomenon have failed to do so in the context of SDB and/or overlooked the potential impact of PulTT. Also, the impact of the drop in intrathoracic pressure during inspiration, affecting afterload and SBP, independently of the changes in left ventricular output, cannot be disregarded. Thus, the above interpretation of the data should be considered as 'food for thought', and as a stimulus for further research regarding the potential influence of PulTT upon the haemodynamic responses to SBD. Nonetheless, it seems extremely probable, given the unquestionable existence of a transit time delay interposed by the pulmonary circulation, that alterations in f_R contribute to changes in the phase relationship between the outputs of the two ventricles, thereby contributing to f_R–dependent behaviours of left ventricular ΔSV ($LVSV_I$ minus LVSVe).

Breathing frequency and resonant behaviour of the cardiovascular control system

It is well established that HRV indices, and particularly RSA, are affected by f_R and tend to maximise at around 6 breaths·min^{-1} (Angelone and Coulter, 1964, Kelman and Wann, 1971, Hirsch and Bishop, 1981, Berger et al., 1989a, Saul et al., 1989, TenVoorde et al., 1995, Cooke et al., 1998, Taylor, Myers, Halliwill et al., 2001, Giardino, Glenny, Borson et al., 2003, Song and Lehrer, 2003). These observations are consistent with the data reported in Chapter 4. Notwithstanding, important inter-individual differences in the f_R at which RSA was maximised were observed by us, and by others, possibly reflecting differences in stature, gender and the volume of individual participant's vasculature (Lehrer, 2007), including their pulmonary vasculature.

Some authors have speculated that the maximisation of HRV at 6 breaths·min^{-1} reflects an attempt of the cardiovagal baroreflex to buffer the ABP oscillation occurring at 0.1 Hz and the existence of resonances in the baroreflex control loop at frequencies close to 0.1Hz (TenVoorde et al., 1995, Julien, 2006, Di Rienzo et al., 2009). However, the increased RSA and HRV does not necessarily indicate increased buffering ability of the cardiac baroreflex at such frequencies. In fact, it has been suggested that when breathing at frequencies close to 0.1 Hz, RSA and

HRV can further magnify respiratory-driven fluctuations in ABP (Taylor and Eckberg, 1996, Elstad et al., 2001, Vaschillo et al., 2002, Tan and Taylor, 2010).

While we found no definitive evidence of the existence of a resonant behaviour linking ABP and *fc* through the cardiac baroreflex, previous studies seem to support the contribution of such phenomena to the amplification of ABP variability at frequencies close to 0.1Hz (De Boer et al., 1987, Lehrer et al., 2000, Vaschillo et al., 2002, Julien, 2006, Vaschillo et al., 2006). It has been suggested that breathing at a specific, individualised, resonant f_R, potentially contributes to an amplification of cardiovascular variability due to an approximate 5-second delay in the baroreflex control of vascular tone; likely determined by the elasticity of the vasculature (Vaschillo et al., 2002), which is regulated by both neural (Fagius and Wallin, 1980, Wallin and Nerhed, 1982, Julien, 2006, Zamir, Goswami, Liu et al., 2011) and myogenic processes (Stefanovska and Bracic, 1999a, b, Zamir et al., 2011). Supporting his theory of resonance, Vaschillo and colleagues described regular phase relationships between *fc* and ABP at very specific, individualised, frequencies (Vaschillo et al., 2002). Under this construct, ABP and *fc* fluctuations are 180º out of phase at the resonant frequency for *fc* (which occurs within the low-frequency range, close to 0.1Hz). This results in perfectly opposite fluctuations in *fc* and ABP, which reinforce each other. In other words, as respiration produces oscillations in *fc* through mechanical and reflex processes, these are further amplified by engagement of baroreflex control of ABP. The baroreflex response produces decreases in *fc* and vascular tone (MSNA) in response to increased ABP, while decreases in ABP result in increased MSNA and *fc*. Subsequently, baroreflex-induced fluctuations in *fc* and MSNA produce and maintain oscillations in ABP, thus contributing to its modulation and amplification at said resonant breathing frequency.

However, there are some caveats that need to be applied to Vaschillo's theory. Firstly, Vaschillo suggests that ABP resonant frequency occurs within the very low-frequency band and that at this frequency *fc* and ABP oscillate in phase with each other, resulting in an inhibition of *fc* oscillations by baroreflex influences, while BPV is increased (Vaschillo et al., 2002). This contradicts the prevalent belief that buffering of BPV occurs fundamentally within the very-low frequency spectral band, while it's resonant frequency lies within the low-frequency band and is intimately related to the occurrence of the so called Mayer-waves (Julien, 2006), as demonstrated by sino-atrial denervation in animals (Di Rienzo, Castiglioni, Parati et

al., 1996, Mancia et al., 1999), mathematical modelling (van de Vooren et al., 2007) and evidence arising from paraplegic (Castiglioni, Di Rienzo, Veicsteinas et al., 2007) and quadriplegic (TenVoorde et al., 1995) individuals.

Secondly, MSNA is believed to display respiratory-dependent pattern, inverse to that of *fc* (i.e. *fc* increases throughout inspiration, while MSNA tends to decrease during inspiration), with most MSNA bursts taking place at the lowest lung volumes (Eckberg et al., 1985, Seals et al., 1990, Seals et al., 1993). To the best of the author's knowledge, no systematic characterisation of the effect of f_R upon the phase relation between respiration and MSNA has been produced to date. However, it is accepted that MSNA during spontaneous, uncontrolled breathing does not differ from controlled paced breathing (Eckberg et al., 1985). Nonetheless, the duration of the respiratory cycle, more particularly the length of the inspiratory phase, might shift the onset of sympathoinhibition (Seals et al., 1990). Therefore, without the characterisation of the impact of f_R upon MSNA and a complete understanding of the phase relationships between, *fc*, ABP and MSNA, a precise description of the mechanisms involved in cardiovascular resonant behaviour remains merely hypothetical.

Thirdly, most depictions of the cardiovascular resonant behaviour seem to disregard the direct contribution of respiratory-mediated changes in MSNA to the Traube-Hering wave[5] amplitude and consider these oscillations in ABP as the logical consequence of respiratory-driven changes in systemic venous return, and subsequent effect upon $\dot{Q}$. It has been demonstrated that respiratory-mediated changes in MSNA are closely associated with Traube-Hering wave amplitude (Towie et al., 2012, Shantsila, McIntyre, et al., 2015). Furthermore, while earlier work has described the respiratory modulation of MSNA in terms of its effects upon total activity (Eckberg et al., 1985, Seals et al., 1990, Seals et al., 1993, St. Croix et al., 1999, Dempsey et al., 2002, Limberg et al., 2013), some studies have characterised the MSNA response to external stimulation in terms of the burst incidence, frequency and strength (Kienbaum, Karlssonn, Sverrisdottir et al., 2001, Shantsila, McIntyre, et al., 2015), and determined that these factors are modulated differently by respiration (Shantsila, McIntyre, et al., 2015) and baroreflex stimulation

[5] Traube-Hering waves are respiratory related oscillations in arterial blood pressure. A more detailed description of respiratory-driven cardiovascular fluctuations can be found in Chapter 2, section 2-4.2 – Oscillations in blood pressure.

(Kienbaum et al., 2001), adding yet another layer of complexity to any interpretation of the contribution of changes in MSNA to resonant behaviour of baroreflex control.

Fourthly, Vaschillo's theory ignores the contribution of central respiratory-sympathetic coupling to the generation of respiratory-related oscillations in MSNA, and consequently of ABP oscillations. Others have recently demonstrated, in human beings, a significant association between Traube-Hering wave amplitude and the amplitude of the preceding respiratory related MSNA bursts (Towie et al., 2012, Shantsila, McIntyre, et al., 2015). Additionally, the inhibitory role of pulmonary stretch receptors upon sympathetic activity during SDB is not considered within Vaschillo's theory. This is particularly relevant in the light of data that suggests a reduction in MSNA burst incidence with SDB (Shantsila et al., 2014b), potentially secondary to increased lung inflation afferent input (Seals et al., 1990, Seals et al., 1993, St. Croix et al., 1999, Khayat, Przybylowski, Meyer et al., 2004).

Fifthly, BPV_{LF} also contains contributions from intrinsic vasomotor rhythmicity, as shown by the persistence of BPV_{LF} after ganglion blockade (Zhang, Iwasaki, Zuckerman et al., 2002). Moreover, BPV may also be dependent on experimental conditions, and vary between individuals with regards to the relative weight of vasomotor and neural/reflex contributions to its genesis, and cannot be assumed to simply reflect changes in MSNA (Parati et al., 1995, Zhang et al., 2002).

Finally, if the so called 'resonant behaviour' was simply a reflection of the baroreceptor control delay, the amplification of the HRV by SDB at specific f_Rs will necessarily need to almost completely obliterate BPV, which does not seem to be suggested by Vaschillo's or most studies that have reported BPV under SDB conditions (TenVoorde et al., 1995, Cooke et al., 1998, Chang et al., 2013, Jones, Asadi, Valipour et al., 2014, Chang et al., 2015). However, in the case of Vaschillo's findings on BPV behaviour during SDB, some methodological peculiarities need to be highlighted. The SDB in the studies by the Vaschillo group employ a biofeedback intervention that does not dictate specific breathing frequencies. Instead, participants are instructed to use any means (respiratory, muscular or mental activity) to voluntarily match their fc response to a sinusoidal signal with a period between 7 and 100s (Vaschillo et al., 2002). Furthermore, in their 2002 study, Vaschillo and colleagues used a small sample of just five cosmonauts. It is known that repeated exposure to high G forces, such as those encountered by pilots and

trainee cosmonauts, can lead to marked adjustments in the baroreflex regulation of blood pressure (Newman, White and Callister, 1998), which might result in altered cardiovascular interactions, when compared to our healthy, untrained, participants.

In Chapter 4 we reported that unlike most others (TenVoorde et al., 1995, Cooke et al., 1998, Chang et al., 2013, Jones et al., 2014, Chang et al., 2015) we encountered much reduced BPV at frequencies lower than 8 breaths·min^{-1}. To the author's knowledge, only one study has reported a similar decrease in the amplitude of SBP oscillations accompanying a reduction in f_R (Sin et al., 2010). Several explanations might be advanced, most involving the contribution of cardiac and vascular baroreflex responses. Elstad and colleagues (2011) suggested that low-frequency fluctuations in HRV determine $\dot{Q}$, while MAP fluctuations at 0.1 Hz are determined by fluctuations in TPR. Importantly, Elstad reported that $\dot{Q}$ and TPR fluctuations are 180^o out of phase at 0.1Hz, meaning that TPR variations potentially counteract within-breath variations in $\dot{Q}$, when breathing at 6 breaths·min^{-1} or below.

The impact of the HRV (and RSA) upon the modulation of respiratory driven BPV should also be considered, as it has been suggested that RSA contributes to buffering left ventricular fluctuations in $\dot{Q}$ and therefore BPV (Elstad, 2012). This is supported by data from Chapter 4 suggesting that RSA counteracts the within-breath fluctuations in LVSV (ΔSV) and that at these low frequencies, SBP and *fc* fluctuations tend towards being in phase opposition. Taken together, all of the above stated factors would conspire to minimise in BPV at low f_R*s*, which reassures that my observations are physiological in origin.

One possible reason for the dissimilarities between my BPV results and those of others is the difference in the extent of control over the independent effects of V_T and f_R upon respiratory modulation of ABP. In the present study, V_T and f_R were controlled rigorously in an upright-reclined position. In contrast, most previous studies have evaluated the cardiorespiratory phase relationships and transfer functions (including BPV) under supine resting conditions (Baselli et al., 1986, Toska and Eriksen, 1993), in response to orthostatic challenges (Baselli et al., 1986, Saul et al., 1991, Taylor and Eckberg, 1996, Cooke et al., 1999, Elstad et al., 2001), carotid baroreflex stimulation (Keyl et al., 2000), paced breathing (Berger et al., 1989a, Saul et al., 1989, Taylor and Eckberg, 1996, Keyl et al., 2000), or following the administration of drugs (Saul et al., 1991, Toska and Eriksen, 1993, Elstad et

al., 2001). One notable exception is Cooke and colleagues' (1998) study, which not only characterised HRV and BPV through a comprehensive range of f_Rs, with and without V_T control (with spectral power peaks for both HRV and BPV occurring at the same f_R close to 0.1 Hz), but also described the phase relation between SBP and *fc* throughout said range. However, unlike us, Cooke's study was performed in the supine position. Previous research has demonstrated that under these conditions HRV (RSA) enhances SBP fluctuations, while in our upright-reclined position these are expected to be buffered (Elstad et al., 2001).

Collectively, the experimental data presented herein seems to suggest that reductions in f_R result in decreased BPV, likely due to the buffering action of RSA upon mechanically driven fluctuation in LVSV. Nonetheless, the amplification of ABP oscillations by the action of a mechanism that is not exclusively dependent of mechanically driven increments in LVSV, remains a strong possibility, as the application of the concept of system resonance cannot be excluded, albeit perhaps not in the simplistic way is has been in the past.

Other factors affecting the cardiovascular response to changes in f_R – rate of acetylcholine hydrolysation

One element that has been referred in the literature as contributing to the breathing frequency dependent response of HRV is the kinetics of acetylcholine (ACh) influences on the sinus node, particularly (but not limited to) the rate of ACh hydrolysation (Eckberg and Eckberg, 1982, Taylor et al., 2001, Song and Lehrer, 2003). These authors have speculated that during spontaneous breathing the amount of ACh released during expiration is limited by its duration. Since the time required for the de-activation of ACh by acetylcholinesterase is a relatively slow (1.5-2s), fixed-rate process that seems to be independent of f_R (Baskerville et al., 1979, Eckberg and Eckberg, 1982), 'quick' f_Rs result in less ACh being released during the expiration and incomplete hydrolisation of the same during inspiration. This creates an 'ACh floor' that limits the effects of the waxing and waning of ACh upon the sinoatrial node. At slower f_Rs, a larger bolus of ACh is released during expiration (contributing to maximal inhibition of sinoatrial note firing and decreased fc_E), while the longer inspiratory time allows for complete inactivation of the larger bolus of ACh (resulting in increased fc_I).

Consequently, longer respiratory cycles result in more complete hydrolysation and inactivation of ACh even if the bolus of ACh released during each expiration is similar, which partially helps to explain the higher RSA at lower f_Rs described in this study, as well as others (Eckberg and Eckberg, 1982, Taylor et al., 2001, Song and Lehrer, 2003).

Like Song and Lehrer (2003) we encountered a more pronounced decrease in fc_E, but without a significantly larger increase in fc_I, as f_R decreased. These changes seem to have occurred in the absence of significant changes in mean *fc*, confirming previous observations that even though breathing might impact the timing of cardiac vagal discharge, it has little impact on the magnitude of such discharge. The amplitude of RSA seems to be determined by several factors, not just by the level of cardiac vagal activity (Brown et al., 1993).

7-4.2 Effects of tidal volume

The HRV and BPV respond inversely to changes in f_R (Table 4-10), with both tending to increase when V_T is augmented (Table 4-11). We previously highlighted the contribution of baroreflex mechanisms to the amplitude of HRV and RSA, in response to reductions in f_R. However, the results from Chapter 4 also showed a relatively unchanged LVSV, $\dot{Q}$, ΔSV, $\Delta\dot{Q}$ and BPV (despite a significant linear increase in $\Delta\dot{Q}$ with the progression in V_T). Simultaneously, the phase relations between changes in lung volume, SBP and *fc* are seemingly unaltered by the increase in V_T. The linear increase in RSA and other HRV indices with larger V_Ts has been repeatedly demonstrated (Freyschuss and Melcher, 1976c, Hirsch and Bishop, 1981, Brown et al., 1993, Taha et al., 1995, Ritz, Thons and Dahme, 2001, Poyhonen et al., 2004) and while it has been widely attributed to baroreflex mechanisms (Davies and Neilson, 1967, De Boer et al., 1987, Toska and Eriksen, 1993, Scheffer, TenVoorde, Karemaker et al., 1994, Karemaker, 2009a, b, c), the lack of effect upon LVSV, SBP and BPV hints at a direct influence of respiratory-induced changes in right atrial filling upon sinus node activity (Freyschuss and Melcher, 1976a, Bernardi et al., 1989, Slovut, Wenstrom, Moeckel et al., 1998, Taylor et al., 2001), and/or increased vagal afferent input to the sinus node arising from the influence of feedback to the cardiovascular control centre from lung stretch receptors (Freyschuss and Melcher, 1976c, Taha et al., 1995). Overall, these results

support the view that the systemic implications of breathing at larger lung volumes are minor compared to the more potent effects of altering f_R.

7-4.3 Effect of changes in intrathoracic pressure

Inspiratory loaded breathing

The addition of an inspiratory impedance (Study 2, Chapter 5) increases venous return throughout inspiration and amplifies within-breath variations in LVSV (∆SV) by significantly increasing $LVSV_E$ while enhancing $\dot{Q}$. This is accompanied by increases in the amplitude of BPV, RSA and HRV. Interestingly, while both BPV and HRV are proportional to the magnitude of the inspiratory resistance being applied, the response of BPV is the most pronounced, and correlates strongly with ∆SV, supporting the mechanical origin of respiratory-driven fluctuations in SBP.

The magnitude of HRV (and RSA) on the other hand, has been linked to a plethora of factors, which have been described previously in this thesis (cf. sections 2-4 and 2-5 in Chapter 2 - Literature Review). Considering the absence of significant differences in fc_E with the addition of inspiratory resisted breathing, and a ~9 beats·min^{-1} (non-significant) increase in fc_I, (underpinning the higher RSA during inspiratory loading), a case can be made for a contribution from sinus node stretch to the chronotropic response to inspiratory resisted SDB (Freyschuss and Melcher, 1976a). Interestingly, findings from this thesis contradict reports from a similar experiment (Freyschuss and Melcher, 1976c), which observed decreased fc_E (but not increased fc_I) during inspiratory resisted SDB. Oddly, in a study from the same year and using a similar respiratory protocol (Freyschuss and Melcher, 1976a), the authors reported simultaneous increases in fc_I and decreased fc_E, leading to enhanced RSA. Like us, Freyschuss and Melcher also clamped f_R and V_T, so the changes in breathing pattern cannot account for the observed differences. Taken together these results seems to suggest that the effect of inspiratory loading upon RSA can be underpinned by changes in fc_I, fc_E or both.

The slight dissimilarities between the response patterns of HRV and BPV to the increase in inspiratory resistance can be interpreted as evidence that the response of HRV, and concomitantly RSA, is influenced by more than just vagal cardiac baroreflex mechanisms. Other potential sources of cardiac chronotropic stimulation during SDB are the cardio-acceleration induced by right atrial stretch (Freyschuss

and Melcher, 1976a, Bernardi et al., 1989), as well as a contribution arising from afferent feedback from pulmonary stretch receptors (Taha et al., 1995).

Effect of increased within-breath left ventricular stroke volume fluctuations

Data arising from this thesis' experimental chapters point to a respiratory-driven enhancement of the within-breath fluctuations in LVSV and to a smaller extent $\dot{Q}$, particularly when combined with inspiratory resisted breathing. The within-breath pattern and amplitude of LVSV is highly related to BPV at the respiratory frequency (Table 4-10). Importantly, the amplification of the within-breath fluctuations in intrathoracic-pressure with the application of inspiratory resistances significantly increased HRV above those values encountered during any other breathing condition (SDB alone; SDB combined with expiratory resistances; simultaneous inspiratory and expiratory resistances). However, the simultaneous application of inspiratory and expiratory resistances proved to generate significantly larger BPV at the respiratory frequency, when compared to expiratory resisted SDB.

Recent studies employing daily bouts of inspiratory resisted SDB show a more pronounced and sustained reduction in ABP in hypertensive individuals when compared to SDB alone, following eight weeks of SDB training (Jones et al., 2010, Sangthong et al., 2016). Other respiratory interventions, using inspiratory and expiratory muscle training, appear to confirm the importance of intrathoracic pressure variations, rather than large respiratory volumes, to sustained reductions in ABP after six weeks of daily respiratory training (Vranish and Bailey, 2015). While these studies have considered the chronic effects of systematic respiratory training with large intrathoracic pressure variations, they support a view that the repeated, daily, amplification of within-breath ABP swings is the most likely triggering factor for any chronic antihypertensive effects of (resisted) SDB.

Our research shows that the use of inspiratory resistances (or combined inspiratory and expiratory resistances) in conjunction with SDB heightens the acute cardiovascular perturbations induced by SDB, without necessarily promoting an acute increase in MAP, as was observed during expiratory resisted SDB. Furthermore, if the objective is to maximise cardiovascular system perturbation, results from Chapter 4 (Study 1) suggest that f_R should be set at a level that potentiates the influence of intrathoracic pressure changes, with inspiratory and

expiratory durations that are slower than, or a harmonic of PulTT. Future research should then focus on unravelling the link between the acute effects of resisted SDB and the development of long-term cardiovascular adaptations. Studies that look into the immediate short-term effect of SDB interventions in the period immediately after the cessation of the resisted SDB stimulus might shed light of potential stimuli.

7-4.4 Effects of different methods of respiratory loading

In contrast to inspiratory resisted SDB, expiratory resisted SDB tended to reduce RSA and HRV, LVSV and $\dot{Q}$, while increasing TPR and MAP. The simultaneous application of resistances to both phases of the respiratory cycle (dual loading) led to similar LVSV and $\dot{Q}$, lower (non-significant) RSA and HRV and higher BPV, when compared to inspiratory resisted SDB. Dual loading also promotes an elevation of MAP and TPR, similar to that observed with isolated expiratory resisted SDB. A similar response is observed during nasal breathing through a single nostril, provided that similar intrathoracic pressures are achieved. In contrast to claims by others (Telles et al., 1994, Raghuraj and Telles, 2008, Pal et al., 2014), there were no significant differences in the acute cardiovascular and autonomic response to SDB between right and left nostril breathing. It is likely that any differences found in previous studies are an artefact of individual differences in nostril anatomy leading to different pressures being generated between nostrils, for the same airflow rate.

The use of inspiratory resisted SDB seems to provide the largest stimulus to the cardiovascular system without triggering a significant acute rise in MAP, which particularly in hypertensive populations, might be safer, and therefore would be a preferred strategy to improve SDB interventions. A recent study has shown anti-hypertensive effects using large inspiratory and expiratory resistances (Vranish and Bailey, 2015), but it remains unclear whether similar effects could be achieved during SDB, with much smaller respiratory resistances. Nonetheless, it does seem that an intervention that combines SDB with inspiratory resistances generates superior and more sustained reductions in ABP, compared with SDB alone, in hypertensive individuals (Sangthong et al., 2016).

7-4.5 Effects of P_aCO_2

Chapter 4 provided no evidence that failing to clamp P_aCO_2 has an important impact on the acute cardiovascular response to SDB when compared to clamped conditions. Breathing with unclamped P_aCO_2 occurred only in the semi-spontaneous breathing conditions used in Chapter 4. For those conditions, either f_R (Protocol 1- *SS*V_T) or V_T (Protocol 2 - *SS*f_R) was the only respiratory parameter being controlled, allowing participants to freely adjust their respiratory frequency (with *SS*f_R) or volume (with *SS*V_T) to a comfortable, spontaneous range. Participants spontaneously maintained minute ventilation ($\dot{V}_E$) at comparable levels to those encountered in the fully clamped conditions conducted at similar f_R or V_T. At the same time, P_aCO_2 levels were within the 'normal' physiological range, varying between ~35 mmHg ($P_{ET}CO_2$) in the semi-spontaneous conditions and ~41 mmHg in the fully controlled conditions, in both experimental protocols (Tables 4-2 and 4-3).

Nonetheless, small but significantly higher values for SBP, DBP, MAP and pulse pressure (PP) were present at f_Rs (clamped V_T) between 6 and 10 breaths·min^{-1} than those observed at 4 breaths·min^{-1} and in the semi-spontaneous condition (SSf_R). These differences could not be explained by a peripheral vasoconstrictor response to elevated P_aCO_2 in the fully controlled conditions as $P_{ET}CO_2$ and TPR remained almost unaltered across all conditions. Furthermore, the 4 breaths·min^{-1} condition was clamped at the same slightly elevated level observed for the higher f_Rs and semi-spontaneous breathing condition (SSf_R). The slight increase in ABP (around 10 mmHg in SBP and 5-6 mmHg for MAP, PP and DBP) between 6 and 10 breaths·min^{-1} was likely associated with a trend towards increased LVSV, $\dot{Q}$ and TPR, and arguably lacks physiological relevance. Thus, the significant pressor response observed for f_Rs ≥ 6 breaths·min^{-1} does not seem to be related to a chemoreflex response. Furthermore, it has been argued that small alterations in P_aCO_2 have negligible effects upon HRV and RSA (Freyschuss and Melcher, 1976a, c, Cooke et al., 1998, Henry et al., 1998) and that the effect of chemoreceptor stimulation upon HRV is mainly indirect and mediated by changes in ABP (Henry et al., 1998). Simultaneously, the effects of chemoreceptor stimulation are larger when baseline ABP is high (Henry et al., 1998), which might help to explain the apparently minor impact of $P_{ET}CO_2$ in our young and healthy individuals.

Previous studies have argued about the need to control for P_aCO_2 when evaluating HRV in human beings, particularly in conscious individuals (Sasano et al., 2002, Poyhonen et al., 2004). We did not encounter any evidence to suggest a significant impact of P_aCO_2 upon HRV during SDB interventions, provided that the P_aCO_2 is maintained within normal physiological limits (35 to 40 mmHg). In all likelihood, differences between my findings and those of others are solely related to the fact that in those studies HRV was evaluated in the presence of significantly larger P_aCO_2 variations than those reported herein (Al-Ani et al., 1996, Henry et al., 1998, Sasano et al., 2002, Poyhonen et al., 2004, Tzeng et al., 2007).

Most SDB interventions only control f_R; under these conditions, alveolar ventilation might increase, and P_aCO_2 decrease, because of a lowering of dead space ventilation. Nonetheless, the increase in V_T likely overrides any potential effect of hypocapnia upon RSA/HRV (Ritz et al., 2001), which is in line with the RSA data from Protocol 2 of Study 1 (Chapter 4), as well as the data arising from both semi-spontaneous conditions in the same study (SSf_R and SSV_T – Chapter 4). A possible avenue to empirically test such statement involves manipulations of dead space ventilation at constant f_R and V_T with an experimental setting similar to the applied in the studies comprised in this thesis.

7-4.6 Summary

In the preceding sections of this chapter, I discussed the main findings of the experimental chapters at the light of my current understanding of the existing literature, having as a framework the model presented in Figure 7-1. The discussion was based on trying to establish a mechanistic link between 'input variables' that were manipulated during the experimental studies comprised in this thesis, and the 'outcome variables' depicted in Figure 7-1.

The novel findings of this thesis can be summarised as follows:

1) The reduction in f_R resulted in an inversion of the normal within-breath pattern in LVSV, likely due to the influence of PulTT;
2) The alteration of the within-breath pattern of LVSV at low f_Rs leads to frequency dependent changes in the phase relationship between respiration

and the ensuing respiratory-driven fluctuations in SBP, which leads to decreased amplitude of BPV at the lowest f_Rs;

3) Increasing V_T has a minor cardiovascular impact compared to the effect of reducing f_R;
4) The generation of progressively more negative intrathoracic pressures with inspiratory resisted SDB amplifies the cardiovascular effects of SDB, likely due to increased inspiratory venous return;
5) The combination of inspiratory and expiratory resistances does not amplify the within-breath effects of inspiratory resisted SDB alone, but acutely increases MAP;
6) The acute cardiovascular response to single-nostril SDB is determined by the variation in intrathoracic pressure generated by a given airflow and not by the nostril being used *per se*.

The links between these 'input variables' and any chronic adaptations to SDB need to be explored in future research, which is considered in section 7-6.

7-5 Limitations

7-5.1 Sample size

The sample size in each of the three studies was relatively small (Chapter 4, *n* = 14 for Protocol 1; Chapter 4, *n* = 11 for Protocol 2; Chapter 5 *n* = 9; Chapter 6, *n* = 14) and therefore vulnerable to the occurrence of Type II statistical errors. There is a possibility that physiologically relevant alterations might have been described as statistically non-significant as a result of both the sample size and the use of conservative Bonferroni *post-hoc* procedures. This is particularly important in Chapters 4 and 5, where the *post-hoc* analysis involved multiple comparisons between all experimental conditions, which greatly reduced the alpha level. While this means that Type I errors were very unlikely in our interventions, it further increased the likelihood of Type II errors, i.e. increased the risk of underreporting significant physiological responses to the interventions. This is especially true for the second experimental chapter (Chapter 5), which had the smallest sample, and the largest number of multiple comparisons of the three studies. This effect was mitigated to some extent in Chapter 6, when a pre-defined number of relevant comparisons were made, based on my research hypothesis. Nonetheless, the reported magnitude of change, particularly for the haemodynamic parameters, was much smaller than other interventions that are known to significantly impact the haemodynamic response, such as exercise. The fact that most variables reached significance suggests that the few reported significant differences in the haemodynamic parameters, with SDB, represent meaningful physiological responses to the applied interventions and support the presence of adequate statistical power. Furthermore, the use of other statistical interventions like (multiple) regression analysis and mechanism analysis (Hopkins, 2003) allowed for the identification of trends and relationships that would otherwise remain obscured by the use of analysis of variance (ANOVA) tests. Whilst it is unarguable that larger samples would have been beneficial for the analysis of the various parameters collected throughout the experimental procedures, the complexity and length of the interventions meant that a) recruitment of participants was highly restrained by their availability to perform several lengthy visits to our laboratory, b) not all the participants were able to complete the procedure (particularly in study 1 – Chapter 4) and c) a small number of tests were either lost or had to be repeated due to technical difficulties.

7-5.2 Measurement errors

Although the Modelflow method of estimating LVSV has been shown to agree strongly with LVSV measured by Doppler ultrasound (van Lieshout et al., 2003), relevant research has indicated that Modelflow-measured LVSV demands calibration against a standard method, such as the Fick principle (van Lieshout, Pott, Madsen et al., 2001) or thermodilution (Jansen, Schreuder, Mulier et al., 2001), if accurate values are required. Nonetheless, LVSV measured through the Modelflow method does track changes in LVSV with the same precision (Jansen et al., 2001, Bogert and van Lieshout, 2005). Similarly, ABP derived non-invasively from infrared photoplethysmography (Finometer) tracks, but it is not identical to, intra-arterial pressure measurements (Bogert and van Lieshout, 2005). Nonetheless, the reconstructed brachial ABP signal that was utilised in my studies meets the Association for the Advancement of Medical Instrumentation (AAMI) criteria (Bos, van Goudoever, van Montfrans et al., 1996, Guelen, Westerhof, Van Der Sar et al., 2003, Schutte, Huisman, van Rooyen et al., 2004, Guelen et al., 2008), which warrants an acceptable error when compared to intra-arterial pressure measurements. Importantly, the Modelflow estimates of LVSV are based on the premise that transmural aortic pressure remains stable in the presence of increased intra-abdominal pressure or extreme pulmonary hyperinflation (Bogert and van Lieshout, 2005), which in the context of the interventions employed within this thesis, might have influenced the results. Nevertheless, the use of the Finometer did allow for continuous, non-invasive monitoring of ABP and cardiac haemodynamics during SDB, without the methodological constraints of other methods, like Doppler ultrasound (which precludes measurements during inhalation due to signal interference resulting from lung inflation), or the need for an invasive intra-arterial pressure measurement. All other equipment used throughout the studies that comprise this thesis were calibrated according to the manufacturers' specifications.

7-5.3 Day to day variability

Considering the magnitude of the change in some analysed cardiovascular variables, compared to other interventions (e.g. during different forms of exercise), and the issues with sample size addressed in section 7-5.1, an evaluation of the within-individual variability of the repeated cardiovascular measures would have been advisable. This test-retest reliability analysis could have strengthened the arguments made in this thesis, if established that the coefficient of variation (or

another measure of within-subject variation) of repeated cardiovascular measures on at least two different occasions is lower than the effects of the interventions employed in this thesis (Hopkins, 2000).

Results from studies of short-term variation of RSA (over the course of days to weeks) have generally produced good to excellent test-retest reliability in adults (Kleiger, Bigger, Bosner et al., 1991, Sinnreich, Kark, Friedlander et al., 1998, Pinna, Maestri, Torunski et al., 2007, Bertsch, Hagemann, Naumann et al., 2012, Borges, Mathewson and Schmidt, 2017). Substantial variation of the magnitude and direction of within-subject change in resting RSA has been reported in the literature (Borges et al., 2017), which translates a well-known 'intrinsic lability' of resting RSA (Sandercock, Bromley and Brodie, 2005).

In contrast to RSA, recent studies on the within-individual variation of *fc*, SBP and DBP in healthy young adults are scarce. One notable exception is the recent study by Borges and colleagues (2017) These researchers reported excellent short-term test-retest reliability for *fc* and SBP, and good test-retest reliability for DBP, particularly when controlled for age and difference in time of day between the test and retest

To the best of the author's knowledge, no study of test-reliability of within-breath cardiovascular variables reported in this thesis (cf. Appendix II) has been performed to date. Establishing the short-term test-retest reliability of cardiovascular measures is critical if they are to be used to clarify the acute cardiovascular and autonomic response to SDB interventions.

7-5.4 Breathing pattern control

The pattern of breathing (thoracic *vs.* diaphragmatic) and its impact on the phase relationship between respiration and subsequent variations in LVSV was not sufficiently considered and addressed in the preparation of the experimental chapters. Unfortunately, we only encountered supporting evidence of a differentiated effect of thoracic *vs.* diaphragmatic breathing, in the form of two papers by Miller and colleagues (2005b, 2005a), close to the end of the project. Such events implied that while we state the relevance of said studies in the

Literature review (Chapter 2) and this section of the thesis, we did not control for the possible effect upon the results herein described.

In short, thoracic breathing resulted in more negative intrathoracic pressure, which lowered right atrial pressure creating a favourable pressure gradient that facilitates venous return during the inspiratory portion of the respiratory cycle. While promoting a similar reduction in intrathoracic pressure, diaphragmatic breathing also increased intra-abdominal pressure, which compromised venous return during inspiration due to its effect upon inferior vena cava resistance. It also amplified venous return during expiration, when the effect of increased intra-abdominal pressure is removed (Miller, Pegelow, et al., 2005b). In addition, the same authors demonstrated that diaphragmatic breathing could cancel the effects of larger intrathoracic pressure variations. Using similar-sized respiratory resistances to the ones applied in Chapters 5 and 6, they showed that a more negative intrathoracic pressure during inspiration had no impact on venous return in the presence of a diaphragmatic breathing pattern, while with thoracic breathing, the inspiratory venous return is amplified beyond what is observed during spontaneous breathing. Finally, the application of simultaneous inspiratory and expiratory resisted breathing in conjunction with diaphragmatic breathing resulted in an inversion of the within-breath pattern of for venous return, when compared to spontaneous diaphragmatic breathing (Miller, Pegelow, et al., 2005a).

These data (Miller, Pegelow, et al., 2005a) highlight the necessity for future studies to investigate the interplay between the phase response of LVSV to changes in f_R and those resulting from changes in breathing pattern (thoracic *vs.* diaphragmatic). While I do not believe that intra-individual changes in breathing pattern were present in our experiments, or affected the amplitude of the within-breath cardiovascular patterns reported, it is questionable whether the data are generalisable, since breathing pattern is known to vary widely between individuals.

7-5.5 Uncoupling of heart rate and HRV

A frequently documented but largely unexplored issue in research studies involving HRV analysis is the inverse relation between most HRV parameters and the actual mean *fc*, such that HRV is lower when mean *fc* is high and vice-versa. This phenomenon is fundamentally due to the inverse relationship between HR and R-R

interval, which is normally used to calculate HRV instead of HR time series (Sacha, 2014). When HRV parameters are calculated from *fc* time series this inverse relation tends to disappear . While other factors like the sinoatrial node action potential dynamics (Monfredi, Lyashkov, Johnsen et al., 2014) might contribute to the relationship between mean *fc* and HRV, the problem is most and foremost mathematical in origin. Thus, for the correct interpretation of HRV parameters it is paramount that the relationship between HR and HRV is considered, as overlooking it can lead to serious misinterpretation of experimental data. Unfortunately, within this thesis, mean *fc* was not taken into consideration when analysing and interpreting the HRV data. Notwithstanding, the absence of statistically significant differences to mean *fc* as the result of the interventions that comprised the experimental chapters of the thesis suggests that the reported changes in HRV were not impacted by mean *fc* fluctuations.

7-6 Directions for future research

7-6.1 Effects of breathing pattern (thoracic vs. diaphragmatic)

Results from this thesis provide evidence that reducing f_R with SDB alters the within-breath pattern (inspiratory *vs.* expiratory) of LVSV. As previously mentioned, future studies addressing the effects of the breathing pattern (thoracic *vs.* diaphragmatic) may extend these findings further by clarifying the impact of fluctuations in intra-abdominal pressure upon the frequency-induced phase changes in left ventricular output. Previous research analysing the effects of breathing pattern, with and without increased intrathoracic pressure variations, have reported important within-breath phase differences in venous return when comparing thoracic with diaphragmatic breathing (Miller, Pegelow, et al., 2005b, a). No study to date has investigated the effects of breathing manner upon the within-breath cardiovascular modulation during (resisted) SDB. An understanding of these issues may contribute to the improvement of future interventions that aim to amplify the acute within-breath cardiovascular oscillations as a potential error signal for the cardiovascular control centres.

7-6.2 Heart-lungs interactions – right vs. left ventricular function response to SDB

Phase alterations in SBP and LVSV with the reduction of f_R, likely reflect the effects PulTT. Notwithstanding, we are not aware of any study that has evaluated the phase relationship between RVSV and LVSV in the context of SDB. More particularly, it has been demonstrated that in spontaneously breathing individuals in the lateral decubital position, RVSV and LVSV are in complete opposite phases and that RSA contributes to minimise the effects of LVSV upon left ventricular $\dot{Q}$ (Elstad, 2012).

Given the alteration of the within-breath phase pattern in LVSV during low frequency SDB, it is unclear how RSA impacts right ventricular $\dot{Q}$ and pulmonary blood flow and how that might influence the pulmonary artery baroreceptors, which are known to trigger an opposing reflex response to those of the systemic circulation baroreceptors (Moore et al., 2011, Hainsworth, 2014).

The lack of noninvasive technologies that allow for simultaneous evaluation of the right and left cardiac function in the presence of large respiratory excursions is perhaps the main constraint to such assessment. Others have used pulsed Doppler

ultrasound to extrapolate RVSV from pulmonary artery flow (Elstad, 2012), but mentioned the impossibility of the measuring RVSV in the presence of large chest movements. Echocardiographic measurements of tricuspid valve flow have been suggested to be a valid option to evaluate right ventricular function, even during exercise (Argiento, Chesler, Mule et al., 2010, Claessen, La Gerche, Voigt et al., 2016), but lack accuracy when compared to cardiac magnetic resonance (Claessen et al., 2016). Cardiac magnetic resonance is the most reliable, but also costly, alternative to evaluate cardiac dynamics in response to SDB. This technique was recently used to characterise the interactions between respiration, RVSV and LVSV, both at rest and during supine exercise (Claessen et al., 2014). Unlike the ultrasonographical methods, cardiac magnetic resonance imaging is not limited by the effects of chest excursion, as long as such excursion is maintained constant throughout several breaths. Thus, it may be possible to evaluate cardiac function even in the presence of large lung volumes associated to (resisted) SDB using cardiac magnetic resonance imaging.

However, if Doppler ultrasound is the only available noninvasive option, brief collection periods with paced breathing at low f_Rs consistent with SDB and small lung volumes may be regarded as an initial approach to understand right vs. left cardiac function relationships during SDB,

7-6.3 Sensitisation and neuroplasticity in response to SDB

Understanding the mechanistic factors that link repeated acute physiological changes induced by the regular practice of SDB with long term cardiovascular adjustments remains a great challenge. In the human body, the stimuli that engage systems involved in the maintenance of homoeostasis often result in progressive increases in the magnitude of the response, when stimulated repeatedly (Johnson, Zhang, Clayton et al., 2015).

It has been hypothesised that in the case of SDB, the repeated stimulation of the arterial baroreflex may produce a chronic increase in baroreflex efficiency (Lehrer, 2007). Evidence of neuroplasticity, defined as persistent morphological and functional changes of a given neural pathway, have been reported for musculoskeletal reflexes (Bawa, 2002), and respiratory neural control (Mitchell and Johnson, 2003). In the particular case of the cardiovascular system, it is believed

that neural remodelling (neuroplasticity) is involved in the development of essential hypertension (Johnson et al., 2015) and several forms of cardiovascular disease, in response to inactivity (Mischel, Subramanian, Dombrowski et al., 2015). Thus, the repeated stimulation of the baroreflex (or other unappreciated neural, molecular or cellular mechanisms) by SDB might underpin the adaptations to SDB, as well as their reversibility.

Even though we have previously referred to the limitations of the traditional time and frequency domain cardiac BRS measurements, BRS has been demonstrated to increase both acutely and chronically following regular practice of SDB in both healthy individuals (Lehrer et al., 2003) and patients with heart failure (Bernardi et al., 2002). This can potentially be regarded as an indicator of sensitisation of the baroreflex control mechanism. Sensitisation phenomena have been the object of recent interest in the context of essential hypertension development and implicate molecular and cellular adjustments in elements of the neural network controlling ABP, resulting in sensitisation of the response to hypertensinogenic stimuli (Johnson et al., 2015).

In the context of SDB, the identification of relevant changes in respiratory, cardiovascular or autonomic variables for a relatively prolonged, period that outlasts the duration of the acute SDB stimulus, must be understood as a first evidence for the existence of any neuroplastic response to SDB. Furthermore, the identification of the brain structures engaged during SDB can further the understanding of both the acute and chronical cardiovascular response to SDB. Recently, novel insights arising from fMRI have shown a strong relation between rostroventral medulla activity, BPV and HRV, likely reflecting the impact of breathing upon baroreflex regulation (Critchley, Nicotra, Chiesa et al., 2015).

7-6.4 Effect of slow and deep breathing upon cerebral blood flow

Other possible lines of investigation are to more fully understand the effects of SDB, with and without the addition of inspiratory resistances, upon blood flow to key organs like the brain or the kidney.

At least one study involving SDB has indicated increased inspiratory CBF (Lucas et al., 2013); a similar finding to that encountered with the use of small inspiratory

resistances in supine, spontaneously breathing, healthy individuals (Aufderheide, Pirrallo, Provo et al., 2005, Convertino, Cooke, et al., 2005, Cooke et al., 2006, Rickards et al., 2007, Rickards et al., 2008, Hayen, Herigstad, Kelly et al., 2013). Important respiratory modulation of cerebrospinal fluid movement has also been described as a response to increasingly negative intrathoracic pressures (Dreha-Kulaczewski, Joseph, Merboldt et al., 2015). This is particularly important in the light of one current theory for the generation of systemic hypertension, based on the existence of a protective 'Cushing mechanism' (Paton, Dickinson and Mitchell, 2009)[6], termed the 'selfish brain hypothesis' (Rodrigues, Hart, Hassan et al., 2015, Warnert, Rodrigues, Burchell et al., 2016). This theory argues that brainstem hypoperfusion may trigger systemic hypertension as part of a defence mechanism aimed at increasing CBF. Brainstem hypoperfusion leads to increased cerebral vascular resistance, which is believed to precede changes in MSNA and the onset of hypertension (Warnert et al., 2016). Reduced CBF and cerebral vessel alterations have been previously reported in hypertensive individuals (Dai, Lopez, Carmichael et al., 2008, Rizzoni, De Ciuceis, Porteri et al., 2009, Waldstein, Lefkowitz, Siegel et al., 2010).

Observations made both at rest and during exercise (50% maximal oxygen uptake) show that $\dot{Q}$ is linearly related to CBF, independently of cerebral autoregulation (Ogoh, Brothers, Barnes et al., 2005). However, with whole body exercise at higher intensities CBF tends to decline, while $\dot{Q}$ increases (Ogoh, Dalsgaard, Secher et al., 2007). Furthermore, this notion does not apply to small muscle mass exercise such as the one legged kneed extensor exercise or to ATP infusion induced increases in leg blood flow, where $\dot{Q}$ increases progressively but CBF remains unchanged. In those scenarios $\dot{Q}$ increases but CBF does not (Ogoh, Brothers, Jeschke et al., 2010, Bada, Svendsen, Secher et al., 2012). This supports that local brain blood flow is dependent upon local factors, rather than MAP and/or $\dot{Q}$. It is thus unclear how any [within-breath] changes in LVSV and $\dot{Q}$ resulting from the use of SDB can impact the regulation of CBF. It is therefore worthwhile investigating if SDB, particularly when used in conjunction with small inspiratory resistances, might bring about beneficial long-term changes in cerebrovascular perfusion and auto-

[6] Cushing's mechanism is a terminology used by Julian Paton, John Dickinson and Graham Mitchell, in their 2009 review, to describe a long term ABP control mechanism that in humans consists of a sustained systemic ABP increase triggered by narrowing of the arteries supplying the brainstem. Such mechanism is based on Harvey Cushing's (early 20th century physiologist) early studies in dogs that showing a relation between brainstem compression and ABP elevation.

regulation. In addition, understanding how CBF is affected acutely by f_R, breathing pattern and the magnitude of intrathoracic pressure variation, can help to refine SDB interventions aimed at promoting large within-breath oscillations in CBF.

Furthermore, the study of changes in cerebrovascular flow and architecture following a period of training with SDB and/or inspiratory resisted breathing might further the understanding of the contribution of the ‘selfish brain’ compensatory mechanism in triggering hypertension in otherwise healthy individuals.

7-7 Novelty and utility of findings

The thesis confirms and expands the known association between SDB and the modulation of within-breath cardiovascular oscillations, providing further insights into the contribution of f_R, V_T and ITP into the complex interplay between respiratory, cardiovascular and autonomic nervous systems. It shows that there is an individualised f_R that triggers the most substantial cardiovascular perturbation and supports the role of ITP changes as a potent modulator of cardiovascular variability.

Breathing against a small inspiratory resistance at an individual (resonant) frequency between 4 and 6 breaths.min^{-1} generates large within-breath variations in fc, SV and $\dot{Q}$ that are thought to represent an error signal to the cardiovascular control centres in the brain. While the studies within this thesis only describe the acute cardiovascular response to a series of respiratory perturbations, they clearly show the potent effect that (inspiratory resisted) SDB can have in generating large within-breath cardiovascular fluctuations without a concomitant raise in mean ABP or TPR. Overall, this thesis suggests that an individualised SDB intervention, used in combination with a small inspiratory resistance, might result in more favourable outcomes than the 'one size fits all' approach seen in other SDB modalities that simply try to drive breathing to a constant frequency (normally 6 breaths.min^{-1}). Strong support to this idea comes from recently published data showing larger reductions in ABP in older hypertensive patients after 8 weeks of daily inspiratory resisted SDB training vs. SDB alone (Sangthong et al., 2016). However, to optimize utilization, it would be reasonable to compare the effects of SDB with different levels of inspiratory resistance, distinct intervention durations (e.g., 10 min vs. 20 min. vs. 30 min) and whether the optimal breathing frequency is altered within one training session or training period.

The present studies accessed the acute cardiovascular effects of different modalities of SDB in a healthy cohort, and the study durations were designed to establish physiological impact and significance of the tested processes in accordance with the study hypotheses. Nonetheless, none of the studies that form this thesis was designed to determine the effects of the findings on long-term clinical outcomes in a hypertensive population. Thus, adequately structured, randomised trials are required to evaluate the clinical benefits of (inspiratory resisted) SDB, as applied in this thesis. Furthermore, the potential of SDB as an anti-hypertensive

treatment intervention must be assessed not only regarding the direct impact upon ABP but also on the effect upon markers of autonomic activity as HRV, BRS, MSNA and plasma catecholamine levels. Additionally, as a wide range of pharmacological agents is routinely used in the management of hypertension, it would be essential to know the effects of these agents when used in combination with SDB.

Considering the complex interrelationships between the cardiovascular, respiratory and autonomic nervous systems, an integrated analysis of all systems will allow for a better understanding of the involved mechanisms and help tailor more effective clinical uses for SDB.

7-8 Conclusions

The findings of the present thesis show that f_R is the most potent determinant of the acute cardiovascular response to unloaded SDB. Changes in lung volume result in minor further augmentation, in the presence of a low f_R. The within-breath pattern of LVSV is inverted for f_Rs close to or below 6 breaths· min^{-1}, possibly due to a delay interposed by the pulmonary circulation, which also impacts the phase relations between respiration and SBP. During SDB, total BPV is reduced, while HRV is increased with progressive lowering of f_R. Increasing V_T has little impact upon BPV, but it promotes an increase in HRV, supporting a contribution of vagal afferent feedback from lungs stretch receptors to the amplitude of RSA.

The addition of small inspiratory resistances to SDB promotes increased within-breath fluctuations in *fc* (RSA), LVSV and left ventricular $\dot{Q}$, in the absence of physiologically relevant changes in mean ABP. The within-breath cardiovascular pattern is associated with increased HRV and BPV, suggesting an important mechanical link between respiratory-driven fluctuation in venous return, within-breath oscillations in left ventricular output, and BPV. Furthermore, increased RSA in the presence of more negative intrathoracic pressure swings, but identical V_Ts, highlight a potential contribution of both mechanical SA node stretch and arterial baroreflex-mediated factors to RSA. Finally, loading different phases of the respiratory cycle resulted in differentiated acute cardiovascular effects, with inspiratory resisted SDB resulting in significantly higher RSA, HRV, BPV, ΔSV and $\dot{Q}$, than expiratory resisted SDB. Furthermore, expiratory resisted SDB resulted in a pressor response for the same variation in intrathoracic pressure, which was not present during inspiratory loading. A lower RSA for higher ABP, suggest a formerly underappreciated contribution of SA node stretch to RSA, and argue against the clinical benefits of expiratory resisted SDB, particularly in hypertensive populations. Finally, it remains to be seen how the acute perturbations characterised in the present thesis relate to the long-term regulation of blood pressure, and indeed, whether it is possible to optimise these to maximise any antihypertensive effects of SDB.

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APPENDICES

Appendix I – Summary table of relevant studies for the understanding the acute cardiovascular effects of SDB

Authors	*n*	Method	Relevance
Dornhorst et al. (1952b)	50 records in individuals with normal circulatory control. Total *n* not reported.	Several postural and respiratory manipulations including apnoea and paced breathing at different f_Rs 6-20 breaths·min^{-1}.	Identification of an f_R-determined phase relation between RESP and ABP. Amplification of ABP swings with the decrease of f_R.
Angelone and Coulter (1964)	1 healthy male adult	Paced breathing from 1-40 breaths·min^{-1} at a fixed V_T	Description of RSA response to different f_Rs with maxima at 6 breaths·min^{-1}. Progressive reduction of RESP-*fc* phase angle with f_R. 180º phase at 10 breaths·min^{-1} and 0º phase for ≤ 4 breaths·min^{-1} (0º representing *fc* starting at the same time as the beginning of expiration).
Freyschuss and Melcher (1976a,b,c)	5 healthy male adults 7 healthy male adults ? healthy male adults	All studies. fixed f_R at 6 breaths·min^{-1} and fixed V_T at 1, 1.5 and 2 L. Interventions included paced breathing with and without NIP and IPPV. Study c) also included INPV.	Increasing V_T increases RSA by impacting both minimum, and maximal *fc* NIP increases *fc*, RVSV, ABP and ventricular filling pressure during inspiration while augmenting RSA. IPPV abolishes RSA and reduces the respiratory variations in venous return, while INPV does not impact RSA. Overall these studies favoured the theory that RSA amplitude was determined by cardiopulmonary reflexes, rather than central or arterial baroreflex mechanisms.
Eckberg and Orshan (1977)	6 healthy adults (5m, 1f).	Fixed V_T at 100% and 150% of spontaneous V_T. Spontaneous f_R. Two intensities of neck suction applied during early inspiration or expiration.	Inspiration inhibits the cardiac baroreflex response to moderate (but not intense) baroreflex stimulation. Increasing V_T reduces the response to inspiratory baroreflex stimuli. Evidence for a central respiratory baroreceptor reflex interaction dependent upon the level of afferent baroreceptor activity and the depth of inspiration (possibly vagal afferent input from SASR).

Eckberg et al. (1980)	6 healthy adults (5m, 1f).	Fixed V_T at 100% of spontaneous V_T. Spontaneous f_R as well as 3, 6, 12 and 24 breaths·min^{-1}. Brief, moderate neck suction applied during early, mid, and late inspiration or expiration with spontaneous breathing and during early inspiration or expiration, with paced breathing.	Maximal cardiac deceleration is observed when baroreflex stimuli is delivered in late inspiration of early expiration. At breathing rates of 3, 6 and 12 breaths·min^{-1}, baroreflex responses are significantly greater during expiration than during inspiration, while at 24 breaths/min, inspiratory and expiratory baroreceptor stimuli resulted in similar increases in RR interval. This study identifies the existence of regular oscillations in human baroreflex responsiveness, determined by breathing, but that cannot be (solely) ascribed to the existence of a central oscillator.
Hirsch and Bishop (1981)	17 healthy adults (10m, 7f)	Paced breathing at a wide range of f_Rs (1-60 breaths·min^{-1}) and V_Ts (0.5 to 3L). A condition with breath-hold is also included.	Established the existence of a linear relationship between V_T and RSA. Determined the existence of individualised f_R-RSA curves, with a plateau at low f_Rs bellow 6 breaths·min^{-1} and a linear inverse relation with increasing f_Rs. RSA observed during spontaneous breathing falls under the individualised curves observed with paced breathing.
Eckberg (1983)	6 healthy adults	Controlled breathing: f_R between 6-24 breaths·min^{-1}; V_T between 1 and 1.5 L.	Cardio-acceleration (RR interval shortening) begins in inspiration for high f_Rs and expiration at slower rates. The RR interval lengthening always initiates in early expiration. RR interval shortening in phase with the start of inspiration at 10 breaths·min^{-1}. A Linear relation between V_T and RSA and an inverse relation between f_R and RSA. The suggestion of phasic respiration-related changes of vagal motoneuron activity during expiration, but that are incompletely expressed at high f_Rs.

Eckberg et al. (1985)	20 healthy adults	Spontaneous breathing (*n*=20); Controlled breathing (*n*=8): a) 12 breaths·min^{-1}, no V_T restriction; b) 12 breaths·min^{-1} while mimicking a). Carotid afferent activity reduced by neck pressure. MSNA was measured.	Reported respiratory fluctuations in MSNA with maximum activity occurring at end-expiration and minimum activity occurring at end-inspiration. Voluntary control of breathing does not impact MSNA. The RR interval and MSNA activity paralleled each other and were related to changes in diastolic pressure. MSNA is more responsive to reductions in carotid baroreceptor afferent activity during expiration than inspiration. This study highlights the existence of parallel phasic changes in spinal muscle sympathetic and medullary cardiac vagal efferent activity, illustrating the presence of spontaneous activity and greater susceptibility to excitation during expiration.
Saul et al. (1989)	18 healthy adults (10m, 8f)	Pseudorandom fluctuations (f_R between 0 and 42 breaths·min^{-1}). Measurements in the upright and supine position.	Onset of inspiration coinciding with the increase in *fc* for f_Rs above 9 breaths·min^{-1}, while for lower f_Rs fc increases precede the start of inspiration. The gain of the transfer function was lower in the upright posture when breathing above 9 breaths·min^{-1} but similar at lower f_Rs.
Seals et al. (1990)	7 healthy adults	a) f_R at 12 breaths.min^{-1} and V_Ts of 30%, 50%, and 70% of inspiratory capacity; and b) simulated exercise hyperpnoea (f_R at 40 breaths.min^{-1}; V_T = 60-70% inspiratory capacity). MSNA was measured.	Within-breath modulation of MSNA was observed during with 30% of inspiratory capacity with most activity occurring during the expiratory phase. The effect was potentiated by paced breathing at 12 breaths.min^{-1} producing strong sympathoinhibition from onset-mid inspiration to early-mid expiration. Duty cycle impacts the onset of sympathoinhibition. Sustained low or high-frequency deep breathing does not alter total minute MSNA compared with spontaneous breathing. These results demonstrate that the depth and pattern of breathing exert marked influences on the within-breath modulation of MSNA in humans but not on the total sympathetic outflow.

Saul et al. (1991)	14 healthy male adults	Pseudorandom fluctuations f_R between 0 and 42 breaths·min^{-1}. Compared purely sympathetic (standing + atropine) and pure vagal (supine + propranolol) modulation of *fc*.	Demonstrated reduced sympathetic control of *fc* for frequencies above 0.1Hz whereas pure vagal modulation of *fc* has higher magnitude at all frequencies. Established mechanical link between respiration, RSA and ABP. The RSA contribution to ABP is significant in the pure vagal condition but not in the pure sympathetic. The mechanical effects of respiration on arterial pressure are seemingly related to flow rather than to the actual lung volume. Mechanical effects of breathing are ampler during systole than diastole and are larger in the standing than supine position.
TenVoorde et al. (1995)	21 healthy adult men and 9 quadriplegic (no sympathetic control) adult men of equivalent age.	Fixed f_R at 4, 6, 10, 15 and 20 breaths·min$^{-1.}$ a) comparison between supine and standing in healthy individuals; b) comparison between healthy and quadriplegic individuals.	a) Standing enhanced the respiratory modulation of SBP, DBP and RSA which maximised at 4 breaths·min^{-1}. RSA maximises at 6 breaths·min^{-1} when supine. Cardiac baroreflex gain is enhanced in the supine position, but such effect tends to be cancelled at the lowest f_Rs. SBP-*fc* phase slightly increases with the decrease in f_R. b) The lack of sympathetic control significantly decreases respiratory modulation of SBP and f*c*, particularly at 6 breaths·min^{-1}. The suggestion of the existence of a resonant effect of the vasomotor baroreflex control loop at 0.1 Hz, directly impacting BPV modulation and indirectly affecting RSA.
Cooke et al. (1998)	10 healthy adults (5m, 5f)	a) spontaneous breathing; b) stepwise breathing (at 3, 6, 9, 12, 15 and 20 breaths·min^{-1}); c) stepwise breathing as above, but with prescribed V_T; d) random-frequency breathing (3-30 breaths·min^{-1}), and e) fixed-frequency breathing (15 breaths·min^{-1}).	HRV and BPV increase with the decrease in $f_{R.}$ Simultaneous control of f_R and V_T reduce HRV during 0.1Hz breathing. Stepwise and random-breathing yielded comparable coherence and RESP-*fc* and SBP-*fc* transfer functions. Indication that stringent control of breathing attenuates low-frequency respiratory modulation of *fc*.

Vaschillo et al. (2002)	5 healthy male adults	HRV biofeedback. Seven f_Rs within the range of 0.6 and 8.4 breaths·min^{-1}.	Describes the use of a biofeedback method for the non-invasive study of baroreflex mechanisms. HRV biofeedback produces large within-breath fluctuations in *fc* and ABP. The highest oscillation amplitudes occur in the range of 3.3-6.6 breaths·min^{-1} for *fc* and 1.2-3.3 breaths·min^{-1} for ABP. Oscillation amplitudes at specific fRs can be explained by resonance among various oscillatory processes in the cardiovascular system. Resonant frequencies show inter-individual variability.
Song and Lehrer (2003)	5 healthy female adults	Fixed f_R at 3, 4, 6, 8, 10, 12, and 14 breaths·min^{-1}.	No change in mean *fc,* RSA increased at 4 and 6 breaths·min^{-1} with the highest values at 4 breaths·min^{-1} and decreased at 3 breaths·min^{-1}. The results are interpreted as reflecting the possible effects of the rate of acetylcholine metabolism and negative resonance at 3 breaths·min^{-1}.
Tzeng et al. (2009)	30 healthy male adults	Cardiovagal BRS evaluated by the Oxford pharmacological method, α-index BRS and sequence BRS, at 15 and 6 breaths·min^{-1}.	Breathing at 6 breaths·min^{-1} showed increased α-index BRS and sequence BRS up-sequences when compared to 15 breaths·min^{-1}, while no changes were seen in BRS measured by the Oxford method. Mathematical BRS indices may not reflect the BRS determined by experimentally driven baroreceptor stimulation.
Sin et al. (2010)	16 healthy male adults	Fixed f_R at 6, 9 and 12 breaths·min^{-1}, with and without sympathetic blockade.	The time interval between RRinterval maximum an expiratory onset is not altered by the change in f_R. while RRinterval minimum shifted from expiratory onset to mid-inspiration with slower breathing. The time interval from inspiration onset to SBP minimum and expiratory onset to SBPmax was not impacted by f_R. SBPmaximum and RRmaximum temporal alignment is maintained irrespective of f_R. RR minimum increasingly lags SBPminimum with the decrease in f_R. Adrenergic blockade does impact the relationship between respiration and RR interval or RSA observed with SDB, suggesting that temporal and phase alterations with SDB are not affected by the cardiac sympathetic activity.

Limberg et al. (2013)	21 adults (13m, 8f)	Fixed f_R at 7, 14 and 21 breaths·min^{-1}	f_R and V_T changes did not affect mean MSNA, blood flow to the forearm, vascular conductance or MAP. Previously described within-breath MSNA pattern was observed. Collectively this study challenges the conception that SDB lowers ABP through inhibition of peripheral sympathetic tone.
Lucas et al. (2013)	16 healthy adults (10m, 6f)	Fixed f_R at 6 breaths·min^{-1} vs. spontaneous breathing. Orthostatic stress with tilt and LBNP.	SDB improves tolerance time to pre-syncope, increased low-frequency oscillations in MAP and cerebral arterial blood velocity and reduced rate of decline in cerebral arterial blood velocity and MAP with orthostatic stress. Orthostatic tolerance is improved by SDB likely due to respiratory-driven fluctuation in venous return and ABP.

ABP – arterial blood pressure; BPV – blood pressure variability; BRS – baroreflex sensitivity; DBP – diastolic blood pressure; f_R – breathing frequency; HRV – heart rate variability; INPV – inspiratory negative pressure ventilation; IPPV – inspiratory positive pressure ventilation; LBNP – lower body negative pressure; MAP – mean arterial pressure; MSNA – muscle sympathetic nerve activity; NIP – negative inspiratory pressure; SBP – systolic blood pressure; RESP – instantaneous lung volume variation; SASR – slowly adapting stretch receptors; RVSV – right ventricular stroke volume; V_T – tidal volume;

Appendix II – List of computed within-breath cardiovascular variables

Variable	Unit	Description
fc_I	beats·min^{-1}	Average *fc* during inspiration
fc_E	beats·min^{-1}	Average *fc* during expiration
RSA	ms	Largest RR interval minus lowest RR interval during an entire respiratory cycle (Chapter 6).
RSA	ms	Largest RR interval during expiration minus lowest RR interval during inspiration.(Chapters 4 and 5).
$LVSV_I$	mL·beat^{-1}	Average LVSV during inspiration
$LVSV_E$	mL·beat^{-1}	Average LVSV during expiration
ΔSV	mL·beat^{-1}	Average difference between $LVSV_I$ and $LVSV_E$
$LVSV_{I/E}$	mL·beat^{-1}	Highest LVSV during expiration minus lowest LVSV during inspiration
$\dot{Q}_I$	L·min^{-1}	Average $\dot{Q}$ during inspiration, calculated as the product of fc_I and $LVSV_I$
$\dot{Q}_E$	L·min^{-1}	Average $\dot{Q}$ during expiration, calculated as the product of fc_E and $LVSV_E$
$\Delta\dot{Q}$	L·min^{-1}	Average difference between Q_I and Q_E
SBP_{Imax}	mmHg	Maximum SBP value recorded during each inspiration. Presented as average value for the epoch
SBP_{Emax}	mmHg	Maximum SBP value recorded during each expiration. Presented as average value for the epoch
$SBP_{I/E}$	mmHg	Highest SBP during expiration minus lowest SBP during inspiration
$DBP_{I/E}$	mmHg	Highest DBP during expiration minus lowest DBP during inspiration
MAP_I	mmHg	Average MAP during inspiration. Calculated as the average value of the raw blood pressure waveform, throughout inspiration
MAP_E	mmHg	Average MAP during expiration. Calculated as the average value of the raw blood pressure waveform, throughout expiration
PP_I	mmHg	Average pulse pressure during inspiration, calculated as SBP_I minus DBP_I
PP_E	mmHg	Average pulse pressure during expiration, calculated as SBP_E minus DBP_E
$PP_{I/E}$	mmHg	Average difference between maximum and minimum pulse pressure throughout the entire epoch
TPR_I	mmHg·min·l^{-1}	Average inspiratory systemic vascular resistance calculated as MAP_I / $\dot{Q}_I$
TPR_E	mmHg·min·l^{-1}	Average inspiratory systemic vascular resistance calculated as MAP_E / $\dot{Q}_E$

Appendix III – Ethical approval

Head of School of Sport & Education
Professor Ian Rivers

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Pedro Vargas
PhD (Sport Sciences) Student
School of Sport and Education
Brunel University

26th July 2013

Dear Pedro

RE45-12 Effects of breathing frequency and tidal volume on the acute cardiovascular and autonomic responses to guided breathing

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University/School Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to grant ethics approval to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for review.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

Dr Richard J Godfrey
Chair of Research Ethics Committee
School Of Sport and Education

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23rd April 2015

Dear Pedro

RE17-14 Title: Integrative approach to investigate the afferent vagal feedback contribution to respiratory induced cardiovascular changes

I am writing to confirm the Research Ethics Committee of the Department of Life Sciences received your application to amend the above mentioned research study. Your amendments have been independently reviewed to ensure they comply with the University Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to you amending your study as proposed.

Any further changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for further consideration.

On behalf of the Research Ethics Committee for the Department of Life Sciences, I wish you every success with your revised study.

Yours sincerely

Richard Godfrey

DR Richard Godfrey
Chair of Research Ethics Committee
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Pedro Vargas
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14th August 2015

Dear Pedro

RE49-14 Title: Impact of resisted breathing modality upon respiratory induced cardiovascular changes

I am writing to confirm the Research Ethics Committee of the Department of Life Sciences received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to grant ethics approval to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee.

On behalf of the Research Ethics Committee for the Department of Life Sciences, I wish you every success with your study.

Yours sincerely

Richard Godfrey

Dr Richard Godfrey
Chair of Research Ethics Committee
Department of Life Sciences

Appendix IV – Participant Information

[Some of the participant information sheets contain information regarding methods and interventions that are not reported in this thesis. These result from either a retrospective decision not to collect such data, or in some cases, a deliberate decision to not present the collected data as doing so would not further the understanding of the topics debated in this thesis. Importantly, no procedures were added form those that have received ethical approval.]

PARTICIPANT INFORMATION

'Effects of different breathing patterns on cardiovascular responses to guided breathing'

This research investigation has been granted full ethical approval by the Brunel University School and Education Research Ethics Committee **(SREC):** Approval #: RE45-12

Contact	Position	Phone	Email
Mr Pedro Vargas, MSc	PhD Researcher	01895 266499	pedro.vargas@brunel.ac.uk
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Dr Richard Godfrey	Chair of the SREC	01895 266473	richard.godfrey@brunel.ac.uk

Recent evidence suggests that practicing deep, slow breathing patterns can reduce blood pressure. These breathing patterns have been practiced for centuries during yoga, **and more recently, through devices that guide users to adopt deep slow breathing**. The purpose of this study is to explore changes in breath volume (tidal volume) and breathing frequency upon the acute response of a range of cardiovascular variables, e.g., heart rate and blood pressure.

Study purpose

The purpose of this study is to explore the impact of a change in breathing volume (tidal volume) or breathing frequency on heart rate and blood pressure.

Study design

You have been invited to participate in this research study as one of 12 participants. The entire experiment will require approximately 4.5h of your time split over 3 sessions on different days (separated by at least 24h and no more than a week). On the first session we will collect some baseline measurements you will learn how to control your breath at a specific rhythm and depth, using a visual feedback device. During session 2 you will perform 5 bouts of controlled breathing at a fixed tidal volume (constant breath depth) and varying breathing frequencies (10, 8, 6 and 4 breaths per minute), plus one bout at your spontaneous breathing frequency. Every bout will last approximately 5 minutes, with 5 minutes of rest between them. Session 3 will also involve 5 bouts of controlled breathing, but this time at a fixed breathing frequency, and with varying tidal volumes (5 minute bouts with 5 minutes rest between each). During the entire trial specific measures of your physiological function will be assessed (see below). In order to determine the between-day variation and accuracy of the collected data, approximately half of the study participants will be required to repeat both session 2 and 3 in five consecutive days, totalizing 10 testing sessions. During the initial testing session

you will be inquired regarding your availability to perform the 10 testing sessions. Irrespective of your answer, you will always be able to enrol in the study, performing the 3 aforementioned testing sessions.

Do I have to take part? Can I change my mind and withdraw from the study?

Your participation in the study is entirely voluntary and at no time should you feel obliged to take part in, or continue the study. You may withdraw from the study at any time, without the need to provide any reason, without subsequent penalty or prejudice, and without affecting your University grades or professional evaluation.

What will happen to me if I take part?

As stated in the 'Study Design' paragraph you will be submitted to a series of bouts of controlled breathing, during which we will be performing a series of physiological measurements.

Pre-test requirements

You will be first required to complete a health questionnaire and sign a consent form to confirm that you're healthy and able to participate in the study. To ensure that external factors do not influence your test results, you will be required to refrain from eating for 2 hours, caffeine for 12 hours and alcohol for 24 hours prior to testing.

Physiological measurements

Your height and weight and lung function will be accessed during your first visit (lung function will be also assessed at the start of subsequent testing sessions and height and weight measurements will not require you to remove any cloths except for your shoes), by performing a series of sub-maximal and maximal breathing manoeuvres into a mouthpiece. A face mask will be used throughout each trial to measure breathing patterns, and the amount of CO_2 and oxygen that you expire, during testing. We will measure your blood pressure and estimate the amount of blood that is pumped out of your heart using two cuffs; one placed on the upper arm and one on the middle finger. A small sensor in the finger cuff will also allow the measurement of the amount of oxygen carried in the blood.

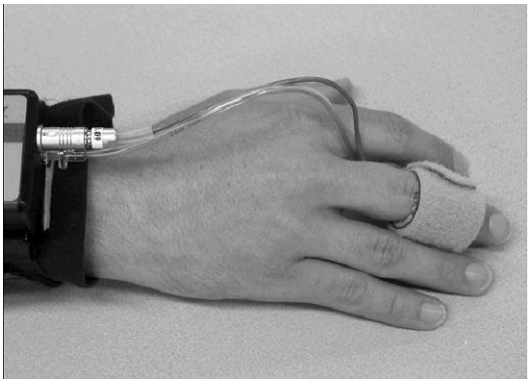

Figure 1 – The finger cuff to be used for the continuous measurement of arterial blood pressure and blood oxygenation.

Heart rate will be measured continuously using a 3-lead ECG whose sensors will be placed on your chest (1 sensor in the vicinity of both shoulders) and lower rib-gage (1 sensor placed about one palm bellow the chest). In order to guarantee proper placement and adhesiveness of the sensors throughout the entire testing sessions (excessive body hair and/or sweat might result in reduced electrode adherence), these might have to be secured with duct tape. This procedure will also eliminate the need for other, less pleasant procedures, like shaving the areas where the electrodes are meant to be placed.

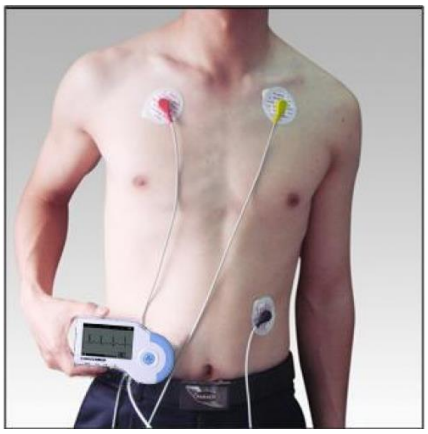

Figure 2 – Placement sites for the 3-lead ECG electrodes.

Breathing function

As above stated, your lung function will be accessed during all of your visits through an instrument called a spirometer. The instrument measures the rate of airflow in and out of your lungs during a maximal breathing manoeuvre. The maximal flow and volume changes that a person can produce provide an indication of how healthy their lungs are. The manoeuvre requires that you inhale until your lungs are full, and then exhale as hard and fast a possible until they are as empty as you can make them. Once empty, you will then fill your lungs as rapidly as possible by inhaling immediately. This manoeuvre will be repeated 3-5 times, with 30-60 rest between each test. During the first testing session the lung function assessment will be performed in a whole body plethysmograph or "body-box". This equipment will allow to quantify of your lung volumes as well as point out the existence of eventual respiratory conditions. A special filter is used to protect the equipment and each user from potential cross-infection between users. There are no risks associated with this test, which is a routine medical assessment.

Figure 3 – Whole body plethysmograph or "body-box"

Exclusion Criteria

This study is aimed at male non-smokers between the ages of 18 and 35 years and with no previous medical history of cardiovascular or respiratory disease (e.g. asthma). Similarly, if you smoke, or if you're currently taking anti-depressants or irritable bowel remedies (e.g., Buscopan®), you cannot to take part in this study. You will also not be able to participate in the study if you present any reaction to the conducting gel used on the ECG or if the "body-box" tests provide evidence of undiagnosed respiratory disease. Finally, if you fail to demonstrate the capacity to control your breathing following a visual cue, during an initial training session (further training sessions might be necessary to overcome this difficulty).

What are the possible disadvantages and risks of taking part?

Controlled breathing

Since you will not be breathing at your spontaneous breathing frequency and amplitude, there is a high likelihood of becoming slightly hypercapnic (excess CO_2 in the blood) or hypocapnic (deficit of CO_2 in the blood). Neither of these conditions represent any known risk to your safety and you are free to interrupt the procedure in the unlikely event that you feel unwell. If you become hypercapnia you will feel a strong urge to breathe more, which can be accompanied by sensations of heat, sweating and flushing. On the other hand if you become hypocapnic, the symptoms may include dizziness. Both sensations will subside quickly once spontaneous breathing is resumed.

What if something goes wrong?

The investigator conducting the experiment is qualified in basic life support, and an individual with advanced life support training will be onsite at all times. In addition, an emergency name and contact telephone number must be provided by all participants in the health check questionnaire. In case of a problem or complaint, contact details for the investigator in addition to the Research Ethics Committee can be found at the top of this document.

Will my taking part in this study be kept confidential? What will happen to the results of the research study?

The researchers hope to publish data collected from this study in scientific journal articles, and/or present the research findings at relevant scientific conferences. No personal information will be used or referred to in the study and you will instead be issued with an identification number. All data will be stored for a maximum of 5 years at the Centre for Sports Medicine and Human Performance, Brunel University, and will not be released without written permission from yourself or unless required by law.

Who is organising and funding the research?

The research is being organized by the Centre of Sports Medicine and Human Performance, Brunel University, and funded by the Portuguese Foundation of Science and Technology as part of Mr. Pedro Vargas' PhD project.

If you have any questions, please contact:

Mr. Pedro Vargas, MSc

Centre for Sports Medicine and Human Performance

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Email: pedro.vargas@brunel.ac.uk

Please retain this information document for your records

PARTICIPANT INFORMATION

'The cardiovascular and autonomic effects of deep and slow breathing'

This research investigation has been granted full ethical approval by the Brunel University Department of Life Sciences Research Ethics Committee **(SREC):** Approval #:

Contact	Position	Phone	Email
Mr Pedro Vargas, MSc	PhD Researcher	01895 266499	pedro.vargas@brunel.ac.uk
Professor Alison McConnell	Professor of Applied Physiology	01895 266480	alison.mcconnell@brunel.ac.uk
Dr Richard Godfrey	Chair of the SREC	01895 266473	richard.godfrey@brunel.ac.uk

The information contained within this document is divided into two parts. Part A provides an overview of the study, whilst part B provides detailed information about the procedures particpants will be requested to undertake.

PART A

Study background

Recent evidence suggests that practicing deep, slow breathing patterns can reduce blood pressure. These breathing patterns have been practiced for centuries during yoga, and more recently, through devices that guide users to adopt deep slow breathing. Our previous studies showed that changes in breath volume (tidal volume) and breathing frequency do have an immediate impact upon some cardiovascular variables, such as blood pressure and heart rate. In this study we intend to explore in more detail, the impact of different breathing patterns (breathing frequency, volume and resistance to inhalation) upon the acute responses of the cardiovascular system. In addition, we wish to manipulate certain conditions that we think might affect responses to deep slow breathing, such as the effect of gravity upon the return of blood to the heart.

Study purpose

The purpose of this study is to explore some of the factors we believe underpin the effect of deep, slow breathing upon heart rate and blood pressure.

Do I have to take part? Can I change my mind and withdraw from the study?

Your participation in the study is entirely voluntary and at no time should you feel obliged to take part in, or continue the study. You may withdraw from the study at any time, without the need to provide any reason, without subsequent penalty or prejudice, and without affecting your University grades or professional evaluation. This study will require 14 healthy, active males aged 18-35.

How long will the study last?

The study will require participants to visit the lab on two occasions. During the first testing visit we will collect some baseline measurements, including echocardiographic imaging (cardiac ultrasound), and you will learn how to control your breathing at a specific rhythm and depth, using a visual feedback device (see Figure 1). You will also be required to ingest a therapeutic dose of a medicine named Buscopan®, to check for any potential hypersensitivity (further information about this medicine can be found in the following pages). The total length of the session will be approximately 1 h.

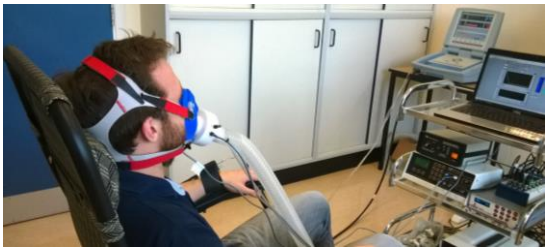

Figure 1. Participant undergoing breathing pacing.

Your second visit will consist of the following parts:

1. **Baseline measurements at rest, preparation and calibration procedures**: 15 min
2. **Phase 1 of the intervention**: 2h
3. **Comfort break**: 20 min
4. **Phase 2 of the intervention** (repetition of steps 1 and 2): 2h15

During **phase 1** you will be requested to perform 3 bouts of controlled breathing at a fixed percentage of your lung capacity and fixed, slow breathing frequencies. Later on, you will be asked to do 2 more bouts at a similar volume and breathing frequency but this time however, you will be breathing against two different inspiratory resistances. The resistances will make inhaling feel slightly harder, but you will still be able to breathe quite freely. The third part of our intervention will involve the repetition of the previous step, with the addition of lower body positive pressure (LBPP), which involves applying pressure to your legs by inflating special trousers that are worn by jet fighter pilots (see figure 4). We will impose mild levels of compression, which shouldn't cause any significant discomfort, or represent any risk for your wellbeing. The next stage of this trial will involve repeating the combination of breathing pattern and LBPP that produced the largest increase in your cardiac response, and at the same time that we will stimulate a nerve branch located in your outer ear using a transcutaneous electrical nerve stimulation (TENS) machine (see figure 5). An overview of the protocol is provided with Figure 2.

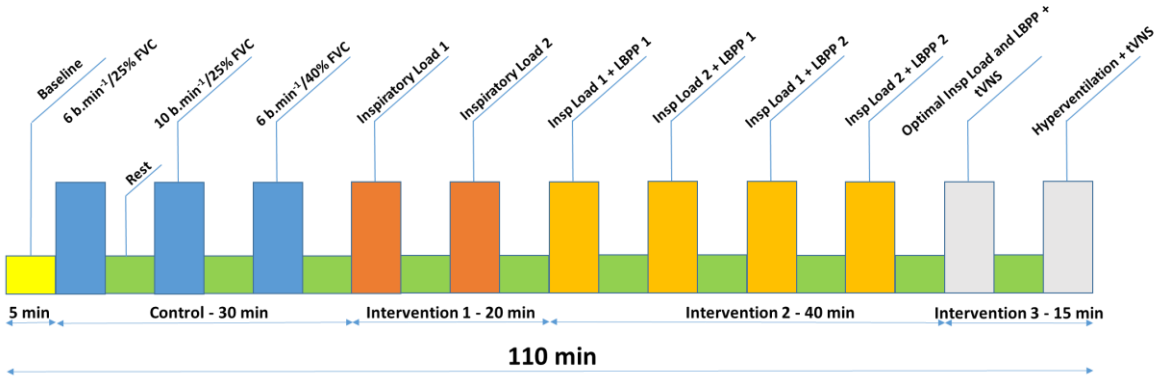

Figure 2- Schematic of study design. General structure of each phase – *Baseline(yellow) – spontaneous breathing; Rest (green) – spontaneous breathing; Control (blue) – device guided breathing; Intervention 1 (red) – device guided inspiratory loaded breathing; Intervention 2 (orange) – device guided inspiratory loaded breathing with lower body positive pressure (LBPP); Intervention 3 (grey) – selected breathing patterns with transcutaneous vagal nerve stimulation (tVNS).*

Each bout of controlled breathing will last approximately 5 minutes, with 5 minutes of rest between them. During the entire trial, specific measures of your physiological function will be assessed. You will be given a 20 min comfort break between the two halves of the protocol. During the break you will be allowed to eat

some fruit (e.g. – banana) and biscuits. You will be allowed to drink approximately 200 ml of water during each phase of the study.

In the second phase of the study, we will ask you to repeat everything you did in the first half, but after having taken a medication that blocks feedback from nerves within the vagus nerve. This will require the ingestion of 20mg of hyoscine butilbromide (Buscopan®). In the UK, Buscopan® is an over the counter treatment for abdominal pain, irritable bowel syndrome and abdominal cramps. As well as treating these conditions, Buscopan® also has an important effect upon the cardiovascular system by blocking the signals that are sent to the brain by a series of receptors in your heart and major blood vessels. The drug is perfectly safe, which is why it can be purchased in any supermarket or pharmacy, without the need to consult a pharmacist. The Buscopan® will be administered orally and the dosage is in line with the therapeutic recommended dose. **Please be advised that this medicine can affect your ability to drive or to operate machines.**

PART B

What will happen to me if I take part?
As previously stated, you will be submitted to a series of bouts of controlled breathing, during which we will be performing a number of physiological measurements. These measurements are described below:

Pre-test requirements
You will be first required to complete a health questionnaire and sign a consent form to confirm that you're healthy and able to participate in the study. To ensure that external factors do not influence your test results, you will be required to refrain from eating for 2 hours, caffeine for 6 hours and alcohol for 24 hours prior to testing. You will also be asked to take a therapeutic dose of Buscopan® a few days prior to undertaking the protocol to test for any side effects (during your first visit). Side effects are rare, but might include:

- Dry mouth;
- tachycardia;
- dyshidrosis (an eczema-like skin condition);
- skin reactions such as urticaria and pruritis (itching);
- difficulty passing urine.

In very rare situations you may experience:

- Anaphylactic reaction and shock;
- Dyspnoea (difficult breathing);
- Allergic reactions (hypersensitivity) such as skin rash and erythema (patchy red skin);

Also, if you suffer from any of the following conditions you will not be allowed to partake in this study:

- allergy or hypersensitivity to any of the ingredients in the medicine;
- fever;
- fructose intolerance;
- glucose-galactose malabsorption problems
- heart problems, including tachicardia;
- myasthenia gravis (condition that provokes fatigue and weak muscles);
- angle glaucoma (abnormally high eye pressure);
- megacolon (dilated colon);
- other gastrointestinal problems;
- sucrase-isomaltase deficiency
- thyrotoxicosis (excess amount of thyroid hormones in the bloodstream);
- urinary problems.

Physiological measurements
Baseline measurements - Your height, weight and lung function will be assessed during your first visit (height and weight measurements will not require you to remove any cloths except for your shoes). Lung function will be assessed by performing a series of maximal breathing manoeuvres into a mouthpiece (cf. figure 3). Cardiac function testing using ultrasonography will also be performed during your first visit (cf. figure 6).

Lung function - As stated above, your lung function will be assessed using an instrument called a spirometer (figure 3). The instrument measures the rate of airflow in and out of your lungs during a maximal breathing manoeuvre. The maximal flow and volume changes that a person can produce provide an indication of how healthy their lungs are. The manoeuvre requires that you inhale until your lungs are full, and then exhale as hard and fast a possible until they are as empty as you can make them. Once empty, you will then fill your lungs as rapidly as possible by inhaling immediately. This manoeuvre will be repeated 3-5 times, with 30-60 rest between each test.

Figure 3 – Handheld Spirometer

During the controlled breathing bouts, a face mask will be used throughout, to measure breathing patterns, and the amount of carbon dioxide and oxygen that you respire (see figure 1).

Cardiovascular function - We will measure your blood pressure and estimate the amount of blood that is pumped out of your heart using two cuffs; one placed on the upper arm and one on the middle finger (figure 4).

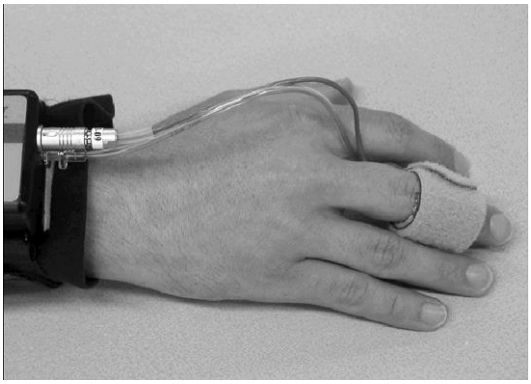

Figure 4 – The finger cuff to be used for the continuous measurement of arterial blood pressure.

Heart rate will be measured continuously using a 3-lead ECG. Sensors will be placed on your chest; 2 sensors in just below your clavicles and 1 sensor placed just above your left iliac crest, in the midaxillary line (figure 5). In order to guarantee proper placement and security of the sensors throughout the entire testing sessions these might have to be secured with tape. Taping will also eliminate the need for other, less pleasant procedures, like shaving the areas where the electrodes are to be placed.

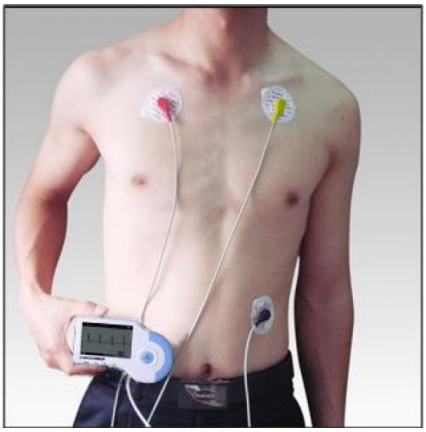

Figure 5 – Placement sites for the 3-lead ECG electrodes.

The amount of blood pumped by the heart per minute (i.e., cardiac output) and on each heartbeat (stroke volume) will be calculated non-invasively from images obtained by ultrasonography of the heart (see figure 6 below).

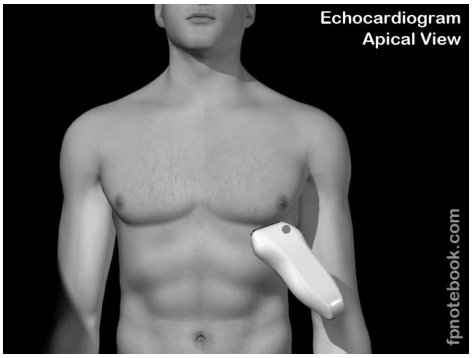

Figure 6: Ultrasound probe positioning for cardiac output measurements. Obtained and edited from http://www.fpnotebook.com/legacy/CV/Rad/TrnsthrcEchcrdgrm.htm

Lower body positive pressure (LBPP) - A slight compression at the legs will be imposed via the inflation of Anti-G trousers, the same that modern jet fighter pilots use (figure 7). This equipment was developed to avoid blood pooling in the legs and facilitate its return to the heart. The maximum inflation pressure of the system is around 50 mmHg, which is much lower than the required pressure necessary to fully occlude circulation. The maximum inflation pressure you will experience is likely to be 20 mmHg.

Figure 7 – Anti-G Trousers

Ear Nerve Stimulation (Transcutaneous Vagal Nerve Stimulation (tVNS)) - Electrical stimulation of the auricular (ear) branch of your vagus nerve will be accomplished through a standard transcutaneous electrical nerve stimulation (TENS) machine. A bespoke ear-clip electrode will be placed in the external part of both ears, more precisely on the tragus (figure 8). Stimulation will be kept at a constant intensity and frequency throughout the entire procedure and sustained for approximately 15 min. This procedure is free from risk or pain, and has no known side-effects. You should experience little or no sensation during stimulation. If at any time you feel a burning sensation or small electric shocks in your ear you should immediately inform the researcher.

Figure 8 – TENS Machine and ear-clip electrode (placed in the tragus).

Vagal blockade - During the final half of the protocol you will be asked to take 20mg of Buscopan®. As previously stated this is an over the counter medication and should not pose any risk for your health.

Figure 9 – Buscopan® medication

Exclusion Criteria

This study is aimed at male non-smokers between the ages of 18 and 35 years and with no previous medical history of cardiovascular or respiratory disease (e.g. asthma). Similarly, if you're currently taking anti-depressants or irritable bowel remedies (e.g., Buscopan®), you cannot take part in this study. You will also not be able to participate in the study if you present any reaction to the conducting gel or sticky pads/tape used on the ECG electrodes, to any of the constituents of Buscopan® or if you suffer from any of the conditions listed in the 'pre-test requirements' section. In addition, if the spirometry tests provide evidence of undiagnosed respiratory disease, you will not be able to take part. Finally, it will also not be possible for you to take part if you fail to demonstrate the capacity to control your breathing following a visual cue.

What are the possible disadvantages and risks of taking part?

Controlled breathing - Since you will not be breathing at your spontaneous breathing frequency and amplitude, there is a high likelihood of becoming slightly hypercapnic (high carbon dioxide in the blood) or hypocapnic (low of carbon dioxide in the blood). Neither of these conditions represent any known risk to your safety at the levels you might experience, and you are free to interrupt the procedure in the unlikely event that you feel uncomfortable or unwell. If you become hypercapnic you will feel a strong urge to breathe more, which can be accompanied by sensations of heat, sweating and flushing. On the other hand if you become hypocapnic, the symptoms may include dizziness. To avoid hypocapnia the breathing circuit will include a re-breathing system that will eliminate the likelihood of becoming hypocapnic. Both sensations will subside quickly once spontaneous breathing is resumed.

Breathing against inspiratory loads - There are no foreseeable risks associated with breathing against inspiratory loads, which are used in the treatment of a number of medical conditions. Inspiratory loading has been implemented in our laboratories for almost 15 years without any adverse events.

Lower body positive pressure (LBPP) - There is no known side-effects or risks associated to the application of LBPP. Notwithstanding this, it is conceivable that the sudden removal of the LBPP might cause gravitational hypotension, i.e., blood rushing away from your head under the force of gravity. This will translate into dizziness and in extreme cases fainting. This is a similar phenomenon to what you might experience when suddenly moving from lying to a standing position. To avoid any negative consequences

of a sudden drop in blood pressure you are advised to remain seated for a few minutes after the release of the LBPP. The maximum pressure generated by the system is just over 50 mmHg, which is much less than the pressure necessary to occlude of arterial circulation. Nonetheless, the system is equipped with a pressure release safety valve.

Transcutaneous vagal nerve stimulation (tVNS) - Transcutaneous electrical nerve stimulation (TENS) is used widely in a range of healthcare applications. The technique is safe and there are no significant adverse effects known. However, skin irritation, or even burns, are fairly common if the applied electrical current is too intense. The currents that will be applied in the present study should be barely perceptible, therefore minimising the risks involved. In the particular case of tVNS, the stimulation of the ear branch of the vagus nerve might elicit some side effects, such as cough, gagging, watery eyes, and fainting. All of these effects are extremely rare and cease once stimulation has stopped. If you report discomfort or burning/electric shock sensation, or if any of the aforementioned responses are observed, tVNS will be ceased immediately.

Vagal blockade - The side effects of Buscopan® might include dry mouth, tachycardia, dyshidrosis (an eczema-like skin condition) and difficulty passing urine. Symptoms like transient hypotension (low blood pressure) or hypertension (high blood pressure) may also occur. You will be seated, and your arterial blood pressure and heart rate will be monitored continuously, so in the unlikely event that a substantial change in blood pressure should arise, this will be apparent immediately. In the unlikely event of substantial hypotension you will be quickly transferred to the supine position without transferring from the reclining chair in which you will be seated. To avoid any discomfort arising from the intake of Buscopan® you will be allowed to drink water throughout the experiment. Buscopan® has reportedly very low toxicity levels, which means that the occurrence of any noxious reaction is highly unlikely. Any side effects or discomforts will be transient and cease as the drug is eliminated from your body.

What if something goes wrong?
The investigator conducting the experiment is qualified in basic life support, and an individual with advanced life support training will be onsite at all times. In addition, an emergency name and contact telephone number must be provided by all participants in the health check questionnaire. In case of a problem or complaint, contact details for the investigator in addition to the Research Ethics Committee can be found at the top of this document.

Pre-participation health check questionnaire
Health and safety within this investigation is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. To identify whether you are eligible to participate in this investigation, we will ask you to fill in the pre-participation health check questionnaire included below.

Benefit of participating in the study
You will gain information on how your body responds to deep and slow breathing and you will have the opportunity to receive lung function information that might useful for your health. You also will have the opportunity to witness some laboratory procedures, such as cardiovascular ultrasonography.

Will I be paid for my participation in the study?
You will not be remunerated for participating in this study, but in case of harm Brunel University has an insurance policy (NHE-01CA29-0013) with public and products liabilities of £30m. In the case of clinical trials the University maintains a comprehensive policy to cover negligent and no fault harm up to a maximum of £10 m.

Will my taking part in this study be kept confidential and what will happen to the results of the research study?
The researchers hope to publish data collected from this study in scientific journal articles, and/or present the research findings at relevant scientific conferences. No personal information will be used or referred to in the study and you will instead be issued with an identification number. All data will be stored for a maximum of 5 years at the Centre for Sports Medicine and Human Performance, Brunel University, and will not be released without written permission from yourself or unless required by law.

Who is organising and funding the research?
The research is being organised by the Centre of Sports Medicine and Human Performance, Brunel University, with the financial support of the Portuguese Foundation of Science and Technology as part of Mr. Pedro Vargas' PhD project.

How can I get information about the study findings?
You will get all information about your results and the study findings by contacting Pedro Vargas (see details below).

Mr. Pedro Vargas, MSc
Centre for Sports Medicine and Human Performance
Brunel University, Uxbridge, UK UB8 3PH
Phone: 01895 266499
Email: pedro.vargas@brunel.ac.uk

Please retain this information document for your records

PARTICIPANT INFORMATION

'Impact of resisted breathing modality upon respiratory induced cardiovascular changes'

This research investigation has been granted full ethical approval by the Brunel University London Department of Life Sciences Research Ethics Committee **(SREC):** Approval #:

Contact	Position	Phone	Email
Mr Pedro Vargas, MSc	PhD Researcher	01895 266499	pedro.vargas@brunel.ac.uk
Professor José Gonzalez-Alonso	Professor of Exercise and Cardiovascular Physiology	01895 267324	j.gonzalez-alonso@brunel.ac.uk
Dr Richard Godfrey	Chair of the SREC	01895 266473	richard.godfrey@brunel.ac.uk

Recent evidence suggests that practicing deep, slow breathing patterns can reduce blood pressure. These breathing patterns have been practiced for centuries during yoga, **and more recently, through devices that guide users to adopt deep slow breathing**. Our previous studies support this idea and showed that changes in breath volume (tidal volume) and breathing frequency do have an immediate impact upon some cardiovascular variables, such as blood pressure and heart rate. In this study we intend to further explore the impact that different breathing patterns (breathing frequency, volume and resistance to inhalation and/or exhalation) might have on the acute regulation of the cardiovascular system.

Study purpose

The purpose of this study is to explore the effect of deep, slow breathing against small respiratory resistances, as well as via the nose, upon heart rate and blood pressure.

Study design

You have been invited to participate in this research study as one of 15 participants. The entire experiment will require approximately 2 h of your time. During the testing session we will also collect some baseline measurements, and you will learn how to control your breathing at a specific rhythm and depth, using a visual feedback device (Cf. Figure 1).

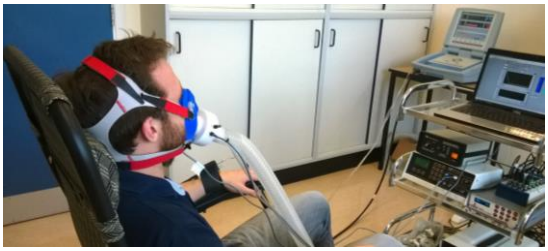

Figure 1. Participant undergoing breathing pacing.

During the testing session you will be requested to perform 9 bouts of controlled breathing at a fixed tidal volume (constant breath depth of 30% of your lung capacity and at a fixed breathing frequency (approx. 5.5 breaths per minute). Six out of those 9 bouts will include breathing against some form of resistance. This resistance will be applied either in the expiratory or inspiratory phases of your breathing cycle, or may be delivered during both phases. These inspiratory or expiratory loads will be delivered either through a flow dependent type of resistance (the quantity of air or air flow that passes through the circuit per unit of time will determine the resistance, just like breathing through a straw) or by a threshold respiratory pressure device (the resistance is determined by an adjustable pressure release valve, making inspiration or expiration only possible if a respiratory pressure above the opening threshold is generated). The final two sets will be single-nostril breathing, with no added respiratory resistance.

Each bout of controlled breathing will last approximately 5 minutes, with 5 minutes of rest between them. During the entire trial, specific measures of your physiological function will be assessed (see below).

Do I have to take part? Can I change my mind and withdraw from the study?

Your participation in the study is entirely voluntary and at no time should you feel obliged to take part in, or continue the study. You may withdraw from the study at any time, without the need to provide any reason, without subsequent penalty or prejudice, and without affecting your University grades or professional evaluation.

What will happen to me if I take part?

As stated in the 'Study Design' section you will be submitted to a series of bouts of controlled breathing, during which we will be performing a number of physiological measurements. These measurements are described below:

Pre-test requirements

You will be first required to complete a health questionnaire and sign a consent form to confirm that you're healthy and able to participate in the study. To ensure that external factors do not influence your test results, you will be required to refrain from eating for 2 hours, caffeine for 6 hours and alcohol for 24 hours prior to testing.

Physiological measurements

Your height and weight and lung function will be accessed during your first visit (height and weight measurements will not require you to remove any clothes except for your shoes). Lung function will be assessed by performing a series of maximal breathing manoeuvres into a mouthpiece. During the controlled breathing bouts, a face mask will be used throughout, to measure breathing patterns, and the amount of carbon dioxide and oxygen that you respire. We will measure your blood pressure and estimate the amount of blood that is pumped out of your heart using two cuffs; one placed on the upper arm and one on the middle finger. A small sensor in the finger cuff will also allow the measurement of the amount of oxygen carried in the blood (figure 2).

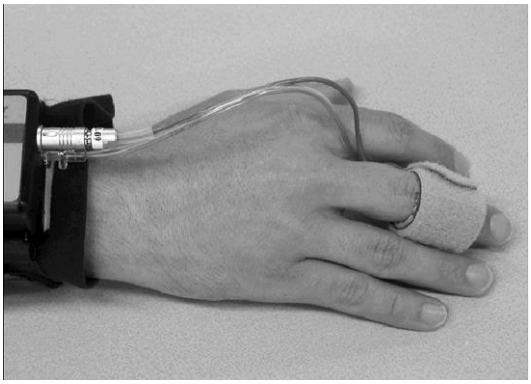

Figure 2 – The finger cuff to be used for the continuous measurement of arterial blood pressure and blood oxygenation.

Heart rate will be measured continuously using a 3-lead ECG. Sensors will be placed on your chest; 2 sensors in just below your clavicles and 1 sensor placed on the lower ribs, just above your left iliac crest. In order to guarantee proper placement and security of the sensors throughout the entire testing sessions these might have to be secured with tape (figure 4). Taping will also eliminate the need for other, less pleasant procedures, like shaving the areas where the electrodes are to be placed.

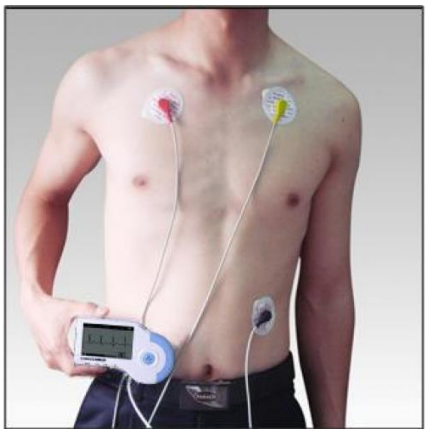

Figure 3 – Placement sites for the 3-lead ECG electrodes.

Breathing function

As stated above, your lung function will be accessed using an instrument called a spirometer (cf. Figure 4). The instrument measures the rate of airflow in and out of your lungs during a maximal breathing manoeuvre. The maximal flow and volume changes that a person can produce provide an indication of how healthy their lungs are. The manoeuvre requires that you inhale until your lungs are full, and then exhale as hard and fast a possible until they are as empty as you can make them. Once empty, you will then fill your lungs as rapidly as possible by inhaling immediately. This manoeuvre will be repeated 3-5 times, with 30-60 rest between each test.

Figure 4 – Handheld Spirometer

Respiratory resistances

The respiratory resistances of approximately 10 cmH_2O will be delivered via two different devices attached to the aforementioned respiratory circuit (cf. Figure 1). The flow dependent respiratory load will be imposed by a bespoke breathing circuit (cf. Figure 2) containing a flow resistor that comprises a number of nylon washers that reduce the internal diameter of the airway, thus creating resistance to flow and generating a precisely calibrated respiratory load.

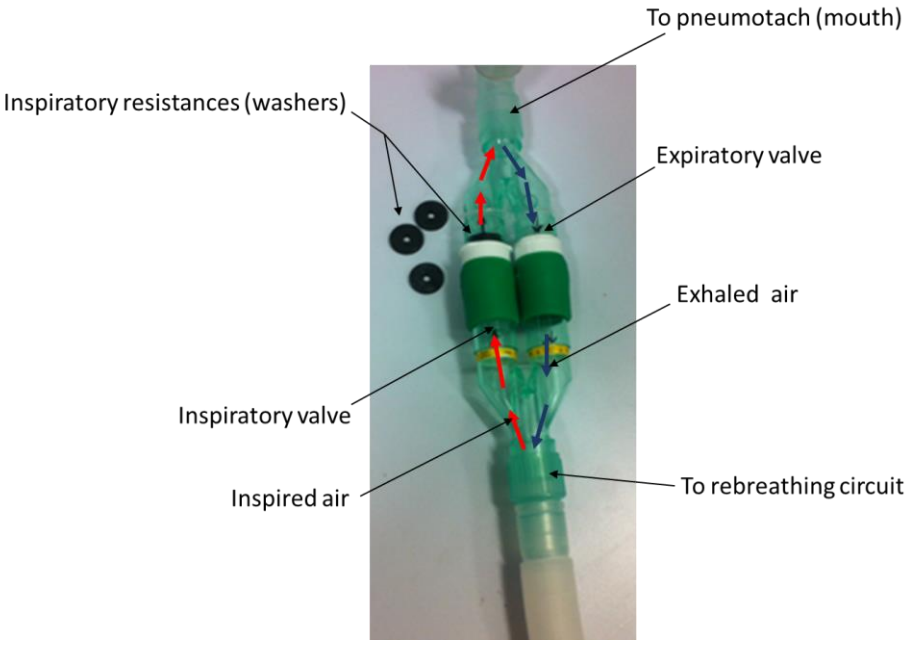

Figure 5 – Flow-dependent respiratory breathing circuit

The threshold respiratory pressure loads will be delivered by a commercially available device (PowerLung®; cf. Figure 6). The load will be adjusted by a spring mechanism that increases/decreases the pressure that the participant will have to generate in order open the built-in inspiratory and expiratory valves.

Figure 6 – Threshold respiratory pressure device (PowerLung®)

Nasal pressure measurements

The single-nostril breathing part of the protocol will involve the use of a nasal probe for nostril pressure measurements (cf. Figure 7).

Figure 7 – Nasal pressure probe

Exclusion Criteria

This study is aimed at male non-smokers between the ages of 18 and 35 years and with no previous medical history of cardiovascular or respiratory disease (e.g. asthma). You will also not be able to participate in the study if you present any reaction to the conducting gel used on the ECG or if you're currently experiencing frequent episodes of nasal bleeding. In addition, if the spirometry tests provide evidence of undiagnosed respiratory disease, you will not be able to take part. Finally, it will also not be possible for you to take part if you fail to demonstrate the capacity to control your breathing following a visual cue.

What are the possible disadvantages and risks of taking part?

Controlled breathing

Since you will not be breathing at your spontaneous breathing frequency and amplitude, there is a high likelihood of becoming slightly hypercapnic (high carbon dioxide in the blood) or hypocapnic (low of carbon dioxide in the blood). Neither of these conditions represent any known risk to your safety and you are free to interrupt the procedure in the unlikely event that you feel unwell. If you become hypercapnic

On the other hand if you become hypocapnic, the symptoms may include dizziness. To avoid hypocapnia the breathing circuit will include a re-breathing system that will eliminate the likelihood of becoming hypocapnic. Both sensations will subside quickly once spontaneous breathing is resumed.

Breathing against respiratory loads

There are no foreseeable risks associated to breathing against respiratory resistances, which are used in the treatment of a number of medical conditions. Respiratory loading has been implemented in our laboratories for almost 15 years without any adverse events.

Single-nostril breathing

The only potential risk of this particular part of the intervention is the forceful insertion of the nasal probe. To mitigate this risk the probe will be inserted by you, the participant. In addition, all the probes, as well as the masks to be used in this study, will always be new or sterilised.

What if something goes wrong?

The investigator conducting the experiment is qualified in basic life support, and an individual with advanced life support training will be onsite at all times. In addition, an emergency name and contact telephone number must be provided by all participants in the health check questionnaire. In case of a problem or complaint, contact details for the investigator in addition to the Research Ethics Committee can be found at the top of this document.

Will my taking part in this study be kept confidential and what will happen to the results of the research study?

The researchers hope to publish data collected from this study in scientific journal articles, and/or present the research findings at relevant scientific conferences. No personal information will be used or referred to in the study and you will instead be issued with an identification number. All data will be stored for a maximum of 5 years at the Centre for Sports Medicine and Human Performance, Brunel University London, and will not be released without written permission from yourself or unless required by law.

Who is organising and funding the research?

The research is being organised by the Centre of Sports Medicine and Human Performance, Brunel University London, with the financial support of the Portuguese Foundation of Science and Technology as part of Mr. Pedro Vargas' PhD project.

If you have any questions, please contact:

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Please retain this information document for your records

Appendix V – Consent form

CONSENT FORM

This research investigation has been granted full ethical approval by the Brunel University Department of Life Sciences Research Ethics Committee **(SREC):** Approval #:

The participant should complete the whole of this sheet		
	Please tick the appropriate box	
	YES	**NO**
Have you read the Research Participant Information Sheet?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Have you received satisfactory answers to all your questions?	☐	☐
Who have you spoken to?		
Do you understand that you will not be referred to by name in any report concerning the study?	☐	☐
Do you understand that you are free to withdraw from the study:		
• **at any time?**	☐	☐
• **without having to give a reason for withdrawing?**	☐	☐
• **(where relevant, adapt if necessary) without affecting your future care?**	☐	☐
Do you agree to take part in this study?	☐	☐
Signature of Research Participant:		☐
Date:		☐
Name in capitals:		
<u>Witness statement</u>		
I am satisfied that the above-named has given informed consent.		
Witnessed by:		
Date:		
Name in capitals:		

Researcher name:	**Signature:**
Supervisor name:	**Signature:**

Appendix VI – Health Questionnaire

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety within this investigation is of paramount importance. For this reason it is essential that we are aware of your current health status before you begin any testing procedures. Additionally, the following questions are designed to establish whether you are suited to take part in this study.

Participant name: …… **Date of birth:** ___/___/_____

Emergency Contact Name:…… **Emergency Contact Tel:**……

Please answer the following questions:	**YES**	**NO**
1. Has your doctor ever diagnosed a heart condition or recommend only medically supervised exercise?	☐	☐
2. Do you suffer from chest pains, heart palpitations, arrhythmia or tightness of the chest?	☐	☐
3. Do you suffer from myasthenia gravis, megacolon, narrow angle glaucoma, thyrotoxicosis, Intolerance to or difficulty digesting sugars, intestinal/urinary obstruction or any other gastrointestinal problems?	☐	☐
4. Do you have known high blood pressure? If yes, please give details (i.e. medication).	☐	☐
5. Have you ever taken Buscopan®? If yes, did you experience any side effects (please give details)?	☐	☐
6. Do you suffer from any lung/chest problem, e.g., asthma, bronchitis, emphysema? If yes, please give details (i.e. medication).	☐	☐
7. Do you suffer from epilepsy? If yes, when was the last episode?	☐	☐
8. Are you currently being medicated with anti-depressants, anti-histamines, antipsychotics, antiarrhythmics, or any drugs for nausea, asthma or Parkison's disease (please give details)?	☐	☐
8. Are you a smoker? If yes, please give number of cigarettes per week.	☐	☐
9. Do you have any known allergy to conductive gel? (i.e. the gel that is used in ECG, Doppler, etc…)	☐	☐

10. Please document your current weekly exercise routine;

Type of exercise (cycling, running, weight training etc)	**Number of sessions/week**	**Duration of session**

If you feel at all unwell as a result of a temporary illness (cold or fever) please inform the investigator. Please note that if your health status changes and in any way affects the answers you provided to the questions above, it is paramount that you notify the investigator immediately.

I have read and fully understand this questionnaire. I confirm that to the best of my knowledge the answers provided are correct and accurate. I am aware of no reasons why I should not participate in physical activity and I am fit and fully able to volunteer for this investigation. I understand I will be taking part at my own risk.

Participant's name & signature:_____Date:_____

Investigator's name & signature:_____Date:_____

Appendix VII – Conference abstracts

Evaluation of the independent influences of breathing frequency and tidal volume upon heart rate variability in healthy men

P. Vargas[1], J. González-Alonso[1] and A. McConnell[1]

[1]Centre for Sports Medicine and Human Performance, Brunel University London, Uxbridge, (UK)

Introduction: Respiratory sinus arrhythmia (RSA), a main component of heart rate variability (HRV), maximises at a breathing frequency (F_R) of 4-6 b.min^{-1} (1), and correlates with cardiac vagal activity (2). The independent influences of F_R and concomitant changes in tidal volume (V_T) and PCO2 upon HRV and autonomic nervous system (ANS) function are unknown. We tested the hypothesis that HRV may be maximised by controlling both F_R and V_T. **Methods:** Nine healthy, recreationally active men participated (27.1±2.6 yr). In part 1 the relationship between F_R was assessed and individual F_R optima identified, whilst maintaining a constant V_T of 30% of vital capacity (VC). In part 2 the optimal F_R identified in part 1 was implemented across a range of V_T. Parts 1 and 2 included a semi-spontaneous condition in which only V_T or F_R were controlled, respectively. Conditions were randomised and made in an upright-reclined position. Mild hypercapnia was maintained under all conditions except semi-spontaneous breathing (GA-200 gas analyser, iWorx Systems Inc.). A biofeedback system (LabView, National Instruments Inc.) specified respiratory flow and duty cycle, which were measured by heated pneumotachograph (Hans Rudolph 3813, Hans Rudolph Inc.), whilst heart rate was measured via 3-lead ECG. Primary outcomes were the standard devi ation of normal R-R intervals (SDNN) and the total power (TP) of the power spectrum density. Repeated measures ANOVA with post hoc pairwise comparisons using Bonferroni correction were used to test differences between conditions and Pearson correlations accessed the inter-relationships. **Results:** In 3 of 9 participants SDNN and TP maximised at 4 b.min^{-1}, whilst the remaining 6 maximised at 6 b.min^{-1}. Significant main effects of F_R and V_T were found for SDNN [$F(5, 40) = 8.195$; $p<0.001$; $F(6, 48) = 13.280$; $p<0.001$] and TP [$F(5, 40) = 11.147$; $p<0.001$; $F(6, 48) = 7.233$; $p<0.001$] respecttively. Effect sizes were moderate for all variables and conditions ($\eta p2=0.475$-0.624). **Discussion:** There were significant independent effects of F_R and V_T, upon SDNN and TP, with maxima at 6 b.min^{-1} for 6/9 participants, and at the highest V_T for all participants. Adopting a higher V_T than that used spontaneously at optimal F_R did not significantly increase HRV. Whether V_T s over 40% VC yield significantly greater HRV remains to be explored.

References

1) Song H & Lehrer PM (2003). Applied Psychophysiol Biofeedback, 28 (1),13-23; 2) Kollai M & Mizsei G (1990). J Physiol, 424 (1), 329-342.

This work was supported by the Portuguese Foundation for Science and Technology - FCT

Presented at the Annual Physiological Society Meeting – Physiology 2014, London, UK (July 2014).

Evaluation of the independent influences of breathing frequency and tidal volume upon respiratory sinus arrhythmia, blood pressure and baroreflex sensitivity in healthy men.

Pedro Vargas[1]; José González-Alonso[1]; Alison McConnell[1,2]

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Keywords: Respiratory sinus arrhythmia, Baroreflex Sensitivity

Background: Slow breathing exerts a potent effect upon blood pressure regulation, but the independent influences of breathing frequency (f_R) and tidal volume (V_T) upon the interplay of respiratory sinus arrhythmia (RSA), mean arterial pressure (MAP) and the baroreflex sensitivity (BRS) are poorly understood. We hypothesised that the f_R and V_T would exert independent effects upon RSA, MAP and BRS. **Methods Part 1:** the relationship between f_R and cardiovascular outcomes was characterised, and the f_R (between 4 and 10 b.min^{-1}) that maximised RSA in each of 14 healthy men was identified. **Part 2:** the optimal f_R identified in part 1 was implemented across a range of V_Ts (20 to 40% FVC). PaCO2 was controlled using added dead-spaces. A bespoke biofeedback system specified the respiratory flow rates. **Results:** RSA, MAP and BRS exhibited a bell-shaped response to changes in f_R, with a peak at 6 b.min^{-1} for all variables. A significant main effect for f_R upon RSA ($p<.05$) and BRS ($p<.05$) was detected, but MAP showed no significant relationship to f_R ($p>.05$). RSA increased linearly with increments in V_T, peaking at the highest V_T tested (40%FVC; $p<.05$). A positive main effect for V_T upon MAP ($p<.05$) was found, but *post-hoc* analysis found no differences between V_Ts ($p>.05$). V_T induced changes in BRS were not significant ($p>.05$). **Discussion:** Independent effects of f_R and V_T upon RSA were found in all participants, whilst only changes in f_R affected BRS. It is possible that V_Ts above 40%FVC might elicit even higher RSA and BRS.

This work was supported by the Portuguese Foundation for Science and Technology - FCT

Presented at the International Society for the Advancement of Respiratory Psychophysiology (ISARP) Congress, Seville, Spain (October 2014).

Acute cardiovascular responses to slow and deep breathing with different modalities of airway loading

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Introduction: Daily bouts of slow, deep breathing (SDB) with added inspiratory resistance have been shown to have a larger antihypertensive effect than SDB alone, in people with hypertension (Jones et al., 2010; Sangthong et al., 2016). We compared acute, with breath (inhalation vs. exhalation) cardiovascular responses to different modalities of respiratory loading during SDB. **Methods:** Fifteen healthy males were tested in a semi-recumbent position. Participants completed randomised, 5 min bouts of SDB at 6 breaths·min^{-1} and a tidal volume of 30% of forced vital capacity. One bout consisted of 5 min of unloaded SDB (UL), remaining bouts combined SDB with flow resistive (FR), or pressure threshold (PT) inspiratory (I) or expiratory (E) loads of ~10 cmH2O, interspersed by 5min of normal breathing. **Results:** Loading type (FR vs. PT) did not influence cardiovascular responses to SDB, data were therefore pooled for subsequent comparisons. Compared with UL (306±SD120 msec), the amplitude of within breath oscillations in heart rate (fc) was smaller during E (249±SD140 msec; P<0.001), but larger during I (435±SD145 msec; P<0.001). Compared with UL (11.6±SD6.2 mL), the amplitude of within breath oscillations in stroke volume (SV) were unchanged during E (15.1±SD6.4 mL), but larger during I (24.0±SD7.3 mL; P<0.001). Compared to both UL (92.3±SD5.8 mmHg) and I (90.7±SD8.8 mmHg), E elicited a significant pressor response (100.1±SD7.2 mmHg; P<0.001), but significantly lower cardiac output (6.2±SD0.85 vs. 6.4±SD0.89 vs. 5.8±SD0.94 l.min^{-1}; P<0.004). **Conclusions:** Inspiratory loading generated the largest within breath perturbation of fc (respiratory sinus arrhythmia) and SV, most likely due to an augmentation of venous return via a more negative intrathoracic pressure. This may contribute, at least in part, to the reportedly more potent antihypertensive effect of SDB with I loading. However, potential underlying mechanisms remain to be resolved.

References

1) Jones, C.U., B. Sangthong, and O. Pachirat. (2010) J Physiother, 56(3): 179-86; 2) Sangthong, B., et al. (2016) Med Sci Sports Exerc. 48(9): 1641-7.

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