The influence of pregnancy upon acute cardiovascular responses to slow and deep breathing

Malika Felton

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy Degree (PhD)

Bournemouth University
March 2021

Abstract

The influence of pregnancy upon acute cardiovascular responses to slow and deep breathing

Malika Felton

Slow and deep breathing (SDB) is a promising intervention that has provided reductions in blood pressure (BP) in primary hypertension when practised daily and has potential as an intervention to treat women who develop hypertension during pregnancy. Before SDB can be introduced in a clinical setting during pregnancy, it is important to understand whether normal cardiovascular changes that accompany pregnancy influence the acute responses to SDB. Additionally, known structural and mechanical differences in the respiratory systems of men and women may also influence cardiovascular responses to SDB. As most published studies include only male participants this has not been fully investigated.

In preparation for a clinical study of SDB with women who develop pregnancy-induced hypertension, this thesis characterised the acute cardiovascular responses to a range of different SDB protocols in three distinct population groups (men, women and pregnant women). Novel analysis techniques were applied to delve deeper into the acute cardiovascular responses, by analysing the inter- and intra-breath phase cardiovascular fluctuations induced by breathing. The results highlight the limitation of using averages to understand the cardiovascular changes induced by SDB. Analysis of within-breath (peak-valley) haemodynamics revealed an increase in the amplitude of oscillations during SDB, whilst the average was unchanged. Respiratory sinus arrythmia tripled during SDB compared with rates during normal breathing across all participant groups. The observed increase of the amplitude of BP oscillations provides clues to potential error signal(s) linking daily practise of SDB to chronic BP reductions. This thesis makes an original contribution to existing knowledge by furthering our understanding of the acute cardiovascular responses to SDB and the need to look more closely at peak-valley haemodynamic oscillations. It provides evidence to support the development of an evidence-based SDB intervention to be used with pregnant women, supporting women-centred care and improving the health and experiences of pregnant women.

Contents

List of Figures

Due to the integrated nature of the thesis there is some repetition of Figures in the thesis:

Figure 3-2 Screenshots of Brythm graphic is the same as Figure 4-1, Figure 7-1, and Figure 1 in Chapter 8 (published paper).

Figure 3-6 Calculations for example cardiovascular variable plot is the same as Figure 4-2 and Figure 7-2.

List of Tables

Acknowledgements

My first thank you in this thesis must go to the participants who took part in the research studies, and those who took the time to participate in the patient and public involvement consultations to support development of the research project. And the second has to be a thank you to the maternity group leaders, on social media and running antenatal classes, who provided with me with access to these participants. Participants and gatekeepers are key to all research and without either of these groups I would not have been able to complete my research and therefore my eternal gratitude goes out to these women.

To my fellow postgraduate researchers and office-mates, I could not have completed this journey without you keeping me sane (as much as possible during a PhD), especially Chloe Casey during the past year while working from home during a global pandemic. Knowing that we were all in this together was invaluable and I wish you all the best of luck completing your own research journeys.

To my mum, who has spent hours listening to me talk about my PhD, and learning a lot more about academia, physiology and maternity topics than she ever needed to, thank you for everything I can't list and for your support throughout. To my aunt Alison, thank you for proof-reading my thesis, you are probably the only person outside of my supervisors and examiners who will ever read it in full! My wonderful group of friends, Anna, Cat, Liv, Liz and Nat, you have been there throughout all the years of studying and life, supporting and cheering me on when I needed it, you got me through. And to Robin, I promise I will now stop talking about my PhD 24/7.

And finally, but by no means the least important, a big thank you to my supervisors, both past and present. My supervisory team has changed along the way, but I would not have been able to complete this without each of you. Vanora, thank you for being the last one standing. Your experience from clinical midwifery was invaluable in helping me question my own viewpoint and supporting my transition into clinical health research. Alison, I appreciate your continued involvement in the project more than I can say and thank you for sticking with me. Steph, thank you for your support in opening my knowledge to the clinical side of maternal research and in setting up the clinical study. I hope we can continue to work together to complete the clinical study. Peter, your support during the NHS Research Ethics Committee and designing the clinical intervention was important to my growth throughout the PhD journey. And lastly Vikram, thank you for joining the team last year, it was an unusual time to join but your feedback and support, bringing a new perspective to the project, has been important to improve my thesis and my PhD.

Author's declaration

Initial results from the data presented in Chapter 5 were presented at the Physiology Conference 2019 (Appendix 1.1). The material in Chapter 8.2 has been published in Hypertension in Pregnancy in 2021 and is submitted as part of the integrated thesis format. All material submitted as part of the integrated thesis format are the author's own work, receiving first authorship for all papers. The data analysed in Chapter 4 was collected as part of a linked project and the author was involved in 33% of the data collection. The set-up of the study, all data analysis and write up of the study in Chapter 4 was undertaken by the thesis author.

Definitions

4Ff_r - Fixed breathing frequency of 4 breaths·min^{-1}.

6Ff_r - Fixed breathing frequency of 6 breaths·min^{-1}.

8Ff_r - Fixed breathing frequency of 8 breaths·min^{-1}.

A

Acute response - Immediate, short-term response to a single session of slow and deep breathing.

Antenatal - Period of pregnancy before the birth.

Aorta - The large, elastic artery that carries blood away from the left ventricle and into the systemic circuit.

Aortic (central) pulse pressure (AoPP) - Pressure difference between central systolic and diastolic blood pressure.

Aortic (central) systolic blood pressure (AoSBP) - Pressure in the aorta.

Arterial blood pressure (ABP) - Pressure in the arteries *(see blood pressure).*

Augmentation Index (AIx) - Ratio of augmentation pressure and pulse pressure; indirect measure of arterial stiffness. ***Equation:*** *AIx = (augmentation pressure ÷ aortic pulse pressure) x 100.*

AIx@HR75 - Augmentation index adjusted for heart rate at 75 beats·min^{-1}.

Augmentation Pressure (AP) - The increase in systolic pressure due to the early return of the reflective wave. Represents pressure at the heart from the reflective wave.

Autonomic nervous system - Centres, nuclei, tracts, ganglia and nerves involved in the unconscious regulation of visceral functions; includes components of the central nervous system and the peripheral nervous system.

Autoregulation - Changes in activity that maintain homeostasis in direct response to changes in the local environment, does not require neural or endocrine control.

B

Baroreceptor reflex/ Baroreflex - A reflexive change in cardiac activity in response to changes in blood pressure.

Baroreceptors - The receptors responsible for detecting changes in pressure.

Baroreflex sensitivity (BRS) - Measure of the autonomic effector response to a given change in arterial pressure, often measured as the relationship between heart rate

fluctuations and blood pressure fluctuations. A measure of autonomic control of the cardiovascular system.

Baseline breathing (B) - Baseline normal (spontaneous) breathing.

Blood pressure (BP) - A force exerted against vessel walls by the blood in the vessels, due to the push exerted by cardiac contraction and the elasticity of the vessel walls. ***Equation:*** *BP = Cardiac output x total peripheral resistance.*

Blood pressure fluctuations/ oscillations - Acute changes in blood pressure in response to a change in internal environment such as breathing frequency or external stressor.

Blood pressure variability - Differences in blood pressure taking over a set period of time.

Brachial artery - Main artery in the arm.

Breathing condition - Protocol for slow and deep breathing exercises.

Breathing frequency - Number of breaths take per minute.

Breath - Full cycle of breathing phases (inspiration and expiration).

Breath phase - Inspiration or expiration phase of breathing.

Breath cycle - Full breathing set of inspiration and expiration phases of breathing.

C

Cardiac output ($\dot{Q}$) - The volume of blood ejected by the left ventricle each minute.

Equation: *Cardiac output = heart rate x stroke volume.*

$\dot{Q}$e	Cardiac output during expiration.
$\dot{Q}$i	Cardiac output during inspiration.
$\dot{Q}\Delta$	Inter-breath phase cardiac output variation ($\dot{Q}$i- $\dot{Q}$e).
$\dot{Q}\Delta$e	Peak-valley cardiac output during expiration ($\dot{Q}$e max – $\dot{Q}$e min).
$\dot{Q}\Delta$i	Peak-valley cardiac output during inspiration ($\dot{Q}$i max – $\dot{Q}$i min).
$\dot{Q}\Delta$PV	Peak-valley cardiac output ($\dot{Q}$i max – $\dot{Q}$e min or $\dot{Q}$i min – $\dot{Q}$e max).
$\dot{Q}\Delta$PVInd	Peak-valley breath independent pulse wave velocity ($\dot{Q}$ max – $\dot{Q}$ min).

Cardiovascular - Pertaining to the heart, blood and blood vessels.

Carotid artery - The principal artery of the neck; one branch the internal carotid provides a major blood supply to the brain.

Carotid body - A group of receptors, adjacent to the carotid sinus, that are sensitive to changes to the carbon dioxide levels, pH, and oxygen concentrations of arterial blood.

Carotid sinus - A dilated segment at the base of the internal carotid artery whose walls contain baroreceptors sensitive to changes in blood pressure.

Central nervous system - The brain and spinal cord.

Chronic adaptations - Habitual or long-term physiological adaptations following repeated sessions of slow and deep breathing.

Chronic heart failure (CHF) - The failure to maintain adequate cardiac output due to cardiovascular problems or myocardial damage.

Chronic hypertension - Hypertension that is present at a women's first antenatal visit during pregnancy, or before 20 weeks, or the women is already taking antihypertensive medication when referred to maternity services. It can be primary or secondary in aetiology.

D

Detraining - Changes in physiological function in response to a reduction or cessation of regular physical training.

Device-guided breathing (DGB) - Slow and deep breathing delivered using an external device.

Diaphragm - The respiratory muscle that separates the thoracic cavity from the abdominopelvic cavity.

Diastolic blood pressure (DBP) - Pressure measured in the walls of an artery when the left ventricle is in diastole. The lowest pressure when the heart is at rest.

- **DBPe** Diastolic blood pressure during expiration.
- **DBPi** Diastolic blood pressure during inspiration.
- **DBPΔ** Inter-breath phase diastolic blood pressure variation (DBPi - DBPe).
- **DBPΔe** Peak-valley DBP during expiration (DBPe max – DBPe min).
- **DBPΔi** Peak-valley DBP during inspiration (DBPi max – DBPi min).
- **DBPΔPV** Peak-valley DBP (DBPi max – DBPe min or DBPi min – DBPe max).
- **DBPΔPVInd** Peak-valley breath independent DBP (DBP max – DBP min).

Duty cycle (T_I/T_{TOT}) - Ratio of inspiration duration to total breath cycle duration.

Dynamic breathing condition (Df_r) - Dynamic breathing frequency using optimisation algorithm.

Dyspnoea - Laboured or difficult breathing.

E

Electro-cardiogram (ECG) - A graphic record of the electrical activities of the heart, as monitored at specific locations on the body surface.

Epoch – A specific period of time that is a subdivision of a period.

Error signal - A signal that represents the difference between the set point value and the actual value of the regulated variable. For example, the resting value of blood

pressure compared with the dynamic value of blood pressure caused by blood pressure fluctuations.

Expiration (e) - Exhalation, breathing out.

F

***f_c* -** See heart rate.

Fetus - An unborn baby from 8 weeks after fertilisation until the time of birth.

F*f_r* - Fixed breathing frequency; in the present thesis study relating to either 4 (4F*f_r*), 6 (6F*f_r*) or 8 (8F*f_r*) breaths·min^{-1}.

Frank-Starling mechanism - The mechanism by which an increased amount of blood in the ventricle causes a stronger ventricular contraction to increase the amount of blood ejected.

G

Gestation - The period of the fetus developing inside the womb between conception and birth.

Gravid - State of carrying young or eggs and being pregnant.

H

Haemodynamics - Relating to the flow of blood within the organs and tissues of the body.

Heart rate (*f_c*) - The frequency the heart pumps per minute.

- ***f_c*e** Heart rate during expiration.
- ***f_c*i** Heart rate during inspiration.
- ***f_c*Δ** Inter-breath phase heart rate variation (*f_c*i - *f_c*e).
- ***f_c*Δe** Peak-valley heart rate during expiration (*f_c*e max – *f_c*e min).
- ***f_c*Δi** Peak-valley heart rate during inspiration (*f_c*i max – *f_c*i min).
- ***f_c*PV** Peak-valley heart rate (*f_c*i max – *f_c*e min or *f_c*i min – *f_c*e max).
- ***f_c*ΔPVInd** Peak-valley breath independent heart rate (*f_c* max – *f_c* min).

Heart rate variability (HRV) - Variation in the time interval between consecutive heartbeats.

Homeostasis - The maintenance of a relatively constant internal environment.

Hypertension - Abnormally high blood pressure. Normally defined as a systolic pressure of 140 mmHg or higher and/or a diastolic pressure of 90 mmHg or higher.

Hypercapnia - An abnormally high plasma PCO_2 commonly as a result of hypoventilation.

Hypertension - A condition in which the blood vessels have persistently raised pressure. Also known as high blood pressure.

Hyperventilation - A rate of respiration sufficient to reduce plasma PCO_2 to levels below normal.

Hypocapnia - An abnormally low plasma PCO_2 commonly as a result of hyperventilation.

Hypoventilation - A rate of respiration insufficient to keep plasma PCO_2 within normal levels.

I

Inspiration (i) - Inhalation, breathing in.

Inspiratory resistance (IR) - Breathing condition using an added inspiratory resistance to breathing at a frequency of 6 breaths·min^{-1}.

M

Mean arterial pressure (MAP) - The average pressure exerted by the blood in the arteries. **Estimated using equation**: MAP = diastolic blood pressure + (0.333 x pulse pressure)

MAPe Mean arterial pressure during expiration.

MAPi Mean arterial pressure during inspiration.

MAPΔ Inter-breath phase mean arterial pressure variation (MAPi - MAPe).

MAPΔe Peak-valley MAP during expiration (MAPe max – MAPe min).

MAPΔi Peak-valley MAP during inspiration (MAPi max – MAPi min).

MAPΔPV Peak-valley MAP (MAPi max – MAPe min or MAPi min – MAPe max).

MAPΔPVInd Peak-valley breath independent MAP (MAP max – MAP min).

Multipara (Multip) - A woman who has given birth at least once before >24 weeks gestation.

Multiple pregnancy – A pregnancy with more than one fetus.

N

Nadir - The lowest point.

National Health Service (NHS) - Publicly-funded healthcare system of the United Kingdom.

Normotensive - Normal levels of blood pressure.

Nulliparous - A woman who has never given birth to a live baby.

P

Parasympathetic - One of the two divisions of the autonomic nervous system, generally responsible for activities that conserve energy and lower the metabolic rate.

Peak-valley (PV) - Difference between maximum and minimum values.

Perturbation - A disturbance or change in a structure or function, as a result of an external influence.

Postnatal/ postpartum - The period after birth.

Pregnancy-induced hypertension (PIH) - New high blood pressure (hypertension) presenting after 20 weeks of pregnancy without significant proteinuria.

Pre-eclampsia - New onset of high blood pressure (hypertension) after 20 weeks of pregnancy with significant proteinuria.

Preterm/ premature - A baby born before 37 weeks of pregnancy.

Primary hypertension - High blood pressure (hypertension) that doesn't have a secondary cause.

Primigravida - A woman who is pregnant for the first time.

Primipara - A woman who is giving birth for the first time.

Proteinuria - Increased levels of protein in the urine.

Pulse pressure (PP) - The difference between systolic and diastolic blood pressures.

Equation: PP = systolic blood pressure minus diastolic blood pressure

- **PPe** Pulse pressure during expiration.
- **PPi** Pulse pressure during inspiration.
- **PPΔ** Inter-breath phase pulse pressure variation (PPi - PPe).
- **PPΔe** Peak-valley pulse pressure during expiration (PPe max – PPe min).
- **PPΔi** Peak-valley pulse pressure during inspiration (PPi max – PPi min).
- **PPΔPV** Peak-valley pulse pressure (PPi max – PPe min or PPi min – PPe max).
- **PPΔPVInd** Peak-valley breath independent pulse pressure (PP max – PP min).

Pulse transit time (PTT) - Time taken for the pulse wave to travel between two sites.

- **PTTe** Pulse transit time during expiration.
- **PTTi** Pulse transit time during inspiration.
- **PTTΔ** Inter-breath phase pulse transit time variation (PTTi - PTTe).
- **PTTΔe** Peak-valley pulse transit time during expiration (PTTe max – PTTe min).
- **PTTΔi** Peak-valley pulse transit time during inspiration (PTTi max – PTTi min).
- **PTTΔPV** Peak-valley PTT (PTTi max – PTTe min or PTTi min – PTTe max).
- **PTTΔPVInd** Peak-valley breath independent PTT (PTT max – PTT min).

Pulse wave analysis (PWA) - Innovative method to measure central blood pressure measures using the analysis of pressure wave reflection characteristics.

Pulse wave velocity (PWV) - The rate at which pressure waves move down a vessel.

PWVe Pulse wave velocity during expiration.

PWVi Pulse wave velocity during inspiration.

PWVΔ Inter-breath phase pulse wave velocity variation (PWVi - PWVe).

PWVΔe Peak-valley PWV during expiration (PWVe max – PWVe min).

PWVΔi Peak-valley PWV during inspiration (PWVi max – PWVi min).

PWVΔPV Peak-valley PWV (PWVi max – PWVe min or PWVi min – PWVe max).

PWVΔPVInd Peak-valley breath independent PWV (PWV max – PWV min).

Q

Q̇ - See cardiac output.

R

Randomised control trial (RCT) - A study in which a number of similar people are randomly assigned to two or more groups to test a specific drug, treatment or other intervention. One group (the experimental group) has the intervention being tested, the other (the control group) has an alternative treatment, a dummy intervention (placebo) or no intervention at all.

Renal resistive index (RRI) - Ratio of peak systolic and end diastolic velocity. Normal = 0.6 and the upper healthy limit is <0.7. Equation: RRI = (Peak systolic velocity minus end diastolic velocity) ÷ peak systolic velocity.

Respiration - The exchange of gases between cells and the environment; includes pulmonary ventilation, external respiration, internal respiration, and cellular respiration.

Respiratory condition - Disease or condition related to the respiratory system such as asthma.

Respiratory pump - A mechanism by which changes in the intrapleural pressures during the respiratory cycle assist the venous return to the heart.

Respiratory sinus arrhythmia (RSA) - Within breath fluctuations in heart rate. Difference between the maximum RR interval during expiration minus minimum RR interval during inspiration.

Rest period (R) - Period of normal breathing between two breathing conditions to allow cardiovascular variables to return to normal levels.

RR interval (RR) - Beat-to-beat interval from an ECG.

S

Singleton pregnancy - A pregnancy with one fetus.

Slow and deep breathing (SDB) - Breathing at a frequency lower than 10 $breaths \cdot min^{-1}$.

Spontaneous breathing condition (Sf_r) - Spontaneous breathing frequency (uncontrolled normal breathing).

Stroke volume (SV) - The amount of blood ejected from the left ventricle during each contraction.

SVe Stroke volume during expiration.

SVi Stroke volume during inspiration.

SVΔ Inter-breath phase stroke volume variation (SVi - SVe).

SVΔe Peak-valley stroke volume during expiration (SVe max – SVe min).

SVΔi Peak-valley stroke volume during inspiration (SVi max – SVi min).

SVΔPV Peak-valley stroke volume (SVi max – SVe min or SVi min – SVe max).

SVΔPVInd Peak-valley breath independent stroke volume (SV max – SV min).

Sympathetic - One of the two divisions of the autonomic nervous system, primarily concerned with the elevation of metabolic rate and increased alertness.

Systolic blood pressure (SBP) - Pressure measured in the walls of an artery when the left ventricle is in systole. The peak pressure when the heart beats.

SBPe Systolic blood pressure during expiration.

SBPi Systolic blood pressure during inspiration.

SBPΔ Inter-breath phase systolic blood pressure variation (SBPi - SBPe).

SBPΔe Peak-valley SBP during expiration (SBPe max – SBPe min).

SBPΔi Peak-valley SBP during inspiration (SBPi max – SBPi min).

SBPΔPV Peak-valley SBP (SBPi max – SBPe min or SBPi min – SBPe max).

SBPΔPVInd Peak-valley breath independent SBP (SBP max – SBP min).

T

T_I/T_{TOT} - See duty cycle.

Tidal volume (V_T) - The volume of air inspired or expired during a normal breathing cycle.

Term (full term) - Considered to be 40 weeks of pregnancy from the first day of the woman's last menstrual period. Normal duration of pregnancy is 37-42 weeks gestation.

Total peripheral resistance (TPR) - The resistance to blood flow, primarily caused by friction with the vascular walls. Equation: TPR = mean arterial pressure ÷ cardiac output.

TPRe Total peripheral resistance during expiration.

TPRi Total peripheral resistance during inspiration.

TPRΔ Inter-breath phase total peripheral resistance variation (TPRi- TPRe).

TPRΔe Peak-valley TPR during expiration (TPRe max – TPRe min).

TPRΔi Peak-valley TPR during inspiration (TPRi max – TPRi min).

TPRΔP Peak-valley TPR (TPRi max – TPRe min or TPRi min – TPRe max).

TPRΔPVInd Peak-valley breath independent TPR (TPR max – TPR min).

Trimester - A time span of 3 months during pregnancy, each marked by different phases of fetal development. First trimester (first 12 weeks), second trimester (13-27 weeks) and third trimester (28 weeks until birth).

V

V_T – See tidal volume.

Chapter 1. Introduction

1.1 Hypertensive disorders of pregnancy

Hypertension is the most common medical disorder during pregnancy (Moser et al. 2012), and hypertensive disorders of pregnancy are the second highest direct cause of maternal deaths worldwide, accounting for 14% of direct deaths (Say et al. 2014). Although frequently referred to as "hypertension in pregnancy" (NICE: National Institute for Health and Care Excellence 2019b; Royal College of Obstetricians and Gynaecologists 2019), caution must be taken when interpreting research and statistics due to the different conditions that are often grouped together, and not always analysed independently, despite the differences between conditions in both aetiology and outcomes. The Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (2000) groups women with high blood pressure into 4 classification groups; 1) chronic hypertension, 2) pre-eclampsia/ eclampsia, 3) Pre-eclampsia super imposed on chronic hypertension and 4) gestational hypertension (transient hypertension of pregnancy if no pre-eclampsia present and blood pressure (BP) returns to normal by 12 weeks post-partum, or chronic hypertension if BP does not return to normal post-partum).

Chronic hypertension is hypertension that was observed prior to pregnancy or before the 20th week of gestation. Pregnancy-induced hypertension (PIH), or gestational hypertension, is a specific hypertensive condition that presents with high BP that was not present before pregnancy and BP that returns to normal following giving birth. The National Institute for Health and Care Excellent (NICE) define PIH as new high blood pressure (≥140 / ≥90 mmHg) presenting after 20 weeks of pregnancy, which was not present before conception, without significant proteinuria (NICE: National Institute for Health and Care Excellence 2019b).

Pre-eclampsia, is a more serious hypertensive disorder of pregnancy, which is characterised by PIH onset with proteinuria (NICE: National Institute for Health and Care Excellence 2019b) and 25% of women with PIH progress to develop pre-eclampsia (Tranquilli et al. 2014). Due to the separate aetiology and differences in physiological changes/outcomes between the conditions, the present thesis will focus on the study of women with PIH, allowing a specific focus of the hypertension element of the condition. It is possible that the findings within this thesis could be applicable to women who have pre-eclampsia, however as pre-eclampsia involves multisystem dysfunction (Karthiga et al. 2019) there are likely to be other factors that interventions targeting a reduction in BP

may not be able to treat. Additionally, control of BP alone does not treat pre-eclampsia (Abbas et al. 2005). Further research would be needed to apply any findings to pre-eclampsia, hence the focus on women who have PIH. Where possible, references are used that studied PIH specifically, and did not group hypertensive disorders of pregnancy together. If these references are not available then it is made clear that the reference includes other types of hypertensive disorders of pregnancy and is not specific to PIH.

In the United Kingdom (UK), PIH affects around 8-10% of all pregnant women and can cause maternal morbidity, stillbirths and neonatal deaths, and perinatal morbidity (NICE: National Institute for Health and Care Excellence 2019b). Additionally, women who experience PIH during pregnancy are at an increased risk of developing cardiovascular disease later in life including stroke, coronary artery disease, cardiac arrhythmias, chronic kidney disease and multimorbidity (Garovic et al. 2020). There may also be an increased risk of developing mental health disorders such as anxiety, postpartum depression and post-traumatic stress disorder (Roberts et al. 2019).

It seems possible that PIH may become an increasing problem, as trends in 'normal' BP during pregnancy have shown a significant rise in diastolic BP (DBP) of 0.26 mmHg every year between 1969 and 2017 and a non-significant increase of 0.12 mmHg every year for systolic BP (SBP) (Loerup et al. 2019). Therefore, although rates of PIH are currently falling, rising BP is a problem for the general pregnant population, which may lead to higher rates of PIH in the future. Predictive modules using risk factors of PIH to calculate rates of hypertension, such as increasing maternal age and obesity, reveal a predicted increase in rates of PIH (Roberts et al. 2015). Additionally, with the reclassification of hypertension by the American Heart Association at levels of ≥130mmHg SBP and ≥80mmHg DBP (Whelton et al. 2018), levels of BP that were once considered normotensive may have greater negative consequences than previously known, which may be applicable during pregnancy, although this has yet to be reviewed.

There is evidence of an increasing trend of early delivery in women with PIH. In data collected between 2001 and 2012, Roberts et al. (2015) observed increasing early delivery before 38 weeks in women who had experienced PIH, compared with trends in normotensive women who showed an increasing percentage of births after 39 weeks. Although the study was undertaken in Australia, the authors suggest findings are generalisable to other high-income countries (Roberts et al. 2015). Preterm birth complications are the leading cause of death among children under 5 years of age (Liu et al. 2016) and the WHO's antenatal care guidelines include strategies to help prevent

preterm birth (World Health Organization 2016). Currently the only cure for PIH is to give birth, with recommended treatments normally involving pharmaceuticals.

The current NICE recommended pharmaceutical treatments for hypertension during pregnancy (chronic, gestational and pre-eclampsia) recommend using labetalol as a first choice (NICE: National Institute for Health and Care Excellence 2019b; Royal College of Obstetricians and Gynaecologists 2019). Alternatively, if labetalol is not suitable then nifedipine or subsequently methyldopa should be considered. Pharmaceutical treatments for PIH should be chosen based on pre-existing treatments, side-effect profiles, risks (including fetal effects) and the women's preference (NICE, 2019). However, there is a paucity of data available for most of the new antihypertensive drugs over the last 20 years due to pharmaceutical companies being reluctant to test medication with pregnant women (Cifkova 2011). As a result of this, despite the general (non-pregnant) use of medications such as methyldopa declining since its introduction more than 50 years ago, it is still one of the preferred choices during pregnancy (Okur et al. 2017). Consequently, treatments that can be trialled specifically in pregnant women are important in widening options for treatment of hypertension during pregnancy.

An alternative treatment for PIH is to give birth early (pre-term), however there are associated risks. The 2017 Cochrane Review (Cluver et al. 2017) compared planned early delivery versus expectant management for hypertensive disorders from 34 weeks gestation to term. The review concluded that early delivery reduced the risk of maternal complications but there was not enough information to draw conclusions on the effects on infant mortality or morbidity. However, planned early delivery was associated with higher levels of admission to neonatal intensive care unit. Although, the authors also noted that evidence was limited and more research is needed to specifically examine the any potential differences between types of hypertensive conditions. For PIH specifically, the optimal time for delivery is argued to be between 38-39 weeks based on the balance of lowest maternal and neonatal morbidity and mortality (Cruz et al. 2012). Neonatal complications from pre-term births can also have long-term consequences, with evidence suggesting significant adverse performance across a range of cognitive and educational measures compared with children born at term (Chan et al. 2016).

In terms of healthcare costs, pre-term births are associated with higher mean cost per infant over the child's first three years of life and these costs decreased with increasing gestational age at birth (Clements et al. 2007). Children born between 32 to 36 weeks gestation had more than double the associated costs compared with children born at term, and children born between 24 to 31 weeks gestation had associated costs over 7

times higher (Clements et al. 2007). Therefore, interventions are needed that can prolong the gestation period by controlling BP, consequently not requiring planned pre-term delivery, and benefiting the development of the baby and its ongoing health during early childhood.

Furthermore, a diagnosis of PIH changes the experience of pregnancy for women and following a diagnosis of hypertension pregnant women feel a lack of control (Roberts et al. 2017) and may experience psychological trauma caused by a lack of information and control (Cowan et al. 2017). This feeling of lacking control could be reduced by providing a treatment method, such as non-pharmacological interventions including slow and deep breathing (SDB), which gives the women back an element of control over their condition. Additionally, improving women's involvement in the management of their disease, fits into the 'women-centred' model that underpins midwifery practice (Royal College of Midwives 2014) by actively involving the women in their care (Lavallee et al. 2018).

Research utilising interventions that include SDB (e.g. yoga, meditation) have shown encouraging reductions in BP that warrant further examination using a more robust intervention than those applied to date (Curtis et al. 2012; Cullins et al. 2013; Rampalliwar et al. 2013; Aalami et al. 2016). Specifically, yoga, which emphasises slow breath control, has been shown to improve pregnancy and fetal outcomes, including the incidence of PIH (Rakhshani et al. 2012). A full review of studies utilising interventions that include SDB during pregnancy will be provided in section 2.2.

Overall, women who have PIH are a promising group in which to examine the potential benefits of SDB. The aetiology of PIH has been linked to dysfunctional breathing (Jerath et al. 2009), in particular, high breathing frequencies (Fischer and Voss 2014). Pregnant women are also normally otherwise healthy, and therefore less likely to be taking certain medications that may impact the effectiveness of SDB. Finally, most pregnant women have an aversion to medication (Twigg et al. 2016) and therefore they have high levels of engagement and are highly motivated to comply with non-pharmacological interventions (Adams et al. 2009). SDB may be an important component of behavioural interventions aimed at reducing BP (Sica 2011) and therefore given pregnant women's potential acceptability of such an intervention there is a need to conduct this potentially beneficial research. However, Band and colleagues note that few trials using digital interventions aimed at pregnant women have conducted in-depth acceptability testing (Band et al. 2019), which is important to evaluate the feasibility of such interventions, and is the reason this thesis is important. Using SDB as an independent intervention has recently gained popularity, but the origins of SDB are found within more integrative

exercises, and these are outlined below to provide perspective on the intervention in question.

1.2 Background to the therapeutic use of slow and deep breathing

Yoga, Qigong and meditation have been practiced for thousands of years, with the original purpose of spiritual enlightenment and correcting supposed imbalances of mind, body and spirit. The common element between these exercises is the regulated breathing and it is theorised that the associated benefits are due to this element of controlled breathing (Gerritsen and Band 2018). Since the 1960s, the benefits have first been researched in yoga (Miles 1964), meditation (Benson et al. 1974) and qigong (Koh 1982). A search of Web of Science showed the number of published clinical trials on meditation, mindfulness, yoga, tai chi or qi gong increased from approximately 20 in 2000 to 250 in 2014, with citations also increasing from 20 in 2000 to 7,112 in 2014 (Gerritsen and Band 2018). Integrated treatments, which include both the general practise of the above exercises and integrated breathing techniques, have subsequently formed part of interventions aiming to improve health. Research studies date back to the 1970s with the first recorded published articles on PubMed using integrated treatments to reduce anxiety (Dillbeck 1977), weight (Madhavi et al. 1985), blood pressure (Silverberg 1990), and low back pain (Cramer et al. 2013).

When comparing different types of exercise, yoga has proved to be the most beneficial for a range of health outcomes (Ross and Thomas 2010), leading to the suggestion that the breathing exercises within yoga provide an additional benefit that is separate from the benefits of exercise alone. The health benefits of breathing techniques, which include changing breathing patterns and breath control, became popular in the 1970s and 80s. Breathing techniques such as Lamaze breathing (Hughey et al. 1978), and Leboyer's 'Art of breathing' (Leboyer 1985) became particularly popular during childbirth. Using breathing exercises as a treatment for respiratory conditions such as asthma are also common place and have been subject to the scrutiny of a recent Cochrane systematic review (Santino et al. 2020), which concluded breathing exercises may increase quality of life and lung function, and decrease hyperventilation symptoms. Breathing exercises used by physiotherapists include breathing retraining, pranayama, Papworth method (breathing with the nose and diaphragm), deep diaphragmatic breathing, thoracic expansion exercises, pursed lip breathing exercises and glossopharyngeal exercises, which often include an element of reducing breathing frequency (Santino et al. 2020).

Pranayama breathing techniques, originating within yoga practice, are nowadays practiced independently from yoga. There are multiple types of pranayama techniques, but most involve reducing breathing frequency and this has developed into a technique known in the research context as “slow and deep breathing” (SDB). In the past 30 years SDB has gained prominence as a standalone intervention, with new delivery methods eliminating the need for technique training and/or a yoga/meditation teacher to be present. Standalone implementation of SDB has produced beneficial health outcomes for a variety of conditions, including hypertension (Grossman et al. 2001), stress and anxiety (Clark and Hirschman 1990), depression (Chung et al. 2010), post-traumatic stress disorder (PTSD; (Descilo et al. 2010), pain threshold and tolerance (Chalaye et al. 2009), chronic pain (Busch et al. 2012) and conflict monitoring (Cheng et al. 2017). However, conditions like menopausal hot flushes (Huang et al. 2015) and overactive bladder syndrome (Huang et al. 2019) did not show improvement after SDB, compared with control groups.

Typical SDB interventions involve reducing breathing frequency to less than 10 breaths·min^{-1} for at least 5 minutes, on at least 3 days per week and a recent meta-analysis found device guided breathing can reduce SBP by 5.3 mmHg and DBP by 2.7 mmHg (Chaddha et al. 2019). Device guided breathing involves using an external pacing device to guide breathing in a more robust way (Rosenthal et al. 2000; Parati and Cerretta 2007), ensuring consistency and accuracy between sessions. When using a device to guide breathing, breath synchronisation, time spent in SDB and average reduction in breathing frequency are not correlated with number of sessions completed (Gavish 2010), showing that experience is not a requirement to achieve successful SDB when using a device to guide breathing. On the other hand, undertaking non-device guided SDB, such as yoga exercises or meditative breathing, requires training and practice to learn how to independently control breathing (Patel 1975).

The most frequently used device guided breathing system is the RESPeRATE product, which was cleared by the Food and Drug Administration (FDA) in the U.S.A. in 2002 and added to the National Health Service (NHS) Drug Tariff List in 2012 in the UK. RESPeRATE provides pacing via headphones, which play a fluctuating musical tone to lower the user’s breathing rate to what the makers claim to be the ‘therapeutic breathing zone’ (≤10 breaths·min^{-1}). Users breathe in time with the musical tones and breathing frequency is monitored using a belt worn around either the chest or upper abdomen. It is recommended to attain 40 minutes or more of SDB per week (40 minutes in therapeutic breathing zone). A full description of how RESPeRATE works can be found in Gavish (2010) and Cernes & Zimlichman (2017). Evaluation of the related clinical trials are

discussed in the Literature Review chapter of this thesis, only an overview is provided in this chapter.

RESPeRATE is recommended by the American Heart Association as an effective treatment for hypertension (Brook et al. 2013). However, there is much debate around this recommendation due to the evidence on which the recommendation was based, specifically there are concerns regarding the influence of manufacturer-sponsored studies and methodological weaknesses (Landman et al. 2014; van Hateren et al. 2015; Zimlichman 2017). Additionally, despite RESPeRATE being included in the UK NHS Drug Tariff List, many localised NHS Clinical Commissioning Groups (CCG) do not support its prescription, citing limited evidence of the long-term effects and the lack of recommendation by NICE, or other national hypertension guidelines. In fact the British Hypertension Society (2012) released a statement that current research has only shown small effects over short durations, which they believe is insufficient evidence to recommend the device for routine use. This scepticism was also reflected in the advice in other countries such as Australia and New Zealand (National Horizon Scanning Unit 2004).

Despite the widespread promotion and the focus of research articles on RESPeRATE, there are a number of disadvantages to the product, and limitations of the evidential support for its marketing claims. Firstly, there are a high number of industry sponsored RESPeRATE studies, which when removed from meta-analyses reduce the magnitude of its effectiveness (Mahtani et al. 2012). Additionally, as RESPeRATE costs ~£250 at the time of writing, it is not an easily accessible or affordable treatment method, especially in the UK where the NHS CCG will currently not cover the cost. It is also a fairly bulky (12.4 x 11.7 x 6.6cm), albeit lightweight (360g), device to carry around for everyday use. Increasing the ease with which users can undertake SDB, such as being able to practice anywhere, would support greater adherence and therefore increase the potential associated benefits. Harnessing the capabilities of portable devices that are more easily integrated into modern busy life is critical to moving the SDB research forward.

Thus, although there are data supporting the antihypertensive effects of SDB, the current evidence is inconsistent; indeed, a recent mainstream media article in The Observer newspaper attested that the 'jury is still out' until better quality, well-controlled human studies are conducted (Fleming 2020). Although SDB has the potential to reduce BP, the current evidence is preliminary, and more consistent support is needed before recommending its use as a behavioural therapy to reduce BP (Sica 2011). Importantly,

the mechanisms by which SDB reduces BP are not fully understood (Gerritsen and Band 2018) and further research is needed to investigate both the short-term (acute) responses to, and long-term effects of, SDB. In order to improve SDB interventions and provide the sufficient evidence required by national governance organisations, a mechanistic understanding of SDB is needed. Once this is achieved, interventions can be designed around the physiological systems that SDB targets, thereby enhancing the potential benefits and/or reducing the 'investment' from users. By using SDB interventions that are tailored to the population in question, this could lead to more consistent outcomes by targeting the physiological pathways that lead to BP reductions.

Finally, feasibility trials for integrating SDB interventions into the healthcare system are lacking for SDB interventions, despite the recommendation that the evaluation and establishment of nonpharmacological treatments for hypertension is a public health priority (Adler et al. 2019). This is especially important with different population groups such as pre-hypertensive individuals and pregnant women with high BP. As outlined in section 1.1, SDB has the potential to be an important nonpharmacological treatment method for PIH, but this has not been trialled. It is vital that researchers investigate how best to deliver SDB, as a therapy, so that users find it both accepted and engaging.

Existing studies of SDB have focussed on populations with primary hypertension, but using SDB as a non-pharmacological intervention to reduce BP in pregnant women could be a significant step forward in saving mothers' lives and/or reducing risk in women who develop PIH. The benefits of drug treatment for mild to moderate high BP (hypertension) during pregnancy (≤160/≤110 mmHg) are uncertain (European Society of Hypertension and European Society of Cardiology 2013) and the American Heart Association suggest alternative approaches are becoming increasingly important in the management of all forms of hypertension (Brook et al. 2013). Therefore, there is a strong argument to support exploring alternative non-pharmacological interventions to reduce BP in pregnant women with hypertension, such as SDB.

In summary, SDB has shown promising reductions in BP following daily use in primary hypertension. Using a device to guide breathing requires less training and monitoring than traditional delivery of breathing exercises (yoga and meditation). The most popular method to deliver SDB (RESPeRATE) has limitations both in the device design itself and the existing evidence to support its use. A SDB intervention has the potential to offer a non-pharmacological treatment method for PIH, but must be tested in this population group, with a full understanding of the changes which SDB produces in pregnant women.

1.3 Outline of the thesis

This thesis combines the need for non-pharmacological treatments for PIH with a novel SDB intervention. The first study compared the acute (short-term) cardiovascular responses to an existing device (RESPeRATE) with those to a new device designed at Bournemouth University. The responses to both devices were compared in men and women, to allow a comparison of possible sex-related differences in the acute cardiovascular responses to SDB (Chapter 4). To understand the mechanisms by which SDB may reduce BP in women who have PIH, normative data in normotensive non-pregnant (Chapter 5) and normotensive pregnant women (Chapter 6) was needed for comparison. Due to the cardiovascular changes caused by pregnancy it is important to understand any potential differences in the acute responses to SDB of healthy pregnant and healthy non-pregnant women, which may influence any long-term adaptations following a SDB intervention (Chapter 7). This study design also allowed the optimal SDB frequency, specific to pregnant women, to be used in a planned, future long-term intervention to explore the feasibility of using SDB with pregnant women who develop PIH (Chapter 8). The planned protocol has been published in *Hypertension in Pregnancy* in 2021 (Felton et al. 2021).

1.4 Impact of the coronavirus pandemic on the research

In 2020, the coronavirus pandemic caused worldwide disruption to both life and research. At this time, the final study of this thesis (investigating the feasibility of using SDB as a treatment method with women who develop PIH), was recruiting from the local NHS maternity unit, but no women had been enrolled into the study. Pregnant women are classed as high risk for COVID-19 infection, especially when combined with existing medical conditions such as hypertension. All NHS research studies not linked to coronavirus were paused, and specifically in our local maternity unit, only 2/11 studies were still open for recruitment by the end of March 2020. Therefore, the decision was made to stop the study and to include only the planned protocol as part of this thesis.

To supplement the data already collected from the laboratory-based studies (investigating acute cardiovascular responses to SDB, presented in Chapters 5 and 6), an additional study was included in the thesis. The data presented in Chapter 4 was collected as part of a linked research study, in which the author (MF) was involved. For narrative reasons, these data have been presented first in the thesis, although the timeframe of data collection was simultaneous with the Chapter 5 data set. The data in Chapter 4 provides a comparison of the cardiovascular responses to different methods

of SDB delivery; an existing device, a new biofeedback device, and a fixed SDB frequency.

The result is a thesis that explores the acute cardiovascular responses to SDB and reports the development and validation of a novel intervention, which is based on scientific data, as well as women's user feedback. To investigate the complex topic of SDB, an interdisciplinary approach is taken, combining physiology and maternal health perspectives. As such, the thesis will be examined by a multi-disciplinary team of examiners and therefore certain terminology may be used that is unfamiliar to one or other examiner. A glossary, including abbreviations, is included on page 17 and where appropriate footnotes are included throughout to explain certain terms in more detail.

Chapter 2. Literature Review

This chapter will provide an overview of the relevant literature related to slow and deep breathing (SDB) and its potential as a treatment method for hypertension that develops during pregnancy. The chapter will start by outlining the respiratory and cardiovascular changes associated with normal pregnancies, comparing these normal adaptations with the different changes observed in hypertensive pregnancies (Section 2.1). Section 2.2 will provide an overview of interventions, which include or have similarities with SDB, and have already been used as interventions during pregnancy. Next, an overview of the evidence of using SDB interventions to reduce blood pressure (BP) in primary hypertension will be presented (Section 2.3), followed by an exploration of the short-term (acute) responses to SDB, which may provide mechanistic explanations for any long-term (chronic) reduction in BP (Section 2.4). The chapter will finish with a summary of the literature and the overall aims for this thesis (Section 2.5).

2.1 Respiratory and cardiovascular changes during pregnancy

A woman's body experiences dramatic changes during pregnancy, with maternal adaptations supporting the development and growth of the fetus (Weissgerber and Wolfe 2006). During pregnancy, the body undergoes intense haemodynamic modifications, such as an increase in blood volume of 30-40% (Heidemann and McClure 2003), but at the same time, hemodynamic stability needs to be maintained to preserve the health of the mother and growing fetus (da Silva Correa et al. 2019).

The most profound physiological changes are those that occur in the cardiovascular system (Carlin and Alfirevic 2008). However, the mechanisms which control adaptations to autonomic cardiovascular modulation during pregnancy are not fully understood (da Silva Correa et al. 2019), especially in relation to hypertensive disorders of pregnancy. Although the aetiology of pregnancy-induced hypertension (PIH) remains unknown, there are known physiological differences in women with PIH compared with normotensive pregnancies (Dudenhausen and Travis 2014). These differences will be outlined below in relation to the physiological changes experienced during a healthy pregnancy.

2.1.1 Respiratory

During pregnancy, the growing fetus expands the uterus upwards changing the shape of the chest (Carlin and Alfirevic 2008). The organ where this process is most felt by women

is the lungs, as their natural resting position is changed throughout pregnancy. The diaphragm ascends up to 5cm (Elkus and Popovich Jr 1992) and to maintain adequate lung volumes and capacity (which are unchanged or undergo minimal decreases during pregnancy), the angle of the ribcage and the circumference of the lower ribcage both increase during pregnancy (Hegewald and Crapo 2011). This anatomic change peaks at 37 weeks gestation (Hegewald and Crapo 2011) and is primarily caused by hormonal changes causing the ligaments of the ribcage to relax (McCormack and Wise 2009).

Despite the changes in diaphragm position, there are no significant changes in respiratory muscle strength (LoMauro and Aliverti 2015) and diaphragm range of movement is increased by 2cm, which is explained by an increased area of the diaphragm next to the ribcage resulting in improved coupling (McCormack and Wise 2009; Hegewald and Crapo 2011). After ~15 weeks of gestation, respiratory rate (breathing frequency) remains steady throughout the remaining pregnancy (Heidemann and McClure 2003), although a recent meta-analysis could not find sufficient data on breathing frequencies during pregnancy to produce normative values (Loerup et al. 2019).

During pregnancy, an increase in minute ventilation and tidal volume is observed (Norwitz et al. 2005; Hegewald and Crapo 2011). The amount of air breathed in (minute ventilation) increases significantly during pregnancy compared with non-pregnant women, peaking during the third trimester and showing a slight dip during the second trimester (McAuliffe et al. 2002). Minute ventilation can increase by up to 30% and is associated with feelings of an increased drive to breathe (McCormack and Wise 2009). Interestingly, this has not been found to increase any further with twin pregnancies (McAuliffe et al. 2002).

Tidal volume increases by ~200 ml due to a reduced functional residual capacity (the amount of gas left in the lungs after normal expiration) (Carlin and Alfirevic 2008). As a result of increases in minute ventilation and tidal volume, the majority of women (70%) experience dyspnoea (shortness of breath) by 30 weeks gestation (LoMauro and Aliverti 2015). However, spirometry testing during pregnancy reveals no significant differences in forced vital capacity, compared with non-pregnant women, suggesting no difference in expiratory airflow resistance during pregnancy (Hegewald and Crapo 2011). A higher than average minute ventilation can be a sign of pre-eclampsia (da Silva et al. 2010) and further decreases in functional residual capacity, oxygenation and changes in airway size occur in the supine position (Hegewald and Crapo 2011). The increase in ventilation also causes an increase in PCO_2 (Weissgerber and Wolfe 2006).

Overall, disorders of breathing may be a mechanism in the development of pre-eclampsia (Jerath et al. 2009) and pregnancy is also linked with a higher incidence of respiratory dysfunctions, such as snoring. There is also an association between snoring and sleep apnoea, alongside higher levels of hypertension, as well as increased incidence of infants born small for gestational age (Franklin et al. 2000; Facco et al. 2017). Consequently, the cardiorespiratory relationship is clearly important in hypertensive pregnancies and should be explored to investigate treatments which can normalise disorders of breathing, and establish if they in turn can affect the associated high blood pressure of hypertension.

2.1.2 Blood pressure

Normative values for blood pressure (BP) during pregnancy are based on general population guidelines, with a lack of separate, pregnant-specific ranges to diagnose high BP. Indeed, NICE Hypertension in Pregnancy guidelines (NICE: National Institute for Health and Care Excellence 2019b) are based predominately on adult guidance (NICE: National Institute for Health and Care Excellence 2019a) in addition to one pregnancy-specific study (CHIPS: Control of Hypertension in Pregnancy Study). This is due to "very little evidence on treatment initiation thresholds for hypertension during pregnancy" (NICE: National Institute for Health and Care Excellence 2019b; page 43). Moreover, the CHIPS Study specifically investigated the treatment thresholds for hypertension, rather than the threshold for hypertension *per se* (Magee et al. 2015; Pels et al. 2018). Guidance values recommended during pregnancy are often based on insufficient evidence, referencing outdated data from textbooks (Loerup et al. 2019). Nonetheless, BP during pregnancy is traditionally described as decreasing during the first trimester before returning to normal in the third trimester (Sanghavi and Rutherford 2014). It has therefore been suggested that normative BP guidelines during pregnancy should be related to gestational age, but this idea has not been widely accepted (Higgins and de Swiet 2001).

Women who experience hypertensive disorders of pregnancy, exhibit BP that rather than returning to normal in the second and third trimesters, continues to rise to hypertensive levels as defined by the NICE guidelines (NICE: National Institute for Health and Care Excellence 2019b). Although, it has been observed that pregnant women who later develop PIH may also exhibit higher than average BP earlier in pregnancy compared with normotensive women (Higgins and de Swiet 2001). However, due to the lack of gestational age specific data these comparisons are difficult to make, and impossible to use at this time for clinical decision making.

BP may decrease by 5 – 10 mmHg during the first trimester of pregnancy and the greatest changes are observed in diastolic blood pressure (DBP) compared with changes in systolic blood pressure (SBP) (Moser et al. 2012). A recent meta-analysis found SBP during pregnancy fluctuates from between 110 mmHg at 10 weeks gestation to 116 mmHg at 40 weeks gestation, a difference of 5.6 mmHg (Loerup et al. 2019). DBP fluctuated from 67 mmHg at 10 weeks gestation to 73 mmHg at 40 weeks gestation, with a nadir at 21 weeks of 66 mmHg (a change of 7.6 mmHg during pregnancy). The reduction in systemic vascular resistance, caused by vasodilation, is offset by an increase in cardiac output, which subsequently reduces DBP more than SBP (Moser et al. 2012). The changes in cardiac output and systemic vascular resistance are outlined below (Section 2.1.3 and 2.1.5).

The most important short-term (acute) mechanism to control arterial BP oscillations during pregnancy is the baroreceptor system, the sensitivity of which is increased during pregnancy (Leduc et al. 1991). In both normotensive and preeclamptic pregnancies arterial blood pressure (ABP) variability is preserved at rest (Eneroth-Grimfors et al. 1994) and there is no difference in SBP response to orthostatic tests between pregnant and non-pregnant women (Ekholm et al. 1993). Consequently, although baseline resting BP differs between normotensive and hypertensive pregnancies, the ability of the cardiovascular system to fluctuate and respond to stressors is maintained during pregnancy and during hypertensive pregnancies.

2.1.3 Hemodynamics: Blood volume, heart rate, stroke volume and cardiac output

Pregnancy is characterised as a high volume, low-resistance cardiovascular state (Abbas et al. 2005). Blood volume increases by 30-40% throughout pregnancy, due to an increase in plasma volume, which reaches a peak between 20-32 weeks before maintaining a steady level during the third trimester (Soma-Pillay et al. 2016; da Silva Correa et al. 2019). Higher blood volumes are also seen in twin pregnancies (Norwitz et al. 2005). The increase in blood volume for singleton pregnancies can be as much as 1.2-1.6 L (Soma-Pillay et al. 2016). The expansion of blood volume increases pre-load and end-diastolic volume, leading to increased stroke volume during pregnancy (Sanghavi and Rutherford 2014), by as much as 25% (Heidemann and McClure 2003).

There is also an increase in ventricular wall thickness, which contributes to and is caused by the higher stroke volume and heart rate during pregnancy (Soma-Pillay et al. 2016; Ngene and Moodley 2017). Left ventricular wall thickness can increase by 28% and wall

mass by 52% (Sanghavi and Rutherford 2014). A recent meta-analysis found heart rate increased from 79 beats·min^{-1} at 10 weeks gestation to 87 beats·min^{-1} at 40 weeks (Loerup et al. 2019), with heart rate starting to increase as early as 5 weeks and peaking at 32 weeks gestation with a ~20% increase (Norwitz et al. 2005). The increase in heart rate is likely a result of counterbalance measures to maintain BP due to the decrease in systemic vascular resistance, as part of the contribution of heart rate to cardiac output (Norwitz et al. 2005). An outline of the cardiovascular variables which contribute to BP is presented in Figure 2-1. Arterial BP is maintained by total peripheral resistance and cardiac output, which in turn is controlled by heart rate and stroke volume. Consequently, any changes 'downstream' to heart rate and stroke volume can affect arterial BP through their contribution to cardiac output.

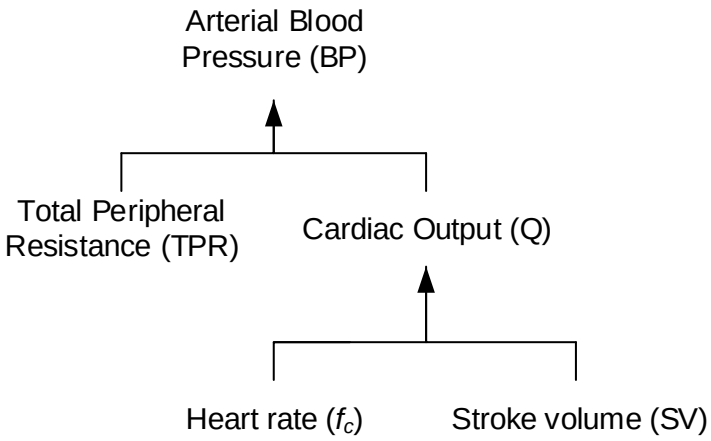


Figure 2-1 Cardiovascular variables contributing to arterial blood pressure

Cardiac output increases during pregnancy between 30-50%, with a peak between 20 and 28 weeks gestation when cardiac output plateaus until delivery (Del Bene et al. 2001; Hegewald and Crapo 2011). Cardiac output is influenced by stroke volume and heart rate (Figure 2-1), and during pregnancy stroke volume is the major determinant of cardiac output, until the second trimester when heart rate takes over (Ngene and Moodley 2017). Twin pregnancies exhibit an increase in cardiac output of up to 20% compared with singleton pregnancies, supported by increases in stroke volume of 15% and heart rate of 3.5% (Kametas et al. 2003).

Comparisons between pregnant and non-pregnant women reveal that the responses to exercise are significantly different; cardiac output is higher for a given exercise intensity during pregnancy, primarily due to an increased stroke volume (Hegewald and Crapo 2011). This shows that baseline levels of cardiac output, stroke volume, and by its nature heart rate, affect maternal responses to external stimuli, such as exercise.

When supine, pregnant women in later gestational ages experience a reduction in venous return due to the pressure of the gravid uterus on the inferior vena cava, leading to a decrease in stroke volume, and consequently cardiac output (Heidemann and McClure 2003). Cardiac output can fall by as much as 25%, compared with a lateral body position (Soma-Pillay et al. 2016). It is therefore important that cardiovascular and respiratory measurements are not measured in the supine positioning, due to the associated haemodynamic changes (Hegewald and Crapo 2011).

2.1.4 Respiratory sinus arrythmia and other cardiovascular oscillations

Respiratory sinus arrythmia (RSA) is reduced during pregnancy and has been shown to exhibit an attenuated response to relaxation compared with non-pregnant women (DiPietro et al. 2012). RSA during pregnancy can be 65% lower than non-pregnant women (Miyazato and Matsukawa 2010) and depressed RSA has been suggested to be a biophysical marker of pre-eclampsia (Lakhno 2016).

As well as RSA, there are other cardiovascular variability measures that are observed in non-pregnant women including BP oscillations at ~0.1 Hz called Mayer waves (outlined in more detail in section 2.4.2). However, although Mayer waves have been found to change during different phases of the menstrual cycle (Lutsenko and Kovalenko 2017) the author is not aware of any studies reporting Mayer waves during pregnancy. Although, high frequency oscillations of SBP increased in women with PIH compared with healthy normotensive pregnant women (Ekholm et al. 1997).

2.1.5 Systemic vascular resistance

Systemic vascular resistance decreases by up to 40% during pregnancy until the middle of the second trimester (Sanghavi and Rutherford 2014) and starts to fall as early as week 6 of gestation (Soma-Pillay et al. 2016). The initial decrease in BP, traditionally observed in pregnancy, is a result of this immediate drop in vascular resistance, which cannot be fully compensated by the accompanying increase in cardiac output (Ngene and Moodley 2017) (see TPR in Figure 2-1). The decrease in systemic vascular resistance is caused by widespread vasodilation (Ngene and Moodley 2017), a result of increasing levels of progesterone and oestrogen throughout pregnancy (Heidemann and McClure 2003).

2.1.6 Autonomic nervous system

Pregnancy is associated with reduced baroreflex sensitivity (BRS) and lower parasympathetic modulation (Blake et al. 2000; Voss et al. 2000; Brooks et al. 2010; Kolovetsiou-Kreiner et al. 2018). There is a move towards more sympathetically mediated changes during normotensive pregnancies, including reduced vagal modulation of the heart caused by increased BP stretching the sinoatrial node (da Silva Correa et al. 2019). This is manifested in increased muscle sympathetic nervous activity (MSNA) by 6 weeks gestation, compared with pre-pregnancy values (Spradley 2018).

Primary hypertension can be caused by sympathetic overactivity and parasympathetic withdrawal (Joseph et al. 2005). In hypertension that develops during pregnancy there is an exaggerated sympathetic nervous system activation prior to the development of hypertension (Spradley 2018). Normal pregnancies show a decrease in parasympathetic cardiovascular control (Ekholm et al. 1993) and an increase in sympathetic activity early in pregnancy (Sanghavi and Rutherford 2014). When this increase in sympathetic activity becomes excessive, hypertensive disorders develop during pregnancy (Sanghavi and Rutherford 2014).

In summary, pregnancy is characterised by substantial changes to both the respiratory and cardiovascular system. Cardiorespiratory interactions may be important in the development of hypertensive disorders of pregnancy, which are associated with increased sympathetic activity. In both normotensive and hypertensive pregnancies, systemic vascular resistance decreases, placing greater importance on cardiac output (and consequently heart rate and stroke volume) to maintain adequate BP during pregnancy.

2.2 Effect of interventions including an element of breath control upon blood pressure during pregnancy

One of the most common ways in which pregnant women practice breathing exercises is during yoga. A 2012 systematic review found that yoga reduces stress, and increases quality of life, autonomic nervous system functioning and labour parameters such as comfort, pain and duration (Curtis et al. 2012). Using the Jadad scale and Delphi List the review only found 6 studies of high quality to include, showing the scarcity of high quality non-pharmacological trials in maternity research. Two hundred and twenty-two studies were excluded because they were cross-sectional studies, qualitative studies, case reports, commentary's or non-academic articles. Overall, yoga has been found to be

beneficial for pregnant women and specifically, practising yoga during pregnancy can increase birth weight with significantly more babies born >2500g, and reduce the incidence of preterm labour (Narendran et al. 2005). Practicing yoga throughout pregnancy also reduces the incidence of PIH compared with walking twice a day, which is the current routine obstetric advice (Narendran et al. 2005; Rakhshani et al. 2012).

One of the first studies to examine the impact of relaxation, and by association controlled breathing, on BP during pregnancy was Little et al. (1984). Following 6 weeks of relaxation therapy, they observed a reduction in hospital admissions and a significant reduction in BP compared with a control group. Relaxation remains popular with women during pregnancy, and pregnant women who develop PIH have benefited from both muscle relaxation (Jacobson method) and deep breathing, compared with a control group (Aalami et al. 2016). After 4 weeks of undertaking slow and deep breathing (SDB; 5-min daily) at 6-10 breaths·min^{-1}, SBP decreased by 10.6 mmHg compared with only 1.5 mmHg in the control group; DBP decreased by 3.6 mmHg and 0.4 mmHg respectively (Aalami et al. 2016). The significant reduction in SBP was observed following just 1 week of SDB, whereas DBP only exhibited a significant reduction after three weeks of practice. This suggests although SDB can produce fast reductions in BP, decreases in DBP take longer to occur.

In a comparison of a SDB intervention, breathing at 4.5 - 7 breaths·min^{-1}, compared with bed rest for women with PIH (Cullins et al. 2013), no difference was found in BP between groups following intervention. However, women in the SDB group had a 35% greater birth weight and gave birth at a gestational age which was 10% greater than the women in the bed rest group. Thus, even if SDB does not reduce BP it has the potential to produce better birth outcomes. Qualitative data from this study also revealed that women felt the SDB helped them fall asleep, calm down and to relax. Therefore, using SDB as an intervention during pregnancy produces additional benefits beyond direct BP reductions, which could reduce stress and produce better birth outcomes for women and their babies.

Mindfulness and meditation exercises, which in their essence involve reducing breathing frequency, have also generated health benefits for pregnant women. Compared with usual care, mindfulness meditation decreases perceived stress scores and increases heart rate variability (Muthukrishnan et al. 2016). Although their study only included normotensive women, the authors suggest that meditation has the potential to modulate sympathetic nervous system activity. Therefore, as PIH is characterised as a disorder

involving sympathetic overactivity, SDB could be beneficial to modulate nervous system activity during pregnancies effected by hypertensive disorders.

2.3 Chronic cardiovascular adaptations following daily intervention of slow and deep breathing

Despite the cardiovascular abnormalities associated with hypertension, a functional component remains operative. This means that acute cardiovascular regulation is still possible and the system can be operated to 'normal' capacity (i.e. at normotensive levels) when it is stimulated appropriately (Calcaterra et al. 2013). Conditions that exhibit a health benefit following SDB are those that are often characterised by sympathetic overactivity and/or stress such as hypertension (Gerritsen and Band 2018). Therefore, if SDB is able to provide the appropriate stimulation to provoke a change in cardiovascular regulation, stimulating a move to functioning at 'normal' capacity, SDB could potentially combat the sympathetic overactivity associated with hypertension by eliciting parasympathetic activity (Calcaterra et al. 2013). The long-term outcomes of SDB interventions are discussed below in the context of the potential mechanistic error signal(s)[1] that might underpin reduction in long-term blood pressure (BP).

Research has progressed from studies examining breathing exercises that are integrated within other modes of exercise such as yoga, to isolating the breathing element and using slow and deep breathing (SDB) as a distinct intervention. SDB has been investigated as a treatment method for a variety of health conditions, but primarily to treat hypertension and the focus of this section will be on the chronic (long-term) cardiovascular adaptations produced by SDB in hypertensive individuals.

SDB used as a distinct intervention is often externally guided, known as device-guided breathing. SDB guided by a device ensures consistency and is easy to master, with user's reduction in breathing frequency, time spent in SDB and breath synchronisation not being correlated with experience and number of sessions already completed (Gavish 2010). The most cited device in the literature is RESPeRATE, but other biofeedback devices exist and have been discussed in more detail by Gavish (2010).

[1] An error signal is a signal that represents the difference between the set point value and the actual value of the regulated variable. For example, the resting value of blood pressure compared with the dynamic value of blood pressure caused by blood pressure fluctuations.

2.3.1 Reductions in blood pressure following daily practice of slow and deep breathing

The most recent meta-analysis of SDB as a treatment for hypertension found an overall reduction in SBP of 5.62 mmHg and 2.97 mmHg for DBP, following daily practice of SDB (Chaddha et al. 2019). However, the overall efficacy of SDB has also been questioned by others (Parati and Cerretta 2007). The impact on 24-hour ambulatory BP has also been inconsistent. However, studies that did not find a reduction in BP were typically short in duration (only 4 weeks) and the authors suggest that reductions in BP may have been seen following a longer intervention (Anderson et al. 2010). This suggestion was supported by a significant reduction in 24-hour SBP following an 8 weeks of SDB (Bazzini et al. 2011).

Additionally, there have been mixed effects on the reduction of BP during pregnancy with some studies finding no significant reductions in BP compared with control groups (listening to music). For example, Altena and colleagues (2009) observed a non-significant reduction of 4.2 mmHg in systolic BP (SBP) compared with a 2.6 mmHg reduction in the control group. Notwithstanding, a reduction of this magnitude could potentially have a clinically meaningful effect. It is also worth noting that mean breathing frequency at the end of the SDB exercise was 8.4 (±3.9) breaths·min^{-1}, i.e. relatively high for SDB (see below), and that 33% of participants did not reach the target breathing frequency of <10 breaths·min^{-1} (Altena et al. 2009). Accordingly, SDB was unlikely to reduce BP in 33% of participants, as they did not achieve the criterion for SDB. A breathing frequency of 8 breaths·min^{-1} may also not be a low enough frequency, compared with the often cited optimal of 6 breaths·min^{-1}, to reduce BP chronically. Grossman et al. (2001) also observed that 23% of participants did not reach the threshold of 10 breaths·min^{-1} and therefore many of the inconsistencies of BP findings may relate to the breathing frequency that was achieved by participants rather than the SDB intervention itself.

Individuals who are experienced in techniques that involve SDB have slower spontaneous breathing frequencies (Spicuzza et al. 2000) and therefore repeated SDB training may reduce long-term spontaneous breathing frequencies (Bernardi et al. 2001), even after only 4 weeks of SDB training (Anderson et al. 2009). Subsequently, when considering the hypothesised link between PIH and dysfunctional breathing (Jerath et al. 2009), if SDB is able to reduce spontaneous breathing frequency and then SDB may specifically target the aetiology of PIH. Additionally, SDB has also produced significant reductions in BP in patients who have both hypertension and obstructive sleep apnea

(Bertisch et al. 2011), which when linked with the higher rates of obstructive sleep apnea in women with PIH outlined earlier in section 2.1.1, provides evidence to support a potential beneficial effect of SDB during pregnancy.

2.3.2 Potential mechanisms for long-term reduction in blood pressure following daily practice of slow and deep breathing

The long-term reduction in BP, following daily practice of SDB, is attributed to changes in mechanical and neural pathways, including in baroreflex sensitivity, heart rate variability, microvascular flow and venous return (Zhang et al. 2009). SDB increases heart rate variability (HRV) and consequently increases baroreflex sensitivity (BRS), which might contribute to BP reduction, accompanied by a chronic decrease in sympathetic nervous system activity when practiced daily (Anderson et al. 2009).

The kidneys regulate BP automatically and chronic hypertension is only maintained when there is sustained impairment of the ability of the kidneys to regulate BP (Anderson et al. 2009). Following 8 weeks of SDB, renal resistive index (RRI) significantly decreased (Bazzini et al. 2011), with significant reductions, compared with control group, observed as early as 1 week (Modesti et al. 2015). The combination of improvements in BRS, reduction of RRI and changes in autonomic nervous system are suggested as key mechanisms in the antihypertensive effects of repeated SDB (Modesti et al. 2015). In normotensive pregnancies renal blood flow increases (Lote 2012), but in hypertensive pregnancies indices of renal venous impedance are significantly higher (Bateman et al. 2004). Consequently, reductions in RRI may also benefit women who develop PIH.

SDB also enhances cardio-respiratory coupling, but in order to have a therapeutic effect, and for long-term health benefits there must be a lasting affect (Dick et al. 2014). Acute responses to SDB do not persist post-intervention, when breathing frequency returns to pre-SDB levels, as shown for HRV (Cheng et al. 2019) and BP (Anderson et al. 2009). SDB is likely to require constant engagement with the exercises to maintain the health benefits, in a similar way to the physiological effects of physical activity and exercise, which are reversable following a period of sedentary behaviour, known as detraining (Mujika and Padilla 2000). Consequently, the acute cardiovascular responses to SDB are central to understanding the mechanisms on which BP is reduced chronically. The acute responses must lead to a re-setting of the cardiovascular system, in one way or another, through repeated exposure to the internal environment created by SDB, and thus by understanding the acute responses to SDB, the error signal(s) which results in chronic BP reduction can be investigated.

2.4 Acute cardiovascular responses to slow and deep breathing

This section will explore the short-term immediate (acute) responses to SDB. During a long-term intervention, SDB acts as an acutely perturbing physiological stimulus, with the adaptations occurring as a result of repeated stimulation (Keerthi et al. 2013). To understand the chronic adaptations induced by SDB it is important to understand the acute response to the repeated stimulus of SDB. The cardiovascular responses to SDB are short-lived post-SDB, returning to baseline levels within 20 minutes (Dick et al. 2014), but the changes within the period of SDB appear sufficient to generate a long-term adaptation.

The cardiovascular system is designed to be adaptable and has inbuilt variability, which in most cases is reflective of a healthy system. Multiple processes, driven by receptors in the heart, lungs and vascular beds, respond to external and internal changes with the aim of maintaining homeostasis and/or responding to the needs placed on the body in different situations. SDB produces a response across the cardiovascular system and these responses are outlined below. Restoration of this healthy cardiovascular variability has been suggested to offer a potential treatment to prolong life (Elstad et al. 2018).

At rest, breathing represents a normal mechanism associated with cardiovascular control via respiratory modulation (Convertino 2019) and it is well established that cardiovascular variables fluctuate with breath phase. For example, during normal inspiration, heart rate increases and BP decreases while the opposite is true during expiration (Chang et al. 2013). The most well-known fluctuation is respiratory sinus arrythmia (RSA), which is the change in heart rate caused by respiratory breath phase. Although this cardiorespiratory coupling is well established, the physiological purpose for the interaction is not fully understood or accepted (Dick et al. 2014). It is not within the scope of this thesis to fully explore RSA's purpose; however, some theories will be discussed in relation to the changes in RSA induced by SDB.

It has been suggested that the long-term BP reductions could be due to the relaxation process itself, rather than the SDB. However, although mental relaxation has been shown to exhibit a reduction in systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate, the reduction was significantly larger in all variables following SDB (Kaushik et al. 2006). Additionally, simply controlling breathing frequency, without reducing it, does not seem to elicit the same cardiovascular response as SDB, since controlled, faster breathing does not produce the same physiological changes as SDB, despite providing the same regularisation of breathing (Bernardi et al. 2002; Pinna et al.

2006; Guzik et al. 2007). By reducing breathing frequency during controlled breathing, breath phase duration is increased, which is not the case for controlled breathing at higher frequencies. Due to the longer duration for each breath phase it is plausible to suggest that heart rate and BP have more time to fluctuate during each prolonged breath phase and therefore greater cardiovascular changes would be produced during SDB compared with controlled higher frequency breathing. Given the interlinked variables within the cardiovascular system, it is also logical to suggest that changes in heart rate and BP would cause, or be caused by, responses in other variables such as stroke volume, cardiac output and total peripheral resistance (Figure 2-1).

There has also been debate regarding the effect of the conscious control of breathing, i.e. that the mental effort required to reduce breathing frequency may alter the physiological response to breathing (Cooke et al. 1998). However, SDB techniques have been modified over the past 20 years and, rather than the traditional techniques which require a concerted effort to count and concentrate on breathing, device-guided breathing techniques require less mental effort. In fact, multiple studies have encountered problems with participants feeling too relaxed and falling asleep (Gavish 2010; Cullins et al. 2013; Adler et al. 2019), which shows that the mental concentration required to follow device guided breathing is not taxing and therefore should not alter the physiological response to SDB.

2.4.1 Respiration

Increases in tidal volume compensate for the lower breathing frequency during SDB (Anderson et al. 2009). Increased tidal volume activates the Hering-Breuer reflex causing a reduction in chemoreflex sensitivity and possible enhancement of baroreflex (Bernardi et al. 2002). The increased tidal volume also activates lung stretch receptors, which increase inhibitory neural impulses, both in frequency and duration or neural impulses (Keerthi et al. 2013). This has an additional effect of reducing BP and sympathetic activity through baroreflex activation as similar control mechanisms are shared by both the respiratory and cardiovascular systems (Joseph et al. 2005).

Respiratory-induced cardiovascular variability can originate from mechanical, neural and metabolic pathways, which arise from volume alternations, cardiopulmonary and arterial baroreceptors, and chemoreceptors (Parati et al. 2008). These pathways can all be altered by modulating breathing. PCO_2 has a role in cardiovascular homeostasis via chemoreceptor reflexes (Anderson et al. 2009) and PCO_2 is decreased by 15% during SDB, which contributes to the decrease in mean arterial pressure (MAP) (Dick et al.

2014). Additionally, the efficiency for oxygen transport at the lungs is increased during SDB, as shown by an increase in SpO_2 at both high and normal altitudes (Bilo et al. 2012; Esposito et al. 2016).

2.4.2 Blood pressure

Despite long-term reductions in BP following long-term practice of SDB, the acute response of BP to SDB is heterogeneous. While normotensive individuals experienced no significant differences in heart rate, SBP or DBP during SDB, hypertensive participants experienced a 8.3 mmHg decrease in SBP at 6 breaths·min^{-1} (Joseph et al. 2005). Other studies observed an acute reduction in SBP of between 2.9 – 9 mmHg for SBP and 2 – 4.9 mmHg in DBP (Bernardi et al. 2002; Anderson et al. 2009; Esposito et al. 2016; Fonkoue et al. 2018; Jette et al. 2019).

The heterogeneity of acute changes in BP during SDB are suggestive of an individual response, which is reflected in the differences between SBP and DBP changes. Opposing responses can be compared between Esposito et al. (2016), who observed a significant reduction in DBP but not in SBP, with DBP reductions of 2.9 mmHg in diabetic participants and 1.7 mmHg in healthy participants; whilst Anderson et al. (2009) found a 6.4 mmHg reduction in SBP, but no change in DBP. Fluctuations in BP are primarily caused by respiratory-induced fluctuations in stroke volume (SV) causing changes in cardiac output (Toska and Eriksen 1993). The SDB responses of SV and cardiac output are outlined below in section 2.4.6.

Some studies have shown that BP regulation is different between the sexes (Wallin et al. 2010). Specifically, women have significantly lower SBP, DBP, MAP, cardiac output and SV and significantly higher heart rate than men (Wallin et al. 2010). There is also a lack of agreement in the literature regarding whether acute reductions of BP during SDB are similar between men and women (Adler et al. 2019), or whether reductions are only exhibited by males (Nili et al. 2017). As men and women use different physiological mechanisms to maintain normal BP (Hart et al. 2009), this may influence their acute response to the internal stimuli generated by SDB. Differences in maintenance of normal BP were observed in the differences in correlations for sympathetic activity and total peripheral resistance and cardiac output between men and women (Hart et al. 2009). Muscle sympathetic nerve activity had no relationship to either total peripheral resistance or cardiac output in women, but exhibited positive relationships in men. Consequently, the cardiovascular responses created by SDB may therefore produce different chronic adaptations in men and women, depending which cardiovascular variables are affected.

The breath phase relationships of BP also change during SDB. Although during normal breathing BP falls during inspiration, when breathing at SDB frequencies, BP tends to increase during inspiration (Parati et al. 2008). Due to the longer duration of breath phase at reduced breathing frequencies, the natural fluctuations of BP peak during different stages of the breath phase cycle, thereby changing the phase relationship of BP during SDB. The amplitude of BP oscillations is inversely proportional to breathing frequency <6 breaths·min^{-1} (Parati et al. 2008) and therefore BP fluctuations could be amplified at breathing frequencies lower than the traditionally utilised 6 breaths·min^{-1}. Acute BP variability is linked to Mayer waves, which are spontaneously occurring oscillations of BP at a frequency of 0.1 Hz (equivalent to 6 breaths·min^{-1}). This frequency of 0.1 Hz is suggested to be one reason that 6 breaths·min^{-1} may be the optimal SDB frequency due to the matching of Mayer waves with breathing frequency at resonance frequencies. Arterial BP oscillations are buffered by heart rate oscillations (Julien 2006) and therefore link with respiratory sinus arrythmia (RSA; outlined below in section 2.4.3).

Under normal conditions, acute intrinsic increases in BP are caused by vasoconstriction, and increased cardiac output, via the sympathetic nervous system (Sharma et al. 2011). An increase in BP inhibits sympathetic activation and activates parasympathetic nerves through arterial baroreceptors (Elstad et al. 2018). If SDB creates an increase in the amplitude of BP oscillations, it is likely the arterial baroreceptors will be activated to a greater extent during SDB. Consequently, acute responses to SDB could reflect activation of the parasympathetic nervous systems.

2.4.3 Heart rate and respiratory sinus arrhythmia (RSA)

Respiratory sinus arrythmia (RSA) is the difference between maximum heart rate during inspiration and minimum heart rate during expiration (or the equivalent for RR interval). Although, average heart rate remains stable during SDB when a small inspiratory resistance is added, the extra effort needed to inhale results in augmentation of sympathetic activity and slight tachycardia (Nuckowska et al. 2019). Synchronisation of heart rate with respiration occurs at 6 breaths·min^{-1} (Parati et al. 2008). During pregnancy, when breathing at 6 breaths·min^{-1}, heart rate is significantly lower, compared with non-pregnant women (Ekholm et al. 1993). There is no difference in nulliparous compared with multiparous women (Ekholm et al. 1993).

During SDB the amplitude of RR internal fluctuations increases at the rate of respiration at 6 breaths·min^{-1} and this in turn increases the amplitude of fluctuations and therefore RSA (Joseph et al. 2005). RSA is a measure of parasympathetic neural control of the

heart (Zhang et al. 2009) and therefore increasing RSA during SDB also switches the dominance to parasympathetic activity, supported by BRS. As RSA can be seen as a reflection of vagal tone (Gerritsen and Band 2018), it could be argued that an increase in RSA is caused by an increase of vagal nerve activity resulting from the SDB. However, there is also debate regarding the specific relationship between RSA and autonomic control and therefore these 'cause and effect' associations should be made with caution (Parati et al. 2006; Eckberg 2009; Karemaker 2009a).

RSA is suggested to maintain cardiac output by opposing the respiration-induced fluctuation in SV. An inverse relationship exists whereby when SV decreases during inspiration heart rate increases and vice-versa during expiration (Elstad 2012). Consequently, by maintaining cardiac output, RSA buffers oscillations in MAP, but may increase variations in SBP (Elstad et al. 2001). This could suggest that while RSA can maintain average BP (MAP) it is unable to fully buffer variations in the peak of BP (SBP). While the arterial baroreceptors impact on RSA, their response is slow, and therefore impulses from arterial baroreceptors are not the only cause of RSA due to the speed in which changes in heart rate occur (Elstad et al. 2001). Consequently, while RSA is increased during SDB, this is not due solely, or even predominantly, to an increase in baroreceptor sensitivity.

Heart rate variability (HRV), of which RSA is one index, is attenuated in people with hypertension (Singh et al. 1998; Terathongkum and Pickler 2004). This reduction in variability associated with hypertension leads to the argument that there would be health benefits from restoring variability which could be produced via device-based approaches (Elstad et al. 2018). SDB has been found to acutely increase HRV accompanying the increase in heart rate (Guzik et al. 2007). Overall, reduced RSA may be a sign of hypertension, but SDB produces an acute increase in RSA, which may be linked with its relationship with both stroke volume, cardiac output and BP.

2.4.4 Baroreflex sensitivity and the autonomic nerve system

Breathing is one of the most powerful modulators of the arterial baroreflex (Sharma et al. 2011); given the important role of the arterial baroreflex in maintaining acute BP, this suggests that respiration can have a large impact on BP. Sympathetic nervous system activity increases during inspiration and decreases during expiration. During SDB baroreflex sensitivity (BRS) varies depending on the respiratory phase and is enhanced during expiration (Parati et al. 2008). SDB is associated with a change in autonomic balance shown by an increase in BRS (Joseph et al. 2005) and a decrease in

sympathetic nerve activity (de Barros et al. 2014). Accompanying small decreases in heart rate, SBP and DBP could enable the increase in BRS to occur due to a relative increase in vagal activity and reduction in sympathetic activity (Bernardi et al. 2002).

BRS is attenuated in people with hypertension, and resets to regulate around a higher pressure range (Sharma et al. 2011). In other patient groups who also exhibit elevated sympathetic nervous activity, such as posttraumatic stress disorder, SDB has been shown to have positive effect by reducing SBP, DBP and MSNA (Fonkoue et al. 2018). Sympathetic tone is reduced and parasympathetic tone is increased during SDB (Wallbach and Koziolek 2018) showing an autonomic shift occurs, from sympathetic to parasympathetic dominance. The baroreceptor reflex is a suggested mechanism for stimulation of the vagal nerve (Gerritsen and Band 2018). This increase in vagal activity could also result in a resetting of the baroreflex to the normotensive ranges, thereby responding to lower, more 'normal' levels of BP. Acute resetting of the cardiac baroreflex is present when an inspiratory resistance is applied to breathing, similar to the effect seen during exercise (Convertino 2019) and the threshold for triggering the baroreflex is lowered when breathing at 6 breaths·min^{-1} (Gerritsen and Band 2018). This resetting allows an elevated cardiac output to be maintained, despite an elevated ABP, which would normally result in a reduction in heart rate and cardiac output.

Evidence to support the activation of the parasympathetic nervous system leading to acute BP changes comes from Pramanik et al. (2009) who found acute BP reductions only occurred in the group without a parasympathetic nervous system blockade, suggesting that it is, at least in part, the vagal activity that produces BP reductions. SDB is a potential method for vagal nerve stimulation (Gerritsen and Band 2018).

Despite, chronic reductions in renal resistive index (RRI) following repeated SDB practice (Bazzini et al. 2011; Modesti et al. 2015), to the best of the author's knowledge, there are no studies which investigate acute responses to SDB. In summary, baroreflex sensitivity is increased during SDB, which may be a sign of increased parasympathetic activity during SDB.

2.4.5 Muscle sympathetic nerve activity

Muscle sympathetic nerve activity (MSNA) reflects tonic sympathetic nervous system activity and acute adjustments of the cardiovascular system in response to perturbation. MSNA increases during expiration and is at its lowest at the end of inspiration/start of expiration (Seals et al. 1993). Differences in the responses to SDB between males and

females have been found; Wallin et al. (2010) observed lower MSNA in women (although not significantly) but detected no correlation between breathing frequency and MSNA in women despite this being observed in men. On the other hand, Adler et al. (2019) observed a reduction in MSNA for both males and females during SDB (Adler et al. 2019). The difference may be explained in the different breathing frequencies utilised in each study; spontaneous breathing (~14 breaths·min^{-1}) in Wallin et al. (2010) and <10 breaths·min^{-1} in Adler et al. (2019). Although MSNA baseline values may differ between men and women, the response to SDB is similar.

MSNA decreases acutely in participants with post-traumatic stress disorder (PTSD) following SDB and also decreases to a greater extent in those with more severe symptoms (Vemulapalli et al. 2019). This may mean that people who have higher levels of sympathetic overactivity experience greater benefits from SDB.

2.4.6 Stroke volume and cardiac output

During inspiration, intra-abdominal pressure increases and intrathoracic pressure decreases, which increases blood flow to the right atrium (venous return) and right ventricle, thereby increasing right ventricular stroke volume (Elstad et al. 2018). This increase in right ventricular stroke volume has an opposing influence on left ventricular stroke volume (SV), which decreases (Harrison et al. 1963). Changes in right- and left-ventricular SV are of equal amplitude (Elstad 2012), suggesting a degree of interdependence.

However, the effects of SDB upon SV have yet to be fully explored in a peer reviewed study. An unpublished PhD thesis (Vargas 2017) found that SDB increased SV, which was attributed to within-breath changes in venous return. At frequencies of ≤6 breaths·min^{-1}, SV during expiration was higher than SV during inspiration, suggesting that the augmented venous return generated during inhalation had time to transit the pulmonary system, bolstering SV during the subsequent expiration. This effect of pulmonary transit time most likely explains individual differences in optimal breathing frequencies and in responses to SDB.

Cardiac output (Q) displays a significant inverse correlation with breathing frequency in men, but not women (Wallin et al. 2010). However, alternative studies observed no difference in cardiac output response to SDB in males and females (Adler et al. 2019). As mentioned above, this difference could also be due to the breathing frequencies

utilised in both studies. Additionally, the inverse relation of heart rate (RSA) with SV reduces respiratory variations in cardiac output (Elstad 2012).

Stroke volume variability was observed to increase when tidal volume was increased from 0.5 L to 0.8 L (Roeth et al. 2014), however it should be noted that breathing frequency was not maintained during tidal volume changes and therefore this would not be classed as a SDB condition. However, as SDB does produce an increase in tidal volume then it would be reasonable to suggest SV variability may increase during SDB.

2.4.7 Different breathing frequencies used for slow and deep breathing

The most common device-guided SDB method is the RESPeRATE system, which produces a dynamically driven breathing frequency < 10 breaths·min^{-1}. The most common fixed breathing frequency explored in the literature is 6 breaths·min^{-1} and this is often touted as the optimal breathing frequency for SDB (Vaschillo et al. 2006). However, there is a paucity of research directly examining the cardiovascular responses to different SDB frequencies. The known studies examining the acute cardiovascular response to different breathing frequencies are summarised in Table 2-1.

Table 2-1 Literature overview of short-term studies examining multiple breathing frequencies

Author	Number of participants	Health condition	Breathing frequency	Duration	Summary outcomes
Anderson et al. (2010)	22 (12 hypertensive. 12 control)	Hypertension & control	Average 8.7 breaths·min^{-1} (<10 breaths·min^{-1} from min 2-15)	15 min (split into 1 min sections for analysis)	Decreasing SBP with decreasing frequency
Bernardi et al. (2014)	102 (81 chronic heart failure. 21 control)	Chronic heart failure (CHF) & control	6, 15 breaths·min^{-1} and Spontaneous	4 min (5 min Spontaneous)	BRS enhanced at 6 breaths·min^{-1} CHF and control
Calcaterra et al. (2013)	133 obese 168 healthy	Children – Obese and healthy	6, 15 breaths·min^{-1} and Spontaneous	Not stated	BRS enhanced SDB for those with higher BMI and insulin resistance
Chalaye et al. (2009)	20	Healthy	6, 16 breaths·min^{-1}, Spontaneous, Video game (distraction spontaneous)	>2min	SDB increases pain threshold and tolerance
Chang et al. (2013)	53	Healthy	8, 12 and 16 breaths·min^{-1}	Not stated	Respiratory peak shifts to LF range in HRV at 8 breaths·min^{-1}
Cooke et al. (1998)	10	Healthy	Stepwise 3, 6, 9, 12, 15, 18 breaths·min^{-1} and Spon (with and without tidal volume control)	2 min at each stepwise stage 5 min Spon	No difference with and without tidal volume control

Author *(cont.)*	Number of participants	Health condition	Breathing frequency	Duration	Summary outcomes
Guzik et al. (2007)	15	Healthy	6, 9, 12, 15 breaths·min^{-1}	5 min	Increased heart rate, HRV and BRS only at 6 breaths·min^{-1}
Joseph et al. (2005)	46 (20 hypertension, 26 control)	Hypertensive & control	6, 15 breaths·min^{-1} and Spontaneous	2 min (5min Spontaneous)	Decrease BP only in hypertensive group at 6 breaths·min^{-1}. BRS increased to values similar to control.
Nuckowska et al. (2019)	20	Healthy	6, 12, 18 breaths·min^{-1}, spontaneous and resistance at 6 breaths·min^{-1}	Protocol 1: 10 min Protocol 2: 5 min	Reduction DBP and MAP during SDB and
Oneda et al. (2010)	27 (14 completed SDB, 13 placebo)	Hypertensive	Average 6.8 breaths·min^{-1} (<10 breaths·min^{-1} average for all 5 min sections)	15 min (split into 3 x 5 min for analysis)	BP decreased both SDB and placebo, MSNA reduced SDB.
Zhang et al. (2009)	13	Healthy	7, 8, 9.5, 11, 12.5, 14 breaths·min^{-1}	Gradual decrease. Total time 15 min (2-3 min per frequency)	PTT and RR interval increased with decreasing frequency

Baroreflex sensitivity (BRS), blood pressure (BP), chronic heart failure (CHF), diastolic blood pressure (DBP), muscle sympathetic nerve activity (MSNA), pulse transit time (PTT), slow and deep breathing (SDB), systolic blood pressure (SBP).

Most of the reviewed literature examined breathing frequencies of 6 breaths·min^{-1} or a variable rate <10 breaths·min^{-1}. Research to date has therefore failed to investigate the differences that alternative SDB frequencies may have on cardiovascular responses, and to fully understand the differences between normal to SDB frequencies. Furthermore, relatively few studies have examined multiple breathing frequencies (Table 2-1). As SDB is considered to be breathing at a frequency less than10 breaths·min^{-1}, then most studies examine only one breathing frequency that would be considered to be SDB (Bernardi et al. 2002; Joseph et al. 2005; Chalaye et al. 2009; Calcaterra et al. 2013; Chang et al. 2013; Nuckowska et al. 2019). Although RESPeRATE creates a dynamic breathing frequency that results in different breathing frequencies over time, only 2 studies report results at different time points during the intervention rather than averaging an epoch of the whole intervention (Anderson et al. 2009; Oneda et al. 2010). These studies examine variables relative to time and cannot therefore link cardiovascular responses to specific breathing frequencies due to the individual nature of the RESPeRATE breathing frequency implementation. Only 2 studies have examined multiple fixed breathing frequencies but this was also within a protocol with a gradually declining breathing frequency, resulting in short periods at each individual frequency (Zhang et al. 2009), or breathing conditions that used breathing frequencies of 9 breaths·min^{-1} and above (Guzik et al. 2007). This overview highlights the large gap in the current understanding of the cardiovascular responses to different fixed breathing frequencies. In particular, the steady state responses, where sufficient time is allowed (at least 5 minutes) for acute responses to mature fully. By understanding how steady-state cardiovascular responses change across a range of fixed breathing frequencies this will provide a better understanding of the mechanisms that may lead to the long-term reduction in BP following SDB.

2.5 Summary

Overall, there is a heterogenous pattern within the literature describing the acute cardiovascular responses to SDB; for example, both increases and decreases in BP have been found during SDB. Understanding the acute responses is important in order to develop an understanding of the mechanisms that result in any chronic reduction in BP following daily practice of SDB. The above literature review revealed that the understanding of the acute responses to SDB is currently limited, with variables often investigated independently, with no acknowledgement of their interaction within the cardiovascular system. Since BP is influenced by the ensemble of changes in total peripheral resistance, cardiac output, SV and heart rate, it is therefore important to

understand the complex interactions of all variables simultaneously to complete the picture of acute cardiovascular responses during SDB.

The baseline cardiovascular differences between both males and females and during pregnancy have been outlined in the literature review above. These differences may influence how the body responds to SDB and therefore warrant further investigation of whether differences between populations changes the acute cardiovascular response to SDB.

2.6 Aims, objectives, and hypothesis

The overall aims of this thesis are to characterise and compare the acute cardiovascular responses to SDB of pregnant women and design a specific slow and deep breathing intervention for women who develop pregnancy-induced hypertension. A series of research questions, objectives and hypotheses were set and were answered systematically in each chapter.

In summary, the objectives for the thesis were to:

1. Identify the acute response in blood pressure and amplitude of blood pressure oscillations during SDB for healthy young men, healthy non-pregnant women and healthy pregnant women.
2. Characterise and compare the response of mechanism-related parameters (e.g. respiratory sinus arrythmia, stroke volume, cardiac output) to SDB for healthy young men, healthy non-pregnant women and healthy pregnant women.
3. Evaluate differences in acute cardiovascular responses to a range of SDB frequencies for healthy non-pregnant women and healthy pregnant women.
4. Design an evidence based SDB intervention for women with PIH.

Chapter 3. General Methods

3.1 Introduction

This chapter outlines a detailed explanation of the general methods (slow and deep breathing delivery, pre-test procedures & physiological equipment and procedures) that are common between the studies that form this thesis. Unique methods that are specific to individual studies are described in the relevant chapters, such as ultrasound measurements of renal resistive index (RRI), central blood pressure measures, and the proposed long-term intervention. The thesis includes three experimental lab-based studies (Chapters 5, 6 & 7) and a proposed clinical study which was postponed due to COVID-19. The first three studies aim to investigate the immediate (acute) responses to slow and deep breathing (SDB) to understand the potential mechanisms by which SDB may lower long-term blood pressure (BP) when practiced daily. They are conducted in normotensive participants in order to understand the different responses to SDB of males and females (Chapter 4), normotensive non-pregnant women (Chapter 5), and normotensive pregnant women (Chapter 6). The final proposed study (Felton et al. 2021) presented in Chapter 8) was planned to include hypertensive pregnant women who would complete the acute SDB responses protocol, in addition to moving the research to a clinical setting using a SDB long-term intervention. The final study would have provided the next step between the theoretical lab-based study in a controlled environment to a real-life pragmatic study, where the greatest impact can be found. Unfortunately, due to COVID-19 it was not possible to complete this experimental study but the proposed protocol is presented in this thesis.

An overview of the thesis structure and chapters is shown in Figure 3-1. Chapter 7 provides a comparison of the data collected in the studies presented in Chapter 5 and 6 (healthy pregnant and healthy non-pregnant women), therefore as it does not have a separate methodology it is not presented in Figure 3-1. The greyed sections of Figure 3-1 show common methods between studies and chapters and it is these sections that are outlined in this General Methods chapter to avoid repetition in subsequent chapters.

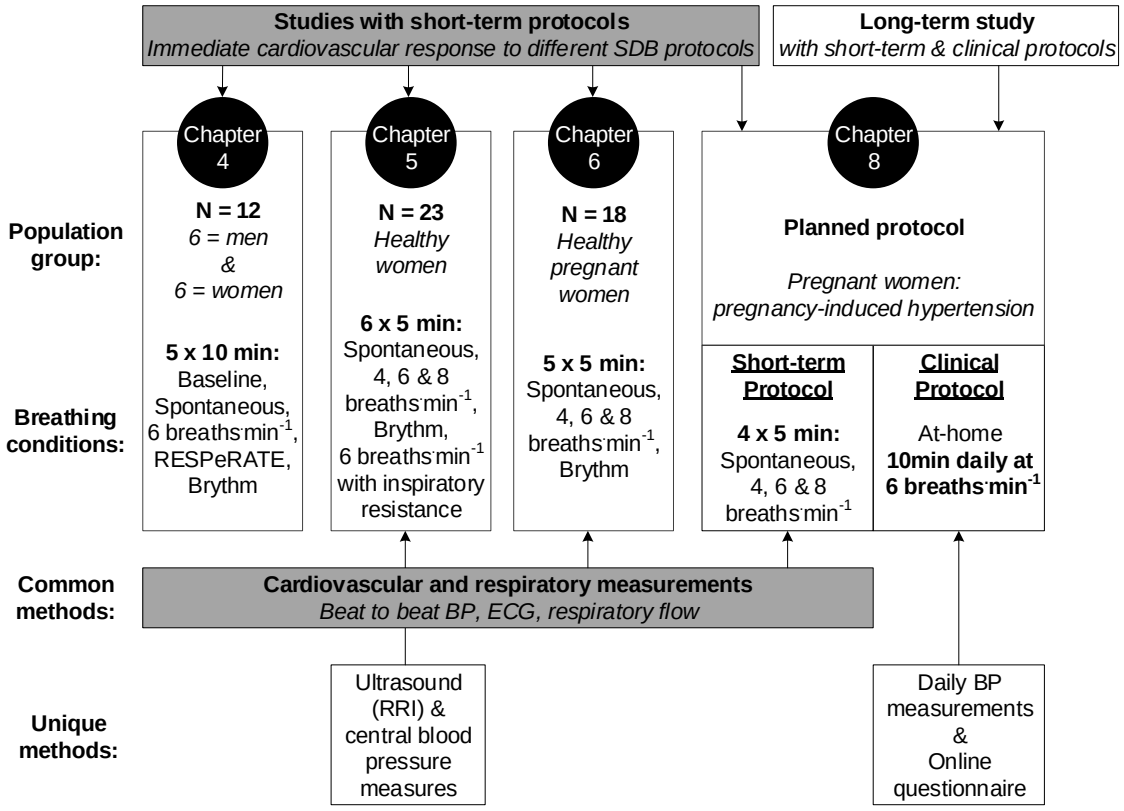


Figure 3-1 Overview of studies comprising this thesis
Blood Pressure (BP); Electrocardiogram (ECG); Renal Resistive Index (RRI).
Chapter 7 contains an integrated paper comparing the results of data collected from Chapters 5 and 6.

3.1.1 Ethics approval

The experimental protocols in Chapters 5, 6 & 7 were approved by Bournemouth University's Research Ethics Committee (Appendix VI) and all experiments conformed to the Declaration of Helsinki. The protocol for the final study (Felton et al. 2021) presented in Chapter 8) was approved by the Hampshire B Research Ethics Committee and Health Research Authority, but due to COVID-19 the study was not completed as part of this thesis. It is included as a protocol paper to show the work completed to date.

3.2 Slow and deep breathing delivery

Participants completed a range of breathing conditions[2] during each study including a spontaneous 'normal' breathing condition. A different set of breathing frequencies and conditions were used in each study and these are justified and outlined separately in each chapter. Figure 3-1 provides an overview of the breathing conditions used in each study. Breathing conditions were ten minutes in duration in Chapter 4 and five minutes

[2] In the context of this thesis breathing conditions means the different breathing exercise protocols that the participants undertook.

in Chapter 5 and Chapter 6. A SDB session of 10-min is commonly used in literature for long-term SDB interventions to reduce BP (Chaddha et al. 2019). The results from Chapter 4 revealed no significant differences in the acute cardiovascular differences between the first and final 5-min for all conditions except RESPeRATE and therefore subsequent studies used 5-min duration to reduce time burden on the participants. An equal period of rest to the breathing protocol length (either ten or five minutes) was undertaken prior to each measurement with participants instructed to breathe normally. An equal rest to breathing ratio is sufficient to allow cardiovascular and respiratory variables to return to baseline levels (Vargas 2017). Controlling tidal volume during SDB has no effect on cardiovascular rhythms and normal end-tidal CO_2 levels are maintained without direct control (Cooke et al. 1998). Additionally, there is no advantage of simultaneously controlling breathing frequency and tidal volume (Vargas 2017) and therefore only breathing frequency was controlled.

For the spontaneous breathing condition, participants were instructed to breathe normally, and no visual feedback was provided to control breathing. The spontaneous breathing condition provided the baseline comparison for the SDB frequencies. Participants also completed a dynamic breathing frequency condition using an optimisation algorithm (McConnell et al. 2017). The optimisation algorithm guides breathing frequency dynamically to a personalised optimum frequency. Further details of the optimisation algorithm are outlined below (3.2.2). As the optimal SDB frequency is widely regarded in the literature to be 6 breaths·min^{-1} to maximise hemodynamic changes (Vaschillo et al. 2006; Russo et al. 2017) this was used as the comparison condition to the dynamic breathing frequencies in all studies. In Chapter 5 and Chapter 6 fixed breathing frequencies of 4 and 8 breaths·min^{-1} were chosen to span the optimal frequency to provide a linear range of frequencies to understand any potential graduated response to SDB and discover differences in lower and higher breathing frequencies based on results from Chapter 4.

3.2.1 Brythm app

All SDB conditions were delivered in the laboratory by Bournemouth University's (BU) Brythm app using an iPad (iPad Pro, 12.9in, 1st Gen). Brythm provides visual feedback to guide the user's breathing frequency, whereby the user inhales when the dome graphic rises and exhales when the dome falls (Figure 3-2 Screenshots of Brythm graphic). The speed of the graphic can be changed to manipulate the user's breathing frequency to a fixed respiratory rate. The set graphic speed is adjusted prior to starting each breathing condition and is consistent throughout each condition. Inspiration and expiration phases were matched to create an equal duty cycle (~0.5). Although the

RESPeRATE research reflects a benefit of prolonged expiration, acute responses to SDB reflect a similar response with different and matched breathing cycles (Herakova et al. 2017).

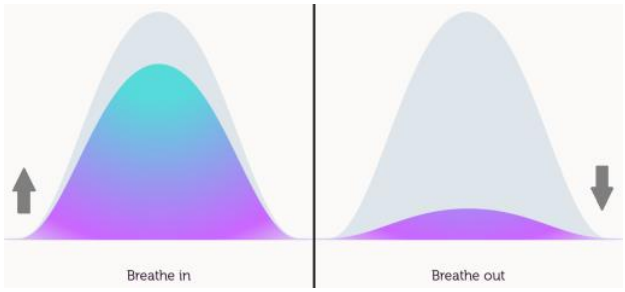


Figure 3-2 Screenshots of Brythm graphic
N.B: Arrows do not appear on app but are shown here to display the direction of graphic movement.

3.2.2 Brythm optimisation algorithm

The Brythm app also has an inbuilt optimisation algorithm (McConnell et al. 2017) which dynamically changes breathing frequency based on the user's physiological response to the breathing. The app responds to data measured from a finger sensor (photoplethysmography; Figure 3-3), which tracks the user's instantaneous physiological responses to their breathing. The optimisation algorithm creates a dynamically driven breathing frequency, which strives to maximise cardiovascular perturbation, using the amplitude of RSA as the controlled variable. The finger sensor is connected via the headphone socket of the iPad.

Figure 3-3 Brythm app finger sensor

For the clinical study (Felton et al. 2021) presented in Chapter 8) it was proposed that the SDB protocol would be delivered using a video graphic instead of the Brythm app, as described below.

3.2.3 Alternative slow and deep breathing delivery method: Video graphic

In accordance with the EU Medical Device Regulations 2017/745 (European Union 2017) and the MHRA guidance regarding medical device stand-alone software including apps (Medicines and Healthcare products Regulatory Agency 2018), Brythm is classed as a medical device due to the intention to treat hypertension. Any research investigating medical devices outside of the legal entity that developed it (in this case BU), is required to notify the relevant national regulatory body. In the UK the regulatory body is the MHRA and submission of a clinical investigation of a medical device requires completion of a large application including in-depth technical documentation and a large processing fee. Additionally, when research is undertaken with the NHS the associated university is required to oversee the management of the project by accepting sponsorship responsibilities for the study. At the time of planning for the final study of this thesis, which required collaboration with the NHS for recruitment of participants, BU was unable to accept sponsorship of studies requiring MHRA notification.

Due to the financial and technical limits restricting PhD research, the challenges outlined above meant that using Brythm to deliver the SDB in the final study of this thesis was unfeasible (Chapter 8). Consequently, to deliver the SDB with pregnant women who would be recruited from the NHS, an alternative method of delivery was needed. Due to the results of the three studies outlined in Chapters 4, 5 and 6 it was concluded there was no difference in the physiological response between the optimisation algorithm and the fixed breathing condition of 6 breaths·min^{-1}. Therefore, there would not be any extra benefit from using the optimisation algorithm compared with fixed breathing at 6 breaths·min^{-1} for the long-term intervention. This decision negated the need for the finger sensor, which tracks the user's physiological response and is needed to run the algorithm. It was therefore possible to use a video of the graphic designed for the app (Figure 3-2) which was set at a fixed breathing frequency of 6 breaths·min^{-1}. Video aids are not classed as a medical device and therefore a video delivering SDB can be used within an NHS study without requiring MHRA notification. A full description of how this video was shared with participants is provided in Chapter 8 (Felton et al. 2021).

3.3 Pre-test procedures

3.3.1 Randomisation procedures

To remove potential order effects breathing conditions in all studies were randomised using a random number generator (www.randomizer.org). The randomised numbers are generated by means of a complex algorithm using the computer's clock. Order was not weighted to have the same condition performed at each order point the same number of

times. An example of the randomisation from Chapter 6 is outlined in Table 3-1, to show the frequency in which breathing conditions were performed in each position order (1st – 5th).

Table 3-1 Example randomisation order: number of times each breathing condition was performed in each order position

Position order	Spontaneous	4 breaths·min^{-1}	6 breaths·min^{-1}	8 breaths·min^{-1}	Dynamic algorithm
1st	6	6	2	3	1
2nd	5	5	4	3	1
3rd	2	2	5	3	6
4th	3	2	4	3	6
5th	2	3	3	6	4

The randomised breathing condition order was set prior to the start of data collection and no changes were possible after this point. The randomised order was linked to a participant number (P01, P02, P03 etc) and each new participant received the next condition order in the list. Participants recruited were booked in at the next available time and the order of conditions was not looked at during the booking process. The investigator (MF) generated the randomised order using the website but after this only accessed the order of breathing conditions on the day of data collection and therefore was not able to make any changes to the order or select people as certain participants. Participants were informed of the order prior to data collection but could not choose or change the order of the breathing conditions. No information was given to participants on the expectation of any effects for the breathing conditions, such as whether a larger response was expected to a specific condition.

3.3.2 Participants, recruitment and pre-test procedures

Participants taking part in studies including short-term protocols (Figure 3-1) were asked to refrain from eating for 2 hours and from caffeine, strenuous exercise and alcohol for 12 hours prior to attending the session at the Cardiorespiratory Research Laboratory at Bournemouth University.

Following consumption of food there is an ingestion related increase in blood flow to the splenic organs which leads to an increase in cardiac output ($\dot{Q}$) to meet the additional demand (Waaler and Eriksen 1992). An increase in stroke volume (SV) contributes to the rise in $\dot{Q}$, with total peripheral resistance (TPR) decreasing to maintain mean arterial pressure (MAP) (Sidery and Macdonald 1994). These cardiovascular responses have been shown to last up to 2 hours (Waaler et al. 1991). Participants could consume water

during the 2 hours prior to data collection as no cardiovascular responses are associated with water consumption (Waaler and Eriksen 1992). Central blood pressure measures are also more reliable when measured in a fasted state (Young et al. 2015) and the waveform measured by finger plethysmography can be altered following consumption of food (Tanaka et al. 2015).

Immediately after consuming alcohol, respiratory sinus arrythmia (RSA) and heart rate (f_c) are decreased, which may suggest that cardiac vagal tone is reduced (Reed et al. 1999). Additionally SV, $\dot{Q}$ and brachial artery diameter are effected immediately after alcohol consumption (Spaak et al. 2008). Systolic (SBP) and diastolic blood pressure (DBP) experience a biphasic effect of alcohol consumption, which can last until the next day (>12 hours) (Bau et al. 2005). Additionally, it is well known that caffeine immediately increases BP (Smits et al. 1985) and the acute response of BP remains even in people who are regular consumers (Lovallo et al. 2004). This is true whether the caffeine is consumed in tea or coffee (Quinlan et al. 1997).

Participants received a participant information sheet (Appendix II) at least 24 hours prior to participating in the study. Written informed consent was obtained from all participants prior to taking part (Appendix III). A health questionnaire was also completed by participants to ensure that they met the inclusion and exclusion criteria for each study (Appendix IV). Inclusion and exclusion criteria are outlined individually in each chapter. All participants were free from any current cardiovascular or respiratory disease such as asthma, Chronic Obstructive Pulmonary Disease (COPD) or hypertension, and were all non-smokers. The exception is that participants outlined in the planned Chapter 8 protocol (Felton et al. 2021) would have been diagnosed with pregnancy-induced hypertension (PIH).

3.4 Equipment and procedures

This section outlines the equipment used to collect the cardiovascular and respiratory data in the short-term responses protocols. Where equipment was used in one study only this is outlined in the relevant chapter. Participants were seated in an upright position, at an approximate angle of 60^{o} for the duration of the data collection. Full equipment set up can be seen in Figure 3-4.

Figure 3-4 Full equipment set up with participant
Note: Photo as example set up only; in all studies blood pressure was measured on the left side of the body.

3.4.1 Anthropometry

Prior to all short-term responses protocols stretch stature was measured using a stadiometer (SECA 213, Germany) and participants were asked to stand barefoot with their feet together, and heels, buttocks and upper part of back touching the stadiometer (International Society for the Advancement in Kinanthropometry 2011). Stature was measured to the vertex of the head while the participant's head was in the Frankford horizontal plane. Body mass was recorded in minimal clothing using calibrated electronic scales (SECA 804, Germany).

3.4.2 Respiratory measures

Respiratory airflow was monitored continuously throughout each breathing condition. Participants wore an oronasal mask that covered both mouth and nose (Oro Nasal 7450 V2 Mask, Hans Rudolph Inc., Kansas, USA) and respired flow rate was measured continuously using a heated pneumotachograph (Model 3700, Hans Rudolph Inc., Kansas, USA) connected to a flow measurement system (RSS 100-HR, Hans Rudolph Inc., Kansas, USA). The respiratory equipment set up can be seen above in Figure 3-4. The flow measurement system was zeroed prior to the start of each breathing condition.

3.4.3 Cardiovascular measures

Heart rate (f_c) was monitored continuously using a 3-lead ECG and non-invasive beat-to-beat arterial blood pressure (ABP) was estimated using a Finometer (Finapres NOVA, Finapres Medical Systems, The Netherlands). The Finometer uses an inflatable finger cuff (Figure 3-5) with inbuilt photo-electric plethysmography to detect finger pulse

pressure waveforms, using the volume-clamp method (Peňáz 1973). The diameter of the artery in the finger pulsates when the heart beats, which causes pulsation in the light detector signal as the blood absorbs the infrared light from the plethysmograph (Finapres 2012). Using the volume-clamp method the diameter of the finger artery is kept constant (clamped) by the cuff rapidly increasing pressure when diameter changes are detected, which prevents the diameter change (Bogert and van Lieshout 2005). Finger cuff pressure therefore equals intra-arterial pressure, however other factors can influence the unloaded diameter requiring regular verification throughout continuous measurements (Bogert and van Lieshout 2005). The Finapres has an inbuilt autocalibration algorithm (Physiocal) which calibrates the finger cuff pressure. Physiocal interrupts the measurement for one heart beat and keeps cuff pressure constant at a level halfway between SBP and DBP to determine the cuff pressure set point to maintain an unloaded diameter (Langewouters et al. 1998). As the calibration interrupts the data collected, due to the maintained pressure intervals, Physiocal was turned off during each breathing condition to maintain uninterrupted data collection but was turned on during each rest period to allow calibration of the finger cuff and ensure accurate measurement.

Figure 3-5 Finapres inflatable finger cuff

The Finapres uses brachial arterial reconstruction technology to correct for the distortions in the pressure waveform as it travels from the brachial artery to the finger. Distortion is caused by increased arterial stiffness at the peripheral arteries, faster transmission of the higher pressure components and wave reflections (Levick 2013) with a difference of 8-10 mmHg between brachial and finger arterial blood pressures for DBP and MAP (Bogert and van Lieshout 2005). The Finapres restores the waveform to the brachial level and allows for differences and changes in height between the finger and heart level by using a height correction unit to correct hydrostatic BP changes (Carlson et al. 2019).

To ensure accurate BP readings from the finger cuff the Finapres uses an upper arm calibration whereby an arm cuff is used on the same arm as the finger cuff (Figure 3-5). The arm cuff uses return to flow calibration whereby when the first pulsation is sensed in

the finger (after cuff inflation) the corresponding arm cuff pressure is recorded. The reconstructed brachial pressure is defined by this recorded measurement and SBP and DBP are both calibrated in this way. The brachial calibration was performed prior to the first breathing condition and halfway through each session; following 3 breathing conditions in the studies outlined in Chapter 5 and Chapter 6, and following 2 conditions in the Chapter 4 and proposed Chapter 8 study. When used with the brachial calibration, the Finapres passes the AAMI ISO81060-2 measurement standards which evaluates performance validation of BP measurement equipment. Additionally, the Finapres BP measures have found to correlate with auscultatory BP measurements in normotensive participants (Carlson et al. 2019). The Finapres (finger and arm cuff) were both set up on the left side of the body in all studies.

The Finapres estimated stroke volume (SV) using the Modelflow method, which computes aortic flow over time using a three-element model (Wesseling et al. 1993). The Finapres uses age, sex, height and weight, which are inputted prior to data collection, to determine pressure-volume, pressure-compliance, and pressure-characteristic impedance relationships (Jansen et al. 2001). This approach produces measurements that show excellent agreement with SV measured by Doppler ultrasound (Van Lieshout et al. 2003) and when blood is withdrawn by phlebotomy (Leonetti et al. 2004).

The Finapres NOVA (or its predecessors the Finapres and Portpres) has limited data on validity of cardiovascular measures during pregnancy. The Portapres overestimated SBP by 5mmHg and underestimated DBP by 3mmHg compared with standard sphygmomanometry in healthy pregnant women (Hehenkamp et al. 2002). In women with pre-eclampsia, SBP was overestimated by 3mmHg and DBP underestimated by 8mmHg. However, it was found to meet the Association for the Advancement of Medical Instrumentation (AAMI) criteria and compares favourably with other non-invasive automated BP monitors (Hehenkamp et al. 2002). Using the Finometer, SBP was also overestimated compared to the Dinamap (an automated oscillometric BP measurement device) (Grindheim et al. 2012). As the majority of BP analysis in this thesis will be within participants, examining responses to SDB compared with normal breathing, overestimations should be consistent across all conditions, and have minimal effect on results. However, reliability data during pregnancy is not available for the Finapres NOVA.

3.4.4 Data acquisition

Analogue outputs from the Finapres NOVA (reconstructed brachial pressure waveform, ECG waveform, SV, SBP, DBP) and the flow meter were sampled continuously at 250Hz

via an analogue to digital converter (NI USB-6218 BNC, National Instruments Inc.) and captured using bespoke acquisition and analysis software (LabView 2015, National Instruments, Inc.). The LabView software corrected for the 4 second delay between the Finapres NOVA output and the respiratory output. A raw data file was created after each condition and summary data files were produced using the LabView software. The summary data files contain beat-by-beat, breath-by-breath and epoch summary data. LabView uses both built-in and bespoke coded sub-routines that calculate mean (Figure 3-6 calculation 1), peak, nadir, and amplitude variations for all cardiovascular and respiratory variables. These calculations were performed for whole breath and within respiratory phases (inspiration & expiration; Figure 3-6 calculations 2 and 3 respectively). Mean data for all variables were calculated in one-minute epochs during each five- or ten-minute condition, for the whole five- or ten-minute epoch and for the 10-min conditions (Chapter 4) into first and final 5-min epochs. Section 3.4.5 provides more detail on the amplitude variation calculations.

The following calculations were applied in LabView, or in subsequent analysis, to calculate the variables used throughout this thesis. Total peripheral resistance (TPR; Equation C) is derived from the measured variables of BP and cardiac output as there is no method available to provide a direct measurement of TPR (Elstad et al. 2011).

Equation A: Cardiac output ($\dot{Q}$) = f_c x SV
Where f_c is heart rate and SV is stroke volume.
Note cardiac output is presented as $ml \cdot min^{-1}$ in this thesis.

Equation B: Mean arterial pressure (MAP) = DBP + (0.333 x PP)
Where DBP is diastolic blood pressure and PP is pulse pressure.

Equation C: Total peripheral resistance (TPR) = MAP / $\dot{Q}$
Where MAP is mean arterial pressure and $\dot{Q}$ is cardiac output.

Equation D: Pulse transit time (PTT) = time peak pulse pressure (finger cuff) – time peak R wave (ECG).
Time difference between pulse detected at heart (from ECG) and detected at the finger (pulse pressure at left index finger).

Equation E: Pulse Wave Velocity (PWV) = path length (m) / PTT (s)
Where path length was measured from sternal notch to the left index finger and PTT is pulse transit time.

Path length for PWV was measured in accordance with Hansen (2010) using the distance measured from sternal notch to the acromiale, added to the distance from the acromiale to the middle of the Finapres finger cuff (left index finger).

3.4.5 'Peak-valley' calculation methods applied to cardiovascular data

Respiratory sinus arrhythmia (RSA) is a variable calculated to determine the amplitude of f_c rhythms using the 'peak-valley' method. In this thesis RSA was calculated using two methods 1) the difference between the average heart rate (f_c) during inhalation (f_ci) and exhalation (f_ce) ($f_c\Delta$; Equation F); 2) the difference in maximum and minimum beat-to-beat intervals (RR) during inhalation and exhalation respectively (RSA; Equation G).

Equation F: $f_c\Delta = f_c\text{i} - f_c\text{e}$
Where f_ci is average heart rate during inspiration and f_ce is average heart rate during expiration.

Equation G: Respiratory sinus arrythmia (RSA) = RRi max – RRe min
Where RRi max is maximum beat-to-beat intervals during inhalation and RRe min is minimum beat-to-beat intervals during exhalation.

In addition to RSA, other cardiovascular variables were also analysed using the 'peak-valley' method, to determine breath related variations induced by SDB. Cardiovascular responses will be grouped into intra- and inter- breath phase responses in the results sections of each chapter (Table 3-2).

Table 3-2 Breath phase analysis calculation

Breath phase analysis	Mean values	Peak-valley analysis
Intra-breath phase response *Within breath phase*	i, e	Δi, Δe
Inter-breath phase response *Between breath phase*	Δ *(Difference in mean values)*	ΔPV
Full breath cycle response *Independent of breath phase*	Mean	ΔPV_Ind

i = Average inspiration. e = Average expiration. Δi = Max I – Min I. Δe = Max E – Min E. Δ = i – e (average inspiration – average expiration). ΔPV = Max I – Min E or Min I – Max (whichever calculation gives largest difference). Mean = mean full breath cycle. ΔPV_Ind = Max – Min (irrespective of breath phase; max value during full breath cycle, min value during full breath cycle).

Inter-breath phase indices (Δ) were quantified as the difference between mean inspiration (i) and mean expiration (e) values (Figure 3-6 calculation 4). Peak-valley (PV) indices were calculated as maximum minus minimum values during inspiration (Δi: Figure 3-6 calculation 6) and expiration (Δe: Figure 3-6 calculation 5). Within-breath phase PV indices (ΔPV) were calculated using maximum inspiration minus minimum expiration, or minimum inspiration minus maximum expiration, depending which calculation gave the largest difference. Figure 3-6 calculation 7 shows an example using the calculation maximum inspiration minus minimum expiration.

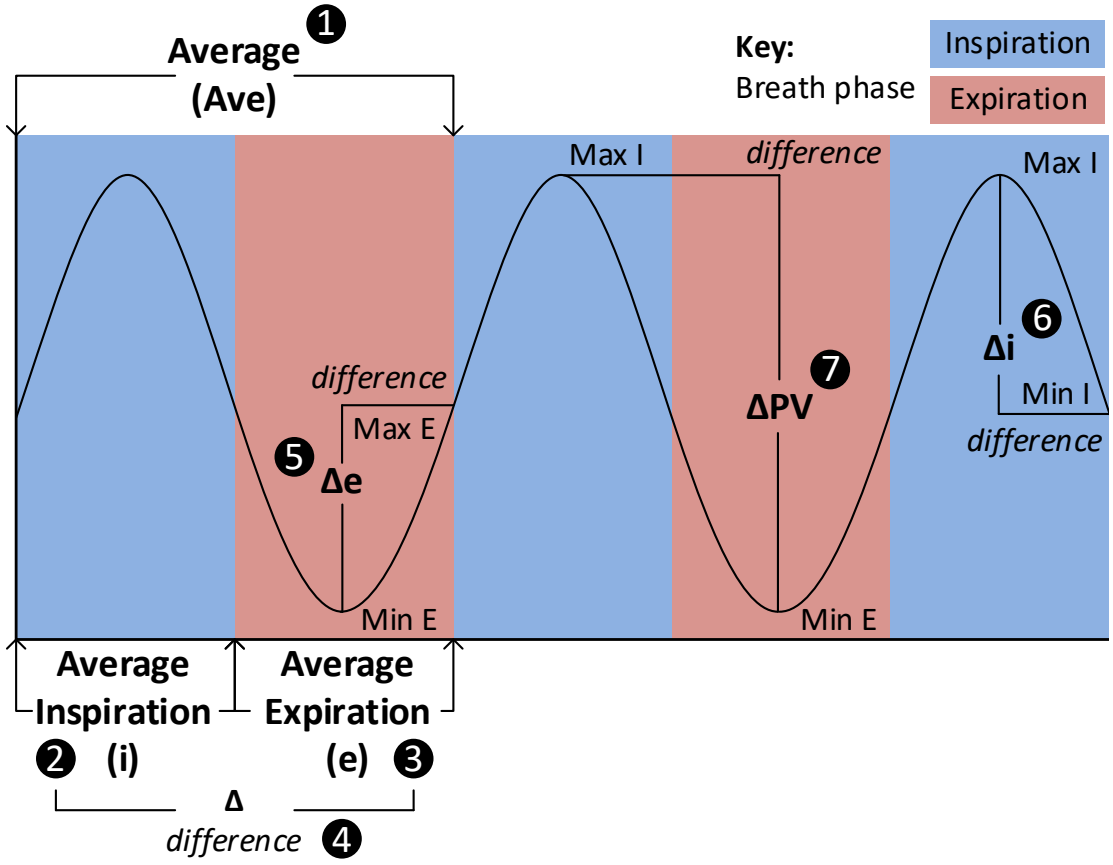


Figure 3-6 Calculations for example cardiovascular variable plot
1) Ave = average of whole breath. 2) i = Average inspiration. 3) e = Average expiration. 4) Δ = i – e (average inspiration – average expiration). 5) Δe = Max E – Min E. 6) Δi = Max I – Min I. 7) ΔPV = Max I – Min E (Note ΔPV calculation varies and can be Min I – Max E depending on which calculation provides largest difference).
Note: Example cardiovascular plot shows arbitrary values, not based on real data, to demonstrate simplified calculations. The point of breath phase change occurs at different points on the sine wave for different variables and during different breathing conditions.

'Breath phase independent' peak-valley calculations (ΔPVInd) were also performed with the maximum and minimum values measured irrespective of the breath phase in which they occurred. Blood pressure fluctuations were calculated as maximum – minimum divided by mean during each breath phase. This method has been used for other cardiovascular variables to calculate respiratory variability (Elstad and Walløe 2015).

In summary, this chapter has described the common elements between methods for the following chapters. A short overview of the methodology is provided in the methods section of each chapter and this chapter can be referred to for more detailed information. Any methods unique to a chapter are outlined fully within the chapter's method section.

Chapter 4. Short-term cardiovascular responses to slow and deep breathing in healthy individuals

4.1 Introduction

The main section of this chapter (4.2) has been prepared as a manuscript as it is intended for publication in European Journal of Applied Physiology. It is presented in the next section as part of the integrated thesis format submission and the supplementary information for the publication is included in section 4.3. Full methodology, including reliability and validity of equipment, procedures and analysis can be found in Chapter 3.

The research questions, objectives and hypothesis for this chapter are outlined below:

Research question

1. Are there differences in the acute cardiovascular responses to an existing SDB device (RESPeRATE) compared with alternative SDB delivery methods?

Objectives

1. Identify whether mechanism-related parameters (e.g. respiratory sinus arrythmia, stroke volume, cardiac output, blood pressures) respond similarly during SDB delivered using an existing device (RESPeRATE) compared with alternative SDB delivery methods.
2. Test a novel method of analysis, which uses peak-valley methods to investigate changes in the amplitude of cardiovascular oscillations.

Hypothesis

1. Alternative SDB delivery methods will produce the same acute cardiovascular responses as RESPeRATE.
2. Peak-valley analysis of cardiovascular oscillations will reveal larger amplitude perturbations than more established methods.

4.2 Integrated paper: Acute cardiovascular responses to slow and deep breathing in normotensive men and women

Introduction

Daily practice of slow and deep breathing (SDB; ≤10 breaths·min^{-1}) has been recommended by the American Heart Association as an effective treatment for hypertension (Brook et al. 2013). Specifically, the RESPeRATE device, which reduces breathing frequency using auditory tones, has been researched extensively as a long-term intervention to reduce blood pressure (BP) in hypertensive individuals (Viskoper et al. 2003; Landman et al. 2013). A recent meta-analysis (Chaddha et al. 2019) found SDB interventions induced a significant reduction of -5.62 mmHg and -2.97 mmHg in systolic BP (SBP) ad diastolic BP (DBP) respectively.

Despite the apparent health benefits associated with SDB, there is a lack of information relating to the mechanism(s) underlying its antihypertensive effect (Gerritsen and Band 2018). Accordingly, these mechanisms remain poorly understood and there is a limited understanding of acute cardiovascular interactions during SDB, including any potential error signal(s) that might underpin its anti-hypertensive effect.

Additionally, those studies that have investigated the mechanistic role of SDB in reducing BP have either excluded women or have not compared the responses of men and women. For example, Yepryntseva and Shekh (2019) included only male participants, whereas Anderson et al. (2009) studied a mixed participant group of men and women (men = 18, women = 26), but used total group analysis for the results, failing to compare results in men and women. In a subsequent paper, Anderson and colleagues (2010) did examine sex differences but in chronic BP changes following SDB, finding reductions in 24-hour BP in women, but not in men.

There are differences between the size, structure and mechanics of the ribcage and lungs of men and women (Sheel et al. 2016), which may influence cardiorespiratory interactions during SDB. For instance, during normal spontaneous breathing women predominantly breathe with their ribcage rather than their diaphragm (LoMauro and Aliverti 2018). It has been suggested that the health benefits associated with SDB are related to diaphragmatic breathing (Gerritsen and Band 2018), which may be promoted during SDB. Thus, men may be more likely to benefit from SDB, due to their propensity to breathe diaphragmatically.

Furthermore, although spontaneous breathing frequencies are similar between men and women, there are differences in BP regulation between the sexes. Specifically, breathing frequency is correlated with cardiac output, heart rate and total peripheral resistance in men, but not correlated in women (Wallin et al. 2010). Additionally, the autonomic response to SDB is different between the sexes (Nili et al. 2017) and different physiological mechanisms are used to maintain normal BP in men and women (Hart et al. 2009). For example, total peripheral resistance and cardiac output were not related to sympathetic activity in women, but had a significant relationship in men, suggesting differences in BP regulation from modulation of sympathetic activity. It is therefore conceivable that sex differences in the interrelationship of the respiratory and cardiovascular systems, as well as sex differences in the physiological mechanisms controlling BP regulation, might result in women responding differently to SDB than men (Anderson et al. 2010).

Recent debate about the appropriate analysis of cardiovascular variability suggests that multi-parametric approaches to analysing multiple variables are needed to provide a more complete picture of the dynamics of cardiovascular variability (Castiglioni and Parati 2011). Previous research has taken a singular approach to the cardiovascular responses during SDB, such as Calcaterra and colleagues who have investigated the acute effects of baroreflex sensitivity and arterial function (pulse wave velocity and augmentation index) following SDB but in separate research studies (Calcaterra et al. 2013; Calcaterra et al. 2014). Since breathing-related fluctuations in variables such as stroke volume and BP are pre-requisites to the generation of any error signal that underpins anti-hypertensive effects of SDB, the present study measured the instantaneous, multi-parameter haemodynamic responses to SDB using RESPeRATE. In addition, responses to RESPeRATE were compared with those of two other SDB conditions, 1) a fixed frequency of 6 breaths·min^{-1}, 2) a dynamic algorithm that maximised respiratory sinus arrhythmia (RSA).

The aim of the present study was to characterise the acute cardiovascular responses to SDB using a number of variables and applying a multi-parametric approach. The responses were compared across different SDB conditions (RESPeRATE, fixed breathing frequency and dynamic algorithm).

Methods

Ethics Approval

The experimental protocol was approved by Bournemouth University's Research Ethics Committee and all experiments conformed to the Declaration of Helsinki. Written informed consent was obtained from all participants prior to participating in the study.

Participants

Twelve participants took part in the study (6 males & 6 females). All participants were non-smokers with no current diagnosis of cardiovascular or respiratory disease. No participants were pregnant at the time of taking part. Participants refrained from eating for 2 hours and from caffeine, strenuous exercise and alcohol for 12 hours prior to data collection.

Slow and Deep Breathing Protocol

Participants completed three controlled breathing conditions and one spontaneous breathing condition in a randomised order. All breathing conditions were 10 minutes in duration with a 10-minute period of normal breathing prior to each measurement. A 10-minute intervention has been used in previous studies of daily SDB using RESPeRATE (Chaddha et al. 2019). Participants rested at baseline for 5 minutes prior to starting the first breathing condition to ensure cardiovascular variables were in a resting state. During the spontaneous breathing condition (Sf_r), participants were instructed to breathe normally and no visual feedback was provided to control breathing. The three SDB conditions were 1) RESPeRATE (Rf_r), 2) a dynamic algorithm driven by RSA (Df_r) and 3) a fixed breathing frequency of 6 breaths·min^{-1} (6Ff_r).

The RESPeRATE device gradually lowers breathing frequency as users breathe in time with a fluctuating musical tone. Breathing frequency is reduced to ≤10 breaths·min^{-1} and is measured using a belt worn around either the chest or upper abdomen. A full description of RESPeRATE can be found in Gavish (2010) and Cernes & Zimlichman (2017). Participants completed the dynamic breathing frequency condition (Df_r) using a novel, bespoke algorithm that guided breathing dynamically to a personalised frequency. The algorithm created a dynamically driven breathing frequency, which strived to maximise cardiovascular perturbation, using the amplitude of RSA as the controlled variable. The algorithm used data measured from a finger sensor (photoplethysmography), which tracked the user's instantaneous physiological responses to their breathing. The finger sensor was connected via the headphone socket of an iPad.

As the optimal SDB frequency is widely regarded in the literature to be 6 breaths·min^{-1} (Cullins et al. 2013; Russo et al. 2017); accordingly, a final condition of 6 breaths·min^{-1} (6Ff_r) was included. Both the dynamic algorithm and 6 breaths·min^{-1} conditions were delivered by Bournemouth University's Brythm app. Brythm provides visual feedback, displayed on an iPad screen, to guide the user's breathing frequency, whereby the user inhales when the dome graphic rises and exhales when the dome falls (Figure 4-1).

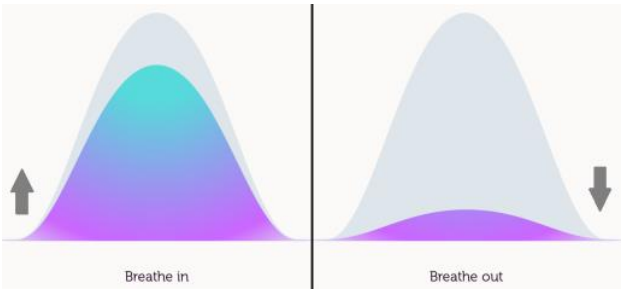


Figure 4-1 Screenshots of Brythm graphic
N.B: Arrows do not appear on app but are shown here to display the direction of graphic movement.

Data Acquisition

Participants were seated in an upright position, at an approximate angle of 60° for the duration of the data collection. Respiratory airflow was monitored continuously throughout each breathing condition. Participants wore an oronasal mask that covered both mouth and nose (Oro Nasal 7450 V2 Mask, Hans Rudolph Inc., Kansas, USA) and respired flow rate was measured continuously using a heated pneumotachograph (Model 3700, Hans Rudolph Inc., Kansas, USA) connected to a flow measurement system (RSS 100-HR, Hans Rudolph Inc., Kansas, USA).

Heart rate (f_c) was monitored continuously using a 3-lead ECG and non-invasive beat-to-beat arterial BP was estimated using a Finometer (Finapres NOVA, Finapres Medical Systems, The Netherlands). The finger cuff derived BP was calibrated using an arm cuff prior to and halfway through data collection. Stroke volume (SV) was calculated by the Finometer using the Modelflow method. Total peripheral resistance (TPR) was calculated as mean arterial pressure divided by cardiac output ($\dot{Q}$). Peripheral pulse transit time (PTT) was calculated from the time delay between the peak of the R wave of the ECG and the peak of the pressure pulse recorded at the finger. End-tidal CO_2 was recorded at the end of each minute using an iWorx CO_2/O_2 Gas Analyzer (GA-200, New Hampshire, USA).

Analogue outputs from the Finapres NOVA (reconstructed brachial pressure waveform, ECG waveform, SV, SBP, DBP) and the respiratory flow meter were sampled continuously at 250Hz via an analogue to digital converter (NI USB-6218 BNC, National

Instruments Inc.) and captured using bespoke acquisition and analysis software (LabView 2015, National Instruments, Inc.). The LabView software corrected for the 4 second delay between the Finapres NOVA output and the respiratory output. Data were recorded during the baseline period (5 minutes), and during each breathing condition (10 minutes; Sf_r, Rf_r, 6Ff_r, Df_r).

Data Analysis

Within the bespoke LabView software, cardiovascular and respiratory parameters were derived breath-by-breath, and minimum, maximum and mean values were calculated for every inhalation and exhalation. Data were calculated in epochs of one-minute, first 5- and final 5-min and the full 10-min for each condition. Data were compared for the three SDB conditions (Rf_r, 6Ff_r, Df_r) and spontaneous breathing (Sf_r).

Respiratory sinus arrhythmia (RSA) was calculated using two methods 1) the difference between the average heart rate (f_c) during inhalation (f_ci) and exhalation (f_ce) ($f_c\Delta$); 2) the difference in maximum and minimum beat-to-beat intervals (RR) during inhalation and exhalation respectively (RSA). RSA is a variable calculated to determine the amplitude of heart rate rhythms using the 'peak-valley' method and in this study the peak-valley method was used to analyse all variables including BP.

Calculated parameters and their derivation are displayed schematically using a sinewave in Figure 4-2 (with corresponding calculation numbers). Inter-breath phase indices (Δ) were quantified as the difference between mean inspiration (i) and mean expiration (e) values for all variables (calculation 4). Peak-valley (PV) indices were calculated as maximum minus minimum values during inspiration (Δi: calculation 6) and expiration (Δe: calculation 5). Inter-breath phase PV indices (ΔPV) were calculated using maximum inspiration minus minimum expiration, or minimum inspiration minus maximum expiration, dependent on which calculation gave the largest difference. Calculation 7 shows an example using the calculation maximum inspiration minus minimum expiration. PV indices irrespective of breath phase, known as peak-valley breath phase independent calculations (ΔPV_Ind), were calculated as the difference between the maximum and minimum values, irrespective of the breath phase in which they occurred (not shown in Figure 4-2).

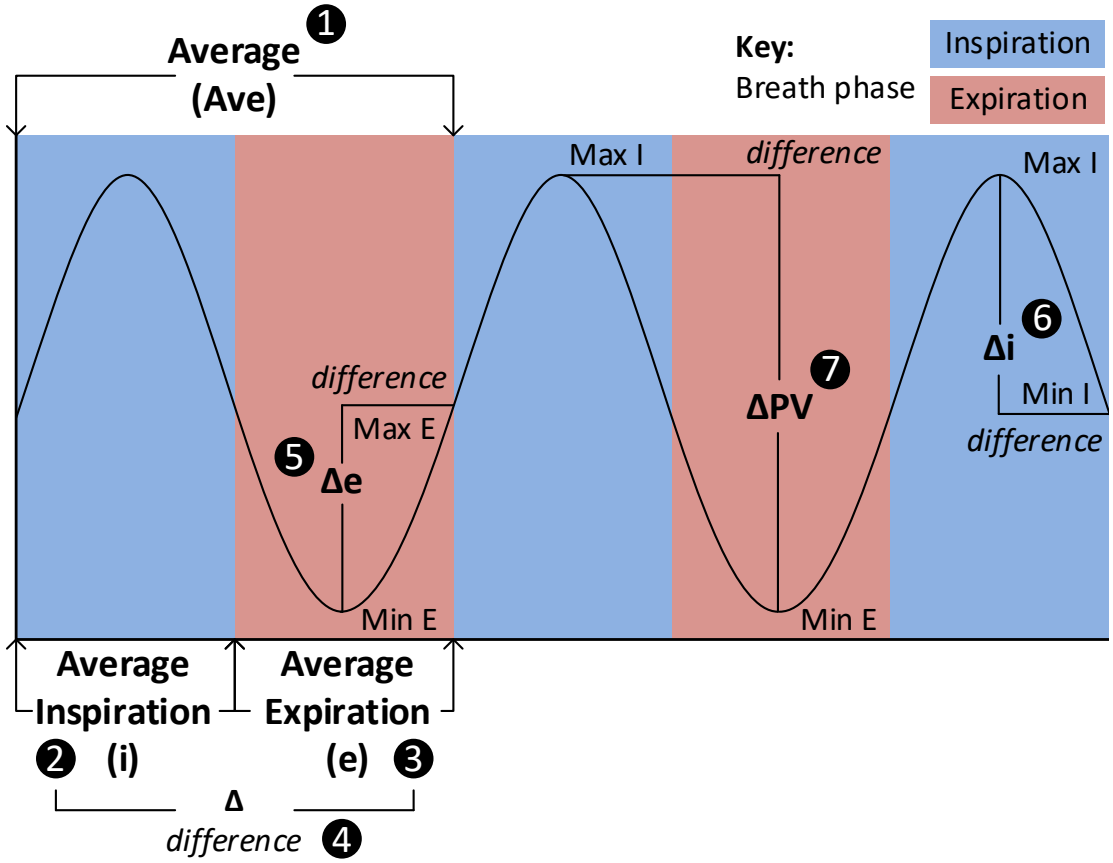


Figure 4-2 Calculations for example cardiovascular variable plot
1) Ave = average of whole breath. 2) i = Average inspiration. 3) e = Average expiration. 4) Δ = i minus e (average inspiration minus average expiration). 5) Δe = Max E minus Min E. 6) Δi = Max I minus Min I. 7) ΔPV = Max I minus Min E (Note ΔPV calculation varies and can be Min I minus Max E depending on which calculation provides largest difference).

Each condition was 10 minutes in duration but the final 5-minute epochs of each SDB condition (Rf_r, 6Ff_r, Df_r) were used for analysis to ensure steady state values were analysed. For spontaneous breathing (Sf_r), the first 5-minute epoch was used, as participants were already in a steady state. Dynamic breathing frequencies were also compared across the full 10-minute condition and between the first- and final-5 minutes.

Values are expressed as means ± SD unless stated otherwise. Statistical analysis was undertaken using SPSS Statistics 24 (IBM Corp.). After normality was confirmed for cardiovascular variables, repeated measures ANOVA with planned pairwise comparisons using Bonferroni corrections were used. Independent samples t-test were used to test for baseline sex differences. Reported p values are those following adjustment for repeated comparisons. For all analyses, *P* was set at 0.05. Due to the large amount of data, additional results (not focused on in this paper) can be viewed in the online supplementary information (calculations 1-4 in Figure 4-2). Where significant differences are stated between breathing conditions, these are calculated using combined male and female data, unless stated otherwise.

Results

Data were collected from 12 participants, but 1 participant was excluded due to failure to adhere to the prescribed breathing conditions. Data for five males and six females were analysed and full descriptive statistics can be seen in Table 4-1. Due to missing data from the Sf_r condition for 2 participants, data from baseline spontaneous measurements were used in place of Sf_r data for these 2 participants, to ensure adequate power was maintained. Before doing so, data integrity checks were performed to ensure the substitution did not affect the study results. Furthermore, for all other participants (n=9), it was confirmed that breathing frequency was not significantly different between baseline and the first 5-min Sf_r condition. There were no significant differences between the baseline data and the first 5-min Sf_r condition for mechanistically meaningful variables.

Table 4-1 Participant characteristics

	Female	**Male**	***P* value**
	n = 6	*n = 5*	
Age (years)	42.0 ± 10.1	40.4 ± 15.9	0.844
Stature (m)	1.66 ± 0.06	1.76 ± 0.04	0.013*
Mass (kg)	71.5 ± 10.9	75.4 ± 9.3	0.546
BMI (kg/m^2)	26.2 ± 5.5	24.4 ± 2.3	0.500
Baseline SBP (mmHg)	118.3 ± 11.4	118.0 ± 8.6	0.958
Baseline DBP (mmHg)	72.2 ± 11.4	69.8 ± 7.0	0.696
Baseline f_r (breaths·min^{-1})	12.5 ± 2.8	12.0 ± 2.8	0.750
Baseline Tidal Volume (L)	0.5 ± 0.2	0.6 ± 0.1	0.472

*Body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), breathing frequency (f_r); *significant difference between groups.*

Respiratory variables

Table 4-2 provides an overview of the respiratory parameters for each condition. There were no significant differences between males and females for any respiratory variables. Breathing frequency during Sf_r was significantly different from all SDB conditions but frequency during SDB conditions were not significantly different from each other. The dynamic algorithm (Df_r) computed the optimal breathing frequency to be 5.5 ± 1.3 breaths·min^{-1} and maintained a steady SDB frequency throughout the 10 minutes with no difference in breathing frequency between first 5- and final 5-min. Whereas RESPeRATE (Rf_r) averaged 6.4 ± 1.9 breaths·min^{-1} during the final 5 minutes, but produced a significantly higher frequency during the first 5 minutes (Figure 4-3; 8.1 breaths·min^{-1}; p=0.02). There was no significant difference in end-tidal CO_2 between any conditions (Table 4-2).

Table 4-2 Respiratory parameters

		S*fr*	R*fr*	6F*fr*	D*fr*	Effect of condition *P* value	Sex x Condition *P* value
fr	*Female*	12.2 ± 4.7	6.0 ± 1.2	6.0 ± 0.1	5.8 ± 1.7		
	Male	12.3 ± 2.5	7.0 ± 2.5	6.0 ± 0.0	5.2 ± 0.4		
	All	**12.3 ± 3.7¥†¤**	**6.4 ± 1.9***	**6.0 ± 0.0***	**5.5 ± 1.3***	<0.001	0.735
V_T	*Female*	0.6 ± 0.2	1.2 ± 0.4	0.9 ± 0.5	1.1 ± 0.5		
	Male	0.6 ± 0.1	1.0 ± 0.4	0.9 ± 0.2	1.1 ± 0.4		
	All	**0.6 ± 0.2¥†¤**	**1.1 ± 0.4***	**0.9 ± 0.3***	**1.1 ± 0.4***	<0.001	0.621
T_I / T_{TOT}	*Female*	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1		
	Male	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.4	0.5 ± 0.0		
	All	**0.4 ± 0.0**	**0.5 ± 0.1**	**0.6 ± 0.3**	**0.5 ± 0.1**	0.129	0.569
End-tidal CO_2	*Female*	4.7 ± 0.6	4.4 ± 0.7	4.7 ± 0.7	4.7 ± 0.8		
	Male	5.3 ± 0.5	5.1 ± 0.5	5.2 ± 0.5	5.3 ± 0.6		
	All	**5.0 ± 0.6**	**4.8 ± 0.7**	**5.0 ± 0.6**	**5.0 ± 0.7**	0.535	0.167

Data represent mean ± SD (female n = 6, male n = 5); Spontaneous breathing (Sf_r), RESPeRATE (Rf_r), 6 breaths·minute^{-1} ($6Ff_r$), optimisation algorithm dynamic breathing frequency (Df_r); Breathing frequency (f_r; in breaths·min^{-1}), tidal volume (V_T; L), duty cycle (T_I/T_{TOT}), end-tidal CO_2 (%); Significantly different from Sf_r (*); Rf_r (¥), $6Ff_r$ (†), Df_r (¤); P<0.05.

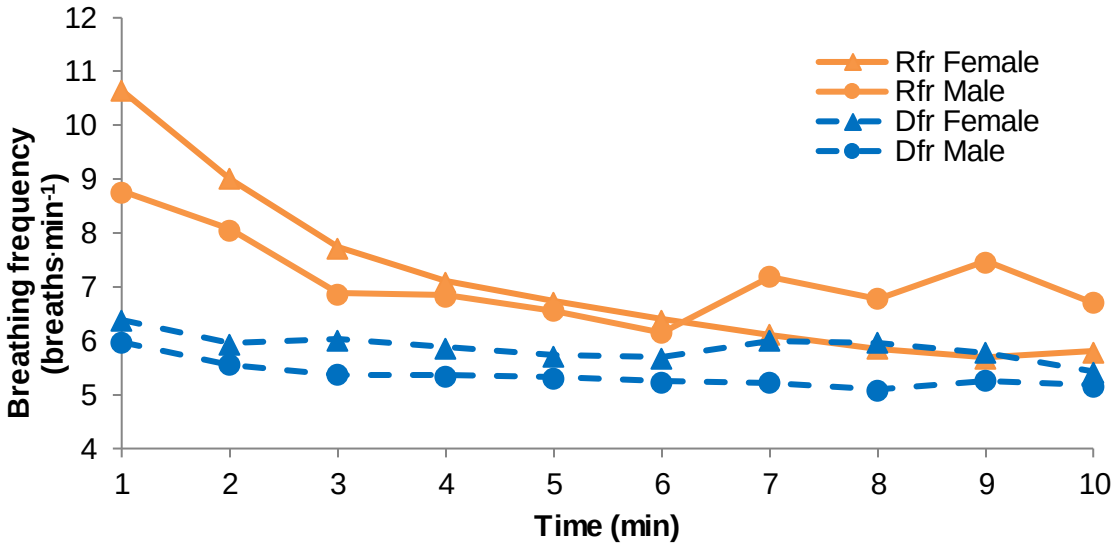


Figure 4-3 Breathing frequency during RESPeRATE (Rf_r) and dynamic breathing frequency (Df_r) conditions
Solid line RESPeRATE (Rf_r), dashed line dynamic algorithm (Df_r); Circle data points - male; Triangle data points - female; Data points represent the average value for the preceding minute (1 min epoch) i.e. data point at 5 min represents average breathing frequency between 4-5min.

Arterial blood pressures

There were no significant differences between males and females for any BP variables. When combining male and female data there were no significant differences for average SBP or DBP between breathing conditions (see supplementary information for data),

however peak-valley amplitude was significant different between Sf_r and all SDB conditions (Table 4-3). All SDB conditions were significantly different from Sf_r for SBPΔi and SBPΔe and between Sf_r and Df_r and 6Ff_r for SBPΔPV. This was reflected in the equivalent DBP values. Peak-valley breath phase independent values (ΔPV _Ind) revealed larger changes for SBP and DBP than peak-valley values (ΔPV).

Table 4-3 Peak-valley differences (±SD) for blood pressure variables (mmHg)

		Sf_r	Rf_r	6Ff_r	Df_r	Effect of condition *P* value	Sex x Condition *P* value
SBPΔi	*F*	3.6 ± 2.6	11.7 ± 3.5	10.5 ± 2.5	11.7 ± 4.5		
	M	3.0 ± 1.6	10.0 ± 5.6	9.5 ± 5.0	11.1 ± 3.6		
	All	**3.4 ± 2.1¥†¤**	**10.9 ± 4.4***	**10.0 ± 3.7***	**11.4 ± 3.9***	<0.001	0.979
SBPΔe	*F*	4.7 ± 2.6	10.0 ± 2.4	10.6 ± 2.1	10.9 ± 4.3		
	M	3.5 ± 2.3	7.0 ± 6.1	6.6 ± 3.2	9.0 ± 5.7		
	All	**4.2 ± 2.4¥†¤**	**8.6 ± 4.5***	**8.8 ± 3.3***	**10.0 ± 4.8***	<0.001	0.611
SBPΔPV	*F*	-9.2 ± 4.1	-6.8 ± 16.0	-5.4 ± 15.8	-11.5 ± 11.8		
	M	-6.6 ± 3.5	-14.3 ± 8.6	-16.1 ± 6.7	17.9 ± 8.3		
	All	**-8.0 ± 3.9**	**-10.2 ± 13.1**	**-10.3 ± 13.2**	**-14.4 ± 10.4**	0.267	0.251
SBPΔPV _Ind	*F*	13.4 ± 3.3	15.9 ± 3.3	17.2 ± 3.9	15.7 ± 5.2		
	M	12.4 ± 3.6	16.2 ± 6.9	17.3 ± 5.1	19.5 ± 7.8		
	All	**12.9 ± 3.3†¤**	**16.0 ± 4.9**	**17.3 ± 4.3***	**17.4 ± 6.5***	0.001	0.150
DBPΔi	*F*	1.9 ± 1.0	7.1 ± 2.9	7.2 ± 2.4	7.6 ± 2.8		
	M	1.0 ± 0.4	5.0 ± 2.8	4.1 ± 1.3	5.3 ± 1.5		
	All	**1.5 ± 0.9¥†¤**	**6.1 ± 2.9***	**5.8 ± 2.5***	**6.6 ± 2.5***	<0.001	0.635
DBPΔe	*F*	2.9 ± 1.1	6.1 ± 2.8	7.2 ± 2.7	6.4 ± 2.1		
	M	1.7 ± 0.8	3.8 ± 2.1	3.5 ± 1.8	4.3 ± 2.3		
	All	**2.4 ± 1.1†¤**	**5.1 ± 2.7**	**5.5 ± 2.9***	**5.4 ± 2.3***	0.001	0.463
DBPΔPV	*F*	-4.2 ± 1.7	-1.4 ± 10.8	1.2 ± 10.3	-4.8 ± 8.3		
	M	-1.2 ± 1.8	-6.5 ± 3.6	-6.7 ± 2.5	-8.0 ± 3.3		
	All	**-2.8 ± 2.3**	**-3.7 ± 8.4**	**-2.4 ± 8.5**	**-6.2 ± 6.4**	0.292	0.096
DBPΔPV _Ind	*F*	7.7 ± 1.4	9.9 ± 3.1	10.6 ± 1.9	9.4 ± 1.3		
	M	6.2 ± 0.2	7.8 ± 2.0	7.7 ± 1.6	9.2 ± 2.6		
	All	**7.0 ± 1.3¤**	**9.0 ± 2.7**	**9.3 ± 2.3***	**9.3 ± 1.9***	0.007	0.288

Data represent mean ± SD (female n = 6, male n = 5); Female (F), Male (M); Spontaneous breathing (Sf$_r$), RESPeRATE (Rf$_r$), 6 breaths·minute^{-1} (6Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$); systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf$_r$ (*); Rf$_r$ (¥), 6Ff$_r$ (†), Df$_r$ (¤); P<0.05.

There were high correlations (>0.8) between SBPΔi and SBP and between SBPΔe and SBP and the DBP equivalents across all breathing conditions. Therefore, percentage change BP oscillations were calculated during inspiration and expiration, producing relative intra-breath phase peak-valley differences (relative Δi and Δe). There were significant differences for all percentage BP oscillations during all SDB variables compared with Sf_r. There were also significant differences for SBP%Δi, SBP%Δe and DBP%Δi between first 5- and final 5-min for Rf_r, but only for SBP%Δi during the Df_r condition, with a larger amplitude of fluctuations in the final 5-min for all variables.

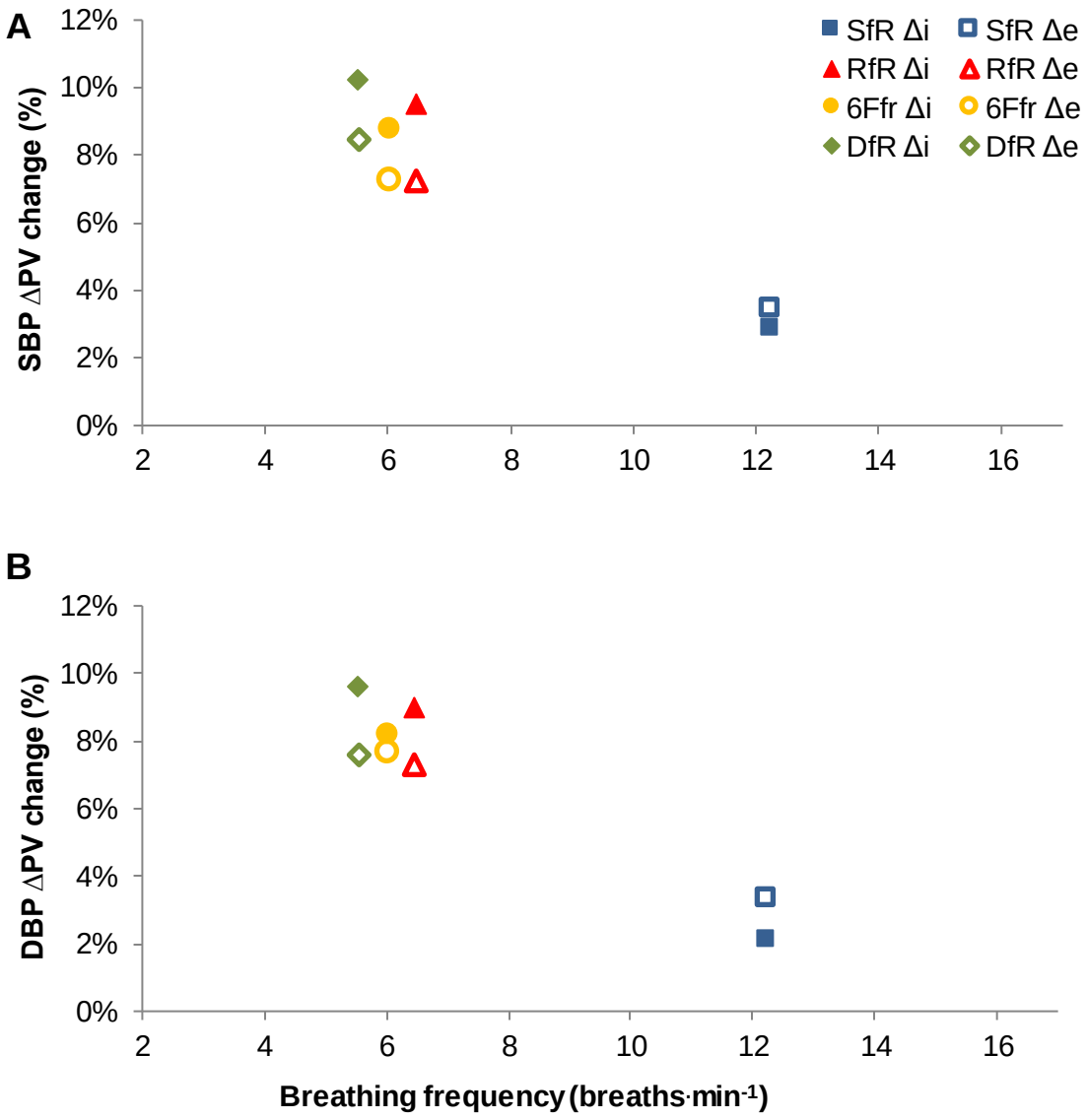


Figure 4-4 Blood pressure oscillations: Relative change of ΔI and ΔE for systolic blood pressure (A) and diastolic blood pressure (B)

Systolic blood pressure (SBP), diastolic blood pressure (DBP); within inspiration difference (Δi), within expiration difference (Δe); Spontaneous breathing (Sf_r), RESPeRATE (Rf_r), 6 breaths·minute^{-1} (6Ff_r), optimisation algorithm dynamic breathing frequency (Df_r). Variable calculated as SBPΔi as a percentage of average SBP during inspiration, or equivalent during expiration and for DBP.

Heart rate and respiratory sinus arrythmia

Average heart rate was significantly higher during 6Ff_r and Df_r, compared with Sf_r, but not during Rf_r (Sf_r 58.6 ± 8.5; Rf_r 60.6 ± 8.5; 6Ff_r 62.4 ± 9.0; Df_r 62.3 ± 9.4 beats·min^{-1}). Whereas, Rf_r and 6Ff_r were significantly different from Sf_r for f_cΔi. Additionally, the amplitude of RSA was significantly different from Sf_r for Rf_r (p=0.05) and Df_r (p=0.018), but not for 6Ff_r (p=0.130; Figure 4-5).

Table 4-4 Mean (±SD) peak-valley differences for heart rate (fc) and respiratory sinus arrythmia (RSA)

		Sf_r	Rf_r	6Ff_r	Df_r	Effect of condition *P* value	Sex x Condition *P* value
	F	4.3 ± 2.5	10.9 ± 5.0	13.4 ± 7.5	13.6 ± 10.3		
$f_c\Delta i$	*M*	2.6 ± 2.9	7.2 ± 4.8	9.3 ± 5.9	9.4 ± 5.3		
	All	**3.5 ± 2.7¥†**	**9.2 ± 5.1***	**11.5 ± 6.8***	**11.7 ± 8.3**	0.004	0.741
	F	6.5 ± 3.9	7.6 ± 4.3	11.6 ± 5.5	9.6 ± 3.7		
$f_c\Delta e$	*M*	3.2 ± 2.2	6.4 ± 3.2	10.3 ± 6.3	10.4 ± 6.5		
	All	**5.0 ± 3.5†**	**7.1 ± 3.7†**	**11.0 ± 5.6*¥**	**10.0 ± 4.9**	<0.001	0.477
	F	-2.1 ± 7.7	11.5 ± 10.6	8.2 ± 17.1	14.2 ± 13.3		
$f_c\Delta PV$	*M*	-1.1 ± 6.8	9.2 ± 7.4	10.6 ± 11.3	13.2 ± 8.1		
	All	**-1.7 ± 7.0**	**10.4 ± 8.9**	**9.3 ± 14.1**	**13.7 ± 10.7**	0.021	0.963
	F	0.09 ± 0.04	0.16 ± 0.05	0.14 ± 0.08	0.15 ± 0.04		
RSA (s)	*M*	0.13 ± 0.13	0.22 ± 0.15	0.26 ± 0.19	0.27 ± 0.17		
	All	**0.11 ± 0.09¥¤**	**0.18 ± 0.10***	**0.20 ± 0.14**	**0.21 ± 0.13***	0.001	0.284

Data represent mean ± SD (female n = 6, male n = 5); Female (F), Male (M); Spontaneous breathing (Sf$_r$), RESPeRATE (Rf$_r$), 6 breaths·minute^{-1} (6Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$); heart rate (f_c; beats·min^{-1}), respiratory sinus arrythmia (RSA; s); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV); Significantly different from Sf$_r$ (*), Rf$_r$ (¥), 6Ff$_r$ (†), Df$_r$ (¤); P<0.05.

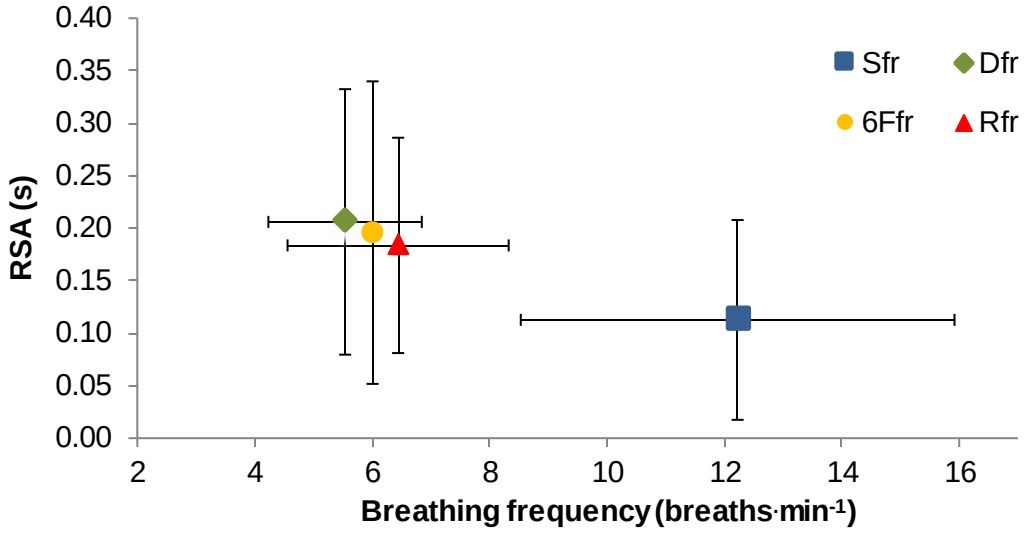


Figure 4-5 Respiratory sinus arrythmia (RSA) response to slow and deep breathing

Data represent mean ± SD (n=11); Spontaneous breathing (Sf_r), RESPeRATE (Rf_r), 6 breaths·minute^{-1} ($6Ff_r$), optimisation algorithm dynamic breathing frequency (Df_r); respiratory sinus arrythmia (RSA; s).

Stroke volume and cardiac output

There was a significant effect of condition upon SVΔi and SVΔe, but paired comparisons revealed no significant differences between breathing conditions (Table 4-5). Intra-breath phase cardiac output ($\dot{Q}$) increased during SDB significantly and was significantly different from Sf_r for $6Ff_r$ for Δi and Δe, and for Df_r for Δi.

Table 4-5 Mean (±SD) peak-valley differences for stroke volume (SV) and cardiac output ($\dot{Q}$)

		Sf_r	Rf_r	6Ff_r	Df_r	Effect of condition *P* value	Sex x Condition *P* value
SVΔi	*F*	5.3 ± 2.5	8.5 ± 3.8	9.5 ± 5.4	10.3 ± 6.2		
	M	5.2 ± 1.0	9.8 ± 8.7	11.2 ± 6.1	10.1 ± 5.6		
	All	**5.3 ± 1.9**	**9.1 ± 6.1**	**10.3 ± 5.5**	**10.2 ± 5.6**	0.006	0.895
SVΔe	*F*	6.7 ± 3.0	9.9 ± 3.9	9.3 ± 1.1	11.1 ± 5.0		
	M	5.7 ± 2.0	7.7 ± 4.3	8.0 ± 4.2	8.2 ± 5.5		
	All	**6.3 ± 2.5**	**8.9 ± 4.0**	**8.7 ± 2.8**	**9.8 ± 5.2**	0.025	0.816
SVΔPV	*F*	-10.4 ± 3.3	-13.7 ± 3.3	-8.5 ± 12.4	-14.2 ± 4.5		
	M	-10.5 ± 4.0	-14.9 ± 10.6	-17.9 ± 9.9	-14.9 ± 9.1		
	All	**-10.4 ± 3.5**	**-14.2 ± 7.1**	**-12.8 ± 11.9**	**-14.5 ± 6.6**	0.384	0.248
SVΔPV_Ind	*F*	11.2 ± 2.7	11.1 ± 2.6	12.1 ± 3.3	13.0 ± 4.8		
	M	14.8 ± 3.4	14.4 ± 8.2	17.2 ± 9.6	14.3 ± 7.0		
	All	**12.8 ± 3.4**	**12.6 ± 5.8**	**14.4 ± 7.1**	**13.6 ± 5.6**	0.440	0.527
$\dot{Q}$Δi	*F*	363.0 ± 301.2	878.2 ± 463.5	943.6 ± 474.3	1042.4 ± 694.5		
	M	304.2 ± 134.2	937.0 ± 760.7	1186.5 ± 764.9	1119.4 ± 734.7		
	All	**336.3 ± 231.3†¤**	**904.9 ± 583.0**	**1054.0 ± 602.1***	**1077.4 ± 677.3***	<0.001	0.820
$\dot{Q}$Δe	*F*	517.8 ± 452.7	821.2 ± 485.3	860.1 ± 363.3	760.8 ± 449.2		
	M	415.9 ± 113.7	686.7 ± 275.4	1020.5 ± 447.2	967.8 ± 535.2		
	All	**471.5 ± 332.3†**	**760.0 ± 391.2**	**933.1 ± 391.2***	**854.9 ± 476.6**	<0.001	0.209
$\dot{Q}$ΔPV	*F*	-751.2 ± 337.6	719.2 ± 1015.5	281.3 ± 1187.0	486.6 ± 1200.3		
	M	-496.7 ± 754.3	-62.8 ± 1259.1	-105.1 ± 1727.6	27.1 ± 1508.6		
	All	**-635.6 ± 549.8**	**363.8 ± 1147.4**	**105.7 ± 1392.5**	**277.8 ± 1299.4**	0.083	0.506
$\dot{Q}$ΔPV_Ind	*F*	842.6 ± 344.6	1034.6 ± 560.9	1086.2 ± 474.4	941.9 ± 584.6		
	M	1010.5 ± 196.8	1112.9 ± 514.1	1485.6 ± 699.5	1368.3 ± 746.6		
	All	**918.9 ± 287.3**	**1070.2 ± 514.5**	**1267.7 ± 593.2**	**1135.7 ± 665.9**	0.037	0.246

Data represent mean ± SD (female n = 6, male n = 5); Female (F), Male (M); Spontaneous breathing (Sf_r), RESPeRATE (Rf_r), 6 breaths·minute^{-1} (6Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); stroke volume (SV; ml), cardiac output ($\dot{Q}$; ml·min^{-1}); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf_r (*); Rf_r (¥), 6Ff_r (†), Df_r (¤); P<0.05.

Total peripheral resistance and pulse transit time

In keeping with the pattern of hemodynamic responses, intra-breath phase total peripheral resistance (TPR) and peripheral transit time (PTT) increased during both phases of respiration (Table 4-6).

Table 4-6 Mean (±SD) peak-valley differences for total peripheral resistance (TPR) and pulse transit time (PTT)

		Sf_r	Rf_r	6Ff_r	Df_r	Effect of condition *P* value	Sex x Condition *P* value
TPRΔi	*F*	1.4 ± 1.2	2.3 ± 1.0	2.3 ± 1.2	2.7 ± 1.8		
	M	1.5 ± 0.6	3.1 ± 1.8	4.3 ± 2.2	3.9 ± 1.9		
	All	**1.4 ± 1.0†**	**2.7 ± 1.4**	**3.2 ± 1.9***	**3.2 ± 1.8**	0.001	0.176
TPRΔe	*F*	1.9 ± 1.7	2.7 ± 1.5	2.7 ± 1.1	2.3 ± 1.4		
	M	2.0 ± 0.6	3.2 ± 1.8	4.5 ± 2.4	4.7 ± 2.8		
	All	**2.0 ± 1.2**	**3.0 ± 1.6**	**3.5 ± 1.9**	**3.4 ± 2.4**	0.004	0.058
TPRΔPV	*F*	-0.1 ± 2.8	-1.7 ± 3.5	-1.4 ± 3.4	-1.8 ± 3.7		
	M	2.3 ± 3.6	-2.0 ± 4.6	-1.3 ± 6.4	-5.7 ± 2.9		
	All	**1.0 ± 3.3¤**	**-1.8 ± 3.9**	**-1.4 ± 4.7**	**-3.6 ± 3.7***	0.037	0.284
TPRΔPV_Ind	*F*	3.1 ± 1.6	3.2 ± 1.5	3.5 ± 1.7	2.9 ± 1.8		
	M	4.9 ± 1.1	4.7 ± 1.0	5.5 ± 2.1	5.6 ± 2.1		
	All	**3.9 ± 1.6**	**3.9 ± 1.0**	**4.4 ± 2.1**	**4.2 ± 2.3**	0.190	0.180
PTTΔi	*F*	11 ± 8	14 ± 4	17 ± 7	16 ± 8		
	M	9 ± 4	19 ± 10	22 ± 10	27 ± 17		
	All	**10 ± 6†**	**16 ± 7**	**19 ± 9***	**21 ± 13**	<0.001	0.104
PTTΔe	*F*	12 ± 8	15 ± 9	18 ± 7	16 ± 5		
	M	10 ± 3	23 ± 11	28 ± 15	33 ± 23		
	All	**11 ± 6¥†**	**19 ± 10***	**23 ± 12***	**23 ± 17**	0.001	0.043
PTTΔPV	*F*	16 ± 10.0	9 ± 18	10 ± 25.0	21 ± 6		
	M	16 ± 6.0	25 ± 10	34 ± 14.3	10 ± 45		
	All	**16 ± 8.0**	**16 ± 17**	**21 ± 23.3**	**16 ± 29**	0.750	0.251
PTTΔPV_Ind	*F*	17 ± 9	16 ± 8	21 ± 8	17 ± 6		
	M	24 ± 9	24 ± 7	32 ± 12	32 ± 18		
	All	**80 ± 9**	**19 ± 8**	**26 ± 11**	**24 ± 14**	0.076	0.480

Data represent mean ± SD (female n = 6, male n = 5); Female (F), Male (M); Spontaneous breathing (Sf_r), RESPeRATE (Rf_r), 6 breaths·minute^{-1} (6Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); total peripheral resistance (TPR; mmHg·min·L^{-1}); pulse transit time (PTT; ms); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf_r (*); Rf_r (¥), 6Ff_r (†), Df_r (¤); P<0.05.

Peak-valley (ΔPV) and peak-valley breath phase independent (ΔPV_Ind)

Comparison of peak-valley values (ΔPV; highest difference between min/max inspiration and expiration; Calculation 7 Figure 4-2) and peak-valley breath phase independent values (ΔPV_Ind; highest difference across breath irrespective of breath phase) reveals

a clear difference in magnitude for some variables, such as SBP. Figure 4-6 shows the last minute of the 6Ff_r condition for 1 female participant; there was synchronisation between respiratory flow and heart rate (A), but asynchrony between inspiratory flow and BP (B). As such, when peak-valley calculations are analysed larger differences are seen when breath phase is excluded from analysis (breath phase independent variables).

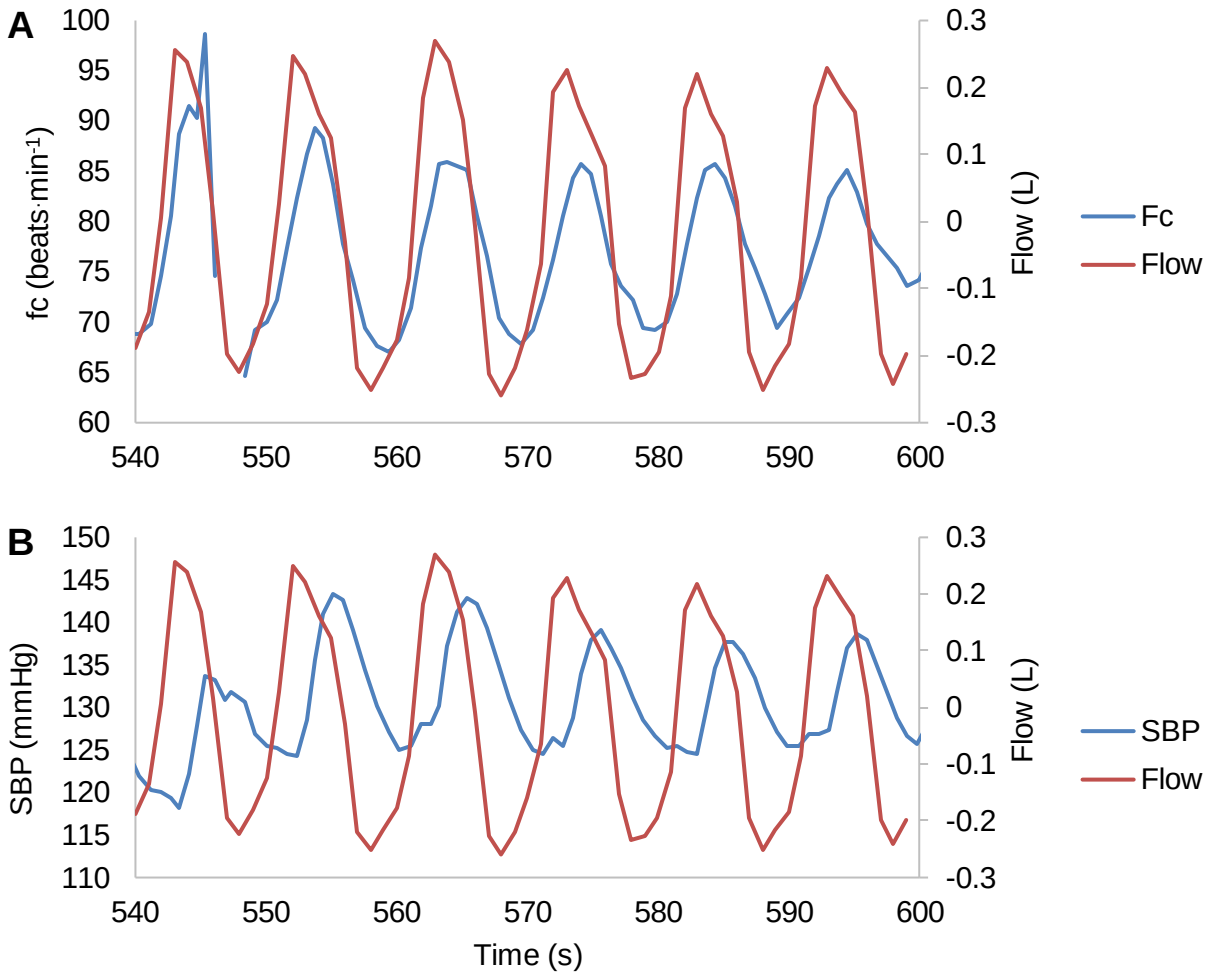


Figure 4-6 Respiratory synchronisation of heart rate (fc) (A) and systolic blood pressure (SBP) (B)
Heart rate (f_c; beats·min^{-1}), systolic blood pressure (SBP; mmHg), inspiratory flow (L: 1 second average). Data for 1 participant during last minute of 6Ff_r condition (6 breaths·min^{-1}).

Discussion

A small subset analysis was performed analysing differences in the acute cardiovascular responses to SDB by sex. No significant differences were found in the responses of men and women and therefore data were pooled for most analyses. Additionally, with small sample sizes for both groups (female *n*=6 & male *n*=5) any comparisons are limited in their statistical power. The results reveal that hemodynamic responses to SDB are similar between males and females, supporting the results of Adler et al. (2019), who found no sex differences in muscle sympathetic nerve activity and vascular sympathetic baroreflex sensitivity when comparing cardiovascular responses to RESPeRATE and spontaneous breathing. The amplitude of cardiovascular oscillations observed in the present study increased during SDB in both male and female participants, with pairwise comparisons revealing no sex differences across any variables. The lack of observed differences in the cardiovascular response to SDB, could be explained by the absence of significant differences between men and women in baseline cardiovascular variables during the spontaneous breathing condition (Sf_r). As baseline values were similar, the variables consequently responded to SDB in the same way regardless of sex. Due to the lack of observed differences between sexes, the following discussion will focus on combined data of males and females.

The main aim of the study was to characterise and compare the multi-parametric response to SDB using RESPeRATE, a fixed breathing frequency of 6 breaths·min^{-1} and a dynamic algorithm driven by RSA. This is the first study to provide a comprehensive characterisation of the acute cardiovascular responses to SDB, including consideration of the inter- and intra- breath perturbations created by breathing, as well as providing a comparison of responses by sex.

The novel analysis presented in this paper highlights the importance of measuring more than simple average values, as only average heart rate showed a significant increase between spontaneous and SDB. Previous research has been limited as it only compared average values, which as our data indicate, overlook the more complex cardiovascular oscillations created by SDB. The novel analysis provides evidence that differences between SDB and spontaneous breathing are only revealed by the peak-valley (Δi, Δe, ΔPV) and peak-valley breath phase independent (ΔPV_Ind) analyses. Therefore, analysis of inter- and intra- breath oscillations is needed to reveal the true cardiovascular perturbation induced by SDB.

These perturbations are markedly observed within BP oscillations and their response to SDB. The SBP oscillations within breath phases increased during SDB by up to 10.2% (11.4 mmHg) during inspiration (SBPΔi) and up to 8.4% (10 mmHg) during expiration (SBPΔe). In comparison, during spontaneous breathing (Sf_r) oscillations were just 2.9% (3.4 mmHg) and 3.4% (4.2 mmHg), respectively. For DBP, oscillations increased during SDB by up to 9.6% (6.6 mmHg) during inspiration and 7.7% (5.5 mmHg) during expiration, compared with fluctuations during Sf_r of 3.4% (1.5 mmHg) and 3.3% (2.4 mmHg), respectively. Thus, SDB generates an increase in the amplitude of BP oscillations during SDB. Interestingly, the largest oscillations were found in the SDB condition with the lowest average breathing frequency (Df_r). The amplitude of BP oscillations increased as breathing frequency was reduced and could perhaps be amplified further at breathing frequencies lower than those assessed in the present study. Extending breath phase duration, allows more time for BP to fluctuate within-breath and provides a possible explanation for the largest fluctuations occurring during the slowest breathing frequency. Fluctuations in BP have been found previously and are potentially linked to cardiorespiratory coupling of respiration, BP and heart rate (Chang et al. 2013; Russo et al. 2017; Nuckowska et al. 2019). This is supported by the RSA data in the present study, which also increased as breathing frequency decreased reaching a peak during Df_r, the lowest breathing frequency. It may also be possible to further increase RSA, using frequencies lower than those used in the present study.

Additionally, during the SDB conditions the largest percentage within-breath BP changes were observed during inspiration, but during spontaneous breathing the largest percentage change was during expiration. This was the same for both sexes. This reflects the known respiratory interactions where BP increases during inspiration when undertaking SDB, but decreases during inspiration during spontaneous breathing, so-called pulsus paradoxus (Parati et al. 2008). The largest oscillations therefore occur in the breath phase in which BP is rising. During inspiration, venous return is increased, which may be amplified by SDB due to a larger amplitude change of intra-thoracic pressure (Russo et al. 2017). The increased BP oscillations during inspiration may therefore be a reflection of the cardiovascular responses to the change in intra-thoracic pressure and subsequent increased venous return during SDB.

A key finding from this study is the higher amplitude of 'breath phase independent' cardiovascular fluctuations, as well as those of the peak-valley intra-breath phase fluctuations. Figure 4-6 shows the mismatch of synchronisation between inspiratory flow and heart rate, and SBP. For heart rate, the peak-valley value (RSA) matches closely the peak-valley breath phase independent values, due to the synchronisation of heart

rate and breathing phase. However, the oscillations of other variables, such as SBP, are misrepresented by inter-breath phase peak-valley values; in Figure 4-6B the minimum and maximum SBP values occur during the same breath phase, which reflects the influence of differing kinetics of the effect of breathing upon heart rate and haemodynamics. If one only considers the instantaneous haemodynamic responses during a given breath phase, then the true amplitude of the perturbations created by SDB are obscured. This is reflected in our statistical analyses, as only ΔPV_Ind values, and not ΔPV, were significantly different between conditions for $\dot{Q}$, SBP and DBP. Therefore, it is important to evaluate breath phase independent values of cardiovascular oscillations, due the nature of acute changes caused by SDB, in order to evaluate the true cardiovascular perturbations. Coherence analysis could further the understanding of this phenomenon, but was beyond the scope of this study.

When comparing between SDB conditions there were no significant differences between the SDB breathing frequencies in the final 5 minutes, which may explain why all three SDB conditions seemed to elicit the same cardiovascular responses compared to spontaneous breathing. This suggests that the 6Ff_r and Df_r conditions induced similar amplitudes of cardiovascular perturbation as RESPeRATE, a device already shown to reduce BP when practiced daily. It seems that the important feature of SDB is that breathing frequency is ~6 breaths·min^{-1}, but not necessarily how this frequency is achieved. Additionally, for ΔPV_Ind values only 6Ff_r and Df_r were significantly different from Sf_r for SBPΔPV_Ind and DBPΔPV_Ind suggesting they may generate slightly superior cardiovascular perturbations to RESPeRATE. Since 6Ff_r and Df_r produce the same error signal(s) as RESPeRATE, it is reasonable to suggest they may produce the same long-term health benefits. Our data indicate that, at the very least, 6Ff_r and Df_r provide alternative methods to implement SDB as an intervention to reduce BP. Indeed, 6Ff_r and Df_r may prove superior to RESPeRATE, since the reduced breathing frequency is experienced for a longer duration, as the conditions either reduce breathing frequency faster (dynamic algorithm) or maintain the same reduced frequency throughout (6 breaths·min^{-1}). For example, RESPeRATE produced an average frequency of 8.1 breaths·min^{-1} during the first 5 min compared with 6.4 breaths·min^{-1} in last 5 min, whilst the dynamic algorithm produced a frequency of 5.8 breaths·min^{-1} (first 5) and 5.5 breaths·min^{-1} (last 5), respectively. Further research is required to determine whether the hemodynamic responses at ~8 breaths·min^{-1} and ~6 breaths·min^{-1} differ, and whether any acute differences reflect changes in the anti-hypertensive effect of SDB. However, there were significantly higher BP oscillations during the final 5-min of RESPeRATE than the first 5-min, showing the potential for different acute cardiovascular responses at higher SDB frequencies.

A final practical consideration is whether the increased 'exposure time' to the optimal SDB frequencies delivered by the 6Ff_r and Df_r conditions could shorten the length of the daily SDB intervention. It is reasonable to suggest if the stimulus (optimal SDB frequency) is applied for a longer duration in these new potential conditions compared with the RESPeRATE condition, then the overall duration of the SDB session could be reduced. The 'active SDB time' would still be the same in the new conditions as during the normal RESPeRATE session, but the overall length of the session could be reduced to remove the time spent above optimal SDB frequencies during RESPeRATE sessions. Further research examining the long-term benefits of these alternative conditions is needed to test this theory.

Limitations

This study did not control for or measure menstrual phase and/or contraceptive phase in the female participants. It has previously been recommended that when testing autonomic function, females should be tested during the early follicular phase of the menstrual cycle or placebo phase of oral contraceptive use (Wallin et al. 2010). However, a previous study found no influence of menstrual cycle or oral contraceptive on the cardiovascular responses to SDB (Nili et al. 2017). Future studies should explore whether menstrual cycle phase influences the cardiovascular response to SDB, specifically at the inter- and intra-breath phase levels.

Conclusion

In conclusion, all three SDB conditions elicit similar cardiovascular responses to each other, when compared with normal breathing. Thus, both the new dynamic algorithm (Df_r) or a fixed frequency of 6 breaths·min^{-1} (6Ff_r) could potentially be used in future studies using a SDB intervention to reduce BP. Future research should examine a range of breathing frequencies to examine if BP oscillations can be maximised at breathing frequencies <6 breaths·min^{-1} and whether SDB at higher frequencies of 8 breaths·min^{-1} (replicating the first 5 min of RESPeRATE) produce the same cardiovascular responses as found in the present study. All future studies should note the importance of looking beyond average responses to examine inter- and intra-breath phase cardiovascular oscillations, especially for BP and RSA, to reflect the true cardiovascular responses to SDB. In this respect, analysis of breath phase independent peak-valley fluctuations of cardiovascular variables seems most appropriate and pragmatic.

4.3 Supplementary material

The following results tables will be included as supplementary information for the publication in European Journal of Applied Physiology.

Table S4-7 Mean values (±SD) for blood pressure variables (mmHg)

		Sf_r	Rf_r	6Ff_r	Df_r	Effect of condition *P* value	Sex x Condition *P* value
	F	117.5 ± 16.8	115.9 ± 14.4	116.0 ± 15.5	115.0 ± 10.5		
SBP	*M*	123.0 ± 5.8	118.7 ± 7.3	118.3 ± 7.9	116.1 ± 10.4		
	All	**120.0 ± 12.8**	**117.2 ± 11.2**	**117.1 ± 12.1**	**115.5 ± 9.9**	0.358	0.857
	F	114.8 ± 16.9	114.2 ± 14.7	114.3 ± 15.4	112.6 ± 11.5		
SBPi	*M*	121.3 ± 5.0	115.3 ± 8.2	113.7 ± 8.2	111.3 ± 11.6		
	All	**117.7 ± 12.8**	**114.7 ± 11.6**	**114.0 ± 12.1**	**112.0 ± 11.0**	0.157	0.445
	F	120.3 ± 16.8	117.6 ± 14.7	117.8 ± 16.2	117.5 ± 9.7		
SBPe	*M*	124.8 ± 6.7	122.1 ± 7.0	122.9 ± 8.2	121.0 ± 9.7		
	All	**122.3 ± 12.8**	**119.7 ± 11.5**	**120.1 ± 12.8**	**119.1 ± 9.4**	0.659	0.992
	F	-5.5 ± 2.3	-3.4 ± 6.3	-3.5 ± 6.2	-4.9 ± 3.4		
SBPΔ	*M*	-3.5 ± 2.1	-6.9 ± 4.5	-9.2 ± 4.6	-9.6 ± 5.1		
	All	**-4.6 ± 2.3**	**-5.0 ± 5.6**	**-6.1 ± 6.0**	**-7.0 ± 4.7**	0.158	0.026
	F	70.5 ± 10.3	70.0 ± 7.4	70.8 ± 10.5	70.4 ± 9.4		
DBP	*M*	72.6 ± 7.7	67.6 ± 8.8	70.7 ± 8.9	69.3 ± 9.8		
	All	**71.4 ± 8.8**	**68.9 ± 7.7**	**70.7 ± 9.3**	**69.9 ± 9.1**	0.412	0.592
	F	69.5 ± 10.2	70.0 ± 8.8	70.5 ± 11.6	69.8 ± 10.0		
DBPi	*M*	72.4 ± 7.6	66.3 ± 8.8	68.9 ± 8.5	67.2 ± 9.8		
	All	**70.8 ± 8.8**	**68.3 ± 8.6**	**69.8 ± 9.9**	**68.6 ± 9.5**	0.326	0.232
	F	71.5 ± 10.5	70.0 ± 6.3	71.0 ± 9.6	71.0 ± 9.0		
DBPe	*M*	72.8 ± 7.7	68.9 ± 8.9	72.4 ± 9.4	71.3 ± 9.8		
	All	**72.1 ± 8.9**	**69.5 ± 7.2**	**71.7 ± 9.0**	**71.2 ± 8.9**	0.482	0.886
	F	-1.9 ± 1.1	-0.1 ± 4.1	-0.5 ± 3.2	-1.2 ± 2.0		
DBPΔ	*M*	-0.4 ± 0.6	-2.6 ± 2.0	-3.5 ± 1.8	-4.1 ± 2.4		
	All	**-1.2 ± 1.2**	**-1.2 ± 3.4**	**-1.9 ± 3.0**	**-2.5 ± 2.5**	0.288	0.031

Data represent mean ± SD (female n = 6, male n = 5); Female (F), Male (M); Spontaneous breathing (Sf_r), RESPeRATE (Rf_r), 6 breaths·minute^{-1} (6Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e).

Table S4-8 Mean values (±SD) for heart rate (f_c)

		Sf_r	Rf_r	$6Ff_r$	Df_r	Effect of condition *P* value	Sex x Condition *P* value
	F	63.6 ± 7.6	65.7 ± 8.0	67.6 ± 7.9	67.3 ± 8.8		
f_c	*M*	52.5 ± 4.8	54.5 ± 3.8	56.3 ± 6.1	56.2 ± 6.2		
	All	**58.6 ± 8.5†¤**	**60.0 ± 8.5**	**62.4 ± 9.0***	**62.3 ± 9.4***	<0.001	0.999
	F	63.6 ± 6.9	68.0 ± 8.7	69.3 ± 9.2	69.4 ± 9.0		
f_ci	*M*	52.0 ± 4.1	56.8 ± 3.4	58.5 ± 5.7	59.0 ± 6.2		
	All	**58.4 ± 8.2¥†¤**	**62.9 ± 8.8***	**64.4 ± 9.3***	**64.7 ± 9.2***	<0.001	0.960
	F	63.8 ± 8.5	63.4 ± 7.6	66.0 ± 7.4	65.2 ± 8.7		
f_ce	*M*	52.9 ± 5.7	52.2 ± 4.9	54.0 ± 7.6	53.3 ± 6.9		
	All	**58.8 ± 9.0**	**58.3 ± 8.5**	**60.5 ± 9.5**	**59.8 ± 9.8**	0.125	0.921
	F	-0.1 ± 2.3	4.6 ± 3.1	3.3 ± 5.2	4.1 ± 2.4		
$f_c\Delta$	*M*	-0.9 ± 3.1	4.5 ± 3.6	4.5 ± 5.7	5.7 ± 4.2		
	All	**-0.5 ± 2.6¥†**	**4.6 ± 3.2***	**3.8 ± 5.2**	**4.9 ± 3.3***	0.005	0.865

Data represent mean ± SD (female n = 6, male n = 5); Female (F), Male (M); Spontaneous breathing (Sf_r), RESPeRATE (Rf_r), 6 breaths·minute^{-1} ($6Ff_r$), optimisation algorithm dynamic breathing frequency (Df_r); heart rate (f_c; beats·min^{-1}); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf_r (*); Rf_r (¥), $6Ff_r$ (†), Df_r (¤); P<0.05.

Table S4-9 Mean values (±SD) for stroke volume (SV) and cardiac output ($\dot{Q}$)

		Sf_r	Rf_r	6Ff_r	Df_r	Effect of condition *P* value	Sex x Condition *P* value
	F	77.5 ± 14.7	74.8 ± 15.5	74.0 ± 13.8	75.1 ± 13.3		
SV	*M*	82.0 ± 13.8	79.1 ± 15.4	79.6 ± 13.6	74.5 ± 14.0		
	All	**79.6 ± 13.8**	**76.7 ± 14.8**	**76.6 ± 13.3**	**74.8 ± 12.9**	0.027	0.205
	F	75.3 ± 14.9	72.8 ±15.4	72.4 ± 12.7	72.8 ± 13.0		
SVi	*M*	79.4 ± 14.3	76.4 ± 14.5	75.6 ± 13.3	71.4 ± 13.9		
	All	**77.1 ± 14.0**	**74.4 ± 14.4**	**73.9 ± 12.4**	**72.2 ± 12.8**	0.013	0.232
	F	79.7 ± 14.7	76.9 ± 15.6	75.7 ± 14.9	77.4 ± 13.7		
SVe	*M*	84.6 ± 13.4	81.7 ± 16.6	83.6 ± 14.6	77.5 ± 14.4		
	All	**82.0 ± 13.6**	**79.0 ± 15.4**	**79.3 ± 14.6**	**77.4 ± 13.3**	0.070	0.173
	F	-4.4 ± 1.5	-4.1 ± 1.2	-3.2 ± 3.1	-4.6 ± 2.4		
SVΔ	*M*	-5.3 ± 3.2	-5.2 ± 4.4	-8.0 ± 5.9	-6.1 ± 4.4		
	All	**-4.8 ± 2.3**	**-4.6 ± 3.0**	**-5.4 ± 5.0**	**-5.3 ± 3.4**	0.668	0.097
	F	4985 ± 1311	4949 ± 1329	5007 ± 1190	5047 ± 1138		
$\dot{Q}$	*M*	4274 ± 626	4274 ± 740	4436 ± 646	4135 ± 647		
	All	**4662 ± 1074**	**4642 ± 1107**	**4748 ± 982**	**4633 ± 1021**	0.454	0.271
	F	4845 ± 1266	4994 ± 1419	5047 ± 1288	5046 ± 1123		
$\dot{Q}i$	*M*	4093 ± 605	4325 ± 782	4397 ± 647	4187 ± 705		
	All	**4503 ± 1050**	**4690 ± 1172**	**4752 ± 1054**	**4656 ± 1015**	0.122	0.746
	F	5134 ± 1359	4893 ± 1253	4975 ± 1095	5052 ± 1156		
$\dot{Q}e$	*M*	4452 ± 701	4225 ± 728	4473 ± 779	4084 ± 636		
	All	**4824 ± 1116**	**4589 ± 1058**	**4747 ± 995**	**4612 ± 1042**	0.078	0.122
	F	-289 ± 129	101 ± 329	72 ± 260	-6 ± 95		
$\dot{Q}\Delta$	*M*	-359 ± 388	99 ± 306	-75 ± 621	103 ± 355		
	All	**-321 ± 265¥†**	**100 ± 303***	**5 ± 441**	**44 ± 241***	0.001	0.642

Data represent mean ± SD (female n = 6, male n = 5); Female (F), Male (M); Spontaneous breathing (Sf_r), RESPeRATE (Rf_r), 6 breaths·minute^{-1} (6Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); stroke volume (SV; ml), cardiac output ($\dot{Q}$; ml·min^{-1}); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf_r (*); Rf_r (¥), 6Ff_r (†), Df_r (¤); P<0.05.

Table S4-10 Mean values (±SD) for total peripheral resistance (TPR) and pulse transit time (PTT)

		Sf_r	Rf_r	6Ff_r	Df_r	Effect of condition *P* value	Sex x Condition *P* value
	F	17.8 ± 3.9	17.9 ± 4.4	17.5 ± 3.1	17.3 ± 3.7		
TPR	*M*	21.2 ± 4.3	20.3 ± 5.2	19.7 ± 2.8	20.9 ± 4.1		
	All	**19.3 ± 4.2**	**19.0 ± 4.7**	**18.5 ± 3.0**	**18.9 ± 4.1**	0.612	0.643
	F	18.0 ± 4.0	17.7 ± 4.3	17.2 ± 3.0	17.1 ± 3.5		
TPRi	*M*	21.9 ± 4.9	19.6 ± 5.2	19.2 ± 2.9	19.9 ± 4.1		
	All	**19.8 ± 4.7**	**18.5 ± 4.6**	**18.1 ± 3.0**	**18.4 ± 3.9**	0.098	0.473
	F	17.6 ± 3.9	18.3 ± 4.5	17.7 ± 3.2	17.6 ± 3.8		
TPRe	*M*	20.4 ± 3.8	21.0 ± 5.4	20.2 ± 3.1	21.8 ± 4.2		
	All	**18.9 ± 3.9**	**19.5 ± 4.9**	**18.8 ± 3.2**	**19.5 ± 4.4**	0.554	0.526
	F	0.4 ± 0.7	-0.6 ± 0.8	-0.5 ± 1.1	-0.5 ± 0.6		
TPRΔ	*M*	1.5 ± 1.9	-1.4 ± 1.4	-0.9 ± 2.0	-1.9 ± 1.5		
	All	**0.9 ± 1.4**	**-0.9 ± 1.2***	**-0.7 ± 1.5**	**-1.1 ± 1.3***	<0.001	0.051
	F	180 ± 23	183 ± 21	180 ± 21	183 ± 24		
PTT	*M*	210 ± 27	212 ± 24	215 ± 27	210 ± 22		
	All	**193 ± 28**	**196 ± 26**	**196 ± 29**	**195 ± 26**	0.445	0.269
	F	182 ± 24	185 ± 22	184 ± 23	183 ± 21		
PTTi	*M*	213 ± 29	215 ± 23	215 ± 23	217 ± 24		
	All	**196 ± 30**	**199 ± 26**	**198 ± 27**	**198 ± 27**	0.499	0.695
	F	177 ± 22	180 ± 21	181 ± 24	177 ± 21		
PTTe	*M*	207 ± 26	209 ± 24	205 ± 22	211 ± 29		
	All	**191 ± 27**	**194 ± 26**	**192 ± 25**	**192 ± 30**	0.646	0.167
	F	5.1 ± 2.7	4.7 ± 2.8	2.7 ± 6.8	6.4 ± 2.4		
PTTΔ	*M*	6.4 ± 3.5	5.3 ± 2.5	10.4 ± 4.6	5.5 ± 8.0		
	All	**5.7 ± 3.0**	**5.0 ± 2.6**	**6.2 ± 6.9**	**6.0 ± 5.4**	0.827	0.208

Data represent mean ± SD (female n = 6, male n = 5); Female (F), Male (M); Spontaneous breathing (Sf$_r$), RESPeRATE (Rf$_r$), 6 breaths·minute^{-1} (6Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$); total peripheral resistance (TPR; mmHg·min·L^{-1}); pulse transit time (PTT; ms); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf$_r$ (*); Rf$_r$ (¥), 6Ff$_r$ (†), Df$_r$ (¤); P<0.05.

4.4 Summary

This chapter has used novel methods of analysis for the first time that reveal the inter- and intra-breath phase responses to SDB. Previous research has focused on average changes during SDB and found mixed responses. However, cardio-respiratory interactions are complex and therefore require a deeper investigation of the full set of cardiovascular responses to SDB. Using this analysis, SDB was observed to cause an increase in the amplitude of BP oscillations. Both the amplitude of BP oscillations relative to mean BP, and RSA increased as breathing frequency decreased, to a maximum at the lowest breathing frequency. The lowest breathing frequency in this study was 6 breaths·min^{-1} and future research should investigate if the amplitude of fluctuations could be increased further at lower breathing frequencies than those undertaken in the present study.

The three SDB frequencies (RESPeRATE, dynamic algorithm and 6 breaths·min^{-1}) produced similar cardiovascular responses, suggesting that alternative delivery methods of SDB could be used to reduce BP with daily SDB practice, using a cheaper alternative to RESPeRATE. There is also potential for the alternative methods to produce a further benefit due to the increased stimulus and duration the user experiences SDB frequencies, without waiting for breathing frequencies to reach the optimal of 6 breaths·min^{-1}. The higher breathing frequency during the first 5 min of the RESPeRATE trial (~ 8 breaths·min^{-1}) may not elicit the full cardiovascular responses of SDB and therefore further investigation of the potential for differing responses to a range of different breathing frequencies is needed. Future studies should investigate a range of frequencies to track the cardiovascular responses from spontaneous normal breathing to different levels/frequencies of SDB. This will allow an evaluation of the optimal breathing frequency for cardiovascular responses based on maximising cardiovascular perturbations, including BP oscillations and RSA.

Chapter 5. Short-term cardiovascular responses to slow and deep breathing in healthy women

5.1 Introduction

Regular practice of slow and deep breathing (SDB) can lower blood pressure in individuals with hypertension (Chaddha et al. 2019). Although significant reductions in blood pressures (BP) have been found (Zou et al. 2017) the mechanisms underpinning the anti-hypertensive effects of SDB remain poorly understood. To date, a limited number of studies have examined the acute cardiovascular responses to SDB, and there is a limited understanding of the cardiorespiratory interactions that might underpin the anti-hypertensive effect of SDB. In particular, the systematic characterisation of the acute responses to a range of SDB frequencies is lacking; no published studies have examined discrete SDB frequencies, with the exception of an unpublished thesis (Vargas 2017). The studies that have compared acute responses to SDB at a range of frequencies have done so using a progressively decreasing SDB protocol, with only short durations at each individual SDB frequency (Anderson et al. 2009; Zhang et al. 2009). Although Guzik et al. (2007) did examine heart rate variability (HRV) and baroreflex sensitivity (BRS) during 5 minute protocols of different SDB frequencies (6 and 9 breaths·min^{-1}) by their own admission the authors did not focus on the physiological meaning of their findings.

Whilst the extant literature suggests SDB at frequencies of ≤6 breaths·min^{-1} generates the greatest perturbation[3] to the cardiovascular system, preliminary research suggests that the optimal perturbing frequency exhibits individual variation (Vargas 2017). Thus, implementing SDB at a personalised breathing frequency, which perturbs each individual's cardiovascular system maximally, could elicit larger anti-hypertensive effects compared with a 'one frequency fits all' SDB model (Vargas 2017).

Characterising the cardiovascular responses to a range of breathing frequencies, including a personalised frequency, may shed light on potential 'error signals' that are responsible for reducing BP following the daily practice of SDB. Chapter 4 also suggested the potential for BP oscillations to be further increased at levels <6 breaths·min^{-1}. As the increase in amplitude of BP during SDB was a novel finding, it has not previously been investigated at different SDB frequencies. Additionally, the data presented in Chapter 4 revealed differences in the cardiovascular response during the

[3] *Perturbation means a disturbance or change in a structure or function, as a result of an external influence.*

RESPeRATE condition between the first 5- and last 5-min, accompanied by a difference in breathing frequency of ~8 breaths·min^{-1} (first 5-min) and ~6 breaths·min^{-1} (last 5-min). However, whether the acute cardiovascular responses are different at higher SDB frequencies (between 6-10 breaths·min^{-1}) has also not been investigated.

Although the link between acute breathing-related cardiovascular perturbations and BP regulation remains unknown, the kidneys are known to be central to regulating BP (Levick 2013). Eight weeks of daily SDB has been shown to reduce renal resistive index (RRI; (Modesti et al. 2015), but it is not known whether there is an immediate change in RRI as a response to SDB. In addition to lowering breathing frequency, adding an inspiratory resistance to SDB has been shown to reduce BP to a greater extent than SDB alone in numerous studies (Jones et al. 2010; Vranish and Bailey 2015; DeLucia et al. 2018; Ubolsakka-Jones et al. 2019).

In summary, the mechanisms by which SDB might reduce BP remain unknown. An essential first step towards addressing this deficit is gaining an understanding of the acute cardiovascular responses to SDB. The purpose of this study was therefore to characterise the acute cardiovascular responses to a variety of SDB conditions, including at a range of breathing frequencies and with added inspiratory resistance. The research questions, objectives and hypothesis for this study are outlined below:

Research questions

1. Using a novel peak-valley analysis method, what are the complex cardiovascular responses to SDB of healthy young women?
2. Are there differences in the acute cardiovascular responses at a range of SDB frequencies for healthy young women?
3. Does adding an inspiratory resistance to SDB amplify cardiovascular responses to SDB for healthy young women?
4. Does SDB elicit acute changes in renal resistive index and/or indices of central blood pressure for healthy young women?

Objectives

1. Characterise the response of mechanism-related parameters (e.g. respiratory sinus arrythmia, stroke volume, cardiac output, blood pressures) during SDB for healthy young women.
2. Characterise acute cardiovascular responses to a range of SDB frequencies in healthy young women.

3. Assess the acute responses of pulse wave velocity, central blood pressure parameters and renal resistive index to SDB for healthy young women.

Hypothesis

1. Peak-valley measures of cardiovascular parameters (respiratory sinus arrythmia, stroke volume and cardiac output) and the amplitude of blood pressure oscillations will increase during SDB for healthy young women.
2. The amplitude of peak-valley fluctuations will increase as SDB frequency decreases for healthy young women.
3. An inspiratory resistance will amplify the amplitude of cardiovascular fluctuations during SDB for healthy young women.
4. Renal resistive index and central blood pressure measures will decrease in response to SDB for healthy young women.

5.2 Specific Methods

5.2.1 Participants

Twenty-three female participants took part in the study from forty one participants who were assessed for eligibility (Figure 5-3). Participants were all of reproductive age as defined by the World Health Organization (2006) in order to match the age range of participants from Chapter 6 who were pregnant women. None of the participants in this chapter were known to be pregnant at the time of participating. As oral contraceptives and the phase of the menstrual cycle may influence BP responses to SDB (Fonkoue et al. 2018), these were tracked in our participants and analysed for significance.

5.2.2 General Design

Participants attended one session at the Cardiorespiratory Research Laboratory at Bournemouth University. Lab conditions were recorded for each session and averages for the study were 24.1 ± 2.9 ^{o}C (range 20.1–31.9 ^{o}C), 991.2 ± 7.3 hPa (975-1000 hPa), 42.8 ± 11.4% (26-68%). Using a within-subject design, participants undertook the breathing conditions in a randomised order. Participants were asked to complete six breathing conditions; spontaneous breathing (Sf_r), 4 (4Ff_r), 6 (6Ff_r), and 8 (8Ff_r) breaths·min^{-1}, optimisation algorithm (Df_r), and 6 breaths·min^{-1} with an added inspiratory resistance (IR). All breathing conditions were five-minutes in duration with a five-minute period of normal breathing prior to each measurement. The reduction in condition length to 5-minutes from the 10-minutes used in Chapter 4 was chosen partly to minimise the burden on participants by reducing the duration of the data collection session, due to the increase of adding 2 additional conditions compared with Chapter 4. This protocol

change was supported by the results of the RESPeRATE study (Chapter 4) which showed a steady state of breathing frequency and key BP variables before 5 minutes of SDB for all but the RESPeRATE condition which was not replicated in this study. As the study aims to explore the immediate responses to SDB, 10-minutes would have shown no additional responses and therefore would have been an unnecessary burden on participants. Additionally shorter durations of 5 minutes (compared with durations of 7 and 9 min) produced the largest increase in HRV during SDB (Cheng et al. 2019). Figure 5-1 shows a schematic of the protocol.

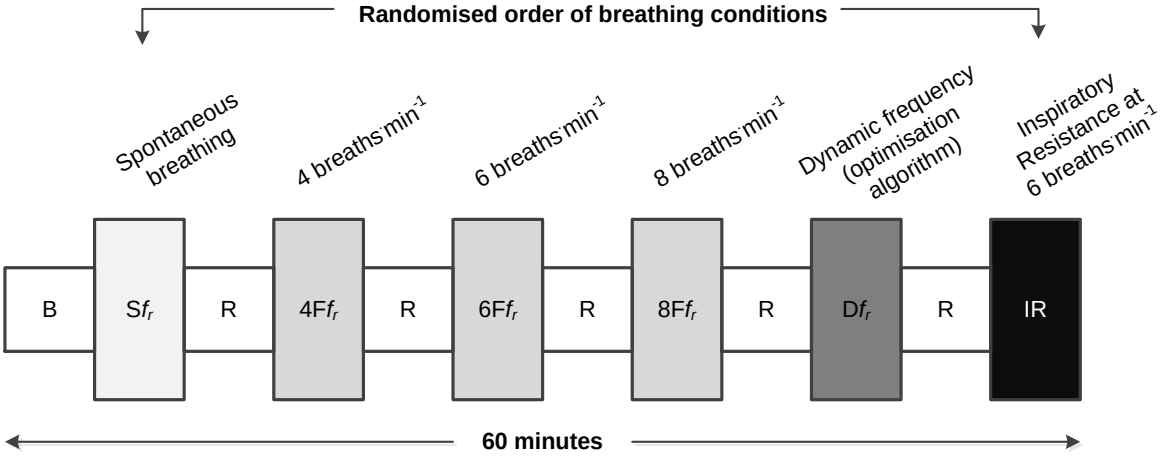


Figure 5-1 Schematic of protocol
All breathing condition and recovery periods were 5-minutes in duration; spontaneous baseline breathing (B), rest periods (R), uncontrolled spontaneous breathing (Sf_r), optimisation algorithm dynamic breathing frequency (Df_r), fixed breathing frequency of 4 breaths·minute^{-1} ($4Ff_r$), 6 breaths·minute^{-1} ($6Ff_r$), 8 breaths·minute^{-1} ($8Ff_r$), inspiratory resistance (IR).

An added inspiratory resistance of 9cm H_2O (IR) was added to the optimal SDB frequency of 6 breaths·min^{-1} using a POWERbreathe Medic Plus (POWERbreathe International Ltd., UK), which was attached to the front of the mask set-up (Figure 5-2).

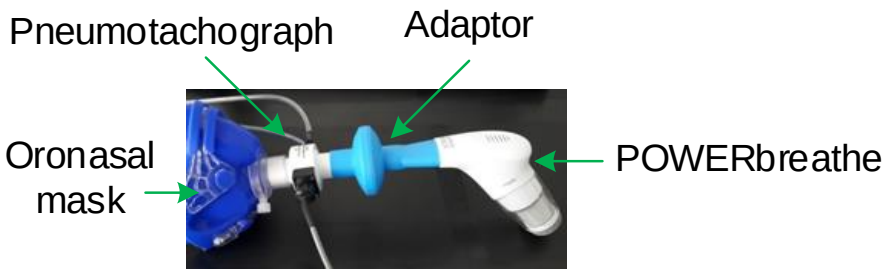


Figure 5-2 Inspiratory resistance set-up (POWERbreathe Medic Plus)

5.2.3 Equipment and procedures

Respiratory airflow, ECG and arterial blood pressure (ABP) were monitored continuously throughout each breathing condition. Participants wore an oronasal mask (Oro Nasal 7450 V2 Mask, Hans Rudolph Inc., Kansas, USA) and respired flow rate was measured continuously using a heated pneumotachograph (Model 3700, Hans Rudolph Inc.,

Kansas, USA) connected to a flow measurement system (RSS 100-HR, Hans Rudolph Inc., Kansas, USA). End-tidal CO_2 was not measured in this study as the results in Chapter 4 revealed no significant differences between SDB and spontaneous breathing conditions.

Heart rate was monitored using a 3-lead ECG and non-invasive beat-to-beat ABP was obtained using finger photoplethysmography (Finapres NOVA, Finapres Medical Systems, The Netherlands). Finapres derived ABP was calibrated using a brachial cuff prior to and halfway through data collection. Analogue outputs from the Finapres NOVA and the flow meter were sampled continuously at 250Hz via an analogue to digital converter (NI USB-6218 BNC, National Instruments Inc.) and captured using bespoke acquisition and analysis software (LabView 2015, National Instruments, Inc.). For more detailed explanation of the data acquisition system see section 3.4.

Additional measures of ABP, including pulse wave analysis (PWA) and pulse wave velocity (PWV), were recorded pre- and post- each breathing condition using a Vicorder (Vascular Complete Model, SMT Medical, Germany). PWA was measured using a brachial cuff and PWV using a neck and femoral cuff. The neck cuff was a 30mm pad that was placed at the level of the left carotid artery, and the femoral cuff a larger oscillometric cuff (100mm) that was placed around the uppermost section of the right thigh. PWV was calculated using the path length of sternal notch to the middle of the femoral cuff.

Renal ultrasound was also performed pre- and post- each condition on a subsection of participants (n=10) to measure renal resistive index (RRI). Due to time constraints of the rest period between conditions, PWV was not measured for participants when ultrasound measures were recorded. Therefore, participants either had ultrasound or PWV measurements recorded, but PWA was recorded for all. To maintain consistency the renal ultrasound measures were always recorded following PWA measurements which were always measured first. The renal ultrasound measurements were undertaken by a trained sonographer. Doppler measurements of kidney blood flow were performed using a Terason t3200 (uSmart 3200T, Terason, Massachusetts, USA) with a curvilinear transducer (5C2, Terason, Massachusetts, USA). Three measurements were recorded for the right kidney during a breath hold and were averaged during analysis.

5.2.4 Statistical analysis

Values are expressed as means ± SD unless stated otherwise. Statistical analysis was undertaken using SPSS Statistics 24 (IBM Corp.). After normality was confirmed

(Shapiro Wilk) repeated measures ANOVA with planned pairwise comparisons using Bonferroni corrections were used. Pairwise comparisons were only viewed when the ANOVA reached significance. If the Mauchly's sphericity condition was violated the Greenhouse-Geisser correction was applied. Reported p values are those following adjustment for repeated comparisons. For all analyses, P was set at 0.05.

The Vicorder blood pressure measurements were calculated as follows for pulse wave analysis (PWA) and pulse wave velocity (PWV):

Equation H: Augmentation Index (AIx) = (AP / AoPP) x 100
Where AP is augmentation pressure and AoPP is aortic pulse pressure.

Equation I: Heart rate adjusted Augmentation Index (AIx75) = (-0.48 x (75 – f_c)) + AIx
Where f_c is heart rate and AIx is Augmentation Index.

Equation J: Pulse Wave Velocity (PWV) = path length / transit time
Where path length was measured from sternal notch to mid femoral cuff.

Renal resistive index (RRI) was calculated by the inbuilt Terason software using the following equation:

Equation K: Renal resistive index (RRI) = (PS - ED) / PS
Where PS is peak systolic velocity and ED is end diastolic velocity.

5.3 Results

Data were collected from 23 participants and 56% of women assessed for eligibility took part (Figure 5-3). Five participants were excluded from the analysis; three due to technical errors in the measurement of respiratory airflow, one due to a failure of the acquisition system to save the signal data, and one because the participant failed to adhere to the prescribed breathing condition. In addition, due to practical problems with implementing the added inspiratory resistance (IR), it was not possible to acquire an accurate respiratory airflow signal for 4 participants. Data from the 14 participants with a full data set revealed no significant difference between the IR and 6Ff_r conditions for any variables, when examining pairwise comparisons following repeated measures ANOVA. Therefore, it was decided to exclude this condition (IR) from the data presented and analysed, to allow analysis of 18 full sets of complete data.

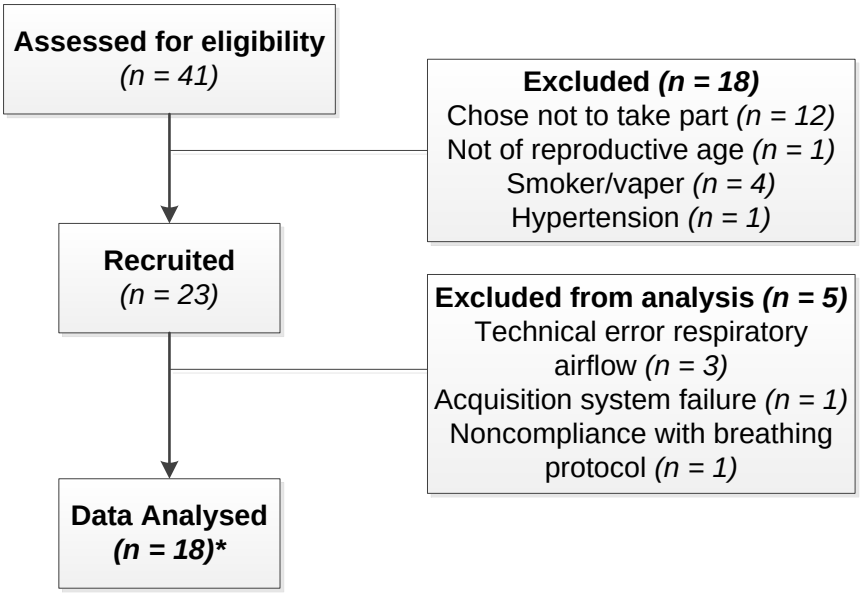


Figure 5-3 Flow chart for number of women who were assessed for eligibility and took part in the study
**Due to problems with respiratory airflow signal for 4 participants, data from the inspiratory resistance (IR) condition was excluded from analysis and the n = 18 data analysed.*

Eighteen participants were included in data analysis (age 30.1 ± 8.8 years; stature 1.66 ± 0.5 m; mass 65.6 ± 10.3kg; BMI 23.9 ± 3.3kg/m^2; systolic BP 113.9 ± 9.1 mmHg; diastolic BP 68.9 ± 8.0 mmHg). Seven participants were taking oral contraception and the average menstrual stage was 16.1 ± 10.1 days. Eight women were in the follicular phase of menstruation and nine in the luteal phase. There were no significant differences found between menstruation cycle phase or contraceptive use for respiratory sinus arrythmia (RSA) and for all SBP and DBP variables ($p>0.05$).

5.3.1 Respiratory variables

Table 5-1 provides an overview of the respiratory parameters for each condition. Duty cycle remained consistent throughout conditions. The optimisation algorithm (Df_r) computed the optimal breathing frequency to be 6.3 ± 1.1 breaths·min^{-1}, which was not significantly different from 6Ff_r ($p>0.05$). All other breathing conditions were significantly different from each for breathing frequency ($p<0.001$).

Table 5-1 Respiratory parameters

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r
f_r	13.3 ± 2.1¥¤†§	8.0 ± 0.0*¤†§	6.3 ± 1.1*¥§	6.0 ± 0.0*¥§	4.0 ± 0.0*¥¤†
V_T	0.4 ± 0.2¥¤†§	0.9 ± 0.4*†§	1.0 ± 0.4*	1.1 ± 0.4*¥	1.3 ± 0.4*¥
T_I / T_{TOT}	0.42 ± 0.0¥¤†§	0.48 ± 0.0*	0.48 ± 0.0*	0.50 ± 0.1*	0.48 ± 0.0*

Data represent mean ± SD (n = 18). Spontaneous breathing (Sf$_r$), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff$_r$), 6 breaths·minute^{-1} (6Ff$_r$), 8 breaths·minute^{-1} (8Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$). Breathing frequency (f_r) in breaths·min^{-1}, tidal volume (V_T) in L, duty cycle (T_I /T_{TOT}); Significantly different from Sf$_r$ (*), 8Ff$_r$ (¥), Df$_r$ (¤), 6Ff$_r$ (†), 4Ff$_r$ (§); P<0.05.

In accordance with the analysis groups outlined in Section 3.4.5 (Table 3-2) all results for each variable are grouped into mean and intra-breath phase responses (mean, mean i, mean e), inter-breath phase responses (Δ), peak-valley intra-breath phase (Δi, Δe), peak-valley inter-breath phase (ΔPV), and peak-valley breath phase independent (ΔPV_Ind). Unless stated otherwise, all data are mean values for the full 5-minute epoch. A reminder that a visual representation of these calculations can be found in section 3.4.5 (Figure 3-6 page 71).

5.3.2 Arterial blood pressures

Mean and intra-breath phase responses

There were no significant differences in mean SBP, DBP, pulse pressure (PP) or mean arterial pressure (MAP) between any of the breathing conditions ($p>0.05$; Table 5-2). There were also no significant difference for mean BP variables during inspiration (calculation 2, Figure 3-6) or mean BP variables during expiration (calculation 3 (Figure 3-6) between breathing conditions ($p>0.05$).

Inter-breath phase responses

Inter-breath phase responses (i.e., difference between mean value during inspiration *vs.* mean value during expiration, calculation 4, Figure 3-6) were significantly different between breathing conditions for SBPΔ ($p<0.001$), DBPΔ ($p<0.001$), PPΔ ($p=0.02$) and MAPΔ ($p<0.001$; Table 5-2). At 4Ff_r, SBPΔ, DBPΔ and MAPΔ were significantly different from all other conditions ($p<0.01$), and MAPΔ during 8Ff_r was also significantly different from Sf_r ($p=0.045$; Table 5-2).

Table 5-2 Mean values (±SD) and inter-breath phase differences (Δ) for blood pressure variables (mmHg)

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	13.3 breaths·min^{-1}		*6.3 breaths·min^{-1}*			
SBP	121.3 ± 17.6	118.0 ± 9.6	115.3 ± 19.9	119.8 ± 13.8	116.2 ± 10.5	0.481
SBPi	118.9 ± 17.4	114.3 ± 9.8	112.3 ± 20.2	117.1 ± 14.7	116.5 ± 10.4	0.475
SBPe	123.8 ± 17.8	121.8 ± 9.6	118.4 ± 20.0	122.7 ± 13.3	115.8 ± 11.1	0.200
SBPΔ	–4.9 ± 2.1§	–7.5 ± 3.4§	–6.0 ± 5.0§	–5.6 ± 5.2§	0.7 ± 5.0*¥¤†	<0.001
DBP	74.7 ± 17.1	73.1 ± 7.3	67.5 ± 14.7	74.1 ± 11.4	69.8 ± 8.7	0.166
DBPi	73.9 ± 17.3	71.8 ± 7.3	67.1 ± 15.4	73.6 ± 11.9	71.9 ± 9.0	0.286
DBPe	75.5 ± 17.0	74.3 ± 7.3	68.0 ± 14.2	74.6 ± 11.1	67.7 ± 8.6	0.064
DBPΔ	–1.5 ± 1.0§	–2.5 ± 1.5§	–0.9 ± 3.4§	–1.0 ± 3.4§	4.2 ± 3.3*¥¤†	<0.001
PP	46.6 ± 7.9	45.0 ± 7.9	47.8 ± 9.6	45.8 ± 7.3	46.4 ± 8.3	0.414
PPi	45.0 ± 7.4	42.4 ± 7.5	45.3 ± 8.8	43.5 ± 7.2	44.6 ± 7.5	0.286
PPe	48.2 ± 8.5	47.5 ± 8.3	50.4 ± 10.6	48.1 ± 7.7	48.1 ± 9.3	0.447
PPΔ	–3.4 ± 2.0	–5.1 ± 2.6	–5.1 ± 3.2§	–4.6 ± 2.8	–3.5 ± 2.9¤	0.002
MAP	88.7 ± 16.9	86.6 ± 7.2	81.9 ± 15.9	87.8 ± 11.7	83.7 ± 8.4	0.238
MAPi	87.4 ± 17.0	84.6 ± 7.4	80.7 ± 16.5	86.7 ± 12.4	85.3 ± 8.8	0.346
MAPe	90.0 ± 16.8	88.5 ± 7.1	83.1 ± 15.4	89.0 ± 11.2	82.1 ± 8.4	0.064
MAPΔ	–2.5 ± 1.1¥§	–4.0 ± 1.9*§	–2.5 ± 3.7§	–2.4 ± 3.8§	3.2 ± 3.6*¥¤†	<0.001

Data represent mean ± SD (mmHg; n = 18). Spontaneous breathing (Sf_r; average 13.3 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 6.3 breaths·min^{-1}); systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial blood pressure (MAP); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

Peak-valley intra-breath phase responses

Intra-breath phase fluctuations in SBPΔi, DBPΔi, and MAPΔi, as well as SBPΔe, DBPΔe, and MAPΔe, were significantly different between the Sf_r and 8Ff_r conditions and the 4Ff_r, 6Ff_r and Df_r conditions (p<0.05; Table 5-3 & Figure 5-4). Following on from the analysis in Chapter 4, relative peak-valley intra-breath phase variables were calculated for Δi and Δe as a percentage of mean i and e for SBP and DBP (Figure 5-4). BP oscillations (relative Δi and Δe for both SBP and DBP) were significantly greater for the 4Ff_r, 6Ff_r and Df_r SDB conditions from both spontaneous (Sf_r) and 8Ff_r conditions, but not significantly different from each other. However, 8Ff_r was also significantly greater than Sf_r.

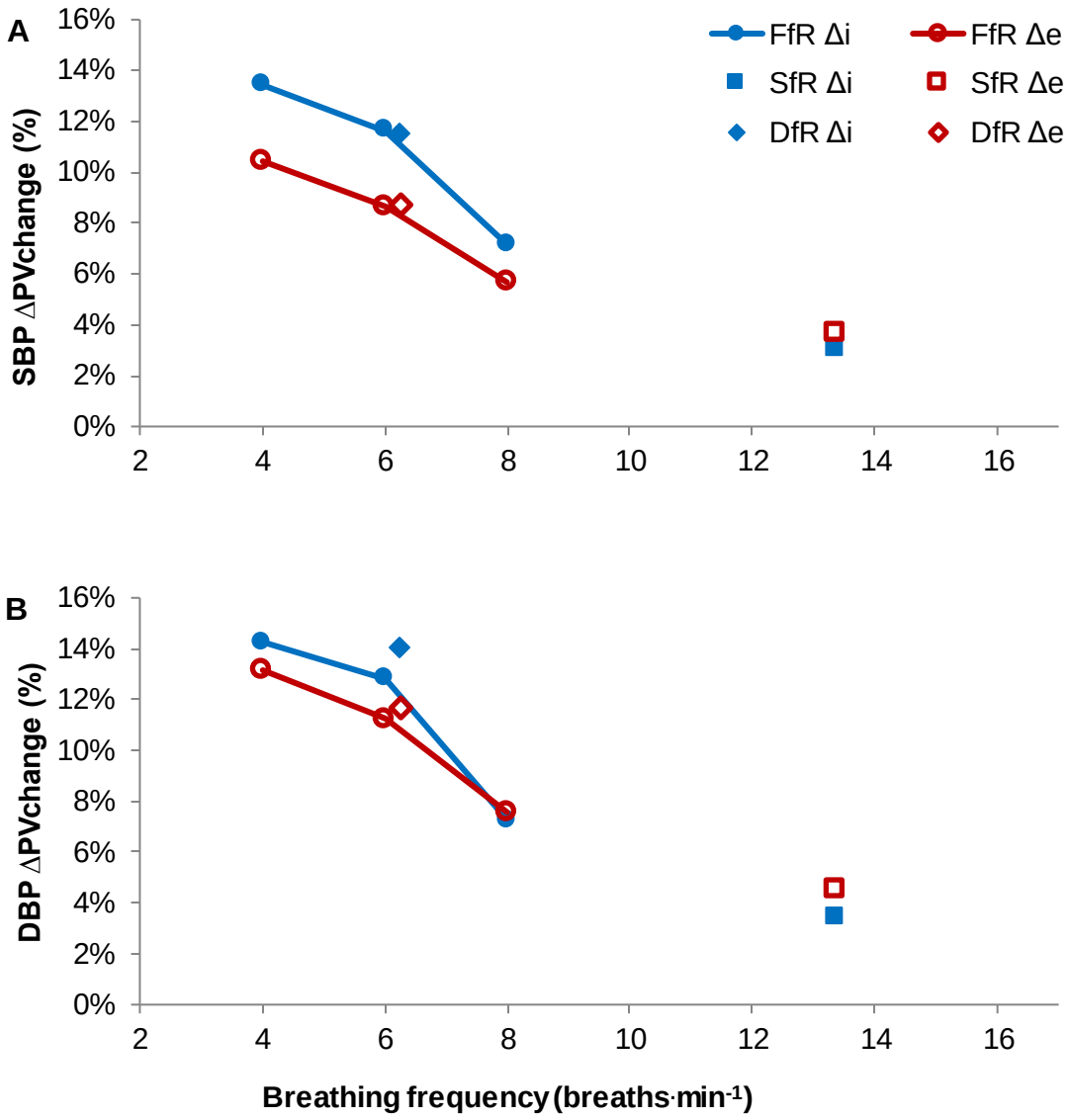


Figure 5-4 Blood pressure oscillations: Relative change of ΔI and ΔE for systolic blood pressure (A) and diastolic blood pressure (B)

Systolic blood pressure (SBP), diastolic blood pressure (DBP); within inspiration difference (Δi), within expiration difference (Δe); Fixed breathing frequency (Ff_f), Spontaneous breathing (Sf_f), optimisation algorithm dynamic breathing frequency (Df_f). Variable calculated as SBPΔi as a percentage of average SBP during inspiration, or equivalent during expiration and for DBP.

Peak-valley inter-breath phase responses

The within-breath peak-valley fluctuations of SBPΔPV, DBPΔPV, and MAPΔPV were significantly different between 4Ff_f and all other conditions, except for SBPΔPV between Sf_f and 4Ff_f ($p<0.05$; Table 5-3). There were no pairwise significant differences for peak-valley breath phase independent (ΔPV_Ind) SBP or DBP between any breathing conditions.

Table 5-3 Peak-valley differences (±SD) for blood pressure variables (mmHg)

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	13.3 breaths·min^{-1}		*6.3 breaths·min^{-1}*			
SBPΔi	3.6 ± 1.7¥¤†§	8.2 ± 2.9*¤†§	12.8 ± 5.4*¥	13.5 ± 4.6*¥	15.5 ± 6.1*¥	<0.001
SBPΔe	4.5 ± 2.5¥¤†§	6.9 ± 2.7*¤†§	10.2 ± 4.6*¥	10.5 ± 4.6*¥	12.1 ± 6.6*¥	<0.001
SBPΔPV	-8.6 ± 3.6	-13.1 ± 7.0§	-11.5 ± 13.6§	-15.3 ± 9.5§	2.3 ± 18.4¥¤†	0.001
SBPΔPV_Ind	15.0 ± 6.1	17.5 ± 5.6	19.1 ± 6.3	19.0 ± 5.1	17.4 ± 6.9	0.014
DBPΔi	2.5 ± 1.2¥¤†§	5.2 ± 1.8*¤†§	8.8 ± 2.9*¥	9.3 ± 3.1*¥	10.0 ± 3.0*¥	<0.001
DBPΔe	3.2 ± 1.6¥¤†§	5.6 ± 2.1*¤†§	7.6 ± 3.1*¥	8.2 ± 3.2*¥	8.8 ± 3.1*¥	<0.001
DBPΔPV	–4.0 ± 2.2¥§	–7.6 ± 2.0*§	–1.9 ± 11.2§	–3.4 ± 11.1§	11.6 ± 7.3*¥¤†	<0.001
DBPΔPV_Ind	9.9 ± 4.4	10.6 ± 2.9	12.1 ± 3.3	12.1 ± 2.5	12.7 ± 3.3	0.014
PPΔi	3.5 ± 1.6¥¤†§	4.8 ± 2.3*†§	6.0 ± 3.1*§	6.3 ± 2.6*¥§	9.2 ± 4.4*¥¤†	<0.001
PPΔe	4.0 ± 2.3†§	5.8 ± 2.8§	6.9 ± 4.3	7.0 ± 3.7*	8.8 ± 5.0*¥	<0.001
PPΔPV	–6.7 ± 4.2	–9.5 ± 5.9	–10.6 ± 7.0	–10.1 ± 6.7	–10.5 ± 7.4	0.034
MAPΔi	2.3 ± 1.3¥¤†§	5.8 ± 2.0*¤†§	9.8 ± 3.5*¥	10.3 ± 3.4*¥	11.2 ± 3.7*¥	<0.001
MAPΔe	3.1 ± 1.7¥¤†§	5.5 ± 1.8*¤†§	7.9 ± 3.0*¥	8.4 ± 3.3*¥	9.1 ± 3.9*¥	<0.001
MAPΔPV	–5.1 ± 1.9¥§	–9.1 ± 2.4*§	–4.8 ± 11.3§	-6.8 ± 10.8§	7.8 ± 11.8*¥¤†	<0.001

Data represent mean ± SD (mmHg; n = 18). Spontaneous (Sf_r; average 13.3 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 6.3 breaths·min^{-1}); systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial blood pressure (MAP); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

5.3.3 Heart rate and respiratory sinus arrhythmia

Mean and intra-breath phase responses

Mean heart rate (f_c) did not differ significantly between conditions (Table 5-4 & Figure 5-6A; p>0.05). Mean values for heart rate during inspiration (f_ci) during 4Ff_r, 6Ff_r and Df_r were significantly different from Sf_r (p<0.01), and significantly different between 6Ff_r and 8Ff_r (p<0.05). During expiration f_ce was significantly different between 8Ff_r, and both 4Ff_r and 6Ff_r (p<0.01), and between 4Ff_r and Sf_r (p<0.01).

Inter-breath phase responses

f_cΔ was significantly different between both Sf_r and 8Ff_r, and all other conditions (Table 5-4; p<0.001).

Peak-valley intra-breath phase responses

All SDB conditions were significantly different from Sf_r for f_cΔi and f_cΔe except for f_cΔe between 4Ff_r and Sf_r.

Peak-valley inter-breath phase responses

For the maximal within-breath fluctuation of f_c (f_cΔPV), Sf_r was significantly different from all SDB conditions ($p<0.01$), and f_cΔPV significantly different between 8Ff_r and both 6Ff_r and Df_r (Table 5-5; $p<0.05$).

Respiratory sinus arrythmia (peak-valley inter-breath phase)

Respiratory sinus arrythmia (RSA, i.e., the maximal amplitude of f_c fluctuations) was significantly higher in all SDB conditions compared with Sf_r ($P<0.001$; Table 5-5). In addition, RSA was significantly different between 8Ff_r and both 6Ff_r ($p<0.001$) and Df_r ($p=0.019$). RSA amplitude increased with decreasing frequency, reaching a zenith at ~6 breaths·minute^{-1} (Figure 5-5). Although group mean RSA is similar for 4Ff_r, 6Ff_r and Df_r, the frequency at which peak RSA occurred differed between individuals, occurring during 4Ff_r for 9 participants, during Df_r for 6 participants and during 6Ff_r for 3 participants.

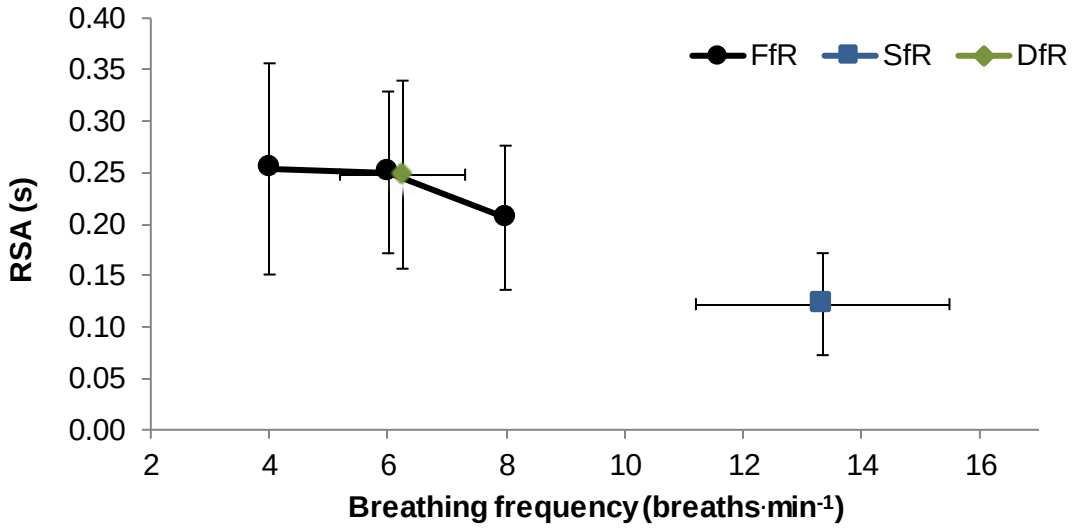


Figure 5-5 Respiratory sinus arrythmia (RSA) response to slow and deep breathing

Values are mean ± SD; Spontaneous breathing (blue ■; Sf_r), fixed breathing conditions (black ●; Ff_r) Brythm algorithm (green ♦; Df_r); respiratory sinus arrhythmia (RSA).

Table 5-4 Mean values (±SD) and inter-breath phase differences (Δ) for heart rate (f_c), stroke volume (SV) and cardiac output ($\dot{Q}$) variables

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	13.3 breaths·min^{-1}		*6.3 breaths·min^{-1}*			
f_c	67.6 ± 10.9	70.7 ± 11.6	70.0 ± 11.0	70.0 ± 10.5	69.5 ± 10.3	0.091
f_ci	67.0 ± 11.3¤†§	72.5 ± 12.1†	74.4 ± 12.5*	74.4 ± 12.0*¥	74.5 ± 11.0*	<0.001
f_ce	68.3 ± 10.6§	68.5 ± 10.8†§	65.8 ± 10.3	65.6 ± 9.2¥	64.6 ± 10.1*¥	0.002
f_cΔ	–1.4 ± 1.4¥¤†§	4.0 ± 2.9*¤†§	8.6 ± 5.4*¥	8.9 ± 4.9*¥	9.9 ± 4.8*¥	<0.001
SV	67.4 ± 19.8	66.7 ± 21.1	68.3 ± 21.3	66.2 ± 22.0	68.5 ± 18.4	0.539
SVi	65.2 ± 19.7	63.5 ± 20.4	64.9 ± 20.4	63.1 ± 20.7	65.1 ± 18.1	0.366
SVe	69.6 ± 20.0	69.9 ± 21.9	71.6 ± 22.4	69.3 ± 23.3	71.9 ± 18.9	0.490
SVΔ	–4.4 ± 2.9	–6.4 ± 3.6	–6.7 ± 4.6	–6.2 ± 4.4	–6.8 ± 3.5	0.188
$\dot{Q}$	4441 ± 1047	4564 ± 1144	4633 ± 1097	4482 ± 1220	4632 ± 1017	0.530
$\dot{Q}$i	4247 ± 1018¤	4476 ± 1181	4707 ± 1184*	4562 ± 1266	4737 ± 1096	0.026
$\dot{Q}$e	4639 ± 1083	4639 ± 1117	4566 ± 1034	4406 ± 1197	4527 ± 952	0.435
$\dot{Q}$Δ	–392 ± 155¥¤†§	–163 ± 250*¤†§	141 ± 307*¥	156 ± 278*¥	210 ± 264*¥	<0.001

Data represent mean ± SD (n = 18). Spontaneous breathing (Sf_r; average 13.3 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 6.3 breaths·min^{-1}); heart rate (f_c; beats·min^{-1}), stroke volume (SV; ml), cardiac output ($\dot{Q}$; ml·min^{-1}); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

5.3.4 Stroke volume

Mean and intra-breath phase responses

The SV, SVi, SVe, and SVΔ did not differ between conditions (p>0.05; Table 5-5 & Figure 5-6B).

Peak-valley intra-breath phase responses

However, peak-valley values for SVΔPV, SVΔi and SVΔe exhibited significant differences between conditions (p<0.001; Table 5-5). In particular, SVΔi was significantly different between 4Ff_r and all other conditions (p<0.01) and between Sf_r and 6Ff_r and Df_r (p<0.05).

Table 5-5 Peak-valley differences (±SD) for heart rate (f_c), stroke volume (SV) and cardiac output ($\dot{Q}$) variables

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	13.3 breaths·min^{-1}		*6.3 breaths·min^{-1}*			
f_cΔ*i*	5.4 ± 2.2$^{¥¤†§}$	14.4 ± 6.9*	15.5 ± 6.2*	15.5 ± 5.9*	15.9 ± 7.1*	<0.001
f_cΔ*e*	7.3 ± 3.2$^{¥¤†}$	14.3 ± 6.0*	14.1 ± 8.4*	13.4 ± 6.7*	10.3 ± 8.3	<0.001
f_cΔPV	–6.6 ± 5.1$^{¥¤†§}$	16.1 ± 8.9$^{*¤†}$	20.7 ± 8.5$^{*¥}$	20.6 ± 7.5$^{*¥}$	21.1 ± 9.5*	<0.001
RSA	0.12 ± 0.05$^{¥¤†§}$	0.21 ± 0.07$^{*¤†}$	0.25 ± 0.09$^{*¥}$	0.25 ± 0.08$^{*¥}$	0.25 ± 0.10*	<0.001
SVΔi	5.1 ± 2.0$^{¤†§}$	7.3 ± 3.9§	7.1 ± 3.5*§	7.7 ± 4.0*§	11.8 ± 4.9$^{*¥¤†}$	<0.001
SVΔe	5.6 ± 2.0$^{¥¤†§}$	8.2 ± 3.1*§	8.9 ± 4.0*§	9.2 ± 3.9*	13.5 ± 6.3$^{*¥¤}$	<0.001
SVΔPV	–8.9 ± 4.2§	–13.1 ± 5.9§	–13.8 ± 6.4	–14.6 ± 6.4	–18.6 ± 6.4$^{*¥}$	0.001
SVΔPV_Ind	11.9 ± 4.2§	13.7 ± 5.9	13.6 ± 6.4	13.4 ± 6.4	16.2 ± 6.4*	0.027
$\dot{Q}$Δi	292 ± 119$^{¥¤†§}$	1092 ± 646*	1107 ± 561*	1129 ± 607*	1073 ± 474*	<0.001
$\dot{Q}$Δe	414 ± 184$^{¥¤†§}$	831 ± 359*	840 ± 469*	787 ± 424*	747 ± 413*	<0.001
$\dot{Q}$ΔPV	–738 ± 203$^{¤†§}$	–638 ± 1153$^{¤†§}$	402 ± 1268$^{*¥}$	493 ± 1208$^{*¥}$	903 ± 923$^{*¥}$	<0.001
$\dot{Q}$ΔPV_Ind	1352 ± 1982	1302 ± 667	1366 ± 1038	1191 ± 565	1018 ± 540	0.489

Data represent mean ± SD (n = 18). Spontaneous breathing (Sf_r; average 13.3 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 6.3 breaths·min^{-1}); heart rate (f_c; beats·min^{-1}), respiratory sinus arrhythmia peak/valley amplitude (RSA; s), stroke volume (SV; ml), cardiac output ($\dot{Q}$; ml·min^{-1}); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

5.3.5 Cardiac output

Mean and intra-breath phase responses

Lower breathing frequencies were associated with an inversion of the within-breath pattern of $\dot{Q}$ (Figure 5-6C); at a breathing frequency of ~6 breaths·min^{-1} and lower (4Ff_r, 6Ff_r & Df_r), $\dot{Q}$i was higher than $\dot{Q}$e.

Inter-breath phase responses

This produced significant differences for QΔ between all SDB conditions compared with Sf_r, as well as between 8Ff_r and all other conditions (p<0.05; Table 5-4).

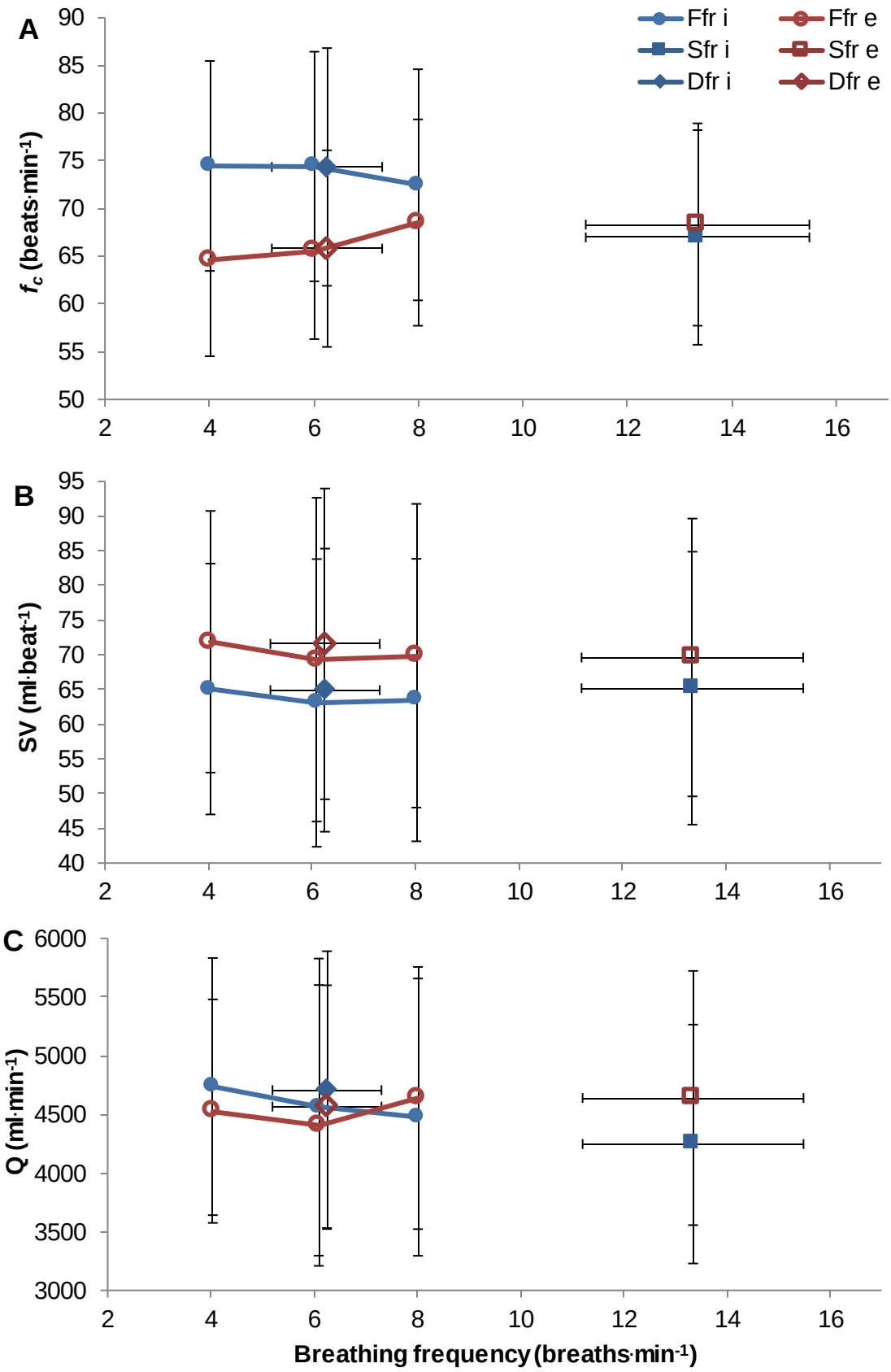


Figure 5-6 Mean intra-breath phase heart rate (fc), stroke volume (SV) and cardiac output (Q̇) variables response to slow and deep breathing

Data represent mean ± SD. Inspiratory (i: solid blue shapes), expiratory (e: open red shapes); uncontrolled spontaneous breathing (Sf_r; ■), fixed breathing frequencies ($4Ff_r$, $6Ff_r$, $8Ff_r$; ●), optimisation algorithm dynamic breathing frequency (Df_r; ♦); A. heart rate (f_c), B. stroke volume (SV), C. cardiac output (Q̇).

5.3.6 Pulse transit time and pulse wave velocity

Mean responses

There were no significant differences between conditions for mean pulse transit time (PTT) or pulse wave velocity (PWV, p>0.05; Table 5-6).

Inter-breath phase responses

PTTΔ at 4Ff_r was significantly different to during 6Ff_r and *8*Ff_r (p<0.001), and significantly different between Df_r and *8*Ff_r (p<0.01; Table 5-6). PWV followed the same pattern (p<0.05) except there was no significant difference between 6Ff_r and *8*Ff_r (p>0.05; Table 5-6).

Table 5-6 Mean values (±SD) and inter-breath phase differences (Δ) for pulse transit time (PTT) and pulse wave velocity (PWV) variables

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p
	13.3 breaths·min^{-1}		*6.3 breaths·min^{-1}*			**value**
PTT	187.2 ± 16.2	188.3 ± 19.3	185.6 ± 16.1	184.5 ± 16.8	184.3 ± 16.8	0.173
PTTi	190.5 ± 16.2	192.8 ± 18.9§	188.8 ± 16.8	188.3 ± 17.3	185.4 ± 17.6¥	0.005
PTTe	184.4 ± 16.3	184.0 ± 19.8	182.4 ± 15.5	180.7 ± 16.5	183.4 ± 16.2	0.440
PTTΔ	6.2 ± 1.6§	8.8 ± 3.9§	6.4 ± 3.2§	7.5 ± 3.7§	2.0 ± 4.3*¥¤†	<0.001
PWV	4.6 ± 0.4	4.6 ± 0.4	4.7 ± 0.4	4.7 ± 0.4	4.7 ± 0.4	0.143
PWVi	4.6 ± 0.4	4.5 ± 0.4§	4.6 ± 0.4	4.6 ± 0.4	4.7 ± 0.4¥	0.002
PWVe	4.7 ± 0.4	4.7 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	0.472
PWVΔ	–0.2 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.2	0.030

Data represent mean ± SD (n = 18). Spontaneous breathing (Sf_r; average 13.3 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 6.3 breaths·min^{-1}); pulse transit time (PTT) in ms, pulse wave velocity (PWV) in m·s^{-1}; mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

Peak-valley intra-breath phase responses

PWVΔI was significantly different during Sf_r to all SDB conditions except 8Ff_r (p0.05; Table 5-7).

Peak-valley inter-breath phase responses

The maximal inter-breath phase fluctuation of PWV (PWVΔPV) was significantly different between all conditions (p<0.05), except between 4Ff_r and 8Ff_r, and 6Ff_r and Df_r (Table 5-7).

Table 5-7 Peak-valley differences (±SD) for pulse transit time (PTT) and pulse wave velocity (PWV) variables

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	13.3 breaths·min^{-1}		*6.3 breaths·min^{-1}*			
PTTΔi	8.2 ± 2.5¤†§	15.7 ± 10.3	18.1 ± 7.1*	19.0 ± 10.5*	17.0 ± 5.7*	<0.001
PTTΔe	11.0 ± 4.1¥¤†	17.2 ± 9.5*	16.8 ± 7.3*	17.1 ± 7.8*	15.0 ± 9.1	0.003
PTTΔPV	13.1 ± 8.4¥¤†	24.5 ± 9.4*	23.0 ± 8.6*	25.3 ± 11.6*	11.2 ± 18.0	0.003
PWVΔi	0.2 ± 0.1¥¤†§	0.4 ± 0.2*	0.5 ± 0.2*	0.5 ± 0.2*	0.4 ± 0.1*	<0.001
PWVΔe	0.3 ± 0.1¥¤†	0.4 ± 0.2*	0.5 ± 0.2*	0.4 ± 0.2*	0.4 ± 0.2	0.001
PWVΔPV	0.4 ± 0.1¥¤†§	–0.7 ± 0.5*†	–0.6 ± 0.3*†	0.6 ± 0.3*¥§	–0.3 ± 0.5*†	<0.001

Data represent mean ± SD (n = 18). Spontaneous breathing (Sf$_r$; average 13.3 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff$_r$), 6 breaths·minute^{-1} (6Ff$_r$), 8 breaths·minute^{-1} (8Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$; average 6.3 breaths·min^{-1}); pulse transit time (PTT) in ms, pulse wave velocity (PWV) in m·s^{-1}; within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf$_r$ (*), 8Ff$_r$ (¥), Df$_r$ (¤), 6Ff$_r$ (†), 4Ff$_r$ (§); P<0.05.

5.3.7 Total peripheral resistance

There was no significantly difference in total peripheral resistance (TPR) between breathing conditions for any mean variables (TPR, TPRi, TPRe). For TPRΔPV, 6Ff_r and Df_r were significantly different from spontaneous breathing (Sf_r).

Table 5-8 Mean values (±SD), inter-breath phase differences (Δ) and peak-valley differences for total peripheral resistance (TPR)

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	13.3 breaths·min^{-1}		*6.3 breaths·min^{-1}*			
TPR	21.5 ± 8.3	20.0 ± 3.7	18.3 ± 3.5	20.9 ± 5.4	18.8 ± 3.5	0.171
TPRi	22.2 ± 8.6	20.0 ± 3.9	17.8 ± 3.6	20.2 ± 5.1	18.9 ± 3.8	0.084
TPRe	20.8 ± 7.9	20.0 ± 3.5	18.8 ± 3.6	21.6 ± 5.7	18.8 ± 3.4	0.233
TPRΔ	1.3 ± 1.1¥¤†	0.0 ± 1.1*¤†	-1.0 ± 0.9*¥	-1.4 ± 1.3*¥	0.0 ± 1.6	<0.001
TPRΔi	1.8 ± 2.2¥†§	4.0 ± 1.9*	3.0 ± 1.2	4.3 ± 2.4*	3.5 ± 1.7*	<0.001
TPRΔe	2.1 ± 2.0†	3.1 ± 1.4	2.8 ± 1.5	3.7 ± 2.3*	3.2 ± 1.3	0.020
TPRΔPV	2.3 ± 3.8¤†	0.8 ± 4.7	-3.3 ± 2.7*	-3.3 ± 5.1*	0.3 ± 4.8	<0.001
TPRΔPV_Ind	6.0 ± 6.3	5.2 ± 2.1	4.4 ± 1.9	5.4 ± 2.7	4.1 ± 1.7	0.192

Data represent mean ± SD (n = 18). Spontaneous breathing (Sf$_r$; average 13.3 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff$_r$), 6 breaths·minute^{-1} (6Ff$_r$), 8 breaths·minute^{-1} (8Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$; average 6.3 breaths·min^{-1}); total peripheral resistance (TPR) in mmHg·min·L^{-1}; mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e), within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf$_r$ (*), 8Ff$_r$ (¥), Df$_r$ (¤), 6Ff$_r$ (†), 4Ff$_r$ (§); P<0.05.

5.3.8 Central blood pressure

Pre- *vs.* post- condition changes in blood pressures were extremely small and showed no significant differences between conditions for any measured parameter (p>0.05; Table 5-9).

Table 5-9 Pre- vs. post- breathing condition differences in blood pressures and renal resistive index (RRI)

	n	**Sf_r**	**8Ff_r**	**Df_r**	**6Ff_r**	**4Ff_r**
		13.3 breaths·min^{-1}		*6.3 breaths·min^{-1}*		
SBP	18	-2.9 ± 8.7	4.8 ± 18.5	0.1 ± 6.7	-2.9 ± 7.9	-1.0 ± 9.0
DBP	18	-1.9 ± 8.4	0.4 ± 12.5	-4.1 ± 5.9	-2.3 ± 10.8	0.3 ± 6.5
PP	18	-1.0 ± 9.7	4.3 ± 13.4	4.2 ± 8.1	-0.6 ± 10.2	-1.3 ± 9.0
AoSBP	18	-2.6 ± 9.5	3.5 ± 18.1	-0.7± 6.4	-2.8 ± 8.1	-1.0 ± 8.6
AIx	17*	0.7 ± 4.1	0.5 ± 9.3	-3.5 ± 8.1	-0.3 ± 3.7	-0.7 ± 3.5
AIx75	17*	1.5 ± 4.2	-0.1 ± 10.7	-3.8 ± 8.4	-0.9 ± 3.8	-0.4 ± 2.8
PWV	9*	-0.3 ± 0.8	-0.2 ± 0.4	-0.4 ± 0.6	-0.3 ± 0.4	-0.4 ± 0.7
RRI	8	-0.03 ± 0.04	0.00 ± 0.03	0.00 ± 0.03	0.01 ± 0.02	-0.01 ± 0.04

*Data represent mean ± SD. Spontaneous breathing (Sf_r; average 13.3 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 6.3 breaths·min^{-1}); Systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg), aortic central systolic blood pressure (AoSBP; mmHg), pulse pressure (PP; mmHg), augmentation index (AIx; mmHg), pulse wave velocity (PWV; (m·s^{-1}), renal resistive index (RRI). *1 participant's data not recorded due to technical error.*

5.3.9 Renal resistive index

Renal resistive index (RRI) showed no difference pre- and post- breathing conditions between any of the conditions (p>0.05; Table 5-9).

Figure 5-7 displays mean heart rate (red line) calculated from the mean during a full breath phase (calculation 1, Figure 3-6), with beat-by-beat heart rate values (green line), to show the fluctuations of heart rate around mean breath values.

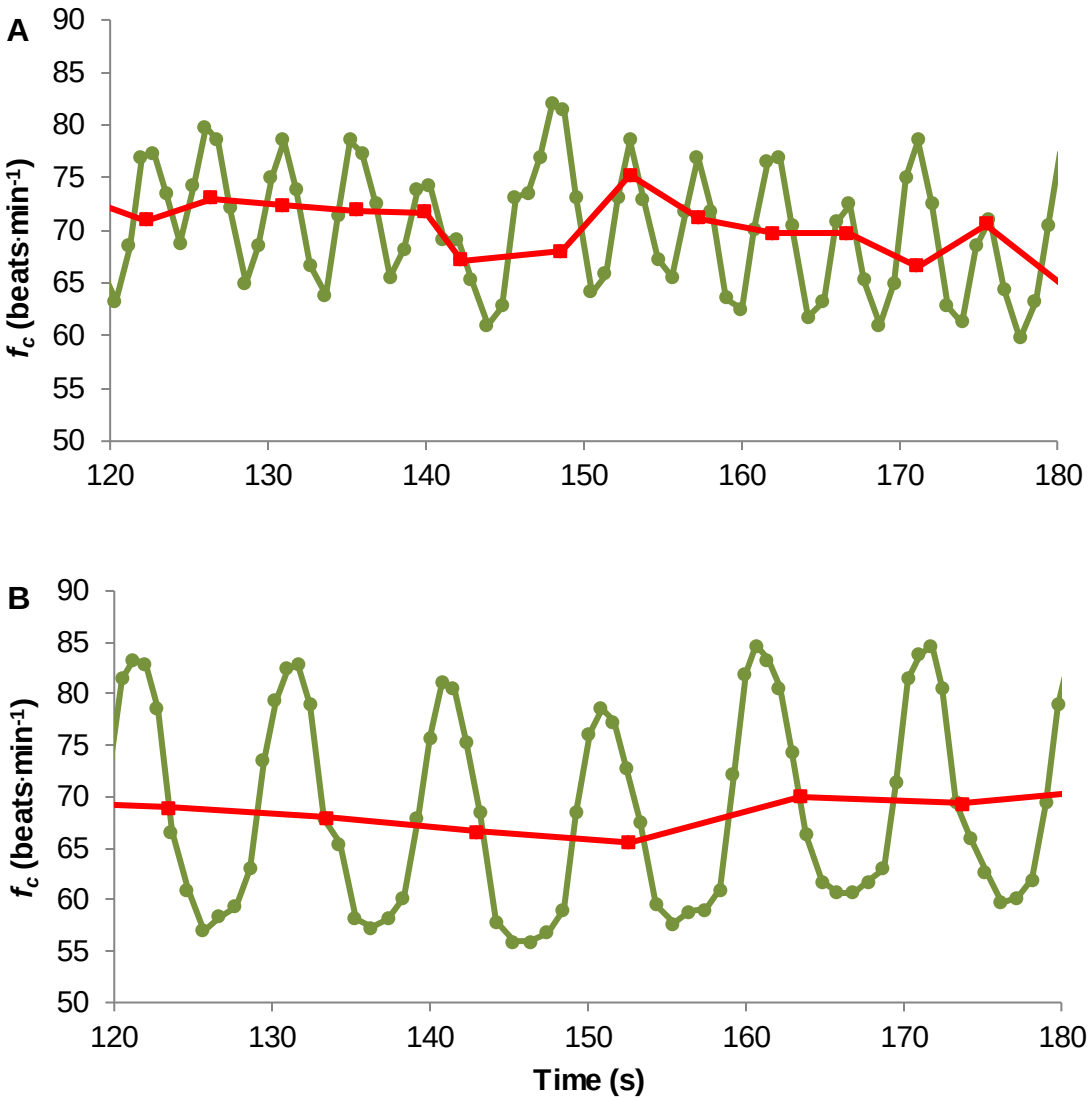


Figure 5-7 Example participant data: beat by beat and mean heart rate (f_c) during spontaneous breathing (A) and fixed breathing frequency of 6 breaths·min^{-1} (B)

Beat by beat heart rate (f_c; green ●) and mean full breath cycle heart rate (red ■) for A.) uncontrolled spontaneous breathing (Sf_r), and B.) fixed breathing frequency at 6 breaths·min^{-1} ($6Ff_r$) during the third minute of data collection (120-180 seconds) for one individual participant.

5.4 Discussion

The main finding of the study was that the influence of SDB is only revealed by a within-breath analysis, using peak-valley analysis. The true magnitude of perturbations created by SDB are obscured when mean values for the entire breath are examined. This is demonstrated by the response of heart rate, which shows no significant effect of breathing frequency upon mean heart rate, but a marked effect of SDB for $f_c\Delta$, $f_c\Delta$PV and RSA. Figure 5-7 illustrates this hidden response by presenting mean f_c during Sf_r and $6Ff_r$ plotted with beat by beat f_c data for an example participant. If only the mean heart

rate is considered (red series) then the true f_c response is obscured and averaged out of existence. In accordance with Nili et al. (2017) no influence of contraceptive pill or menstrual cycle phase was observed for the cardiovascular responses to SDB, supporting the use of the full data set for analysis, without separate analysis groups.

In accordance with previous studies (Anderson et al. 2009; Zhang et al. 2009) the amplitude of RSA increased as breathing frequency declined. This was due to changes both in f_ci and f_ce, but without a significant change in mean heart rate (f_c). Interestingly, although RSA plateaued at ~6 breaths·min^{-1}, for 50% of participants maximum RSA occurred during 4Ff_r. This supports the notion that individual differences exist in responsiveness to SDB and indeed Vargas (2017) found individual variation in the breathing frequency which produced the largest cardiovascular perturbations. Despite this, the individualised breathing condition used in this study (Df_r) did not produce significantly larger RSA values, or produce larger cardiovascular perturbations than the fixed breathing frequency of 6 breaths·min^{-1}. An explanation may be that because the average frequency for Df_r was 6.3 breaths·min^{-1}, and therefore was not significantly different from the fixed frequency of 6 breaths·min^{-1}, Df_r could not produce a larger cardiovascular response as RSA was already maximised at this level. Df_r uses RSA to drive breathing frequency and therefore as the data shows that RSA maximises at ~6 breaths·min^{-1} there was nothing to drive the algorithm to reduce breathing frequency further. Consequently, building on the results from Chapter 4, RSA was not further amplified at lower breathing frequencies and ~6 breaths·min^{-1} may the lowest breathing frequency to maximise perturbations, beyond which further increase in amplitude are not possible. The Df_r in Chapter 4 did produced peak RSA (0.21s) at 5.5 breaths·min^{-1}, however peak RSA in Chapter 4 was lower than the peak RSA produced in the present study (0.25s), supporting the theory that peak RSA is produced ~6 breaths·min^{-1}.

The finding that mean BP values do not change during SDB is in agreement with results found previously in normotensive participants (Joseph et al. 2005), and supports the need for researchers to look beyond mean values, as BP oscillations exhibited significant increases during SDB. As mean ABP measured during SDB were not significantly different it is unsurprisingly that PWA and PWV measures were also found not to be significantly affected by SDB. The cardiovascular system seems to be able to maintain mean ABP during SDB, including at a central level (AoSBP), through the peripheral vasculature. AIx and AIx75 were found to decrease following SDB in children, with no change in PWV, however it should be noted that these values both increase with age (Calcaterra et al. 2014) and therefore direct comparisons are limited due to differing

baseline values. Higher baseline levels may influence the response to SDB and explain the lack of response observed in this study.

No response of BP to SDB is discernible when mean values are examined, which accords with previous studies of normotensive individuals (Bernardi et al. 2002). However, the novel analytical approach used in this study revealed oscillations in ABP that were not apparent within the mean data in all variables. The intra-breath phase analyses undertaken in this study revealed fluctuations of SBP during inspiration (SBPΔi) of up to 13.3% (15.5 mmHg) and up to 10.4% (12.1 mmHg) during expiration (SBPΔe). During Sf_r fluctuations in SBPΔi were only 3% (3.6 mmHg) and 3.6% (4.5 mmHg) in SBPΔe. This is mirrored in the response of DBP, with maximum changes of up to 13.9% (10 mmHg) during inspiration (DBPΔi) and up to 12.9% (8.8 mmHg) during expiration (DBPΔe). In comparison fluctuations in DBPΔi were 3.3% (2.5 mmHg) and 4.3% (3.2 mmHg) in DBPΔe during Sf_r. The largest amplitude of BP oscillations occurred during 4Ff_r showing that BP oscillations increase as breathing frequency reduces. Following the future research suggestions from Chapter 4, where the lowest breathing at 5.5 breaths·min^{-1} produced the largest BP oscillations, this present study revealed that further reductions in breathing frequency to 4 breaths·min^{-1} are able to increase amplitude of BP oscillations further, unlike RSA.

The SDB-induced amplification of fluctuations in both SBP & DBP, within inspiration and expiration, suggest that fluctuations in ABP are adequately minimised during normal breathing and to a certain extent at higher frequencies of SDB (8Ff_r). However, at frequencies ≤6 breaths·min^{-1} BP fluctuations can no longer be managed by countermeasures and changes in other cardiovascular variables that stabilise ABP. In normal breathing conditions it is suggested the synchronisation of SV with heart rate stabilises ABP across breath phases (Elstad et al. 2018), as cardiac output is maintained across breath phases due to respiratory-induced changes in heart rate (RSA) counteracting the decrease in SV which occurs during inspiration (Elstad 2012). Within the SV/RSA synchronisation it is believed RSA is the main mechanism stabilising ABP, as MAP fluctuations are increased when RSA is removed using a parasympathetic blockade (Toska and Eriksen 1993). Through RSA's relationship with cardiac output, RSA can influence oscillations in MAP (Elstad et al. 2011). The results in the present study show mean heart rate and RSA plateau ≤ 6 breaths·min^{-1} and therefore have potentially reached their maximum amplitude, meaning they can no longer contribute to system regulation and assist in maintaining cardiac output and therefore BP. Without being able to call on heart rate to buffer breathing-induced fluctuations in cardiac output, there are no control measures to prevent the larger fluctuations in BP caused by SDB as

seen in this study. Although mean BP is maintained, the fluctuations during breath phases (inspiration and expiration) are not controlled during SDB, and consequently the amplitude of BP oscillations increases.

Research into resistant hypertension has utilised baroreceptor activation therapy, providing repeated stimulation of the baroreceptors, as a treatment method for high blood pressure (Cracchiolo et al. 2021). Baroreflex sensitivity has been observed to increase acutely during SDB (Guzik et al. 2007) and consequently SDB could use the same mechanisms for chronic BP reduction as baroreceptor activation therapy. The aforementioned increase in amplitude of BP oscillations could provide the stimulation of baroreceptors which is produced by electrical stimulation during baroreceptor activation therapy. Consequently, one mechanism contributing to the anti-hypertensive effect of SDB could be the acute intra-breath phase fluctuations in BP. In addition to the acute hemodynamic changes SDB extends the duration of both breath phases such that fluctuations with a fixed time constant are afforded longer to reach their peak, which would inevitably increases their amplitude, assuming the system has not yet reached its saturation point. For example, whilst breathing at 6Ff_r, SBPi fluctuates from 110.8 to 124.3mmHg and SBPe from 116.9 to 127.4mmHg. Oscillations in the cardiorespiratory system are lost in many diseases and therefore it is logical, but currently unproven, that re-establishing these fluctuations may be beneficial to numerous clinical conditions including hypertension (Elstad et al. 2018). In hypertension, following chronic adaptation, the body's control circuits are adjusted to a higher set point (Wallbach and Koziolek 2018). It is possible BP oscillations of the amplitude found in this study could be sufficient to create an 'error signal' that resets these circuits to a lower, more normal set point, reducing BP chronically.

No significant differences in RRI were found, which also supports this study's findings that responses to SDB are only visible in the inter- and intra-breath phase analysis, with mean values not reflecting the full cardiovascular response. This may be amplified by the technique needed to measure RRI (recorded during a breath hold), which negates any possible inter- and intra- breath responses to SDB. It seems that measuring variables during a breath hold, or as a full breath cycle mean, will be unlikely to reveal any cardiovascular responses to SDB.

Previous research has suggested that breathing through an inspiratory resistance can increase cardiovascular perturbations (Vargas 2017). However, the present study found no significant differences for any cardiovascular variables between 6Ff_r and the same frequency with an added IR. The lack of amplification of cardiovascular response with an

added resistance has been reported previously, with no added benefits of inspiratory resistance observed compared with breathing at the same SDB frequency (Nuckowska et al. 2019). Previous studies using IR to reduce BP following daily practice have included a control condition where participants are instructed to just breathe deeply without breathing frequency being measured to ensure compliance (Ubolsakka-Jones et al. 2017; Ubolsakka-Jones et al. 2018). Therefore, there is a question on whether the deep breathing control condition is fully optimising cardiovascular perturbations at an optimal SDB breathing frequency, without the inspiratory load, and therefore whether the inspiratory load is simply creating the optimal conditions to increase perturbations, in lieu of the optimal breathing frequency. This suggests that if breathing frequency is truly optimised at ~6 breaths·min^{-1} the cardiovascular perturbations are already maximised and that adding an inspiratory resistance produces no extra benefit, except in conditions where the breathing frequency has not reached optimal levels.

Finally, when examining the differences between breathing at 8 breaths·min^{-1} and lower breathing frequencies, observations revealed significant differences between 8Ff_r and other SDB conditions (4Ff_r, 6Ff_r, Df_r) for variables such as RSA and both absolute and relative BP oscillations. Nevertheless, 8Ff_r was significantly different from Sf_r, for most variables which showed a response to SDB conditions, revealing there was an attenuated response to SDB. However, cardiovascular fluctuations during 8Ff_r were lessened compared with lower SDB breathing frequencies, suggesting that although there is a cardiovascular response to breathing at 8 breaths·min^{-1}, it does not produce optimal conditions to produce maximum cardiovascular perturbations. This has a potential impact on the effectiveness of the RESPeRATE device, as within Chapter 4 it produced an average breathing frequency of ~8 breaths·min^{-1} in the first 5-min. The data from the present study therefore suggests that the first 5-min of RESPeRATE may not maximise cardiovascular perturbations and consequently alternative SDB conditions such as 6Ff_r and Df_r could produce enhanced health benefits, as a result of larger cardiovascular fluctuations during the breathing interventions.

5.5 Conclusion

The data suggest that personalising breathing frequency during SDB using a method driven by RSA amplitude may not always maximise cardiovascular perturbations. However, the data confirm that SDB of ≤6 breaths·min^{-1} is needed to maximise the BP fluctuations that may provide the error signal underpinning the antihypertensive effects of regular practice of SDB. SDB frequencies ~8 breaths·min^{-1} produce attenuated responses to SDB and therefore interventions which utilise SDB at these levels may not

produce optimal results for health benefits compared with lower breathing frequencies. Analysis of the inter- and intra-breath phase fluctuations are vital to gaining an understanding of the true nature and magnitude of cardiovascular perturbations created by SDB.

This study addressed the need to understand the cardiovascular responses in healthy young women and should be replicated in other populations who have clinically diagnosed hypertension. It will be important to investigate whether the responses to SDB are different in people who have higher resting BP. Specifically, a condition exists which is unique to pregnancy whereby high BP develops in previously healthy women; so-called pregnancy-induced hypertension (PIH). These women often have no other confounding health variables that affects the researcher's ability to pinpoint potential mechanisms of change, and therefore PIH is a logical next step to understanding the cardiovascular responses to SDB. However, given the physiological changes that pregnancy itself brings, it is important to understand how pregnancy *per se* influences the cardiovascular responses to SDB. Therefore, an understanding of how the physiological changes during pregnancy affect women's cardiovascular responses to SDB in a normotensive population is needed, so that data is available to act as a baseline comparison when examining the SDB responses of women who develop PIH.

5.6 Summary

A full understanding of the complex cardiovascular responses of healthy non-pregnant women to SDB has now been completed. Due to the physiological differences and adaptations associated with pregnancy, as outlined in section 2.1 it is important to explore whether these adaptations influence the responses of pregnant women to SDB. Pregnancy increases heart rate, stroke volume, cardiac output, and these higher baseline levels may affect the ability of the body to respond acutely to the stimuli generated by SDB.

Chapter 6. Short-term cardiovascular responses to slow and deep breathing in healthy pregnant women

6.1 Introduction

Breathing exercises are an integral part of most antenatal classes undertaken during pregnancy, where relaxation and deep breathing to support labour and manage contractions is taught. Additionally, practices that incorporate controlled breathing such as yoga and meditation are popular during pregnancy and produce health benefits associated with reducing stress levels, autonomic nervous system functioning and labour parameters (Curtis et al. 2012; Muthukrishnan et al. 2016). Initial evidence suggests yoga is well suited to pregnancy (Satyapriya et al. 2009), however robust randomised controlled trials (RCTs) are lacking in this area (Curtis et al. 2012) and it is not known which elements of yoga (exercise or breathing) cause the physiological adaptations that result in measurable health benefits.

Slow and deep breathing (SDB), using biofeedback pacing is a new type of breathing exercise that has been used in the general population to reduce blood pressure (BP) when practiced daily (Chaddha et al. 2019; see Chapter 2.3). During pregnancy a specific type of hypertension can develop called pregnancy-induced hypertension (PIH). NICE (NICE: National Institute for Health and Care Excellence 2019b) define PIH as "new hypertension presenting after 20 weeks of pregnancy without significant proteinuria" and the only known cure for PIH is to give birth. Specific controlled SDB has not yet been evaluated as a treatment method for women who develop PIH, however a number of related studies indicate the potential for benefit. For example, muscle relaxation techniques (Jacobson method) and SDB to a non-specific range of 6-10 breaths·min^{-1} performed daily for 4 weeks reduced both systolic BP and diastolic BP for pregnant women with PIH (Aalami et al. 2016). Although some studies found no direct change in BP following breathing exercise interventions, variables related to delivery (childbirth) were improved, including a 35% higher birth weight and 10% greater gestational age at delivery compared with the control group (Cullins et al. 2013). The greater gestational age at delivery is likely linked to the higher birth weight in the intervention group but this correlation was not examined or taken into consideration. It should also be noted that this study was not a randomised control trial. Consequently, confounding factors were unlikely to be evenly disrupted across groups and therefore it is not known whether participant characteristics in this group would be associated with a higher risk of pre-term birth or low birth weight. Other interventions, such as those including yoga, which includes elements of SDB, also reduce stress levels, improve

quality of life, autonomic nervous system functioning and labour parameters such as comfort, pain and delivery duration in pregnant women (Curtis et al. 2012).

The mechanisms that lead to any anti-hypertensive effects of SDB in the general population are not fully understood, but preliminary research to understand the acute cardiovascular response to SDB was presented in Chapters 4 and 5. Understanding the immediate cardiovascular responses to SDB can provide an indication to the potential error signal(s) that could elicit BP reductions. The acute cardiovascular responses to SDB have been characterised in healthy women in Chapter 5, using novel inter- and intra-breath phase analyses. Among other variables, respiratory sinus arrythmia (RSA) was shown to be amplified significantly during SDB, as were the amplitude of BP oscillations. Increases in the amplitude of BP oscillations during SDB were only revealed by using within- and between-breath analyses. These revealed SDB induces BP oscillations across the respiratory cycle in the region of 5-10% for both SBP and DBP. Due to the known cardiovascular and respiratory changes that women experience during pregnancy (see section 2.1) the cardiovascular response to SDB may differ in pregnant women.

It is not known whether differing haemodynamics and breathing mechanics between healthy non-pregnant and healthy pregnant women influence the response to SDB. It is important to understand the acute response of healthy pregnant women to SDB, before exploring the acute and chronic effects of SDB with women who develop PIH. The present study aims to characterise the acute cardiovascular response to SDB in healthy pregnant women, using a similar experimental design and novel inter- and intra-breath phase analysis used in Chapters 4 and 5 with healthy non-pregnant women. The research questions, objectives and hypothesis for this study are outlined below:

Research questions

1. Using a novel peak-valley analysis method, what are the complex cardiovascular responses to SDB of healthy pregnant women?
2. Are there differences in the acute cardiovascular responses at a range of SDB frequencies for healthy pregnant women?

Objectives

1. Characterise the response of mechanism-related parameters (e.g. respiratory sinus arrythmia, stroke volume, cardiac output, blood pressures) during SDB for healthy pregnant women.

2. Characterise acute cardiovascular responses to a range of SDB frequencies in healthy pregnant women.

Hypothesis

1. Peak-valley measures of cardiovascular parameters (respiratory sinus arrythmia, stroke volume and cardiac output) and amplitude of blood pressure oscillations will increase during SDB for healthy pregnant women.
2. The amplitude of peak-valley fluctuations will increase as SDB frequency decreases for healthy pregnant women.

6.2 Specific Methods

6.2.1 Participants

Eighteen pregnant women participated in the study. Participants were recruited from local antenatal groups, Facebook groups, and events such as expectant parent evenings, 'bump to baby' shows and specialised markets selling second-hand baby and maternity related items. Specifically, the NCT (National Childbirth Trust) and other local antenatal groups promoted the study on social media and during their local antenatal classes.

Participants were pregnant women at >20 weeks gestation at the time of data collection. This eligibility criterion was chosen to match the gestation in weeks of participants who are diagnosed with pregnancy-induced hypertension (planned participants for Chapter 8). All participants were also carrying singleton pregnancies due to the physiological differences associated with multiple pregnancies such as increased blood volume (Norwitz et al. 2005), heart rate, stroke volume, and increases in BP prominent after 20 weeks gestation (Kametas et al. 2003), which may change the cardiovascular response to SDB. Multiple pregnancies may also require an increase in the use of the accessory muscles of respiration, which may affect the ability to perform SDB or change the respiratory-cardiovascular interactions (Norwitz et al. 2005). All women were nulliparous (never given birth to a live baby). For full inclusion and exclusion criteria see the general methods chapter (section 3.3.2).

6.2.2 General Design

Participants attended one session at the Cardiorespiratory Research Laboratory at Bournemouth University. Laboratory conditions were recorded for each session; 24.0 ± 3.6 ^{o}C (range 19.1 – 28.6 ^{o}C), 994.3 ± 18.9 hPa (959 – 1050 hPa), 42.4 ± 9.7% (25 – 61%). Using a within-subject design, participants performed the breathing conditions in

a randomised order. Participants completed five breathing conditions; spontaneous breathing, 4, 6 and 8 breaths·min^{-1} and a dynamic frequency controlled by an optimisation algorithm. All breathing conditions were five-minutes in duration with a five-minute period of normal breathing prior to each measurement. Participants performed the five breathing conditions in a randomised order (see Figure 6-1 for a schematic of the protocol).

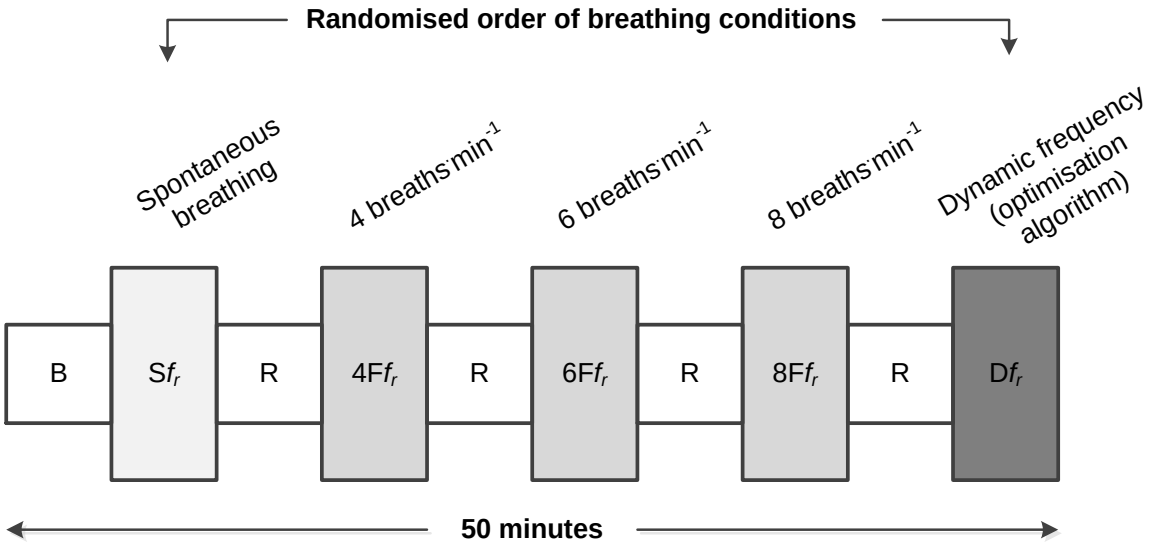


Figure 6-1 Schematic of protocol
All breathing condition and recovery periods were 5-minutes in duration; spontaneous baseline breathing (B), recovery periods (R), uncontrolled spontaneous breathing (Sf_r), fixed breathing frequency of 4 breaths·minute^{-1} ($4Ff_r$), 6 breaths·minute^{-1} ($6Ff_r$), 8 breaths·minute^{-1} ($8Ff_r$), optimisation algorithm dynamic breathing frequency (Df_r).

Using a fetal monitor was considered to monitor the fetus during the study protocol but currently available equipment such as the Novii Wireless Patch System and Meridian M110 Fetal Monitor are only approved for monitoring fetus >36 weeks gestation. Study participants were recruited from 20 weeks gestation and therefore the majority of participants could not have been monitored under the equipment's approved usage. Research during clinical trials has successfully monitored a fetus at 26 weeks, but given the investigator's lack of clinical experience in midwifery and fetal monitoring it was decided that this could cause concern if monitoring of the fetus was not successful at these lower gestational ages.

The intervention (SDB) was not considered to be a risk to the fetus. Controlled breathing is commonly taught during pregnancy, and breathing techniques such as Lamaze breathing specifically recommend slow breathing (Hughey et al. 1978). Yoga, which involves breathing are a controlled and slow rate, does not significantly change fetal heart rate compared with either rest or meditation (Gavin et al. 2020). Although breathing at a frequency of 7.5 breaths·min^{-1} for 10 minutes produced a decrease in fetal heart rate of 4.5 beats·min^{1} (Vasundhara et al. 2018), fetal heart rate did not reach levels of

bradycardia <110 beats·min^{1} which would be considered a sign of fetal distress. Additionally, feto-placental circulation and fetal cardiac function are not significantly affected by breathing conditions such as obstructive sleep apnoea (Robertson et al. 2020).

After completing the protocol, participants were asked which breathing condition they preferred. The question was framed in the context of choosing the breathing condition they would prefer to use if they were asked to perform the SDB daily at home until they gave birth. Following the data collection session participants submitted their antenatal appointment BP measurements up until they gave birth to ensure that no participants subsequently developed hypertension. The submitted BP measurements were recorded by their midwife during their routine antenatal appointments. The measurements were submitted via an online form (Online Surveys) or via e-mail.

6.2.3 Equipment and procedures

Respiratory airflow, ECG and arterial blood pressure (ABP) were monitored continuously throughout each breathing condition. Participants wore an oronasal mask (Oro Nasal 7450 V2 Mask, Hans Rudolph Inc., Kansas, USA) and respired flow rate was measured continuously using a heated pneumotachograph (Model 3700, Hans Rudolph Inc., Kansas, USA), connected to a flow measurement system (RSS 100-HR, Hans Rudolph Inc., Kansas, USA).

Heart rate was monitored using a 3-lead ECG and non-invasive beat-to-beat ABP was obtained using finger photoplethysmography (Finapres NOVA, Finapres Medical Systems, The Netherlands). Finapres derived ABP was calibrated using a brachial cuff prior to and halfway through data collection. Analogue outputs from the Finapres NOVA and the flow meter were sampled continuously at 250Hz via an analogue to digital converter (NI USB-6218 BNC, National Instruments Inc.) and captured using bespoke acquisition and analysis software (LabView 2015, National Instruments, Inc.). For more detailed explanation of the data acquisition refer to Chapter 3.4.

6.2.4 Statistical analysis

Values are expressed as means ± SD unless stated otherwise. Statistical analysis was undertaken using SPSS Statistics 25 (IBM Corp.). After normality (Shapiro Wilk) was confirmed repeated measures ANOVA with planned pairwise comparisons using Bonferroni corrections were used. Reported p values are those following adjustment for repeated comparisons. For all analyses, P was set at 0.05.

6.3 Results

Data were collected from 18 participants. One participant was excluded as she failed to adhere to the prescribed breathing condition and therefore data are presented for 17 participants (age 32.0 ± 5.4 years; stature 1.67 ± 0.8 m; mass 84.1 ± 13.4kg: systolic BP 118.2 ± 7.7 mmHg; diastolic BP 71.9 ± 7.9 mmHg; gestational age 31.4 ± 5.2 weeks). Figure 6-2 presents a flow chart of the number of women who were excluded, withdrew and participated in the study, with 36% of women taking part after being assessed for eligibility. The 8 women who actively chose not to take part contacted the investigator to remove themselves from the list of potential participants. Half of these women did not give a reason for not taking part, 3 women chose not take part due to time constraints and 1 because of illness.

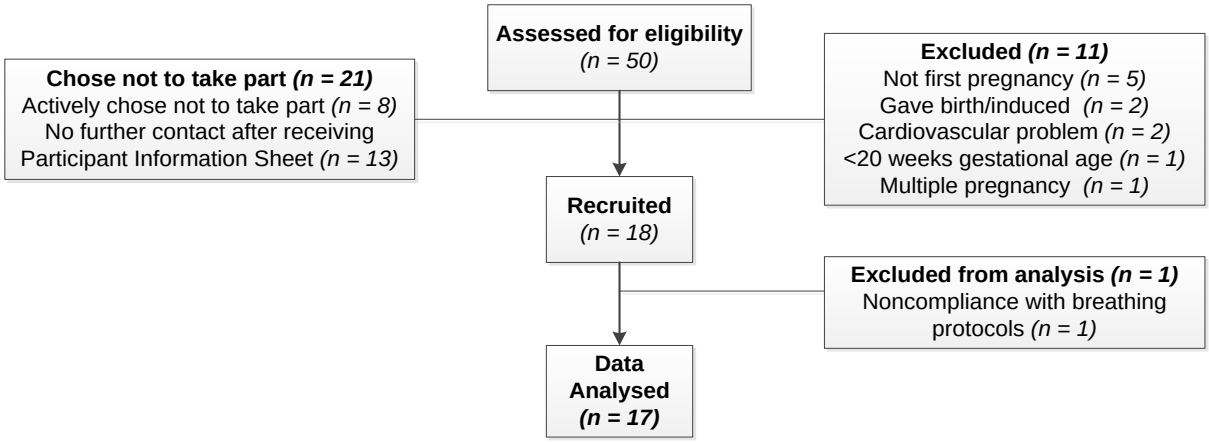


Figure 6-2 Flow chart of pregnant women who participated, were excluded, and withdrew

Note: One women is duplicated in the above numbers, she was excluded for not being above 20 weeks gestation at first contact, she subsequently suffered a miscarriage and participated in the study during a subsequent pregnancy.

6.3.1 Recruitment

The majority of the women were made aware of the study through Facebook (Figure 6-3). However, the investigator (MF) conducted a number of public engagement events such as the Dorset Bump 2 Baby Show and attended antenatal workshops in person.

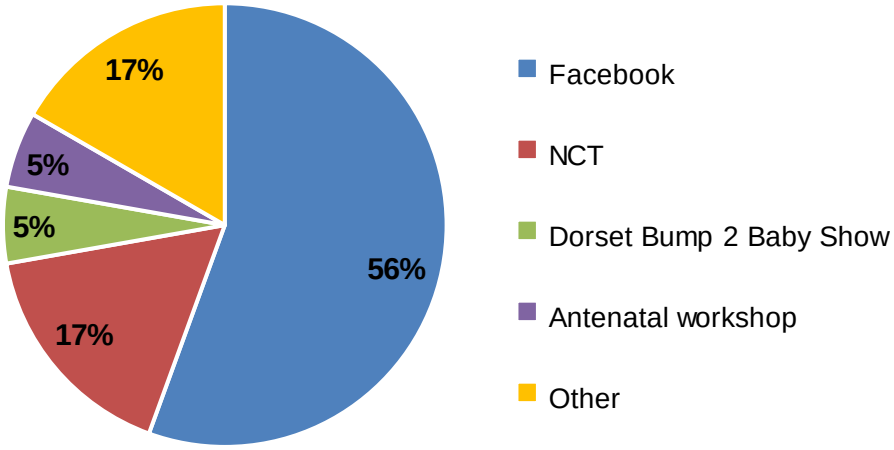


Figure 6-3 Source of participant recruitment
n = 18. National Childbirth Trust (NCT), Antenatal workshop is a local series of workshops for expectant mothers and their partners. Other includes women where the source of recruitment was unknown, i.e. unknown leaflet and through word of mouth with unknown origin.

Despite high engagement with the Facebook groups, only 43% of women who made contact following a Facebook promotional post took part in the study. Additionally, only 20% of women who provided their contact details at the Dorset Bump 2 Baby Show took part in the study, with 60% not engaging in any further contact after the event.

6.3.2 Normative respiratory and cardiovascular data during pregnancy

Due to the lack of normative respiratory and cardiovascular data during pregnancy, Table 6-1 presents average cardiovascular data for 17 pregnant participants during the 5-min spontaneous breathing condition (Sf_r).

Table 6-1 Normative respiratory and cardiovascular data in pregnancy

Cardiovascular variable	Average
Breathing frequency *(breaths·minute^{-1})*	14.2 ± 2.7
Tidal volume *(L)*	0.8 ± 0.5
Systolic blood pressure *(mmHg)*	122.1 ± 12.5
Diastolic blood pressure *(mmHg)*	74.5 ± 10.2
Pulse pressure *(mmHg)*	47.6 ± 6.0
Mean arterial pressure *(mmHg)*	88.8 ± 10.6
Heart rate *(beats·min^{-1})*	80.7 ± 10.1
Stroke volume *(ml)*	82.3 ± 9.8
Cardiac output *(l·min^{-1})*	6.6 ± 0.9
Pulse wave velocity *(m·s^{-1})*	5.1 ± 0.5

Normative respiratory and cardiovascular data from spontaneous breathing condition (Sf_r; 5-min average). n=17; gestational age 31.4 ± 5.2 weeks.

6.3.3 Respiratory variables

Seventeen participants were included in the data analysis. Table 6-2 provides an overview of the respiratory parameters for each condition. Duty cycle (T_I / T_{TOT}) remained consistent throughout conditions. The optimisation algorithm (Df_r) produced a significantly different breathing frequency from all other breathing conditions ($p<0.001$) with the optimum (maximal RSA) occurring at a breathing frequency of 7.0 ± 1.1 breaths·min^{-1}. All other breathing conditions were significantly different from each other.

Table 6-2 Respiratory parameters

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r
f_r	14.2 ± 2.7¥¤†§	8.0 ± 0.1*¤†§	7.0 ± 1.1*¥†§	6.0 ± 0.0*¥¤§	4.0 ± 0.0*¥¤†
V_T	0.8 ± 0.5¤†§	1.1 ± 0.3§	1.1 ± 0.3*§	1.5 ± 0.7*	1.6 ± 0.6*¥¤
T_I / T_{TOT}	0.54 ± 0.4	0.52 ± 0.2	0.48 ± 0.1	0.47 ± 0.0	0.56 ± 0.3

Data represent mean ± SD (n = 17). Spontaneous breathing (Sf_r), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r). Breathing frequency (f_r; breaths·min^{-1}), tidal volume (V_T; L), duty cycle (T_I / T_{TOT}); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

Breathing frequency during the Sf_r condition was not correlated with gestational age (R^2=0.14) and neither was the average optimal breathing frequency based on RSA maximisation (Df_r; R^2=0.11).

6.3.4 Arterial blood pressures

Mean and intra-breath phase responses

There were no significant differences in mean systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) or mean arterial pressure (MAP) between any of the breathing conditions ($p>0.05$; Table 6-3). However, for Df_r and 6Ff_r, mean DBP and MAP during expiration were significantly lower than Sf_r ($p=0.021$).

Inter-breath phase responses

Inter-breath phase responses (i.e., difference between mean value during inspiration *vs.* mean value during expiration, calculation 4, Figure 3-6) were significantly different between breathing conditions for SBPΔ ($p<0.001$), DBPΔ ($p<0.001$) and MAPΔ ($p<0.001$). All SDB breathing conditions less than 8 breaths·min^{-1} were significantly different from Sf_r (Table 6-3).

Table 6-3 Mean values (±SD) and inter-breath phase differences (Δ) for blood pressure variables (mmHg)

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	14.2 breaths·min^{-1}		*7.0 breaths·min^{-1}*			
SBP	122.1 ± 12.5	117.1 ± 17.4	115.4 ± 13.9	117.1 ± 10.7	117.2 ± 15.8	0.347
SBPi	119.8 ± 12.5	115.6 ± 18.1	114.9 ± 14.4	118.0 ± 11.5	118.7 ± 15.5	0.519
SBPe	124.3 ± 12.5	118.5 ± 16.8	116.0 ± 13.8	116.3 ± 10.3	115.7 ± 16.3	0.062
SBPΔ	-4.5 ± 1.8¤†§	-2.9 ± 3.8†§	-1.1 ± 4.1*†§	1.7 ± 4.7*¥¤	3.0 ± 2.2*¥¤	0.000
DBP	74.5 ± 10.2	73.2 ± 12.8	69.7 ± 10.3	71.6 ± 7.3	71.3 ± 12.3	0.289
DBPi	73.2 ± 9.9	72.4 ± 13.2	69.9 ± 10.7	72.9 ± 7.9	72.9 ± 12.0	0.561
DBPe	75.8 ± 10.4¤†	74.1 ± 12.4	69.5 ± 10.0*	70.3 ± 7.0*	69.7 ± 7.0	0.021
DBPΔ	-2.5 ± 0.9¤†§	-1.7 ± 1.8¤†§	0.3 ± 2.1*¥†§	2.6 ± 2.5*¥¤	3.2 ± 2.2*¥¤	<0.001
PP	47.6 ± 6.0	43.8 ± 7.5	45.8 ± 6.9	45.6 ± 7.7	45.8 ± 6.1	0.232
PPi	46.6 ± 6.2	43.2 ± 7.8	45.1 ± 6.7	45.1 ± 8.0	45.7 ± 6.3	0.255
PPe	48.6 ± 6.0	44.5 ± 7.5	46.4 ± 7.4	46.0 ± 7.6	46.0 ± 6.1	0.187
PPΔ	-1.9 ± 1.5§	-1.2 ± 2.4	-1.4 ± 3.0	-0.9 ± 2.8	-0.2 ± 2.1*	0.015
MAP	88.8 ± 10.6	86.4 ± 13.9	83.4 ± 11.1	85.2 ± 7.7	85.1 ± 13.2	0.322
MAPi	87.2 ± 10.4	85.3 ± 14.4	83.4 ± 11.5	86.4 ± 8.4	86.7 ± 12.8	0.569
MAPe	90.3 ± 10.7¤†	87.4 ± 13.4	83.5 ± 10.8*	84.1 ± 7.4*	83.5 ± 13.6	0.030
MAPΔ	-3.1 ± 1.1¤†§	-2.0 ± 2.3¤†§	-0.1 ± 2.5*¥†§	2.3 ± 3.0*¥¤	3.2 ± 2.0*¥¤	<0.001

Data represent mean ± SD (mmHg; n = 17). Spontaneous breathing (Sf_r; average 14.2 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 7.0 breaths·min^{-1}); systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg), pulse pressure (PP; mmHg), mean arterial blood pressure (MAP; mmHg); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

Peak-valley intra-breath phase responses

Percentage change BP oscillations were calculated during inspiration, producing relative intra-breath phase peak-valley differences (Δi) as a percentage of average BP during inspiration (i). The equivalent analysis for variables during expiration were also calculated. There were significant increases in the amplitude of BP oscillations during all SDB variables compared with Sf_r (Figure 6-4).

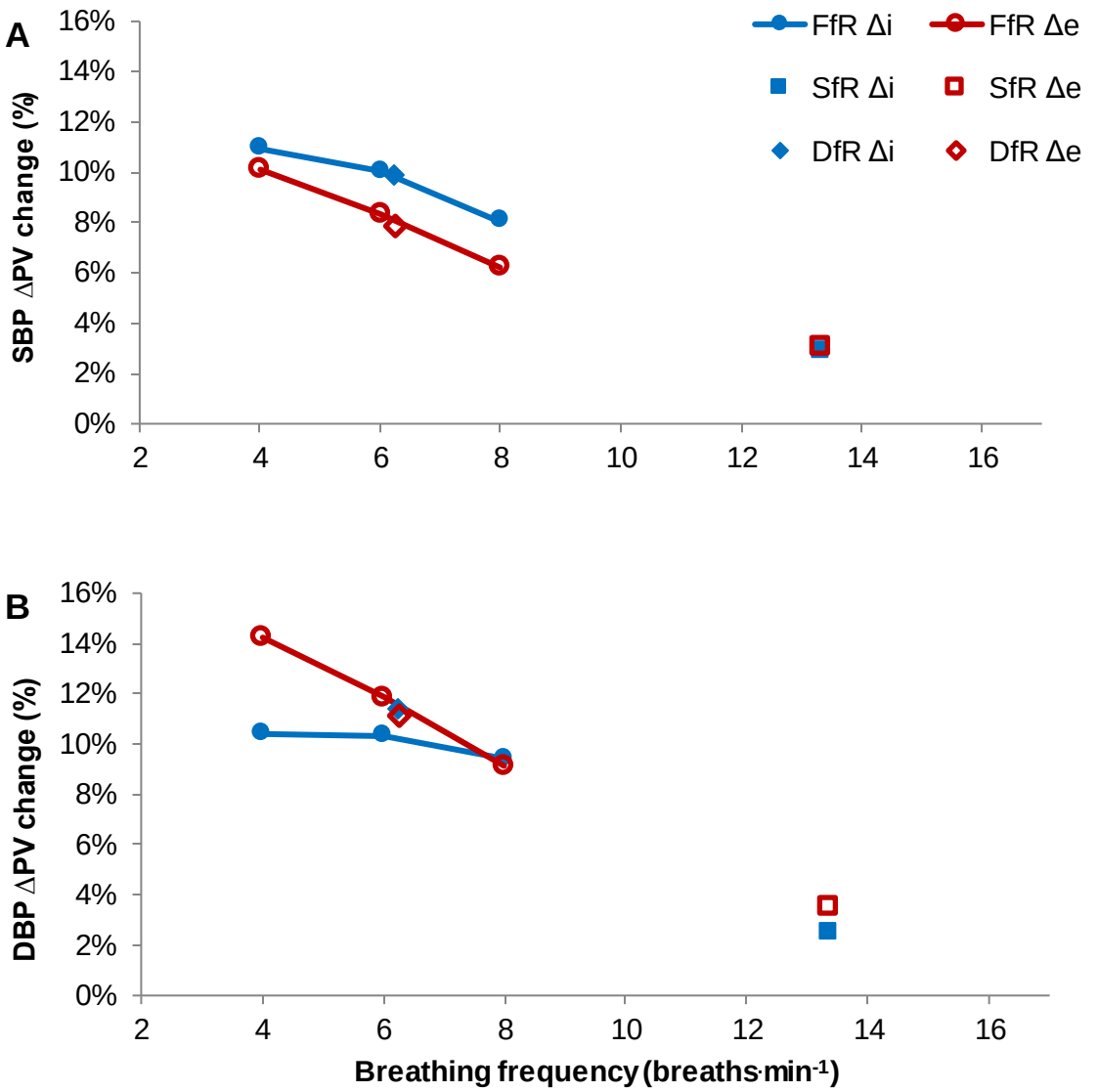


Figure 6-4 Blood pressure oscillations: Relative change of ΔI and ΔE for systolic blood pressure (A) and diastolic blood pressure (B)

Systolic blood pressure (SBP), diastolic blood pressure (DBP); within inspiration difference (Δi), within expiration difference (Δe); Fixed breathing frequency (Ff_r), Spontaneous breathing (Sf_r), optimisation algorithm dynamic breathing frequency (Df_r). Variable calculated as SBPΔi as a percentage of average SBP during inspiration, or equivalent during expiration and for DBP.

Table 6-4 Peak-valley differences (±SD) for blood pressure variables (mmHg)

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p
	14.2 breaths·min^{-1}		*7.0 breaths·min^{-1}*			value
SBPΔi	3.4 ± 1.5¥¤†§	9.1 ± 2.6*¤†	11.2 ± 3.7*¥	11.7 ± 3.8*¥	12.9 ± 4.9*	<0.001
SBPΔe	3.7 ± 1.6¥¤†§	7.4 ± 2.5*¤†	9.0 ± 2.4*¥	9.7 ± 3.0*¥	11.7 ± 6.1*	<0.001
SBPΔPV	-7.7 ± 2.6§	-8.5 ± 9.0§	-3.7 ± 13.1§	0.8 ± 14.7§	12.3 ± 9.8*¥¤†	<0.001
SBPΔPV_Ind	12.9 ± 4.7§	13.9 ± 3.7	15.9 ± 3.1	15.9 ± 3.8	17.6 ± 6.0*	0.033
DBPΔi	1.8 ± 0.8¥¤†§	6.6 ± 2.2*	7.7 ± 2.7*	7.5 ± 3.5*	7.6 ± 3.1*	<0.001
DBPΔe	2.7 ± 1.3¥¤†§	6.7 ± 2.2*†	7.6 ± 1.8*	8.3 ± 2.2*¥	9.9 ± 4.0*	<0.001
DBPΔPV	-4.6 ± 1.7†§	-6.1 ± 6.7†§	0.7 ± 9.3§	5.3 ± 9.1*¥	11.4 ± 3.4*¥¤	<0.001
DBPΔPV_Ind	7.9 ± 2.8†§	9.7 ± 2.8	10.3 ± 2.4	10.4 ± 2.7*	12.8 ± 5.5*	0.005
PPΔi	2.9 ± 1.1¤†§	4.1 ± 1.5†§	4.9 ± 1.6*§	5.6 ± 1.8*¥§	7.8 ± 3.0*¥¤†	<0.001
PPΔe	2.7 ± 1.2¥¤†§	4.4 ± 1.8*§	4.9 ± 1.5*	5.1 ± 1.4*	6.4 ± 1.9*¥	<0.001
PPΔPV	-3.9 ± 3.3	-2.3 ± 6.5	-2.6 ± 7.4	-2.2 ± 7.7	0.3 ± 9.1	0.111
MAPΔi	2.0 ± 1.0¥¤†§	7.2 ± 2.3*¤	8.6 ± 2.9*¥	8.6 ± 3.5*	8.8 ± 3.6*	<0.001
MAPΔe	2.8 ± 1.3¥¤†§	6.6 ± 2.1*¤†	7.8 ± 1.9*¥	8.5 ± 2.5*¥	10.1 ± 4.6*	<0.001
MAPΔPV	-5.3 ± 1.8†§	-6.7 ± 7.1†§	-1.6 ± 10.1§	3.6 ± 10.8*¥	11.9 ± 3.7*¥¤	<0.001

Data represent mean ± SD (mmHg; n = 17). Spontaneous (Sf_r; average 14.2 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 7.0 breaths·min^{-1}); systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg), pulse pressure (PP; mmHg), mean arterial blood pressure (MAP; mmHg); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

6.3.5 Heart rate and respiratory sinus arrythmia

For f_cΔ, all SDB conditions except 4Ff_r were significantly higher than Sf_r (p<0.001; Table 6-5).

Respiratory sinus arrythmia (peak-valley inter-breath phase)

Respiratory sinus arrythmia (RSA) was significantly higher for all SDB conditions compared with Sf_r (p<0.001; Figure 6-5).

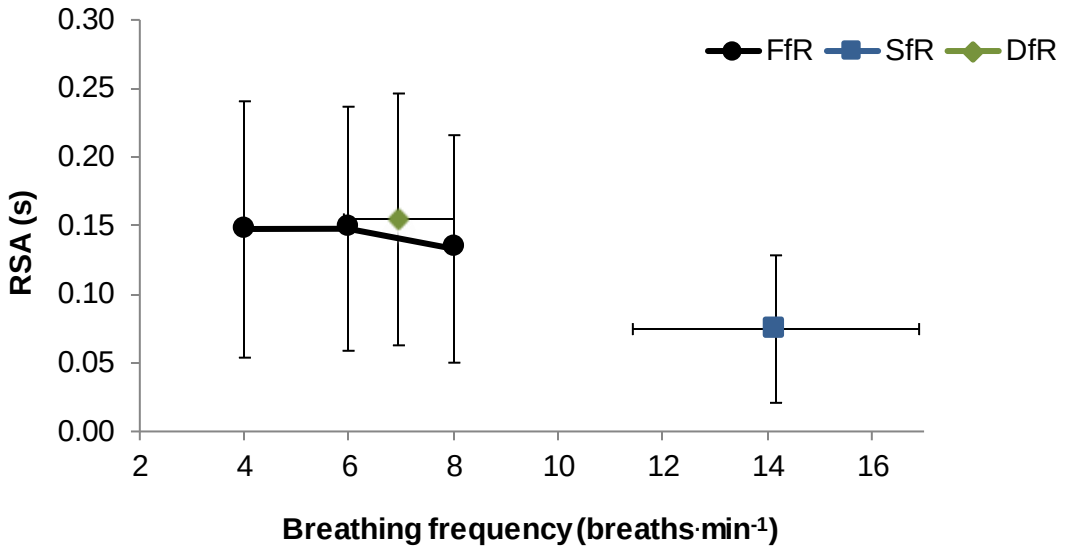


Figure 6-5 Respiratory sinus arrythmia (RSA) response to slow and deep breathing

Values are mean ± SD; Spontaneous breathing (blue ■; Sf_r), fixed breathing conditions (black ●; Ff_r) Brythm algorithm (green ♦; Df_r); respiratory sinus arrhythmia (RSA).

Table 6-5 Mean values (±SD) and inter-breath phase differences (Δ) for heart rate (f_c), stroke volume (SV) and cardiac output (Q̇) variables

	Sf_r *14.2 breaths·min^{-1}*	**8Ff_r**	**Df_r** *7.0 breaths·min^{-1}*	**6Ff_r**	**4Ff_r**	**p value**
f_c	80.7 ± 10.1	82.6 ± 9.8	80.3 ± 9.3	81.7 ± 8.6	79.9 ± 8.4	0.123
f_ci	80.4 ± 10.5¥¤†	85.2 ± 10.0*	83.9 ± 8.8*	85.1 ± 7.5*	82.1 ± 7.3	<0.001
f_ce	80.9 ± 9.8	79.7 ± 9.8	76.4 ± 10.1	78.1 ± 10.0	77.7 ± 9.9	0.009
f_cΔ	-0.5 ± 2.4¥¤†	5.5 ± 3.3*	7.4 ± 4.7*	7.0 ± 5.0*	4.3 ± 5.1	<0.001
SV	82.3 ± 9.8	81.4 ± 10.4	82.5 ± 10.0	82.7 ± 9.2	83.1 ± 10.2	0.920
SVi	80.3 ± 9.5	80.9 ± 10.5	81.9 ± 9.9	82.3 ± 9.2	82.7 ± 10.0	0.640
SVe	84.2 ± 10.3	81.8 ± 10.8	83.0 ± 10.8	83.1 ± 9.9	83.5 ± 10.7	0.854
SVΔ	-3.9 ± 3.0	-0.9 ± 4.2	-1.1 ± 5.7	-0.8 ± 4.9	-0.7 ± 4.2	0.027
Q̇	6596 ± 923	6652 ± 1075	6564 ± 981	6706 ± 1027	6573 ± 863	0.728
Q̇i	6427 ± 953¥¤†	6858 ± 1090*	6855 ± 1034*	6995 ± 990*	6749 ± 884	0.001
Q̇e	6765 ± 913¤§	6447 ± 1077	6268 ± 958*	6427 ± 1079	6399 ± 864*	0.003
Q̇Δ	-339 ± 286¥¤†§	411 ± 278*¤	588 ± 351*¥	568 ± 238*§	350 ± 257*†	<0.001

Data represent mean ± SD (n = 17). Spontaneous breathing (Sf_r; average 14.2 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 7.0 breaths·min^{-1}); heart rate (f_c; beats·min^{-1}), stroke volume (SV; ml), cardiac output (Q̇; ml·min^{-1}); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

6.3.6 Stroke volume and cardiac output

Stroke volume (SV) showed no significant differences between breathing conditions for any mean or intra-breath phase variables (p>0.05; Table 6-5). All SDB conditions were significantly different from Sf_r for SVΔi and SVΔe (p<0.001; Table 6-6). All SDB conditions were significantly different from Sf_r for all cardiac output peak-valley measures (Q̇Δi, Q̇Δe, Q̇ΔPV; p<0.001).

Table 6-6 Peak-valley differences (±SD) for heart rate (f_c), stroke volume (SV) and cardiac output (Q̇) variables

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	14.2 breaths·min^{-1}		*7.0 breaths·min^{-1}*			
f_cΔi	4.5 ± 2.9¥¤†§	8.4 ± 5.0*	9.3 ± 5.2*	9.6 ± 5.8*	11.3 ± 5.7*	<0.001
f_cΔe	6.1 ± 4.4¥¤	10.5 ± 5.2*	9.9 ± 4.6*	9.7 ± 4.0	11.0 ± 6.0	0.004
***f_c*ΔPV**	-3.1 ± 7.4¥¤†§	13.4 ± 6.8*	15.3 ± 7.8*	15.1 ± 6.8*	11.2 ± 12.7*	<0.001
RSA	0.07 ± 0.05¥¤†§	0.13 ± 0.08*	0.15 ± 0.09*	0.15 ± 0.09*	0.15 ± 0.09*	<0.001
SVΔi	5.8 ± 2.0 ¥¤†§	9.0 ± 2.8 *†§	10.7 ± 3.7 *§	11.0 ± 3.3 *¥§	15.3 ± 5.0 *¥¤†	<0.001
SVΔe	5.2 ± 1.9 ¥¤†§	7.9 ± 2.3 *§	9.2 ± 2.3 *§	9.6 ± 2.3 *§	12.5 ± 3.7 *¥¤†	<0.001
SVΔPV	-8.0 ± 6.5	-2.9 ± 12.1	-3.4 ± 15.1	-1.7 ± 14.8	-0.4 ± 17.7	0.119
SVΔPV_Ind	13.0 ± 4.0§	13.4 ± 4.1§	15.5 ± 5.6	15.6 ± 4.6	18.0 ± 5.2*¥	0.002
Q̇Δi	586 ± 293¥¤†§	1151 ± 348*	1340 ± 468*	1292 ± 422*	1453 ± 300*	<0.001
Q̇Δe	563 ± 265¥¤†§	920 ± 243*	1012 ± 336*	1040 ± 355*	1164 ± 479*	<0.001
Q̇ΔPV	-745 ± 663¥¤†§	1181 ± 732*	1432 ± 870*	1431 ± 842*	1452 ± 901*	<0.001
Q̇ΔPV_Ind	1198 ± 341	1382 ± 302	1602 ± 553	1496 ± 373	1426 ± 282	0.006

Data represent mean ± SD (n = 17). Spontaneous breathing (Sf_r; average 14.2 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r; average 7.0 breaths·min^{-1}); heart rate (f_c; beats·min^{-1}), respiratory sinus arrhythmia peak/valley amplitude (RSA; s), stroke volume (SV; ml), cardiac output (Q̇; ml·min^{-1}); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

6.3.7 Pulse wave velocity and total peripheral resistance

Pulse wave velocity (PWV) and total peripheral resistance (TPR) were not significantly different for mean variables (Table 6-7). Peak-valley TPR (TPRΔ) was significantly higher during 8Ff_r, Df_r, and 6Ff_r compared with Sf_r (p<0.001).

Table 6-7 Mean values (±SD) and inter-breath phase differences (Δ) for pulse wave velocity (PWV) and total peripheral resistance (TPR) variables

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	14.2 breaths·min^{-1}		*7.0 breaths·min^{-1}*			
PWV	5.1 ± 0.5	5.1 ± 0.5	5.0 ± 0.4	5.0 ± 0.5	5.0 ± 0.5	0.081
PWVi	5.0 ± 0.5	5.0 ± 0.6	4.9 ± 0.4	5.0 ± 0.5	5.0 ± 0.5	0.205
PWVe	5.2 ± 0.5†§	5.1 ± 0.5†	5.1 ± 0.5	5.0 ± 0.5*¥	5.1 ± 0.5*	0.004
PWVΔ	-0.14 ± 0.1†	-0.10 ± 0.2†§	-0.11 ± 0.1†	-0.02 ± 0.1*¥¤	-0.02 ± 0.1¥	<0.001
TPR	13.8 ± 3.1	13.4 ± 3.1	13.1 ± 2.6	13.1 ± 2.4	13.2 ± 2.2	0.419
TPRi	14.0 ± 3.0¤†	12.8 ± 2.9	12.5 ± 2.6*	12.7 ± 2.3*	13.1 ± 2.2	0.020
TPRe	13.7 ± 3.2	14.0 ± 3.2	13.7 ± 2.7	13.5 ± 2.6	13.3 ± 2.3	0.600
TPRΔ	0.2 ± 0.7¥¤†	-1.2 ± 0.7*§	-1.2 ± 0.6*§	-0.8 ± 0.7*§	-0.2 ± 0.4¥¤†	<0.001

Data represent mean ± SD (n = 17). Spontaneous breathing (Sf$_r$; average 14.2 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff$_r$), 6 breaths·minute^{-1} (6Ff$_r$), 8 breaths·minute^{-1} (8Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$; average 7.0 breaths·min^{-1}); pulse wave velocity (PWV; m·s^{-1}), total peripheral resistance (TPR; mmHg·min·L^{-1}); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf$_r$ (*), 8Ff$_r$ (¥), Df$_r$ (¤), 6Ff$_r$ (†), 4Ff$_r$ (§); P<0.05.

PWV and TPR were significantly higher during inspiration for intra-breath phase peak-valley analysis for all SDB conditions compared with Sf$_r$.

Table 6-8 Peak-valley differences (±SD) for pulse wave velocity (PWV) and total peripheral resistance (TPR) variables

	Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	p value
	14.2 breaths·min^{-1}		*7.0 breaths·min^{-1}*			
PWVΔi	0.2 ± 0.1¥¤†§	0.6 ± 1.3*†§	0.3 ± 0.1*	0.4 ± 0.2*¥	0.5 ± 0.2*¥	<0.001
PWVΔe	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.4	0.3 ± 0.1	0.5 ± 0.2	0.088
PWVΔPV	-0.32 ± 0.2	-0.05 ± 1.4	-0.42 ± 0.5	-0.14 ± 0.5	-0.14 ± 0.6	0.158
TPRΔi	1.1 ± 0.6¥¤†§	1.8 ± 0.8*	2.0 ± 0.8*	2.0 ± 0.9*	2.2 ± 0.7*	<0.001
TPRΔe	1.1 ± 0.6¤†§	1.7 ± 1.1†	2.0 ± 1.2*	2.2 ± 1.1*¥	2.2 ± 1.0*	<0.001
TPRΔPV	0.4 ±1.7¥¤†	-2.8 ± 1.3*	-2.9 ± 1.2*	-2.5 ± 1.7*	-0.7 ± 2.7	<0.001

Data represent mean ± SD (n = 17). Spontaneous breathing (Sf$_r$; average 14.2 breaths·min^{-1}), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff$_r$), 6 breaths·minute^{-1} (6Ff$_r$), 8 breaths·minute^{-1} (8Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$; average 7.0 breaths·min^{-1}); pulse wave velocity (PWV; m·s^{-1}), total peripheral resistance (TPR; mmHg·min·L^{-1}); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV); Significantly different from Sf$_r$ (*), 8Ff$_r$ (¥), Df$_r$ (¤), 6Ff$_r$ (†), 4Ff$_r$ (§); P<0.05.

6.3.8 Antenatal blood pressure measurements

Blood pressure measurements recorded during antenatal appointments were submitted from 15/17 participants (Figure 6-6), and 58.8% of participants provided data up until either week 40 gestation or until they gave birth. From the submitted data no participants developed hypertension after participating in the data collection session.

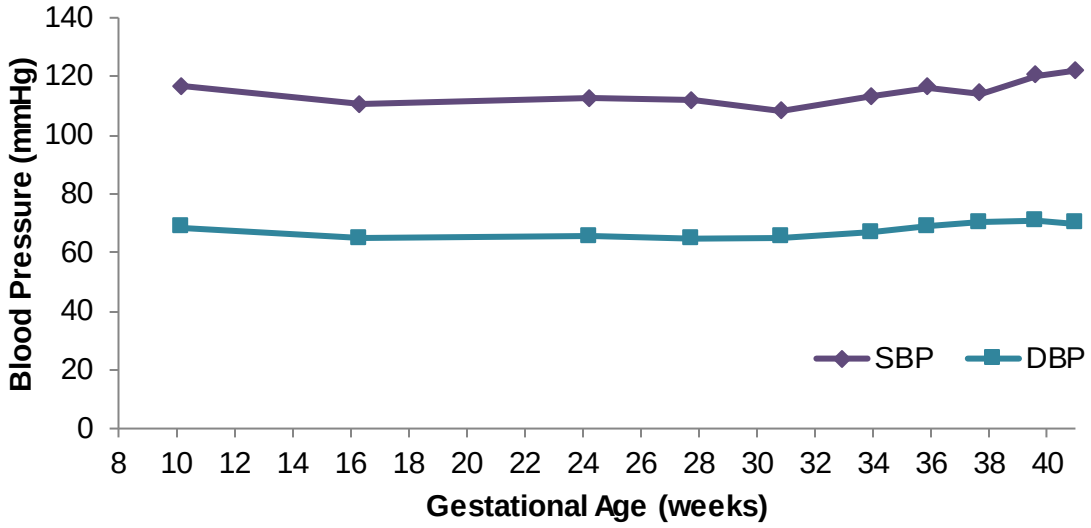


Figure 6-6 Average blood pressure measured during routine antenatal appointments

Data represent mean; week 10 (n=15), week 16 (n=15), week 25 (n=15), week 28 (n=14), week 31 (n=14), week 34 (n=13), week 36 (n=12), week 38 (n=11), week 40 (n=6), week 41 (n=1). Systolic blood pressure (SBP purple ♦), diastolic blood pressure (DBP blue ■).

6.3.9 Preferred breathing condition

The majority of participants (10/18) picked the 6Ff_r condition as their preferred breathing condition to perform at home daily breathing (Figure 6-7) and another 4 participants chose 6Ff_r as their second preferred breathing condition where they had no preference between 2 conditions as their favourite. All 18 participants have been included in the analysis of preferred breathing condition as the excluded participant's noncompliance of the breathing condition does not exclude them from making a judgement on their preference.

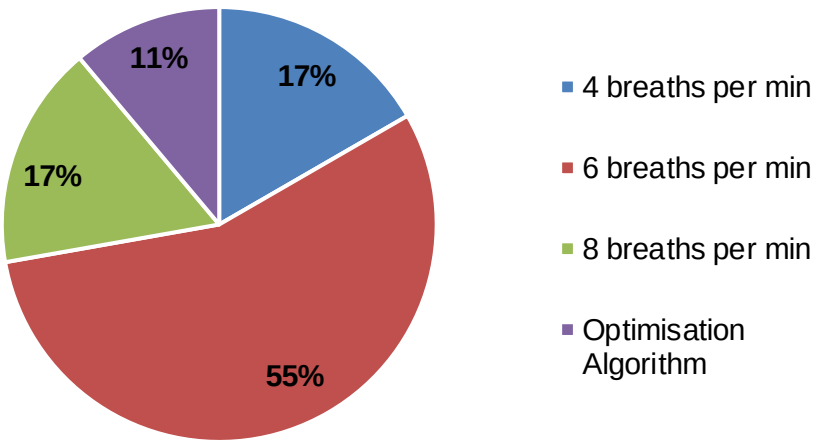


Figure 6-7 Preferred breathing condition

The breathing condition participants (n=18) felt most comfortable breathing at, and would choose to use if they were asked to continue undertaking the breathing exercises daily at home.

There was no correlation between gestational age and preferred breathing frequency (R^2=0.00; Figure 6-8). Where Df_r was chosen, the average breathing frequency during this condition was used in Figure 6-8. There was also no correlation between the preferred breathing frequency and the position of the preferred breathing frequency in the randomised order (R^2=0.15), i.e. preferred breathing frequency was not always performed at the start or end of the protocol.

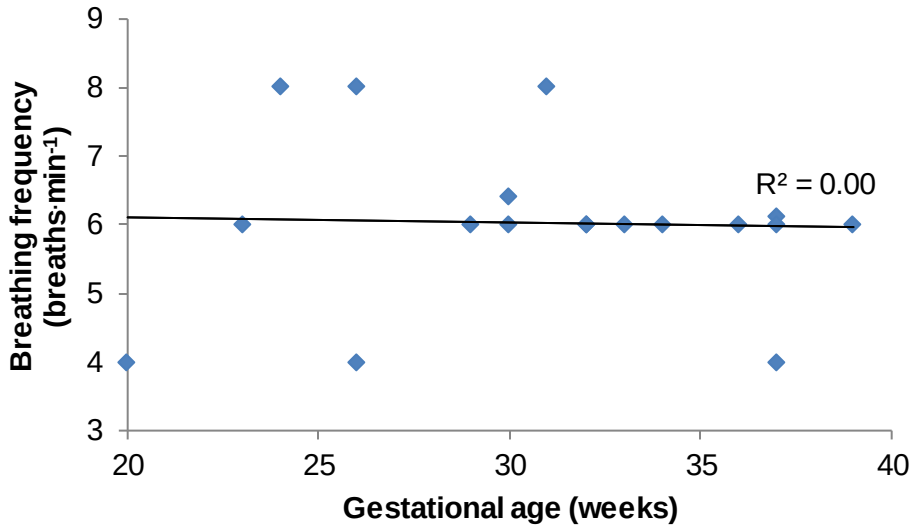


Figure 6-8 Correlation between gestational age and preferred breathing frequency

6.4 Discussion

This discussion will focus on the specific responses of healthy pregnant women to SDB, compared with those reported in existing literature, where breathing exercises have been implemented during pregnancy. On the whole, the principal variables of interest responded similarly to those of healthy non-pregnant women, but a detailed comparison of the data from pregnant (Chapter 6) and healthy non-pregnant women (Chapter 5) can be found in the integrated paper in Chapter 7.

This study adds to the literature reporting normal cardiovascular data during healthy pregnancies. Although sample size is low, Table 6-1 provides a starting point on which to build to a data set of normal data during pregnancy. This does not yet exist in the literature and textbooks such as Anatomy and Physiology for Midwives (Coad et al. 2020) do not always use cited references associated with their normative values. The data set provided by this study show that, on average, women of 31.4 (± 5.2) weeks gestational age have a spontaneous breathing frequency of 14.2 ± 2.7 breaths·min^{-1}, which is ~1 breaths·min^{-1} higher than the breathing frequency of non-pregnant women in Chapter 5.

Although spontaneous breathing frequency was not correlated with gestational age, given the wide range of gestational ages (20-39 weeks), more data is required to confirm this. Evaluation of breathing frequency is important for monitoring health while on hospital wards, and the accuracy of it is important for calculation of early warning scores (Jones et al. 2020). However, if breathing frequency is different across gestational ages then this should be fed into early warning scores as a relative comparison for baseline datal. Consequently, more data is needed on 'normal' breathing frequencies across trimesters and gestational ages.

As expected, heart rate, stroke volume and cardiac output were all significantly higher for pregnant women than non-pregnant women. All BP measures were similar between pregnant and non-pregnant women, and the traditional 'dip' in BP during the first and second trimester was not seen in the data from antenatal BP measures. Recent evidence suggests that the traditionally expected drop in BP may not occur in every pregnancy (Salles et al. 2015), with steady SBP and DBP values throughout pregnancy. However, there was a slight decrease in BP from ~31 weeks gestation, which is in accordance with meta-analysis data showing a slight increase in SBP and DBP from 30 weeks gestation onwards (Loerup et al. 2019). Accordingly, it can be concluded that the sample of pregnant women in the present study were typical of normotensive pregnancies.

The RSA values observed in the present study are higher than were reported in a previous study where pregnant women performed relaxation for 18-minutes, both at baseline (Sf_r) and during the SDB/relaxation interventions (DiPietro et al. 2008). However, the RSA response to SDB mirrored the pattern found during relaxation, with both SDB and relaxation eliciting an approximate doubling of RSA measured at baseline/spontaneous breathing. Depressed RSA has been associated with hypertensive disorders of pregnancy (Lakhno 2016) and although the depression of RSA is not the cause of hypertension, it reflects the functional state of the autonomic nervous system (Buchner 2018), and as there is an overactivity of the sympathetic arm during PIH, RSA depression may reflect a deterioration in the balance of the autonomic nervous system. Thus, if daily practice of SDB can convert the acute increase in RSA into a long-term increase in RSA, this may be beneficial for pregnant women.

There are a handful of studies examining the acute responses of pregnant women to breathing exercises. For example, one study showed that SBP increased significantly during 6 minutes of paced breathing. However, the increase in SBP was only around 2.6 mmHg, whilst the increase for DBP was 1.5mmHg and no change in heart rate was seen (0.4 beats·min^{-1}) (Monk et al. 2011). In the present study, there was no significant

increase in mean SBP or DBP; changes were only observed in the peak-valley BP variables. This discrepancy between studies is likely to be attributable to differences in design; in the study by Monk and colleagues (2011), the slow paced breathing alternated between periods of breathing at 30 breaths·min^{-1}, 20 breaths·min^{-1} and 10 breaths·min^{-1}, all of which were faster than the highest frequency tested in the present study.

It is reasonable to suggest that gestational age might influence the ability to expand tidal volume during SDB and thus the preferred SDB frequency. No correlation between preferred breathing frequency chosen by participants and gestational age was observed, suggesting that women did not find SDB more difficult during the later stages of pregnancy. Indeed, the three women who preferred 4Ff_r (the lowest breathing frequency) participated at a wide range of gestational ages of 20, 26 and 37 weeks gestation. However, this finding might be explained by the fact that these women had pregnancies that were either <28 weeks or >36 weeks. There is more pressure on the diaphragm after 28 weeks gestation in the second trimester (Hegewald and Crapo 2011) which may make SDB harder to achieve and therefore lower breathing frequencies are harder to achieve or less comfortable. Moreover, during first time pregnancies, from 37 weeks onwards there is a drop in the bump, known as lightening (Coad et al. 2020), which might make it easier to breathe at the lower frequencies following lightening. These physiological changes during different stages of gestation may explain why these participants were most comfortable breathing at the lowest frequency (4Ff_r).

It is interesting, but unsurprising, that the only group from which a participant was recruited (from the 8 antenatal groups who shared the study information with pregnant women) was the group that the investigator (MF) was able to attend in person to discuss the project. Additionally, the only event (from 3), where information was provided to women, yielding participants, was the event the investigator attended in person. This demonstrates the importance of face to face contact with potential participants. The exception to this was the NCT classes, from which 3 participants were recruited; however, the NCT has been an ongoing collaborator with the project and the antenatal teachers were enthusiastic about the research and encouraged women to take part. Additionally, although Facebook yielded the most participants, less than half of those who made contact following a Facebook promotion participated in the study. This shows that although Facebook is a good method for sharing the research to a wider group of women this does not necessarily translate to participation in research (Arcia 2013). Most maternity Facebook groups also have a majority of users who already have children, as confirmed by a local parent and user of some of the groups utilised in this study. As these women would be excluded from participation in this study, promoting the research to

them is of no benefit to the project and therefore adds a limitation to using maternity Facebook groups as a recruitment source. Additionally, first time mothers often join these local groups after their baby is born and therefore finding a place where pregnant women group together is difficult, outside of clinical settings. The next study (Chapter 8) will recruit directly from the local NHS maternity ward, and therefore access to participants will be streamlined to one organisation. For specific groups of pregnant women, in future it may be easier to access participants through local NHS Trusts for other research studies. The importance of meeting potential participants in person should not be overlooked when planning recruitment strategies for pregnant women.

Finally, the data from this chapter show a similar response to SDB as non-pregnant women in Chapter 6, and this will be statistically analysed in the next chapter. Now that the acute cardiovascular responses of both healthy non-pregnant and healthy pregnant women have been characterised research can move onto investigating the differences in cardiovascular responses in women who develop pregnancy-induced hypertension. Comparison values during healthy pregnancies are important to differentiate between normal physiological adaptations and responses during pregnancy to those associated with cardiovascular disease. Therefore, more normative maternal data should be collected, recorded and published to support researchers in their investigations.

6.5 Conclusion

In conclusion, RSA more than doubled during all SDB conditions compared with spontaneous breathing, which is reflective of the capacity of SDB to modulate vagal activity. The amplitude of BP oscillations also increased in pregnant women to a similar level as non-pregnant women. The potential for BP oscillations to activate the baroreceptors, and provide repeated stimulus during daily practice, is present in pregnant women and therefore the data reflects no reason why SDB could not produce the same health benefits in pregnant women as observed in non-pregnant women.

6.6 Summary

The short-term cardiovascular responses to SDB have been characterised in healthy non-pregnant and healthy pregnant women. The next chapter will present a manuscript combining the results from Chapters 5 and 6, providing an in-depth comparison of the responses. Mechanistically, data from this study provides a better understanding of the within-breath changes that may create error signal(s), which provides a platform from which to examine responses to SDB of pregnant women who develop hypertension.

Chapter 7. Comparison of short-term cardiovascular responses to slow and deep breathing in non-pregnant and pregnant women

7.1 Introduction

The main section of this chapter (7.2) has been prepared as a manuscript as it is intended for publication and will be submitted to the European Journal of Applied Physiology. The manuscript will synthesise data presented in Chapters 5 & 6, necessitating a degree of overlap between those chapters and the current chapter. However, this is justified because the direct comparison of cardiovascular responses between healthy pregnant and healthy non-pregnant women is an important output of this thesis. There is a need to understand how women respond to slow and deep breathing (SDB) and whether the normal physiological adaptations caused by pregnancy change the cardiovascular response to SDB. Characterising these differences may lead to a better understanding of how SDB reduces long-term blood pressure (BP), supporting the development of an evidence-based SDB intervention, designed specifically to treat high BP during pregnancy.

A full explanation of the methods for each study can be found in Chapter 3 and sections 5.2 and 6.2. The research questions, objectives and hypothesis are outlined below:

Research questions

1. Do healthy pregnant women exhibit the same acute cardiovascular responses to SDB as healthy non-pregnant women?
2. Is there a difference in the acute cardiovascular response of healthy pregnant women to different SDB frequencies as healthy non-pregnant women?

Objectives

1. Identify whether mechanism-related parameters (e.g. respiratory sinus arrythmia, stroke volume, cardiac output, blood pressures) respond similarly during SDB for healthy non-pregnant women and healthy pregnant women.
2. Evaluate whether the acute cardiovascular responses to a range of SDB frequencies are similar for healthy non-pregnant women and healthy pregnant women.

Hypothesis

1. Healthy pregnant women will exhibit the same acute physiological responses to SDB across different breathing frequencies as healthy non-pregnant women.

7.2 Integrated paper: Acute cardiovascular responses to slow and deep breathing in normotensive non-pregnant and pregnant women

Introduction

Slow and deep breathing (SDB) is recommended by the American Heart Association for use as an adjunctive treatment for hypertension (Brook et al. 2013). A recent meta-analysis of studies of SDB in primary hypertension found that following daily practice of SDB reductions of up to 5.26 mmHg for systolic blood pressure (SBP) and 2.97 mmHg for diastolic blood pressure (DBP) were observed (Chaddha et al. 2019). However, there is limited understanding of the acute cardiovascular responses to SDB, which produce the error signal(s) to reduce blood pressure (BP) chronically, as well as a lack of research investigating the underlying mechanisms (Gerritsen and Band 2018).

A recent study (Felton et al. 2021 – in preparation)[4] revealed that SDB increased the amplitude of respiratory sinus arrythmia (RSA) and BP oscillations, with maximal amplitudes occurring at 6 breaths·min^{-1}. However, 6 breaths·min^{-1} was the lowest breathing frequency assessed and it is unknown whether lower breathing frequencies could increase the amplitude of cardiovascular oscillations further. To date, previous studies that have compared cardiovascular responses to SDB at a range of frequencies have done so using a SDB protocol that reduced breathing frequency dynamically, with only short durations at each individual SDB frequency (Anderson et al. 2009; Zhang et al. 2009). A systematic characterisation of the acute cardiovascular responses to a range of steady-state SDB frequencies is therefore needed. This may also shed light on the potential error signal(s) responsible for the anti-hypertensive effect of SDB following daily practice.

Felton et al. (2021)[4] found that there was no difference between the acute cardiovascular responses of men and women to SDB. However, pregnancy induces a series of cardiovascular adaptations, which may change the acute response to SDB, compared with those of non-pregnant women. During pregnancy, baseline cardiovascular

[4] Integrated paper presented in Chapter 4

measures such as heart rate, stroke volume and cardiac output are increased above normal non-pregnant levels (Soma-Pillay et al. 2016). It is possible that these changes in baseline cardiovascular function may influence the acute cardiovascular response to SDB. Additionally, the health benefits and reductions in BP associated with SDB are suggested to be related to diaphragmatic breathing (Gerritsen and Band 2018), however during pregnancy the diaphragm is forced upwards by as much as 5 cm (Elkus and Popovich Jr 1992), which may limit its mobility and the ability to perform SDB and/or achieve any associated health benefits.

The need to understand the acute responses to SDB during pregnancy is important due to a specific condition called pregnancy-induced hypertension (PIH). PIH is defined as high blood pressure, presenting after 20 weeks of pregnancy, which was not present prior pregnancy (NICE: National Institute for Health and Care Excellence 2019b). PIH occurs in up to 15% of pregnancies (James and Nelson-Piercy 2004) and there is an increased risk for obstetric complications for these women (Scantlebury et al. 2013). There is potential for SDB to offer an effective treatment for PIH (Felton et al. 2021)[5], and women who develop PIH are a promising group in which to investigate SDB as a potential treatment method. Firstly, many pregnant women are highly motivated to adhere to and undertake non-pharmacological interventions (Adams et al. 2009) as many have an aversion to medication (Twigg et al. 2016). The aetiology of PIH has also been linked to high breathing frequencies (Fischer and Voss 2014) and dysfunctional breathing (Jerath et al. 2009). SDB may be an important component of behavioural interventions aimed at reducing BP (Sica 2011) and therefore pregnant women are an ideal group to investigate the use of SDB to treat hypertension.

Prior to undertaking an intervention there is a need to characterise and understand the acute responses to SDB of pregnant women and whether these differ from non-pregnant women. This normative and baseline data is needed as a comparison before moving forward to use SDB with women who develop PIH. The characterisation of acute cardiovascular responses can also support the development of SDB interventions designed specifically for pregnant women, based on their measured acute cardiovascular responses, including recognition of any preferences for specific breathing frequencies. Consequently, the present study compared the acute cardiovascular responses of non-pregnant and pregnant women to SDB at a range of breathing frequencies.

[5] Integrated paper presented in Chapter 8. Published 2021 in Hypertension in Pregnancy.

Methods

All experiments conformed to the Declaration of Helsinki and the experimental protocol was approved by Bournemouth University's Research Ethics Committee. Written informed consent was obtained from all participants prior to participating in the study.

Participants

Forty-one women participated in the study: 23 healthy non-pregnant women and 18 healthy pregnant women. All non-pregnant participants were of reproductive age as defined by the World Health Organization (2006) and all pregnant women were over 20 weeks gestation. Participants were recruited from the local student and staff population and using local antenatal and maternal groups including social media. All pregnant women were nulliparous and were carrying single pregnancies. Participants diagnosed with any cardiovascular or respiratory disease were excluded, as were smokers and women who vaped. All participants were normotensive at the time of data collection and the pregnant women submitted regular BP measurements until birth to confirm they did not subsequently develop high BP during their pregnancy.

Participants attended one session at the Cardiorespiratory Research Laboratory at Bournemouth University. Prior to the data collection session participants refrained from eating for 2 hours and from caffeine, strenuous exercise and alcohol for 12 hours. Average lab conditions during data collection were 24.0 ± 3.2 ^{o}C, 992.6 ± 13.5 hPa, 42.6 ± 10.6%.

Slow and Deep Breathing Protocol

Participants completed five[6] breathing conditions in a randomised order; spontaneous breathing (Sf_r), 4 (4Ff_r), 6 (6Ff_r), and 8 (8Ff_r) breaths·min^{-1}, and a dynamic frequency using an optimisation algorithm (Df_r), which maximised respiratory sinus arrythmia (RSA). All breathing conditions were 5-minutes in duration with a 5-minute break of normal breathing between each measurement. All SDB conditions were delivered using Bournemouth University's Brythm app, which delivers either fixed breathing frequencies (4Ff_r, 6Ff_r, 8Ff_r) or uses a novel, bespoke algorithm to deliver a personalised dynamic frequency (Df_r). The bespoke algorithm maximises cardiovascular perturbation, using the amplitude of RSA as the controlled variable. Changes in RSA are measured from a finger

[6] The non-pregnant participant group completed 6 breathing conditions with the addition of an inspiratory resistance condition, where participants breathed at 6 breaths·min^{-1} with an inspiratory resistance. The results of this condition are not presented in this chapter as there is no comparison data with pregnant women.

sensor (photoplethysmography), connected via the headphone socked of an iPad. The app displays visual feedback on an iPad screen to guide breathing; user's inhale when the dome graphic rises and exhale when the dome falls (Figure 7-1).

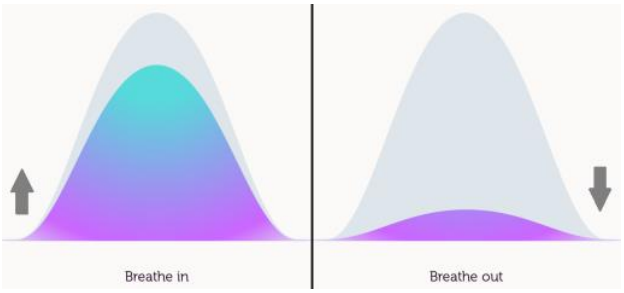


Figure 7-1 Screenshots of Brythm graphic
N.B: Arrows do not appear on app but are shown here to display the direction of graphic movement.

Breathing frequencies of 4 and 8 breaths·min^{-1} were chosen to bookend the widely reported 'optimal' breathing frequency of 6 breaths·min^{-1} (Cullins et al. 2013; Russo et al. 2017), in order to explore cardiovascular responses at a wider range of SDB frequencies. Following completion of the protocol, the pregnant participants were asked which breathing condition they felt most comfortable breathing at and would choose to use if they were asked to continue undertaking the breathing exercise daily until birth.

Data Acquisition

During each breathing condition, respiratory airflow, ECG and arterial blood pressure (ABP) were monitored continuously. Participants were seated in an upright position, at an approximate angle of 60^{o}. Respired flow rate was measured continuously using a heated pneumotachograph (Model 3700, Hans Rudolph Inc., Kansas, USA), connected to a flow measurement system (RSS 100-HR, Hans Rudolph Inc., Kansas, USA) while participants wore an oronasal mask (Oro Nasal 7450 V2 Mask, Hans Rudolph Inc., Kansas, USA).

A 3-lead ECG measured heart rate continuously, whilst non-invasive beat-to-beat ABP was obtained using finger photoplethysmography (Finapres NOVA, Finapres Medical Systems, The Netherlands). Finapres derived ABP was calibrated using a brachial cuff prior to and halfway through data collection. Analogue outputs from the Finapres NOVA and the flow meter were sampled continuously at 250Hz via an analogue to digital converter (NI USB-6218 BNC, National Instruments Inc.) and captured using bespoke acquisition and analysis software (LabView 2015, National Instruments, Inc.). The LabView software corrected for the 4 second delay between the Finapres NOVA output and the respiratory output. Stroke volume (SV) was calculated using the Modelflow method by the Finometer. Total peripheral resistance (TPR) was calculated as mean

arterial pressure divided by cardiac output ($\dot{Q}$). Pulse wave analysis (PWV) was calculated as the distance between sternal notch and Finometer finger cuff divided by pulse transit time (Hansen 2010). Pulse transit time was calculated as the time delay between the peak of the R wave of the ECG and the peak of the pressure pulse recorded at the finger.

Data Analysis

The LabView bespoke software calculated and analysed variables beat-by-beat and breath-by-breath, including the minimum, maximum and mean values for each inhalation and exhalation breath phase. Data were averaged across each 5-minute breathing condition.

Values are expressed as means ± SD unless stated otherwise. Statistical analysis was undertaken using SPSS Statistics 26 (IBM Corp.). After normality was confirmed (Shapiro Wilk) repeated measures ANOVA with planned pairwise comparisons using Bonferroni corrections were used. Between group (pregnant and non-pregnant) comparisons used independent samples t-tests. Reported p values are those following adjustment for repeated comparisons. For all analyses, P was set at 0.05.

Respiratory sinus arrhythmia (RSA) was calculated using two methods 1) the difference between the average heart rate (f_c) during inhalation (f_ci) and exhalation (f_ce) ($f_c\Delta$); 2) the difference in maximum and minimum beat-to-beat intervals (RR) during inhalation and exhalation respectively (RSA). RSA is a variable calculated to determine the amplitude of heart rate rhythms using the 'peak-valley' method, which was also used to analyse all variables including BP in the present study.

The following calculations of variables are displayed on an example sinewave in Figure 7-2 (with corresponding calculation numbers). Inter-breath phase indices (Δ) were quantified as the difference between mean inspiration (i) and mean expiration (e) values for all variables (calculation 4). Peak-valley (PV) indices were calculated as maximum minus minimum values during inspiration (Δi: calculation 6) and expiration (Δe: calculation 5). Inter-breath phase PV indices (ΔPV) were calculated using maximum inspiration minus minimum expiration, or minimum inspiration minus maximum expiration, dependent on which calculation gave the largest difference. Calculation 7 shows an example using the calculation maximum inspiration minus minimum expiration. PV indices irrespective of breath phase, known as peak-valley breath phase independent calculations (ΔPV_Ind), were calculated as the difference between the maximum and minimum values, irrespective of the breath phase in which they occurred.

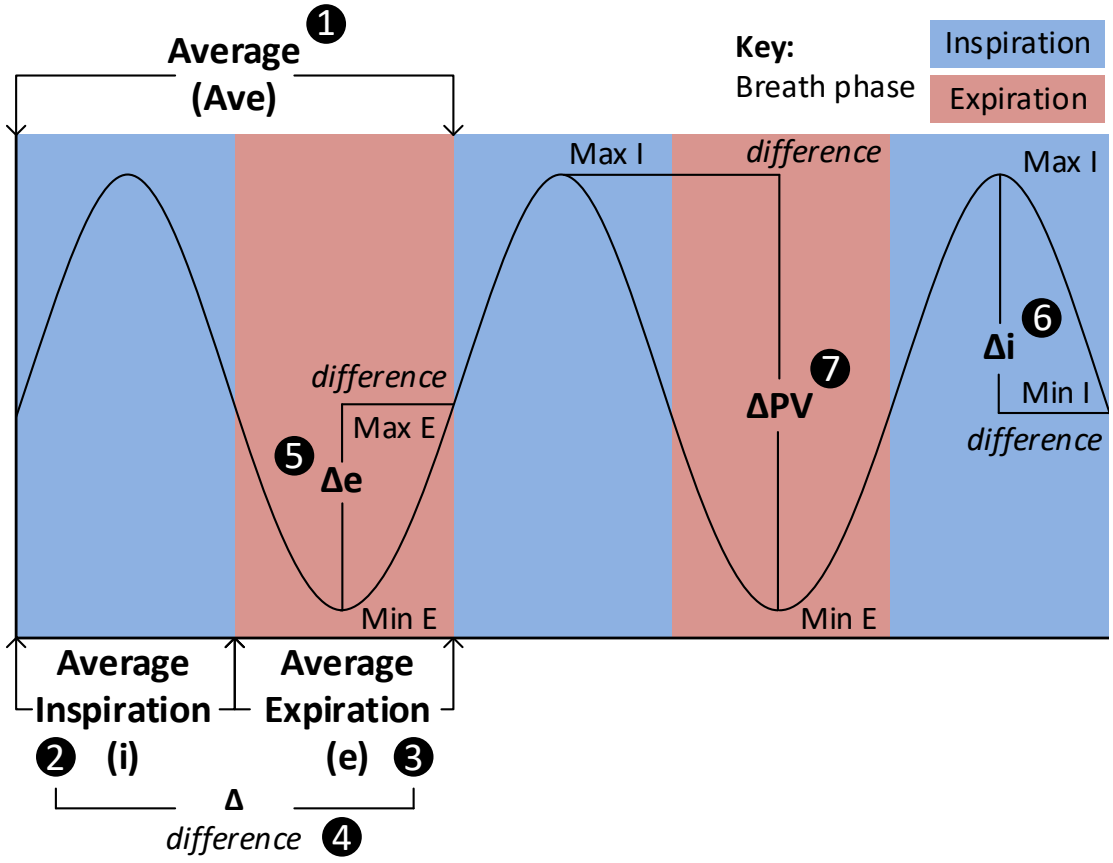


Figure 7-2 Calculations for example cardiovascular variable plot
1) Ave = average of whole breath. 2) i = Average inspiration. 3) e = Average expiration. 4) Δ = i – e (average inspiration – average expiration). 5) Δe = Max E – Min E. 6) Δi = Max I – Min I. 7) ΔPV = Max I – Min E (Note ΔPV calculation varies and can be Min I – Max E depending on which calculation provides largest difference).

Results

Data were collected from 41 participants. Six participants were excluded from the analysis; three due to technical errors in the measurement of respiratory airflow, two because the participant failed to adhere to the prescribed breathing condition and one due to failure of the acquisition system to save the signal data. Consequently, data analysis was performed on data from 18 non-pregnant women and 17 pregnant women (Table 7-1). There were no significant differences in age, stature, systolic blood pressure (SBP) or diastolic blood pressure (DBP) between non-pregnant and pregnant participants. Mass was significantly greater (28%) in pregnant women, accounted for by the growing fetus.

Table 7-1 Participant characteristics

	Non-pregnant	Pregnant	*P* value
	n = 18	*n = 17*	
Age (years)	30.1 ± 8.8	32.0 ± 5.4	0.455
Stature (m)	1.66 ± 0.5	1.67 ± 0.8	0.706
Mass (kg)	65.6 ± 10.3	84.1 ± 13.4	<0.001*
Baseline SBP (mmHg)	113.9 ± 9.1	118.2 ± 7.7	0.141
Baseline DBP (mmHg)	68.9 ± 8.0	71.9 ± 7.9	0.265
Gestational age (weeks)	N/A	31.4 ± 5.2	N/A

*Systolic blood pressure (SBP), diastolic blood pressure (DBP); *significant difference between groups.*

Respiratory variables

Table 7-2 shows the respiratory parameters for both groups. Breathing frequency (f_r) was not significantly different between pregnant and non-pregnant women for any breathing conditions, including spontaneous breathing. The dynamic breathing frequency (Df_r) was significantly different from 6 breaths·min^{-1} for pregnant women (p=0.02), but not for non-pregnant women. All other breathing frequencies were significantly different from each other. Sf_r was not correlated with gestational age (R^2=0.14) and neither was the average optimal breathing frequency based on RSA maximisation during Df_r (R^2=0.11).

Tidal volume was significantly higher for pregnant women during spontaneous breathing (Sf_r, p=0.015), but not during any SDB conditions. Duty cycle remained consistent throughout conditions and was not significantly different between groups or between breathing conditions.

Table 7-2 Respiratory parameters

		Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r
f_r	*Non-pregnant*	13.3 ± 2.1¥¤†§	8.0 ± 0.0*¤†§	6.3 ± 1.1*¥§	6.0 ± 0.0*¥§	4.0 ± 0.0*¥¤†
	Pregnant	14.2 ± 2.7¥¤†§	8.0 ± 0.1*¤†§	7.0 ± 1.1*¥†§	6.0 ± 0.0*¥¤§	4.0 ± 0.0*¥¤†
V_T	*Non-pregnant*	0.4 ± 0.2¥¤†§	0.9 ± 0.4*†§	1.0 ± 0.4*	1.1 ± 0.4*¥	1.3 ± 0.4*¥
	Pregnant	0.8 ± 0.5¤†§	1.1 ± 0.3§	1.1 ± 0.3*§	1.5 ± 0.7*	1.6 ± 0.6*¥¤
T_I / T_{TOT}	*Non-pregnant*	0.42 ± 0.0¥¤†§	0.48 ± 0.0*	0.48 ± 0.0*	0.50 ± 0.1*	0.48 ± 0.0*
	Pregnant	0.54 ± 0.4	0.52 ± 0.2	0.48 ± 0.1	0.47 ± 0.0	0.56 ± 0.3

Data represent mean ± SD (non-pregnant n = 18, pregnant n = 17); Spontaneous breathing (Sf_r), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); breathing frequency (f_r) in breaths·min^{-1}, tidal volume (V_T) in L, duty cycle (T_I /T_{TOT}); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

Arterial blood pressures

There were no significant differences for mean SBP or DBP between breathing conditions or between groups (see supplementary data). SBP and DBP peak-valley amplitude during breath phase (maximum minus minimum values) were significantly greater during both inspiration (Δi) and expiration (Δe) for all SDB conditions compared with spontaneous breathing (Table 7-3). This was true for both pregnant and non-pregnant women ($p<0.001$). The only significant difference in SBPΔPV between pregnant and non-pregnant groups was for the 6Ff_r condition ($p=0.001$).

Peak-valley breath phase independent values (ΔPV_Ind) were higher for both pregnant and non-pregnant women compared with peak-valley analysis linked to breath phase (ΔPV).

Table 7-3 Peak-valley differences (±SD) for blood pressure variables (mmHg)

		Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	Effect of condition *P* value	Group difference P value
SBP Δi	*NP*	3.6 ± 1.7¥¤†§	8.2 ± 2.9*¤†§	12.8 ± 5.4*¥	13.5 ± 4.6*¥	15.5 ± 6.1*¥	<0.001	
	P	3.4 ± 1.5¥¤†§	9.1 ± 2.6*¤†	11.2 ± 3.7*¥	11.7 ± 3.8*¥	12.9 ± 4.9*	<0.001	*0.925*
SBP Δe	*NP*	4.5 ± 2.5¥¤†§	6.9 ± 2.7*¤†§	10.2 ± 4.6*¥	10.5 ± 4.6*¥	12.1 ± 6.6*¥	<0.001	
	P	3.7 ± 1.6¥¤†§	7.4 ± 2.5*¤†	9.0 ± 2.4*¥	9.7 ± 3.0*¥	11.7 ± 6.1*	<0.001	*0.592*
SBP ΔPV	*NP*	-8.6 ± 3.6	-13.1 ± 7.0§	-11.5 ± 13.6§	-15.3 ± 9.5§	2.3 ± 18.4¥¤†	0.001	
	P	-7.7 ± 2.6§	-8.5 ± 9.0§	-3.7 ± 13.1§	0.8 ± 14.7§	12.3 ± 9.8*¥¤†	<0.001	*0.005*
SBP ΔPV_Ind	*NP*	15.0 ± 6.1	17.5 ± 5.6	19.1 ± 6.3	19.0 ± 5.1	17.4 ± 6.9	0.014	
	P	12.9 ± 4.7§	13.9 ± 3.7	15.9 ± 3.1	15.9 ± 3.8	17.6 ± 6.0*	0.033	*0.089*
DBP Δi	*NP*	2.5 ± 1.2¥¤†§	5.2 ± 1.8*¤†§	8.8 ± 2.9*¥	9.3 ± 3.1*¥	10.0 ± 3.0*¥	<0.001	
	P	1.8 ± 0.8¥¤†§	6.6 ± 2.2*	7.7 ± 2.7*	7.5 ± 3.5*	7.6 ± 3.1*	<0.001	*0.118*
DBP Δe	*NP*	3.2 ± 1.6¥¤†§	5.6 ± 2.1*¤†§	7.6 ± 3.1*¥	8.2 ± 3.2*¥	8.8 ± 3.1*¥	<0.001	
	P	2.7 ± 1.3¥¤†§	6.7 ± 2.2*†	7.6 ± 1.8*	8.3 ± 2.2*¥	9.9 ± 4.0*	<0.001	*0.553*
DBP ΔPV	*NP*	-4.0 ± 2.2¥§	-7.6 ± 2.0*§	-1.9 ± 11.2§	-3.4 ± 11.1§	11.6 ± 7.3*¥¤†	<0.001	
	P	-4.6 ± 1.7†§	-6.1 ± 6.7†§	0.7 ± 9.3§	5.3 ± 9.1*¥	11.4 ± 3.4*¥¤	<0.001	*0.097*
DBP ΔPV_Ind	*NP*	9.9 ± 4.4	10.6 ± 2.9	12.1 ± 3.3	12.1 ± 2.5	12.7 ± 3.3	0.014	
	P	7.9 ± 2.8†§	9.7 ± 2.8	10.3 ± 2.4	10.4 ± 2.7*	12.8 ± 5.5*	0.005	*0.130*

Data represent mean ± SD (non-pregnant n = 18, pregnant n = 17); Non-pregnant (NP), Pregnant (P); Spontaneous breathing (Sf_r), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

A high correlation (>0.8) was observed between SBPΔi and SBP and between SBPΔe and SBP, including DBP equivalents, across all breathing conditions. To reveal the change in the amplitude of BP oscillations relative to mean BP, percentage change BP oscillations were calculated during each breath phase (peak-valley difference (Δi or Δe) as a percentage of average BP during corresponding inspiration or expiration (Figure 7-3). All SDB conditions were significantly different from Sf_r for all percentage BP oscillations (%SBPΔi, %DBPΔi, %SBPΔe, %DBPΔe) for both non-pregnant and pregnant women. There were no significant differences between groups.

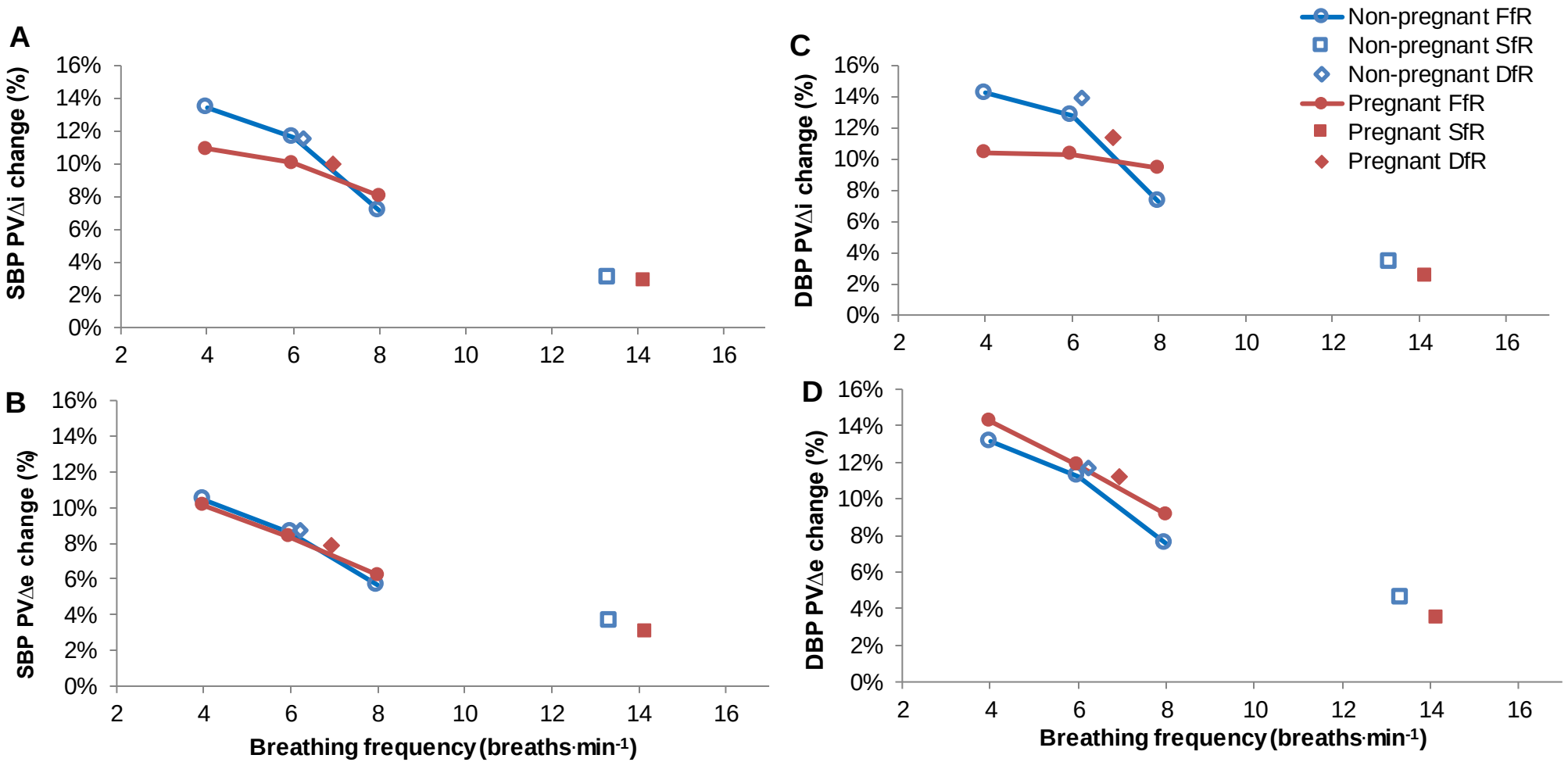


Figure 7-3 Blood pressure oscillations: Relative change for systolic blood pressure of Δi (A), Δe (B) and diastolic blood pressure of Δi (C), Δi (D)
Systolic blood pressure (SBP), diastolic blood pressure (DBP); within expiration difference (Δi); within expiration difference (Δe); Fixed breathing frequency (Ff_r) Spontaneous breathing (Sf_r), optimisation algorithm dynamic breathing frequency (Df_r). Variable calculated as SBPΔi as a percentage of average SBP during inspiration, or equivalent during expiration and for DBP.

Antenatal appointment recorded BP data (available for 58.8% of participants), revealed that no pregnant participants who submitted data developed hypertension following participating in the data collection session (defined as SBP <140 mmHg and/or DBP <90 mmHg).

Heart rate and respiratory sinus arrythmia

Peak-valley amplitude changes in heart rate during inspiration were significantly different between pregnant and non-pregnant women for all SDB conditions, except Sf_r (Table 7-4). There was also a significant increase for mean heart rate between non-pregnant and pregnant women, and for mean heart rate during inspiration and expiration for all conditions (see supplementary data). Peak-valley amplitude during expiration (f_cΔe) and inter-breath phase (f_cΔPV) were significantly higher during all SDB conditions compared with Sf_r for both pregnant and non-pregnant participants (Table 7-4).

Table 7-4 Peak-valley differences (±SD) for heart rate (f_c) and respiratory sinus arrythmia (RSA)

		Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	Effect of condition *P* value	Group difference P value
f_c	*NP*	5.4 ± 2.2¥¤†§	14.4 ± 6.9*	15.5 ± 6.2*	15.5 ± 5.9*	15.9 ± 7.1*	<0.001	
Δi	*P*	4.5 ± 2.9¥¤†§	8.4 ± 5.0*	9.3 ± 5.2*	9.6 ± 5.8*	11.3 ± 5.7*	<0.001	*0.002*
f_c	*NP*	7.3 ± 3.2¥¤†	14.3 ± 6.0*	14.1 ± 8.4*	13.4 ± 6.7*	10.3 ± 8.3	<0.001	
Δe	*P*	6.1 ± 4.4¥¤	10.5 ± 5.2*	9.9 ± 4.6*	9.7 ± 4.0	11.0 ± 6.0	0.004	*0.145*
f_c	*NP*	-6.6 ± 5.1¥¤†§	16.1 ± 8.9*¤†	20.7 ± 8.5*¥	20.6 ± 7.5*¥	21.1 ± 9.5*	<0.001	
ΔPV	*P*	-3.1 ± 7.4¥¤†§	13.4 ± 6.8*	15.3 ± 7.8*	15.1 ± 6.8*	11.2 ± 12.7*	<0.001	*0.044*
RSA	*NP*	0.12 ± 0.1¥¤†§	0.21 ± 0.1*¤†	0.25 ± 0.1*¥	0.25 ± 0.1*¥	0.25 ± 0.1*	<0.001	
(s)	*P*	0.07 ± 0.1¥¤†§	0.13 ± 0.1*	0.15 ± 0.1*	0.15 ± 0.1*	0.15 ± 0.1*	<0.001	*0.002*

Data represent mean ± SD (non-pregnant n = 18, pregnant n = 17); Non-pregnant (NP), Pregnant (P); Spontaneous breathing (Sf_r), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); heart rate (f_c; beats·min^{-1}), respiratory sinus arrythmia (RSA; s); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

RSA was significantly lower for the pregnant women, compared with non-pregnant women for all breathing conditions (p<0.001). RSA during SDB for pregnant women increased to a level similar to that observed during spontaneous breathing (Sf_r) for non-pregnant women (Figure 7-4) and plateaued (saturated) at 6 breaths·min^{-1} (6Ff_r) for both groups. The maximum amplitude of RSA during SDB (≤6 breaths·min^{-1}) was 2.1 times higher than RSA during Sf_r for both the non-pregnant and pregnant group, albeit lower in absolute terms for pregnant women.

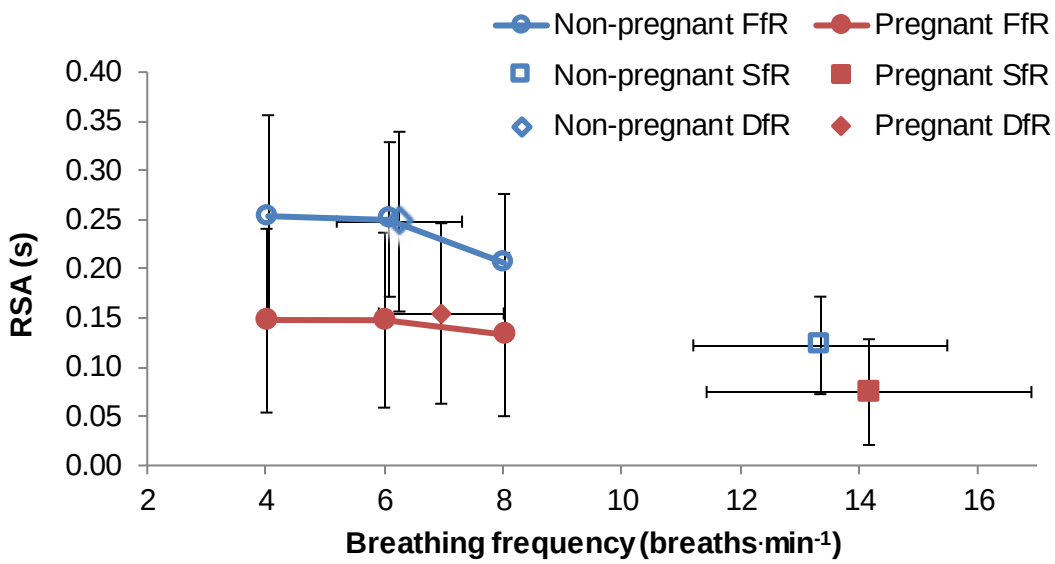


Figure 7-4 Respiratory sinus arrythmia (RSA) response to slow and deep breathing

Values are mean ± SD; Respiratory sinus arrhythmia (RSA); Fixed breathing frequency (Ff_r) Spontaneous breathing (Sf_r), optimisation algorithm dynamic breathing frequency (Df_r).

Stroke volume and cardiac output

Mean stroke volume (SV) and cardiac output ($\dot{Q}$) were significantly higher for pregnant participants than non-pregnant participants (supplementary data). Peak-valley amplitude for SV and $\dot{Q}$ during inspiration and expiration ($SV\Delta i$, $SV\Delta e$, $\dot{Q}\Delta i$, $\dot{Q}\Delta e$) were significantly different during all SDB conditions compared with Sf_r for non-pregnant and pregnant participants (Table 7-5). The only exception was $SV\Delta i$, which was not significantly different between $8Ff_r$ and Sf_r for non-pregnant women. Peak-valley SV was significantly different between pregnant and non-pregnant women during all SDB conditions but not during Sf_r ($p<0.005$). Peak-valley breath phase independent values were higher for SV and $\dot{Q}$, compared with peak-valley values linked with breath phases for both non-pregnant and pregnant women.

Table 7-5 Peak-valley differences (±SD) for stroke volume (SV) and cardiac output (Q̇)

		Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	Effect of condition *P* value	Group difference P value
SVΔi	*NP*	5.1 ± 2.0¤†§	7.3 ± 3.9§	7.1 ± 3.5*§	7.7 ± 4.0*§	11.8 ± 4.9*¥¤†	<0.001	
	P	5.8 ± 2.0¥¤†§	9.0 ± 2.8*†§	10.7 ± 3.7*§	11.0 ± 3.3*¥§	15.3 ± 5.0 *¥¤†	<0.001	*0.014*
SVΔe	*NP*	5.6 ± 2.0¥¤†§	8.2 ± 3.1*§	8.9 ± 4.0*§	9.2 ± 3.9*	13.5 ± 6.3*¥¤	<0.001	
	P	5.2 ± 1.9¥¤†§	7.9 ± 2.3*§	9.2 ± 2.3*§	9.6 ± 2.3*§	12.5 ± 3.7 *¥¤†	<0.001	*0.827*
SV ΔPV	*NP*	-8.9 ± 5.5§	-13.1 ± 8.0§	-13.8 ± 8.5	-14.6 ± 7.6	-18.6 ± 6.9*¥	0.001	
	P	-8.0 ± 6.5	-2.9 ± 12.1	-3.4 ± 15.1	-1.7 ± 14.8	-0.4 ± 17.7	0.119	*0.002*
SVΔPV _Ind	*NP*	11.9 ± 4.2§	13.7 ± 5.9	13.6 ± 6.4	13.4 ± 6.4	16.2 ± 6.4*	0.027	
	P	13.0 ± 4.0§	13.4 ± 4.1§	15.5 ± 5.6	15.6 ± 4.6	18.0 ± 5.2*¥	0.002	*0.361*
QΔi	*NP*	292 ± 119¥¤†§	1092 ± 646*	1107 ± 561*	1129 ± 607*	1073 ± 474*	<0.001	
	P	586 ± 293¥¤†§	1151 ± 348*	1340 ± 468*	1292 ± 422*	1453 ± 300*	<0.001	*0.063*
QΔe	*NP*	414 ± 184¥¤†§	831 ± 359*	840 ± 469*	787 ± 424*	747 ± 413*	<0.001	
	P	563 ± 265¥¤†§	920 ± 243*	1012 ± 336*	1040 ± 355*	1164 ± 479*	<0.001	*0.018*
Q̇ ΔPV	*NP*	-738 ± 203¥¤†§	-638 ± 1153¤†§	402 ± 1268*¥	493 ± 1208*¥	903 ± 923*¥	<0.001	
	P	-745 ± 663¥¤†§	1181 ± 732*	1432 ± 870*	1431 ± 842*	1452 ± 901*	<0.001	*<0.001*
QΔPV _Ind	*NP*	1352 ± 1982	1302 ± 667	1366 ± 1038	1191 ± 565	1018 ± 540	0.489	
	P	1198 ± 341	1382 ± 302	1602 ± 553	1496 ± 373	1426 ± 282	0.006	*0.409*

Data represent mean ± SD (non-pregnant n = 18, pregnant n = 17); Non-pregnant (NP), Pregnant (P); Spontaneous breathing (Sf_r), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); stroke volume (SV; ml), cardiac output (Q̇; ml·min^{-1}); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV); breath phase independent peak-valley difference (ΔPV_Ind). Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

Total peripheral resistance and pulse wave velocity

Table 7-6 shows a significant increase in TPRΔi and TPRΔe during SDB compared with Sf_r for both pregnant and non-pregnant women.

Table 7-6 Peak-valley differences (±SD) for total peripheral resistance (TPR) and pulse wave velocity (PWV) variables

		Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	Effect of condition *P* value	Group difference P value
TPR Δi	*NP*	1.8 ± 2.2¥†§	4.0 ± 1.9*	3.0 ± 1.2	4.3 ± 2.4*	3.5 ± 1.7*	<0.001	
	P	1.1 ± 0.6¥¤†§	1.8 ± 0.8*	2.0 ± 0.8*	2.0 ± 0.9*	2.2 ± 0.7*	<0.001	*0.001*
TPR Δe	*NP*	2.1 ± 2.0†	3.1 ± 1.4	2.8 ± 1.5	3.7 ± 2.3*	3.2 ± 1.3	0.020	
	P	1.1 ± 0.6¤†§	1.7 ± 1.1†	2.0 ± 1.2*	2.2 ± 1.1*¥	2.2 ± 1.0*	<0.001	*0.006*
TPR ΔPV	*NP*	2.3 ± 3.8¤†	0.8 ± 4.7	-3.3 ± 2.7*	-3.3 ± 5.1*	0.3 ± 4.8	<0.001	
	P	0.4 ±1.7¥¤†	-2.8 ± 1.3*	-2.9 ± 1.2*	-2.5 ± 1.7*	-0.7 ± 2.7	<0.001	*0.115*
TPRΔPV_Ind	*NP*	6.0 ± 6.3	5.2 ± 2.1	4.4 ± 1.9	5.4 ± 2.7	4.1 ± 1.7	0.192	
	P	2.5 ± 1.1	3.0 ± 1.4	2.9 ± 1.2	2.8 ± 1.4	2.6 ± 1.1	0.159	*0.002*
PWVΔi	*NP*	0.2 ± 0.1¥¤†§	0.4 ± 0.2*	0.5 ± 0.2*	0.5 ± 0.2*	0.4 ± 0.1*	<0.001	
	P	0.2 ± 0.1¥¤†§	0.6 ± 1.3*†§	0.3 ± 0.1*	0.4 ± 0.2*¥	0.5 ± 0.2*¥	<0.001	*0.194*
PWVΔe	*NP*	0.3 ± 0.1¥¤†	0.4 ± 0.2*	0.5 ± 0.2*	0.4 ± 0.2*	0.4 ± 0.2	0.001	
	P	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.4	0.3 ± 0.1	0.5 ± 0.2	0.088	*0.528*
PWV ΔPV	*NP*	0.4 ± 0.1¥¤†§	-0.7 ± 0.5*†	-0.6 ± 0.3*†	0.6 ± 0.3*¥†§	-0.3 ± 0.5*†	<0.001	
	P	-0.32 ± 0.2	-0.05 ± 1.4	-0.42 ± 0.5	-0.14 ± 0.5	-0.14 ± 0.6	0.158	*0.002*

Data represent mean ± SD (non-pregnant n = 18, pregnant n = 17); Non-pregnant (NP), Pregnant (P); Spontaneous breathing (Sf_r), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); total peripheral resistance (TPR; mmHg·min·L^{-1}), pulse wave velocity (PWV; m·s^{-1}); within inspiration difference (Δi), within expiration difference (Δe), inter-breath phase peak-valley difference (ΔPV), breath phase independent peak-valley difference (ΔPV_Ind). Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

Preferred breathing condition

Fifty five percent of pregnant participants preferred the 6Ff_r condition. Additionally, another 4 participants chose 6Ff_r as their second preferred condition, where they had little preference between 2 conditions as their favourite. There was no correlation between preferred breathing frequency and gestational age (R^2=0.00).

Discussion

The present study builds on work from Felton et al. (2021 – in preparation[7]) to characterise acute cardiovascular responses to SDB, including an analysis of the inter- and intra-breath phase perturbations created by breathing. The first set of analyses show that heart rate, stroke volume and cardiac output were significantly higher in pregnant

[7] Integrated paper presented in Chapter 4

women during spontaneous breathing (Sf_r), which is in agreement with the known adaptations caused by pregnancy (Sanghavi and Rutherford 2014). Pregnant women had higher cardiac output and stroke volume at equivalent breathing frequencies, compared with non-pregnant women, which is consistent with the higher cardiovascular response seen during aerobic exercise in pregnant women (Hegewald and Crapo 2011).

Although heart rate was higher in the pregnant group, their RSA was significantly lower for all breathing conditions, being just 58% the value observed in non-pregnant women during spontaneous breathing. This observation is consistent with the 65% difference found by Miyazato and Matsukawa (2010). SDB caused a significant increase in RSA for both non-pregnant and pregnant women compared with spontaneous breathing (Sf_r); relative RSA (maximum RSA compared with baseline RSA) increased by a maximum of 48% and 47%, respectively. Therefore, although absolute maximum RSA was higher in the non-pregnant group (0.25 v. 0.15 s), the response to SDB created almost a 50% increase in the amplitude of RSA. This is consistent with the absolute RSA response to relaxation, which was also lower in pregnant women compared with no pregnant women (DiPietro et al. 2012). Thus, during SDB breathing, the present study found a similar relative (%) increase in RSA amplitude, despite a lower absolute RSA amplitude.

SDB increased RSA in pregnant women to levels similar to the RSA observed during spontaneous breathing for non-pregnant participants, revealing the ability of SDB to return RSA to pre-pregnancy levels. As an attenuated RSA has been suggested as a biophysical marker of pre-eclampsia (Lakhno 2016), the ability of an intervention to increase RSA during pregnancy is promising. Attenuated RSA *per se* is not the cause of hypertension, but reflects the functional state of the autonomic nervous system (Buchner 2018), and as there is an overactivity of the sympathetic arm during PIH, changes in RSA may reflect an improvement in the balance of the autonomic nervous system. Although it should be noted that RSA's ability to reflect the autonomic nervous system is queried (see point: counterpoint series) (Eckberg 2009; Julien et al. 2009; Karemaker 2009b, 2009a), and therefore any suggestions of relationships must be carefully interpreted. Whether SDB can increase long-term RSA in pregnant women after daily SDB practice is unknown but needs investigating.

RSA is a well-established physiological parameter, which is calculated using the peak-valley method to quantify acute changes in heart rate induced by the two phases of breathing. RSA is quantified irrespective of the breath phase in which the heart beat was recorded, but the kinetics of the heart rate response to breathing are such that the peak of heart rate almost always occurs during inspiration, whilst the trough occurs during

expiration. However, the kinetics of haemodynamic responses are slower than for heart rate, with the peaks and troughs induced by each breath phase often occurring in the next (opposite) breath phase. The present study sought to reveal this phenomenon, as well as overcoming it, but using two different peak-valley methods of analysis, 1) peak-valley amplitude calculated with respect to breath phase; 2) breath phase independent peak-valley amplitude (akin to RSA). This approach reveals the true magnitude of perturbations created by SDB, as well as the influence of response kinetics upon this amplitude. When only mean values are examined, the results mask the complex response including the increase in the amplitude of oscillations that occur to maintain homeostasis during SDB.

The amplitude of BP oscillations (both SBP and DBP) during inspiration and expiration increased as breathing frequency reduced, reaching a peak at 4 breaths·min^{-1} (4Ff_r), which was up to 4 and a half times higher (14.3%, 10 mmHg) than during spontaneous breathing (Sf_r; see Figure 7-3). This supports the data from Felton et al. (2021 – in preparation[8]), which suggested that the amplitude of BP oscillations may be further increased at breathing frequencies below 6 breaths·min^{-1}. However, although the amplitude of BP oscillations was further increased below 6 breaths·min^{-1}, there was no significant difference at 4Ff_r from the response during 6Ff_r suggesting minimal differences between SDB conditions. The difference was only an average 1.2 mmHg (±0.7 mmHg) between 4Ff_r and 6Ff_r, which is unlikely to produce a meaningful clinical difference between the two conditions if used as a long-term SDB condition.

Total peripheral resistance was significantly lower in pregnant women compared with non-pregnant women in the present study, which is most likely attributable to vasodilation that occurs during pregnancy (Ngene and Moodley 2017). Levels of BP are reliant on the balance between total peripheral resistance and cardiac output. Therefore, to maintain BP during pregnancy, cardiac output is increased to counteract the decreased total peripheral resistance (Moser et al. 2012). In the present study there was no significant differences in BP between pregnant and non-pregnant women, despite the lower total peripheral resistance, due to a significantly higher cardiac output in the pregnant women group.

Interestingly, although an increased tidal volume was expected in pregnant women (McAuliffe et al. 2002), this was only observed during spontaneous breathing (Sf_r), where tidal volume was double that of non-pregnant women. There were no significant

[8] Integrated paper presented in Chapter 4

differences in tidal volume between non-pregnant and pregnant women during any SDB conditions, suggesting an ability of the respiratory system of pregnant women to adapt comfortably to reduced breathing frequencies in a similar way to non-pregnant women. Tidal volume in the pregnant women group increased significantly as breathing frequency was reduced below 8 breaths·min^{-1}.

Finally, there were limited differences in the cardiovascular responses observed in the present study between the SDB conditions of 4Ff_r, 6Ff_r and Df_r, however for many variables (such as percentage amplitudes of BP oscillations) the 8Ff_r condition did not deliver a significantly different cardiovascular response compared with spontaneous breathing (Sf_r). This suggests that 8 breaths·min^{-1} may be too high a breathing frequency to elicit the full cardiovascular response of SDB. As a group, the eighteen pregnant women chose 6 breaths·min^{-1} (6Ff_r) as their preferred breathing frequency if they were asked to continue with the SDB exercise daily until birth Additionally, there was no correlation between gestational age and preferred SDB frequency, or for optimal breathing frequency derived from the bespoke optimisation algorithm, suggesting that all SDB frequencies are manageable at all stages of gestation. Therefore, the present study suggests that future studies should utilise 6 breaths·min^{-1} (6Ff_r) for SDB interventions with pregnant women, which provides a good compromise between the optimising physiological responses and participant preference.

Conclusion

The present study adds to growing evidence that the analysis of inter- and intra-breath phase haemodynamic oscillations are vital to reveal the true extent of the cardiovascular perturbations created by SDB. The cardiovascular responses to SDB are similar in healthy pregnant and healthy non-pregnant women, with no significant differences in relative amplitude of BP oscillations and a similar relative increase in RSA from baseline values (Sf_r). RSA is attenuated during spontaneous breathing in pregnant women, but can be increased acutely to non-pregnant levels by SDB. The data support future studies investigating the long-term changes to RSA, BP and other cardiovascular variables following daily practice of SDB using a breathing frequency of 6 breaths·min^{-1}.

7.3 Supplementary material

The following results tables will be included as supplementary information for the publication in European Journal of Applied Physiology.

Table 7-7 Mean values (±SD) and inter-breath phase differences (Δ) for blood pressure variables (mmHg)

		Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	Effect of condition *P* value	Group difference P value
SBP	*NP*	121.3 ± 17.6	118.0 ± 9.6	115.3 ± 19.9	119.8 ± 13.8	116.2 ± 10.5	0.481	
	P	122.1 ± 12.5	117.1 ± 17.4	115.4 ± 13.9	117.1 ± 10.7	117.2 ± 15.8	0.347	*0.921*
SBPi	*NP*	118.9 ± 17.4	114.3 ± 9.8	112.3 ± 20.2	117.1 ± 14.7	116.5 ± 10.4	0.475	
	P	119.8 ± 12.5	115.6 ± 18.1	114.9 ± 14.4	118.0 ± 11.5	118.7 ± 15.5	0.519	*0.681*
SBPe	*NP*	123.8 ± 17.8	121.8 ± 9.6	118.4 ± 20.0	122.7 ± 13.3	115.8 ± 11.1	0.200	
	P	124.3 ± 12.5	118.5 ± 16.8	116.0 ± 13.8	116.3 ± 10.3	115.7 ± 16.3	0.062	*0.538*
SBPΔ	*NP*	–4.9 ± 2.1§	–7.5 ± 3.4§	–6.0 ± 5.0§	–5.6 ± 5.2§	0.7 ± 5.0*¥¤†	<0.001	
	P	-4.5 ± 1.8¤†§	-2.9 ± 3.8†§	-1.1 ± 4.1*†§	1.7 ± 4.7*¥¤	3.0 ± 2.2*¥¤	0.000	*0.001*
DBP	*NP*	74.7 ± 17.1	73.1 ± 7.3	67.5 ± 14.7	74.1 ± 11.4	69.8 ± 8.7	0.166	
	P	74.5 ± 10.2	73.2 ± 12.8	69.7 ± 10.3	71.6 ± 7.3	71.3 ± 12.3	0.289	*0.942*
DBPi	*NP*	73.9 ± 17.3	71.8 ± 7.3	67.1 ± 15.4	73.6 ± 11.9	71.9 ± 9.0	0.286	
	P	73.2 ± 9.9	72.4 ± 13.2	69.9 ± 10.7	72.9 ± 7.9	72.9 ± 12.0	0.561	*0.846*
DBPe	*NP*	75.5 ± 17.0	74.3 ± 7.3	68.0 ± 14.2	74.6 ± 11.1	67.7 ± 8.6	0.064	
	P	75.8 ± 10.4¤†	74.1 ± 12.4	69.5 ± 10.0*	70.3 ± 7.0*	69.7 ± 7.0	0.021	*0.961*
DBPΔ	*NP*	–1.5 ± 1.0§	–2.5 ± 1.5§	–0.9 ± 3.4§	–1.0 ± 3.4§	4.2 ± 3.3*¥¤†	<0.001	
	P	-2.5 ± 0.9¤†§	-1.7 ± 1.8¤†§	0.3 ± 2.1*¥†§	2.6 ± 2.5*¥¤	3.2 ± 2.2*¥¤	<0.001	*0.134*

Data represent mean ± SD (non-pregnant n = 18, pregnant n = 17); Non-pregnant (NP), Pregnant (P); Spontaneous breathing (Sf_r), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff_r), 6 breaths·minute^{-1} (6Ff_r), 8 breaths·minute^{-1} (8Ff_r), optimisation algorithm dynamic breathing frequency (Df_r); systolic blood pressure (SBP), diastolic blood pressure (DBP); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf_r (*), 8Ff_r (¥), Df_r (¤), 6Ff_r (†), 4Ff_r (§); P<0.05.

Table 7-8 Mean values (±SD) and inter-breath phase differences (Δ) for heart rate (f_c), stroke volume (SV) and cardiac output ($\dot{Q}$) variables

		Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	Effect of condition *P* value	Group difference P value
f_c	*NP*	67.6 ± 10.9	70.7 ± 11.6	70.0 ± 11.0	70.0 ± 10.5	69.5 ± 10.3	0.091	
	P	80.7 ± 10.1	82.6 ± 9.8	80.3 ± 9.3	81.7 ± 8.6	79.9 ± 8.4	0.123	*0.001*
f_c *i*	*NP*	67.0 ± 11.3¤†§	72.5 ± 12.1†	74.4 ± 12.5*	74.4 ± 12.0*¥	74.5 ± 11.0*	<0.001	
	P	80.4 ± 10.5¥¤†	85.2 ± 10.0*	83.9 ± 8.8*	85.1 ± 7.5*	82.1 ± 7.3	<0.001	*0.003*
f_c *e*	*NP*	68.3 ± 10.6§	68.5 ± 10.8†§	65.8 ± 10.3	65.6 ± 9.2¥	64.6 ± 10.1*¥	0.002	
	P	80.9 ± 9.8	79.7 ± 9.8	76.4 ± 10.1	78.1 ± 10.0	77.7 ± 9.9	0.009	*0.001*
f_c *Δ*	*NP*	–1.4 ± 1.4¥¤†§	4.0 ± 2.9*¤†§	8.6 ± 5.4*¥	8.9 ± 4.9*¥	9.9 ± 4.8*¥	<0.001	
	P	-0.5 ± 2.4¥¤†	5.5 ± 3.3*	7.4 ± 4.7*	7.0 ± 5.0*	4.3 ± 5.1	<0.001	*0.240*
SV	*NP*	67.4 ± 19.8	66.7 ± 21.1	68.3 ± 21.3	66.2 ± 22.0	68.5 ± 18.4	0.539	
	P	82.3 ± 9.8	81.4 ± 10.4	82.5 ± 10.0	82.7 ± 9.2	83.1 ± 10.2	0.920	*0.008*
SVi	*NP*	65.2 ± 19.7	63.5 ± 20.4	64.9 ± 20.4	63.1 ± 20.7	65.1 ± 18.1	0.366	
	P	80.3 ± 9.5	80.9 ± 10.5	81.9 ± 9.9	82.3 ± 9.2	82.7 ± 10.0	0.640	*0.002*
SVe	*NP*	69.6 ± 20.0	69.9 ± 21.9	71.6 ± 22.4	69.3 ± 23.3	71.9 ± 18.9	0.490	
	P	84.2 ± 10.3	81.8 ± 10.8	83.0 ± 10.8	83.1 ± 9.9	83.5 ± 10.7	0.854	*0.026*
SVΔ	*NP*	–4.4 ± 2.9	–6.4 ± 3.6	–6.7 ± 4.6	–6.2 ± 4.4	–6.8 ± 3.5	0.188	
	P	-3.9 ± 3.0	-0.9 ± 4.2	-1.1 ± 5.7	-0.8 ± 4.9	-0.7 ± 4.2	0.027	*<0.001*
$\dot{Q}$	*NP*	4441 ± 1047	4564 ± 1144	4633 ± 1097	4482 ± 1220	4632 ± 1017	0.530	
	P	6596 ± 923	6652 ± 1075	6564 ± 981	6706 ± 1027	6573 ± 863	0.728	*<0.001*
$\dot{Q}i$	*NP*	4247 ± 1018¤	4476 ± 1181	4707 ± 1184*	4562 ± 1266	4737 ± 1096	0.026	
	P	6427 ± 953¥¤†	6858 ± 1090*	6855 ± 1034*	6995 ± 990*	6749 ± 884	0.001	*<0.001*
$\dot{Q}e$	*NP*	4639 ± 1083	4639 ± 1117	4566 ± 1034	4406 ± 1197	4527 ± 952	0.435	
	P	6765 ± 913¤§	6447 ± 1077	6268 ± 958*	6427 ± 1079	6399 ± 864*	0.003	*<0.001*
$\dot{Q}\Delta$	*NP*	–392 ± 155¥¤†§	–163 ± 250*¤†§	141 ± 307*¥	156 ± 278*¥	210 ± 264*¥	<0.001	
	P	-339 ± 286¥¤†§	411 ± 278*¤	588 ± 351*¥	568 ± 238*§	350 ± 257*†	<0.001	*<0.001*

Data represent mean ± SD (non-pregnant n = 18, pregnant n = 17); Non-pregnant (NP), Pregnant (P); Spontaneous breathing (Sf$_r$), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff$_r$), 6 breaths·minute^{-1} (6Ff$_r$), 8 breaths·minute^{-1} (8Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$); heart rate (f_c; beats·min^{-1}), stroke volume (SV; ml), cardiac output ($\dot{Q}$; ml·min^{-1}); mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf$_r$ (*), 8Ff$_r$ (¥), Df$_r$ (¤), 6Ff$_r$ (†), 4Ff$_r$ (§); P<0.05.

Table 7-9 Mean values (±SD) and inter-breath phase differences (Δ) for total peripheral resistance (TPR) and pulse wave velocity (PWV) variables

		Sf_r	8Ff_r	Df_r	6Ff_r	4Ff_r	Effect of condition *P* value	Group difference P value
TPR	*NP*	21.5 ± 8.3	20.0 ± 3.7	18.3 ± 3.5	20.9 ± 5.4	18.8 ± 3.5	0.171	
	P	13.8 ± 3.1	13.4 ± 3.1	13.1 ± 2.6	13.1 ± 2.4	13.2 ± 2.2	0.419	*<0.001*
TPRi	*NP*	22.2 ± 8.6	20.0 ± 3.9	17.8 ± 3.6	20.2 ± 5.1	18.9 ± 3.8	0.084	
	P	14.0 ± 3.0¤†	12.8 ± 2.9	12.5 ± 2.6*	12.7 ± 2.3*	13.1 ± 2.2	0.020	*<0.001*
TPRe	*NP*	20.8 ± 7.9	20.0 ± 3.5	18.8 ± 3.6	21.6 ± 5.7	18.8 ± 3.4	0.233	
	P	13.7 ± 3.2	14.0 ± 3.2	13.7 ± 2.7	13.5 ± 2.6	13.3 ± 2.3	0.600	*<0.001*
TPRΔ	*NP*	1.3 ± 1.1¥¤†	0.0 ± 1.1*¤†	-1.0 ± 0.9*¥	-1.4 ± 1.3*¥	0.0 ± 1.6	<0.001	
	P	0.2 ± 0.7¥¤†	-1.2 ± 0.7*§	-1.2 ± 0.6*§	-0.8 ± 0.7*§	-0.2 ± 0.4¥¤†	<0.001	*0.015*
PWV	*NP*	4.6 ± 0.4	4.6 ± 0.4	4.7 ± 0.4	4.7 ± 0.4	4.7 ± 0.4	0.143	
	P	5.1 ± 0.5	5.1 ± 0.5	5.0 ± 0.4	5.0 ± 0.5	5.0 ± 0.5	0.081	*0.014*
PWVi	*NP*	4.6 ± 0.4	4.5 ± 0.4§	4.6 ± 0.4	4.6 ± 0.4	4.7 ± 0.4¥	0.002	
	P	5.0 ± 0.5	5.0 ± 0.6	4.9 ± 0.4	5.0 ± 0.5	5.0 ± 0.5	0.205	*0.007*
PWVe	*NP*	4.7 ± 0.4	4.7 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	0.472	
	P	5.2 ± 0.5†§	5.1 ± 0.5†	5.1 ± 0.5	5.0 ± 0.5*¥	5.1 ± 0.5*	0.004	*0.029*
PWVΔ	*NP*	–0.2 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.2	0.030	
	P	-0.14 ± 0.1†	-0.10 ± 0.2†§	-0.11 ± 0.1†	-0.02 ± 0.1*¥¤	-0.02 ± 0.1¥	<0.001	*0.006*

Data represent mean ± SD (non-pregnant n = 18, pregnant n = 17); Non-pregnant (NP), Pregnant (P); Spontaneous breathing (Sf$_r$), fixed breathing frequency of 4 breaths·minute^{-1} (4Ff$_r$), 6 breaths·minute^{-1} (6Ff$_r$), 8 breaths·minute^{-1} (8Ff$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$); total peripheral resistance (TPR) in mmHg·min·L^{-1}, pulse wave velocity (PWV) in m·s^{-1}; mean inspiration (i), mean expiration (e), inter-breath phase difference (Δ; i minus e); Significantly different from Sf$_r$ (*), 8Ff$_r$ (¥), Df$_r$ (¤), 6Ff$_r$ (†), 4Ff$_r$ (§); P<0.05.

7.4 Summary

A comprehensive characterisation of the cardiovascular responses to SDB in healthy non-pregnant and healthy pregnant women has been completed. The data reveal that, although there are baseline cardiovascular differences between pregnant and non-pregnant women, including depression of RSA, the responses to SDB are similar. Specifically, RSA increases by a similar relative amount between non-pregnant and pregnant groups (2.1 times greater). Additionally, when the amplitude of BP oscillations is calculated relative to mean BP there are no significant differences between pregnant and non-pregnant groups across all breathing frequencies, reflected in similar absolute BP oscillation values. Overall, pregnancy does not appear to attenuate the response of key cardiovascular variables to SDB.

With the understanding that SDB produces similar responses in pregnant women as it does in non-pregnant women the next step is to replicate this study in women who develop high BP during pregnancy (pregnancy-induced hypertension; PIH). There are additional cardiovascular adaptations as a result of the underlying pathophysiology of PIH that may change the response to SDB from that of normotensive pregnant women. It is important to understand any differences in acute responses in pregnant women with PIH and how they may link to the error signals and mechanisms underpinning long-term reductions in BP.

Finally, the feasibility of using SDB as a treatment method for PIH must be investigated to test whether pregnant women will accept and adhere to the intervention, in addition to whether it has health benefits, either prophylactically, or after a diagnosis of PIH. This chapter has revealed that the optimal breathing frequency is 6 breaths·min^{-1}, and this should be used in future studies using SDB as an intervention in pregnant women. The next chapter will outline a proposed protocol to investigate the acute responses to SDB in pregnant women who develop PIH and trial the feasibility of using SDB as a daily intervention to reduce BP.

Chapter 8. Effects of slow and deep breathing on reducing obstetric intervention in women with pregnancy-induced hypertension: A feasibility study protocol

8.1 Introduction

The next step following the mechanistic understanding gained in the preceding chapters is to understand how pregnant women who develop pregnancy-induced hypertension respond to slow and deep breathing (SDB) and to test both the short- and long-term effects of SDB. Ethical approval for this study was received from the Hampshire B Research Ethics Committee alongside Health Research Authority (HRA) approval in Dec 2019. However, in the first few months of 2020 the world was hit by a global pandemic when coronavirus spread across the world. This unprecedented situation coincided with the set up and recruitment phase of this study and consequently recruitment for this study was put on hold in March 2020.

On 16th March pregnant women were classed as high risk by the UK government and on 23rd March the country went into lockdown. With a subsequent extended lockdown period it was clear that the study could not be completed within the time restrictions of PhD research, with an unknown date when maternity research could restart. The local maternity unit had only 2/11 existing research studies open for recruitment during the coronavirus outbreak. It is hoped this research study will be conducted with future funding as part of a post-doc project. The protocol for this study has been published in Hypertension in Pregnancy (Felton et al. 2021) and the published version is presented overleaf. References for the published article are listed at the end of the article, and are not replicated in the thesis reference list, unless cited elsewhere in the thesis.

The research questions and objectives for this chapter are outlined below:

Research questions

1. Is a daily SDB intervention accepted and adhered to by women with pregnancy-induced hypertension?
2. Does a daily programme of SDB reduce long-term blood pressure and/or obstetric intervention in women with pregnancy-induced hypertension?
3. Do women with pregnancy-induced hypertension exhibit the same acute cardiovascular responses to SDB as normotensive pregnant women?

Objectives

1. Design an evidence-based SDB intervention for women with pregnancy-induced hypertension.
2. Evaluate the adherence and recruitment rates to a daily SDB intervention with pregnant women with pregnancy-induced hypertension.
3. Assess blood pressure changes and obstetric intervention rates following completion of the SDB intervention.
4. Assess if women accept SDB as a treatment method for pregnancy-induced hypertension.
5. Identify whether mechanism-related parameters (e.g. respiratory sinus arrythmia, stroke volume, cardiac output) respond similarly to SDB for normotensive pregnant women and women with pregnancy-induced hypertension.
6. Identify whether acute changes in blood pressure and amplitude of blood pressure oscillations during SDB are similar for normotensive pregnant women and women with pregnancy-induced hypertension.
7. Evaluate whether the acute cardiovascular responses to a range of SDB frequencies are similar for normotensive pregnant women and women with pregnancy-induced hypertension.

8.2 Integrated paper: Effects of slow and deep breathing on reducing obstetric intervention in women with pregnancy-induced hypertension: A feasibility study protocol

The following pages present the published manuscript.

8.2 Integrated paper: Effects of slow and deep breathing on reducing obstetric intervention in women with pregnancy-induced hypertension: A feasibility study protocol

See: Felton, M., Hundley, V. A., Grigsby, S. and McConnell, A. K., 2021. Effects of slow and deep breathing on reducing obstetric intervention in women with pregnancy-induced hypertension: A feasibility study protocol. *Hypertension in Pregnancy*, 40 (1), 81-87 https://eprints.bournemouth.ac.uk/35051/

8.3 Additional protocol material

Due to the nature of publishing research articles there were elements of this research study that were not published in the protocol (section 8.2). To provide all the relevant information for this thesis, additional material relating to this protocol are outlined below.

8.3.1 Recruitment prior to COVID-19 shut down

Although no-one had been recruited into the study at the date of pausing the study, the recruitment period was open, and 5 women had been approached. Unfortunately, the ANDA midwives were not able to record the reasons women declined to take part due to their busy schedules on the ward. However, initial feedback from the research midwife, following discussion with one woman, was that the pregnant women was already finding it hard to breath sometimes and she felt like the mask pictured in the PIS would be claustrophobic. It was the picture in the PIS that had put her off from taking part. Unfortunately, as the mask is essential for verifying the breathing frequency during the short-term protocol it is not possible to remove this from the research protocol. However, during future RCTs the protocol would not include the short-term lab-based session, instead focusing on the at-home SDB intervention, and therefore this woman may have been willing to take part.

The inclusion and exclusion criteria for this study were originally set to match the criteria for the previous studies of pregnant women without high blood pressure (Chapter 6). However, due to slow recruitment for the study it was decided to make an amendment to the study and expand the criteria to allow multips to take part (women who have previously been pregnant). The justification for this was that blood pressure was not found to be significantly different between nulliparous and parous women across all stages of gestation (Loerup et al. 2019). An amendment was submitted via IRAS to the NHS Research Ethics Approval and was approved in March 2020 after the study had been paused. Consequently, recruitment rates with the new inclusion and exclusion criteria have not been tested.

8.4 Patient and Public Involvement: Conducting maternal research during the coronavirus pandemic

Patient and public involvement (PPI) has been an ongoing process integrated into the development stage of the SDB intervention. Prior to the PhD, (and undertaken independently from the author), PPI was undertaken with the National Childbirth Trust

(NCT) to consultant with pregnant women and practitioners about their views of using a SDB intervention to reduce BP during pregnancy. PPI was also undertaken during the development of the patient-facing documentation for the SDB intervention trial, to ensure paperwork was designed with the end-user in mind. Pregnant women were also involved in the decision of which SDB frequency to use for the long-term intervention protocol, where participant preference was noted during data collection in Chapter 6.

In 2020, following the pausing of the majority of maternity research including the clinical protocol outlined in section 8.2, PPI was undertaken by the author with pregnant women and new mothers to explore their views of participating in research during the coronavirus pandemic. The aim of the PPI consultation was to discuss the proposed project with more women to explore their views on the general research topic, specific protocol (section 8.2), and most importantly to find out their views of taking part in research during the coronavirus pandemic. The latter discussions included what processes and reassurances researchers can put in place to make women feel more comfortable taking part in research, and our research project specifically.

Women were recruited using the local network of maternity groups developed during the recruitment for Chapter 6, and through the local Dorset Maternity Matters Facebook page (www.facebook.com/DorsetMaternityVoices). Dorset Maternity Matters is run by the NHS Dorset Clinical Commissioning Group (CCG), which is responsible for the maternity services in Dorset. A promotional poster/leaflet was shared on social media and women contacted the investigator if they were interested in taking part. The only inclusion criterion was that women were currently pregnant or had given birth since April 2020, and therefore had experienced being pregnant during the coronavirus pandemic.

Twelve women contacted the author expressing an interest in taking part in the PPI and five women participated in the consultation. One woman who was booked to attend was unable to join the consultation on the day. Two PPI consultations were set up, one with pregnant women (*n=3)* and one with new mothers (*n=2)* to ensure that all views were represented. Due to the ongoing restrictions related to COVID-19 the consultation was undertaken on Zoom. In the pregnant group, 1 woman was experiencing her first pregnancy, 1 woman had a previous miscarriage, and 1 woman already had a child. The mother with a child had experience of pre-eclampsia during her first pregnancy, but no other women in either group had experiences of high blood pressure previously. The pregnant women were an average of 24 weeks pregnant and 31.7 years old at the time of consultation. The women in the new mother's group both had 1 child each who was an average of 5.5 months old, and the mothers were an average of 27 years old.

Following discussions of the clinical protocol outlined in section 8.2, all the women said they would be interested in taking part in the intervention study if approached to do so. However, this is not unsurprising given the opt-in nature of the consultation, with women choosing to take part and therefore already expressing an interest in this type of research. The new mothers were asked to consider the questions and frame their responses in the context that they would be currently pregnant if they took part in the project.

> *"It's just obviously a good idea if you can avoid taking extra drugs during pregnancy, then it's a good study."* ***Pregnant woman***

All pregnant women and half of the new mothers were more concerned about taking part in research during the coronavirus pandemic and would be more cautious about whether to take part in research, especially where it involved face-to-face interaction.

> *"I know some friends of mine were absolutely terrified, and carry on being terrified. You know, now that they've got their babies. It's just like a different kind of fear….you know with the government we have been told we were vulnerable."* ***New mother***

As the current protocol includes an initial meeting at Bournemouth University, women discussed whether they would be comfortable attending a university campus. There was a split between the new mothers and pregnant women, with new mothers expressing that they would feel comfortable attending a university campus, whereas the pregnant women would not want to attend a university campus during coronavirus restrictions. The concerns around attending campus related mainly to the women having to mix in spaces with undergraduate students (such as main reception areas and toilets) and the possibility of contact tracing and having to isolate from 'checking in' using a QR code system in a university building used by potentially hundreds of students each day. Concerns were specifically related to being around too many people and having to use or walk through busy areas.

> *"I think I'd rather if we have to attend campus, I don't know, earlier in the morning or in the evening. Maybe when there's not loads of students there because obviously we're trying to protect ourselves from, you know, exposures to too many people."* ***Pregnant woman***

Some of the women had been unable to continue working during the coronavirus pandemic due to their pregnancy and underlying conditions. They felt conflicted that if they couldn't attend their workplace then maybe they shouldn't attend a university campus, which could put them at risk. The specific concern was what if they caught COVID-19 during the visit, and how their workplace would view this given the allowances made by their employer for shielding during the pandemic.

> *"It's really weird because normally I would have no worries about it whatsoever, but my work have gone out of the way to help me work from home. It would feel then irresponsible to then go oh yeah well I went to the uni and I caught it [coronavirus] there."* ***Pregnant woman***

Women discussed that they would feel more comfortable attending campus if certain measures were in place. Firstly, to minimise potential contact and mixing with students the suggestion was made to have a separate less busy entrance to enter the room and also to have separate toilets that were not used by all students. This would be achievable in our current set-up as there is a private corridor and separate outside entrance for use by the clinical laboratory. There is also a dedicated toilet facilitates only used by staff and participants who have access to the corridor. Women stressed the importance of including all information regarding cleaning and wearing of personal protective equipment (PPE) in the participant information sheet, even if it seemed obvious that these processes would be followed. Adequate ventilation of the room was also raised as a comforting factor in attending the initial meeting, and extra precautions such as leaving equipment to rest for 48 hours between participants was welcomed as reassuring.

> *"…and sharing what you've got in place [procedures]. Like, I know it sounds really really obvious, but just reminding people I think will add to feeling comfortable coming in [to campus]…the more information you can share the better"* ***New mother***

The current protocol includes the short-term responses to SDB protocol, which requires access to equipment at the university campus. However, future studies may only include an induction session to receive instructions on accessing the SDB intervention and consenting to participate in the trial. Women were therefore asked where else they would feel comfortable to have the research induction session; 80% of women would be happy to have the induction inside their own home, 80% would be happy if it was outside in their own home (garden) and 100% of women would be happy to have the session virtually over Zoom or an equivalent online platform.

In conclusion, although pregnant women are more concerned and wary about taking part in research during the coronavirus pandemic there is still an appetite to participate in research if appropriate considerations and processes are put in place. Specifically, women would feel more comfortable when attending university campus if the time spent in communal areas shared with undergraduate students was minimised, such as a separate entrance rather than the main reception area and using dedicated and separate toilet facilities. Information regarding cleaning procedures and PPE should be made clear in the participant information sheet and should be updated and reviewed regularly in line with current guidelines. Future studies which do not require use of specialist equipment during induction visits should explore moving induction meetings online.

8.5 Summary

In summary, the clinical protocol outlined in this chapter was paused due to COVID-19, but the author has created a plan to re-start the study as a post-doc project following completion of this PhD. An application for an NIHR Advanced Fellowship has been submitting, to support funding of the project and development of the author as a health researcher.

It is unfortunate that the coronavirus pandemic stopped the clinical study being completed as part of this PhD thesis. It would have provided the next step in the research, bridging the gap from the laboratory-based, mechanistic investigations characterising acute responses to SDB, to trialling SDB as intervention in pregnant women, with real-world clinical implications. As a feasibility study it would have provided evidence for acceptance and adherence in a pregnant population, and initial clinical evidence on whether a SDB intervention was worth pursuing as a non-pharmacological treatment for PIH. As it stands, the protocol is designed, ethical approval remains in place and the clinical study is ready to be resumed when additional funding is secured.

Chapter 9. Discussion

9.1 Introduction and overview

This chapter will review the main findings of the thesis and evaluate them in the context of the existing state of knowledge. An interpretation of the findings in relation to their mechanistic potential for reducing chronic blood pressure (BP) is presented alongside the clinical implications of these findings. Finally, the directions for future research based on the new state of knowledge informed by this thesis are outlined.

The unique nature of this thesis is that the research integrates two separate disciplines, applied human physiology and clinical health research in the maternity field. By using an interdisciplinary approach, this thesis provides the physiological evidence to support the development of a clinical health intervention designed specifically for pregnant women.

The aim of this thesis was to characterise and compare the acute cardiovascular responses to slow and deep breathing (SDB) of pregnant women and design a specific SDB intervention for women who develop pregnancy-induced hypertension (PIH). The objectives set to meet the aim of the thesis are summarised below and can be viewed in full for each study in the relevant chapters.

1. Identify the acute response in blood pressure and amplitude of blood pressure oscillations during SDB for healthy young men, healthy non-pregnant women and healthy pregnant women.
2. Characterise and compare the response of mechanism-related parameters (e.g. respiratory sinus arrythmia, stroke volume, cardiac output) to SDB for healthy young men, healthy non-pregnant women and healthy pregnant women.
3. Evaluate differences in acute cardiovascular responses to a range of SDB frequencies for healthy non-pregnant women and healthy pregnant women.
4. Design an evidence based SDB intervention for women with PIH.

9.2 Discussion of the key findings in relation to existing literature

9.2.1 Novel analysis of cardiovascular responses

This study is the first to use the peak-valley method of analysis across all cardiovascular variables to measure the range of complex responses to SDB. The data from all studies presented in this thesis revealed minimal cardiovascular responses to SDB when

examining simple averages of cardiovascular variables, either across the full breath or during individual breath phases (inspiration/expiration). However, using peak-valley analysis to evaluate differences between minimum and maximum values, reflecting the fluctuations caused by SDB, revealed significant increases during inspiration, expiration and between breath phases. SDB increased the amplitude of the respiratory sinus arrythmia (RSA), and of oscillations in stroke volume (SV), cardiac output, systolic blood pressure (SBP), diastolic blood pressure (DBP) and total peripheral resistance (TPR) in men, women and pregnant women. The novel analysis presented in this thesis highlights the importance of measuring more than the mean values for cardiovascular variables, since the mean overlooks the deeper and more complex cardiovascular responses. The true magnitude of perturbations created by SDB are only revealed when analysing peak-valley (Δi, Δe, ΔPV) and breath phase independent peak-valley (ΔPV_Ind) at an inter- and intra-breath phase level. Figure 5-7 (page 116) demonstrates this hidden response for an example participant, by displaying mean heart rate (red series) during Sf_r and 6Ff_r plotted with beat-by-beat heart rate data. In this example, the true heart rate response is masked and averaged out of existence when only mean heart rate is considered. Due to the dynamic nature of blood pressure (BP), Parati et al. (1995) highlighted the importance of examining the fluctuations around the average BP as early as 1995, arguing that it is within these fluctuations that substantial insight is uncovered into the mechanisms of cardiovascular control. However, the peak-valley method used in this thesis has not previously been used.

9.2.2 Amplitude of blood pressure oscillations and respiratory sinus arrythmia

An important example of the perturbations revealed by the novel analysis employed in this thesis is the true amplitude of BP oscillations induced by SDB, which has not been reported previously. Previous studies have only examined average BP during or after SDB, for example Herakova et al. (2017) and Mori et al. (2005). For all participant groups, mean SBP or DBP were not significantly different during SDB compared with normal spontaneous breathing (Sf_r). However, the amplitude of BP oscillations, calculated as the change in BP during breath phase (inspiration (Δi)/expiration (Δe)) relative to mean BP (corresponding mean BP during inspiration/expiration breath phase), was significantly higher during SDB. The range of SBP oscillations observed during SDB was between 8.0 - 13.4% (9.1 - 15.5mmHg) during inspiration (SBPΔi) and 5.7 - 10.4% (6.9 - 12.1mmHg) during expiration (SBPΔe). In comparison, during spontaneous breathing (Sf_r) oscillations were between just 2.9 - 3.0% (3.4 - 3.6mmHg) during inspiration and 3.0 - 3.6% (3.7 - 4.5mmHg) during expiration. For DBP, amplitude of BP oscillations increased during SDB by between 7.3 - 14.3% (5.2 - 10.0mmHg) during inspiration

(DBPΔi) and 7.3 - 14.2% (5.1 - 9.9mmHg) during expiration (DBPΔe), compared with Sf_r BP oscillations of between 2.1 - 3.5% (1.5 - 2.4mmHg) and 3.3 - 4.6% (2.4 - 3.2mmHg) respectively. Overall, SDB led to a roughly three-fold increase in the amplitude of BP oscillations, compared to spontaneous breathing.

Variability within cardiovascular parameters and the body's ability to react to different stressors is a sign of good health (Shaffer and Ginsberg 2017). Reductions in heart rate variability (HRV) are associated with numerous conditions related to autonomic dysfunction (Camm et al. 1996), whilst high HRV is associated with aerobic training (da Silva et al. 2015). As cardiovascular oscillations are attenuated or lost in many diseases, it is therefore reasonable to suggest that re-establishing fluctuations in people who have clinical conditions such as hypertension may provide health benefits (Elstad et al. 2018). The body uses variables such as heart rate to make adjustments to the cardiovascular system in order to maintain homeostasis, an equilibrium and steady state within the body. BP oscillations have been observed to have anti-hypertensive effects in dogs (Nafz et al. 2000), but not to date in humans. However, it follows that a similar effect could be seen in humans, although this has yet to be observed. It was suggested that BP oscillations may result in a change in renal haemodynamics through renal fluid and sodium excretion (Nafz et al. 2000). As the kidneys play a major role in maintaining BP levels, then changes in renal BP management could be a pathway towards long-term BP change. Indeed, renal resistive index (RRI) has been shown to increase following 4-weeks of SDB (Bazzini et al. 2011; Modesti et al. 2015), but no acute changes in RRI were observed in this thesis (Chapter 5).

Fluctuations in BP are potentially linked to cardiorespiratory coupling of respiration, BP and heart rate (Chang et al. 2013; Russo et al. 2017). Fluctuations in BP are normally caused by an internal cardiovascular response to external perturbations. The body uses the autonomic nervous systems to oppose the response to external stimuli, in an attempt to maintain homeostasis. The aim of homeostasis is to adjust BP back to a reference "set point" (Parati et al. 2006) which it considers to be its normal state. Due to the variables that contribute to the production of BP, and therefore effect fluctuations in BP, heart rate and variations in heart rate can influence BP fluctuations directly. To take a step back, BP fluctuations will be caused by either variations in total peripheral resistance (TPR) or cardiac output (Q; see Figure 9-1).

Changes in TPR need more time to develop due to the nature of their reaction time to external stimuli and therefore rapid fluctuations in BP are likely caused by cardiac output. As cardiac output is affected by variations in heart rate and/or stroke volume then either

could influence BP. It is heart rate that has been suggested to be the main driver of BP variability due to the respiration synchronous RSA (Elstad et al. 2001). Alternatively, SV is increased during SDB as a result of increased venous return, caused by the lower intrathoracic pressure during SDB (Harada et al. 2014; Russo et al. 2017). The increase in amplitude of RSA may act as a counteracting measure to oppose respiratory-induced SV changes, to maintain cardiac output. In support of this theory, BP oscillations increase as breathing frequency reduces, to peak at the lowest breathing frequency measured (4 breaths·min^{-1}). Our data show RSA becomes saturated ≤6 breaths·min^{-1} and therefore RSA is unable to act as a buffer for SV induced fluctuations, leading to peak amplitude of BP oscillations at this level. These mechanisms are outlined in more detail below.

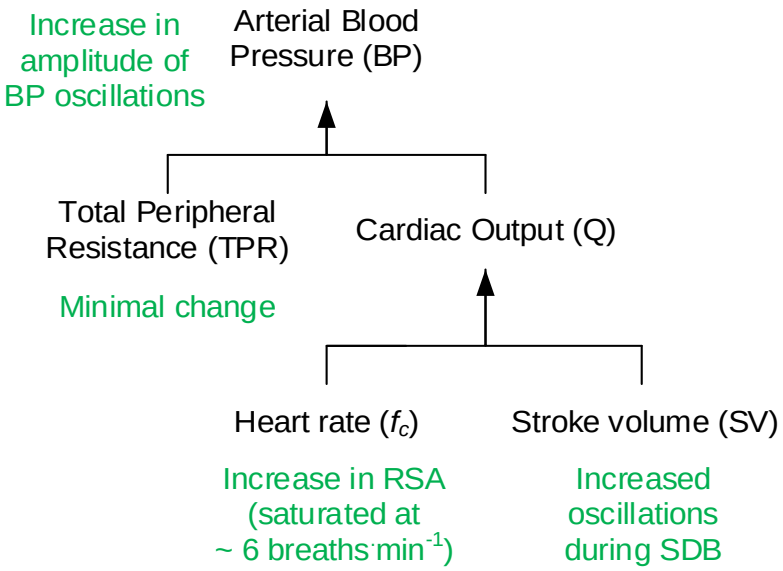


Figure 9-1 Cardiovascular variables contributing to arterial blood pressure during slow and deep breathing
N.B. Figure updated from Figure 2-1 to reflect cardiovascular responses to slow and deep breathing

The amplitude of RSA increased in all studies as breathing frequency declined, showing our population groups responded similarly to participants from previous studies (Anderson et al. 2009; Zhang et al. 2009). Although there was no significant change in mean heart rate, changes in heart rate during inspiration and expiration resulted in an increase in RSA amplitude. There were no significant differences between RSA during the 4Ff_r and 6Ff_r breathing conditions in any participant groups, showing a plateau at 6 breaths·min^{-1} (6Ff_r), and the amplitude of RSA reached saturation point at the lower breathing frequencies of 4 breaths·min^{-1} (4Ff_r). Despite this, peak RSA for 35% of non-pregnant and pregnant women occurred during 4Ff_r, the most common condition for peak RSA to occur.

The physiological function of RSA is still hotly debated, but Elstad et al. (2015) suggest that the main function of RSA is to stabilise arterial BP, by working as a central feed-

forward mechanisms, whereby RSA reduces the mechanical effect respiration has on BP fluctuations. It is still unclear whether heart rate oscillations such as RSA contribute to BP oscillations by generating or by buffering the BP oscillations to produce Mayer waves (Castiglioni and Parati 2011). The results from this thesis suggest that SDB produces a physiological state where RSA is maximised and cannot further regulate BP at breathing frequencies of ~6 breaths·min^{-1}. Central feed-forward mechanisms require a balance between the effect of respiration on venous return to the heart and the neural reflexes that stabilise arterial BP. Venous return increases during inspiration and RSA may act as a buffer against this (Elstad et al. 2015) by creating an inverse relationship during respiration between heart rate and stroke volume (Toska and Eriksen 1993). An inverse relationship also exists between cardiac output and TPR, with a counteracting effect produced to maintain homeostasis for BP, and for cardiac output in the former case. For example, heart rate increases to counteract the decrease in SV during inspiration to maintain cardiac output. However, variations in heart rate (specifically RSA) and subsequently cardiac output do not efficiently buffer BP oscillations (Elstad et al. 2011). The main source of respiratory fluctuations in MAP are as a result of variations in SV, leading to changes in cardiac output (Toska and Eriksen 1993). Elstad et al. (2011) also observed that variations in cardiac output are not sufficient to alleviate MAP oscillations, which it is argued, are predominantly produced by variations in TPR. However, the data from this thesis suggest that, acutely, observed increases in amplitude in both TPR and cardiac output may contribute to the increase in amplitude of BP oscillations. RSA amplifies BP oscillations and through cardiac output is unable to buffer BP oscillations under physiological challenge such as tilt test (Elstad et al. 2011). SDB breathing could be sufficient to present a physiological challenge and therefore produce a state where RSA cannot buffer BP fluctuations.

Overall, the relationship between RSA and BP oscillations may be influenced by the level of mechanical effects that respiration has on arterial BP. The relationship whereby RSA acts as a buffer, may only come into effect when external stimuli inputting into the cardiovascular system are greater than those in supine resting humans (Taylor and Eckberg 1996). The mechanical effects of respiration on stroke volume and cardiac output during SDB are greater than normal breathing in supine humans, and therefore could produce a sufficient stimulus to trigger RSA's role as a buffer to maintain BP.

It is interesting that the amplitude of BP oscillations increased as breathing frequency was reduced, to a maximum amplitude of BP oscillation at 4 breaths·min^{-1}. BP oscillations are calculated as the difference between minimum and maximum values during breath phase (inspiration/expiration) and are therefore dependent on the change in BP during

the breath-phase. As breathing frequency reduces, breath phase duration increases, providing more time for BP to fluctuate within-breath phase and providing a possible explanation for the higher levels of amplitude of BP oscillations at lower breathing frequencies. This can be linked back to the observed RSA saturation point at 6 breaths·min^{-1}, resulting in a plateau of RSA but BP fluctuations increasing and peaking at 4 breaths·min^{-1}. However, there was no significant difference between the SDB conditions <8 breaths·min^{-1} for amplitude of BP oscillations, suggesting minimal differences between cardiovascular responses and potentially no meaningful clinical difference between SDB frequencies when used as part of a long-term intervention, providing the frequency is less than 8 breaths·min^{-1}. SDB frequencies in the literature are defined as <10 breaths·min^{-1}, however these findings suggest that breathing frequencies may need to be lower than < 8 breaths·min^{-1} to elicit the full cardiovascular perturbations. This has implications for the RESPeRATE device specifically, as the average breathing frequency in Chapter 4 for the first 5 minutes was 8.1 breaths·min^{-1} , supporting previously observed average frequencies of 8.4 breaths·min^{-1} (Altena et al. 2009). Consequently, individuals using RESPeRATE may not gain the full benefits from SDB if their breathing frequency remains above 8 breaths·min^{-1}.

Acute responses to SDB immediately change cardiovascular variables and fluctuations during the SDB sessions such as the amplitude BP oscillations and RSA outlined above. The changes to these variables are easy to examine and measure during non-invasive data collection, however, long-term changes in BP caused by SDB are related to more complex factors that change the BP set point (Anderson et al. 2010). A resetting of the autonomic nervous system has been suggested to occur following pranayama breathing (a form of breath control used in yoga) (Keerthi et al. 2013), but the mechanism by which this is achieved has not been understood fully. The findings of this thesis suggest that the increase in amplitude of BP oscillations during SDB may be one of the error signals that contributes to the reset process and may thus offer an explanation for the effectiveness of yoga reducing hypertension. During the development of hypertension the body's control circuits are adjusted to a higher set point (Wallbach and Koziolek 2018), and therefore interventions that can restore this set point to a lower, healthy level could provide benefits and/or alleviation of the condition. BP fluctuations are caused by a cardiovascular response to an external perturbation, in this case the SDB condition which causes cardiorespiratory coupling. To oppose the fluctuations in BP, neural control mechanisms are activated to maintain homeostasis, adjusting BP back to a reference set point (Parati et al. 1995). The body's various set points (e.g., BP, heart rate, blood glucose, arterial PCO_2, etc.) which maintain homeostasis are internally set but can be changed over time. A simple example of this is resting heart rate; following training of

the heart through exercise, the heart becomes more efficient at ejecting blood (stroke volume) and pumps out more blood with each beat. Thus, to maintain the required cardiac output, the heart can pump fewer times per minute to produce the same flow of blood around the body. Consequently, the body resets resting heart rate at a lower level, as the body aims to undertake the least amount of work possible to maintain homeostasis. Using this frame of thinking regarding the body's adaptability, the body may also be able to re-set resting BP at lower levels, after it has experienced the training of daily SDB sessions. During each SDB session the body adapts to the SDB by increasing the amplitude of oscillations, which could lead to a resetting of BP to normotensive levels (see section 9.2.3 for full further discussion of this point). Consequently, the ability of SDB to increase the amplitude of BP oscillations, and therefore increase the fluctuations of acute BP, could be a potential mechanism for the chronic reduction in BP observed following daily practice. However, caution must be used when discussing the relationship of cardiovascular variabilities to measures of autonomic function. The relationship is complex and still relatively undiscovered, leading to much debate in the field and a cautious basis on which to build theories of chronic BP adaptations (Parati et al. 2006).

The frequency of BP oscillations is clinically relevant as it is significantly related to end organ damage during hypertension (Parati et al. 1997), however the amplitude of BP oscillations is still an under-researched topic. BP oscillations are often calculated as standard deviations over 24-hour ambulatory measurements (Parati et al. 1994), not instantaneous BP fluctuations, as occurs during SDB and was measured in this thesis. Therefore, the data presented in the thesis should be carefully compared with previously collected BP variability data.

9.2.3 Potential restoration of autonomic imbalance by slow and deep breathing

Research into resistant hypertension, i.e., high BP that does not respond to traditional pharmacological intervention, has branched into bioelectronic medicine, by using approaches such as invasive vagus nerve stimulation and baroreflex activation therapy (Cracchiolo et al. 2021). Both approaches aim to modulate autonomic nervous system activity using electrical stimulation of different elements of the autonomic nervous system (Lauder et al. 2020). Hypertension is associated with overactivity of the sympathetic nervous system and therefore interventions that can reduce sympathetic activity and increase parasympathetic activity have the potential to reduce BP. When the baroreflex is activated by electronic stimulation (as part of baroreflex activation therapy) increased

vagal tone is observed (Gassler and Bisognano 2014), suggesting an increase in parasympathetic activity.

Following 6 months of baroreceptor activation therapy, using an implanted stimulation device, SBP decreased by 26.0 ± 4.4 mmHg and DBP by 12.4 ± 2.5mmHg (Hoppe et al. 2012). The device is implanted under the skin in the pectoral region and the lead system is tunnelled from there to wrap round the bilateral carotid bulbs in the neck (see Gassler and Bisognano (2014) for detailed description and diagram). After 2 years of use, 50% of patients had reached the target office SBP of <140mmHg, and 58% of participants had decreased the number of antihypertensive medications they were prescribed by at least one, due to confirmed BP levels at or below target levels (Wallbach et al. 2020). Mechanisms associated with baroreflex activation therapy are potentially its ability to reset the operating set-point of the system regulating BP (Gassler and Bisognano 2014). It has been suggested that by providing sustained activation of the baroreceptors, baroreflex activation therapy may reduce long-term BP by chronically suppressing central sympathetic outflow (Iliescu et al. 2014). Activation of the baroreceptors may also restore cardiac rhythmicity by shifting cardiac autonomic balance and improving spontaneous baroreflex sensitivity (Iliescu et al. 2014).

During SDB, baroreflex sensitivity has been observed to increase acutely (Lewis et al. 2018) and in hypertensive patients can return to levels found in normotensive patients (Joseph et al. 2005). The aforementioned oscillations in BP, which are increased during SDB, may provide a similar mechanistic pathway to reduce BP as baroreflex activation therapy. The baroreceptors are responsible for detecting changes in BP and subsequently signal the brain to activate homeostatic mechanisms to buffer beat-to-beat fluctuations in BP. This thesis revealed an increase in beat-to-beat fluctuations in BP during SDB, which would subsequently increase the activation of the baroreceptors due to the increased number and amplitude of BP changes. Consequently, it is plausible that increased amplitude of BP oscillations caused by SDB, which in turn increase activation of the baroreceptors, could provide the same error signal that leads to reduced BP following baroreflex activation therapy.

Baroreflex activation is currently considered an investigational therapy, with a paucity of evidence and therefore is not yet recommended for routine treatment of hypertension (Bhatt et al. 2019; Lauder et al. 2020). Additionally, because of the unapproved nature of the intervention and its use only within limited clinical trials, the recruitment criteria to undertake the therapy excludes women who are pregnant (Wallbach et al. 2020). Consequently, although baroreflex activation therapy has not been tested on PIH, it has

been suggested that baroreflex activation therapy may provide the greatest clinical benefit where hypertension is associated with overactive sympathetic activity (Iliescu et al. 2014), such as in PIH. Therefore, the potential shared mechanisms for baroreflex activation therapy are likely to be applicable to the treatment of PIH, in addition to resistant hypertension. Electromechanical device-based therapies, although promising, are limited by the invasive nature of the intervention (Lauder et al. 2020), and safety concerns due to the surgery required to implant the devices and subsequent related complications. If SDB can produce similar activation of the baroreflex through non-invasive intervention, then SDB has the potential to become a leading non-pharmacological intervention in the treatment of hypertension.

9.2.4 Differences between acute cardiovascular responses to slow and deep breathing in males and females, and pregnant and non-pregnant women

There were no differences in the acute cardiovascular responses to SDB observed between males and females, or between non-pregnant and pregnant women. The data presented in Chapter 4 showed that despite physiological differences between men and women, women's cardiovascular response to SDB was similar to the responses observed in men. Thereafter, an analysis of non-pregnant women showed that despite differences in baseline cardiovascular variables, the response to SDB was not altered fundamentally by pregnancy (Chapter 7).

During pregnancy, RSA values were depressed under all conditions, as observed in Chapter 6. However, SDB was able to increase RSA to levels found during spontaneous breathing in non-pregnant women. During SDB in pregnant women, RSA doubled compared with spontaneous breathing, matching previous observations during relaxation (DiPietro et al. 2008). A reduced RSA is linked with hypertensive disorders of pregnancy (Lakhno 2016) and the future clinical study outlined in Chapter 8 will distinguish whether RSA is further reduced with women who have PIH, compared with healthy pregnancies (Felton et al. 2021). The short-term protocol study will also evaluate whether the RSA response to SDB is maintained in women with PIH and if the amplitude of RSA doubles because of reducing breathing frequency, independent of baseline RSA. The data observed in this thesis suggest no reason why RSA would not increase during SDB in women with PIH, even if baseline RSA is depressed. The response to SDB between non-pregnant and pregnant women was similar, despite reductions in baseline RSA in pregnant women. Therefore, the findings of this thesis support progressing this line of research, as the cardiovascular responses to SDB have been confirmed to be similar during pregnancy compared with responses in non-pregnant women.

Previous research studies investigating chronic effects of SDB on BP have focused on male participants or have not separated data by sex for analysis, such as the majority of studies included in the meta-analysis of SDB by Chaddha and colleagues (2019), who included participants who were predominately men. Although this thesis was not able to perform a long-term SDB intervention due to COVID-19, there is a suggestion from the acute cardiovascular responses studies that there are no differences between men and women. However, due to limited sample sizes between groups there is not enough evidence to provide a definite conclusion on this and larger trials would be needed. Consequently, it is plausible to suggest that long-term cardiovascular changes will be similar in women to those previously observed in men and that this research is worth continuing. Likewise, pregnant women respond in the same way to SDB as non-pregnant women and it is theorised that the chronic adaptations to continued practise of SDB will therefore be similar to the observed BP reductions in non-pregnant populations.

9.2.5 Lack of normative cardiovascular pregnancy data

During the literature review for this thesis a lack of normative cardiovascular and respiratory data during pregnancy was found. As recently as 2019, there was insufficient data on spontaneous breathing frequencies throughout pregnancy to produce normative values (Loerup et al. 2019). Importantly, despite acceptance that BP changes throughout pregnancy depend on gestational age and trimester, the normative values for BP during pregnancy are not categorised into different trimester ranges but use a 'one size fits all' approach across all gestational ages. The idea for normative BP guidelines to be related to gestational age is not new, but has not been widely accepted (Higgins and de Swiet 2001). Accordingly, the NICE Hypertension in Pregnancy guidelines (NICE: National Institute for Health and Care Excellence 2019b) for normal BP levels and ranges of BP to define hypertension are predominately based on non-pregnant adult guidance (NICE: National Institute for Health and Care Excellence 2019a).

The preliminary normative data compiled during this thesis was collected from only 18 participants, and therefore the dataset could not be considered a representative sample of all pregnancies on which to base the development of normative guidelines. However, the data presented in Chapter 6 could provide the start of a database of normative cardiovascular data and shows the ease in which physiologists can collect and analyse these data. Working with midwives, who routinely collect key cardiovascular data during antenatal appointments and on-ward monitoring, presents a unique opportunity to build on the current limited set of data. Currently, teaching textbooks for student midwives (Coad et al. 2020; Rankin 2020) do not cite peer-reviewed references for their normative

data or the source of the data is unclear. Producing a bank of normative data would support future research, especially when investigating maternal health conditions during pregnancy. A lack of comparative data for normal pregnancies is an issue when researchers want to investigate differences between healthy pregnancies and those affected by cardiovascular disease. Without a baseline comparison, researchers cannot be sure whether observed changes in the cardiovascular system are due to the pregnancy itself or the condition in question.

Specifically for PIH research, women who develop PIH later in pregnancy exhibit higher than average BP (but not yet at hypertensive levels) early in their pregnancy compared with women who remain normotensive throughout pregnancy (Higgins and de Swiet 2001). Only by knowing what 'normal' BP values should be, across trimesters and different gestational ages, can differences be discerned for hypertensive disorders of pregnancy. Observed differences during routine antenatal appointments could lead to further investigation with additional clinical screening to assess risk of PIH. If women can be identified earlier as high risk for developing hypertension during pregnancy, then extra measures could be put in place, including the potential to use SDB as a preventative measure, which will be discussed later in section 9.4.

An example of the clinical implications of not having access to normative data is the obstetric early warning systems, which are widely used in the UK but with varied consistency across maternity units. Sub-optimal care and detection of physiological problems occur when there is a lack of uniformity across the early warning scales, and the important physiological parameters are not agreed upon (Isaacs et al. 2019). Obstetric early warning systems have been found to use a large range of normal vital signs with significant variations across different NHS Trusts and maternity units, which causes uncertainty regarding thresholds and escalation leading to a discrepancy of practice between units in the UK (Smith et al. 2017). The early warning systems are designed to reduce maternal morbidity and mortality, but without agreed upon normative values they cannot be effective.

9.2.6 Implications of findings for optimal breathing frequency and methods of implementation of clinical SDB interventions

RESPeRATE is the main device used to guide SDB in the literature and is recommended by the American Heart Association as an alternative treatment for high BP (Brook et al. 2013). Chapter 4 compared RESPeRATE with a fixed breathing frequency of 6 breaths·min^{-1} (6Ff_r) and a novel, bespoke algorithm (Df_r) driven by RSA maximisation.

When comparing the final 5 minutes of each breathing condition, there was no significant difference in cardiovascular responses to SDB between any SDB conditions, but there was also no significant difference in breathing frequency. The studies reported in Chapters 5 and 6 revealed that the increase in cardiovascular perturbations predominately occurred at breathing frequencies less than 8 breaths·min^{-1}, with the 8Ff_r condition not producing a significantly different response from spontaneous normal breathing (Sf_r). Consequently, it seems that the important element to produce maximum perturbation of the cardiovascular system is that the breathing frequency is < 8 breaths·min^{-1}, but that the method used to achieve the breathing frequency is unimportant (fixed or dynamic frequency). It is reasonable to suggest these alternative approaches to implementing SDB (4Ff_r, 6Ff_r, Df_r) may produce the same long-term health benefits as RESPeRATE, given that they all produce the same acute responses and therefore most likely the same error signal(s) that produce the chronic reduction in BP. As for subjective preference, pregnant women found 6Ff_r the most comfortable condition to undertake and would choose this breathing frequency to use in a daily intervention if given the choice.

Research on the dose-response relationship of SDB has not been undertaken systematically, either for acute or chronic SDB interventions, but it is important to understand if differences exist in cardiovascular responses to different doses of SDB (Sica 2011). The observed data (Chapter 7) for both amplitude of BP oscillations and RSA show differences only during breathing frequencies below 8 breaths·min^{-1}, suggesting that not all SBD frequencies are equal in their effect. The literature defines SDB as a breathing at a rate less than 10 breaths·min^{-1} and indeed RESPeRATE's therapeutic breathing zone is set at 10 breaths·min^{-1} to match this. However, this could be sub-optimal, given that the results from this thesis reveal that not all SDB conditions below 10 breaths·min^{-1} produce the same acute cardiovascular response. The data revealed that cardiovascular responses to SDB at 8 breaths·min^{-1} are on the whole not significantly different from spontaneous normal breathing (Sf_r). In Chapter 4, during the first 5 minutes of data collection breathing frequency during RESPeRATE (Rf_r) was 8.1 breaths·min^{-1} and consequently the full impact of SDB could be lost during the first 5 minutes of using RESPeRATE. This is reflected in the significantly lower amplitude of BP oscillations detected during the first 5-minutes of Rf_r compared with the final 5-minutes.

Consequently, although the RESPeRATE device is currently recommended by the American Heart Associate as a treatment method for high BP, this thesis has outlined potential limitations to the individualised breathing frequency generated by

RESPeRATE. RESPeRATE recommends 10 minutes of SDB a day in the therapeutic breathing zone, however as the results from this thesis show it can take time for breathing frequency to be reduced to satisfactory levels. Sica (2011) recommends that RESPeRATE should be used for 15 minutes a day, which would allow the first 5 minutes to be above optimal breathing frequency, while still providing 10 minutes of SDB frequency <8 breaths·min^{-1}. In previous studies using 15 minutes SDB duration, a breathing frequency of <10 breaths·min^{-1} was only achieved for an average 11.5 minutes (±1 minute) of the total session (Anderson et al. 2009). Although using a longer total duration solves the problem of optimal breathing frequency being met for a sufficient time, the duration of the full intervention is increased compared with an intervention where the SDB could be delivered fully within 10-minutes of SDB at a breathing frequency <8 breaths·min^{-1}. Alternative delivery methods suggested in this thesis such as the fixed breathing frequency of 6 breaths·min^{-1} provide SDB from the very first breath and reduce additional burdens on participants by limiting the intervention time to 10-minutes. While 5-minutes of time saved daily does not seem a lot, this adds up to 35 minutes each week and 2 hours 35 minutes across a month, which could be saved by utilising a more effective SDB treatment method. Adherence to interventions is a problem for all research, and even over a period of just 1 week of SDB full adherence was not achieved with 9% of sessions not completed (Cheng et al. 2019). Using the example of cardiac rehab programs, which require behavioural change modifications to participate in the intervention, barriers to adherence include time limitations and needing to see benefits from the time participants do input (Daly et al. 2002). Consequently, by reducing duration of the SDB session and therefore required time input from participants, adherence could potentially be increased. Although fixed breathing frequencies of 6 breaths·min^{-1} have not been trialled for long-term responses, the acute cardiovascular responses were similar to RESPeRATE and therefore it is logical the long-term responses would also be similar.

The novel, bespoke algorithm used in the dynamic breathing frequency condition (Df_r) was designed to maximise cardiovascular perturbations using RSA as the controlled variable. However, average RSA for non-pregnant and pregnant women was the same for all SDB conditions <8 breaths·min^{-1}. Additionally, when analysing the condition in which individual peak RSA occurred, Df_r accounted for only 28% of participants across the two studies (Chapters 5 and 6) in which a range of frequencies were used (35% = 4Ff_r. 28% = 6Ff_r. 28% = Df_r. 10% = 8Ff_r). This analysis shows that the dynamic algorithm could not maximise RSA beyond the increases in amplitude caused by other SDB conditions. An explanation may be that the average breathing frequency during Df_r (non-pregnant women = 6.3 breaths·min^{-1} and pregnant women = 7.0 breaths·min^{-1}) was not

significantly different from the breathing frequency during the 6Ff_r condition and therefore RSA was saturated and unable to be maximised further. Therefore, this thesis found no added benefit of personalising breathing frequency during SDB and it is not recommended to continue with this condition for use in long-term SDB interventions. This will also reduce the cost of the intervention, as the finger sensor is only needed to dynamically change breathing frequency. The fixed breathing frequencies (such as 6Ff_r) can be used without the finger sensor and associated cost (~£45).

Additionally, the Df_r condition was not preferred more than other breathing conditions by pregnant women, so personalisation does not affect perceived effort or levels of comfort when undertaking SDB during pregnancy. In fact, breathing frequency during Df_r was higher in pregnant women than non-pregnant women and 2 pregnant participants had an average breathing frequency >8 breaths·min^{-1}. As outlined above, this level of SDB may not produce the desired beneficial effect to its maximum potential and therefore if the Df_r condition had been used as the SDB intervention in the pregnant cohort of participants for long-term daily use, it may not have produced the desired outcomes in some participants who did not achieve a breathing frequency <8 breaths·min^{-1}.

There was no correlation between preferred breathing frequency and gestational age for pregnant women, and therefore it is suggested that SDB frequencies as low as 4 breaths·min^{-1} are comfortable for pregnant women during all trimesters of pregnancy. Consequently, based on the cardiovascular responses observed in this thesis, the breathing frequencies suggested for use as part of SDB interventions in pregnant women should be either the 4Ff_r or 6Ff_r conditions. As preference from the majority of women was 6Ff_r it is recommended that 6 breaths·min^{-1} is the optimal SDB intervention to be trialled in pregnant women.

9.2.7 Designing an evidence-based slow and deep breathing intervention for pregnant women who develop pregnancy-induced hypertension

The evidence produced by this thesis has provided a solid foundation on which to build a specifically designed SDB intervention for pregnant women who have developed pregnancy-induced hypertension (PIH). Importantly, the data from this thesis has explored a variety of breathing frequencies, which could be used as SDB interventions in pregnant women. All conditions used in Chapter 6 (4, 6 and 8 breaths·min^{-1} and the dynamic algorithm) were well tolerated in pregnant women across gestational ages and the full 5 minutes were completed for all conditions by every participant. A combination of both the cardiovascular responses to SDB and the subjective data from pregnant

women suggest that 6 breaths·min^{-1} is the optimal breathing frequency to trial in pregnant women. Although such an intervention has yet to be trialled in pregnant women, the PPI work conducted as part of this thesis suggest women would value such an intervention, and showed interest in the video graphic delivering SDB at 6 breaths·min^{-1}.

Unfortunately, due to COVID-19 the designed protocol for piloting SDB as a treatment method for PIH was paused. However, the protocol has been peer-reviewed and published in the *Hypertension in Pregnancy* journal in 2021. A National Institute for Health Research (NIHR) Advanced Fellowship application has been submitted, to support the re-starting of this study and continuation of the research project.

9.3 Strengths and limitations of the research

Strengths of the thesis

The biggest strength of this research is that it bridges the gap between physiological and clinical maternal health research. The interdisciplinary approach links two disciplines, which are both similar but very independent and distinct. The research provides an opportunity to translate the physiological laboratory research, aimed at understanding the physiological mechanisms behind clinical changes, into an evidence-based clinical intervention. The author's experiences of conducting the research presented in this thesis has further underpinned the need for interdisciplinary approaches in maternal health research. While applied physiology research publishes a wide range of normative data related to a varied range of sports and physical activities, including even those new and niche exercises such as stand up paddleboarding (Schram et al. 2016), there is a lack of normative cardiovascular data in maternal health. While there is no doubt that these data exist, they are currently not being collated, analysed or made accessible. Current midwifery textbooks and teachings to student midwives rely on observations and 'what is generally accepted' rather than published data (Coad et al. 2020; Rankin 2020). Loerup et al. (2019) could not find enough data to perform a meta-analysis on normal breathing frequencies during pregnancy, and were therefore unable to conclude whether breathing frequencies differ from those of non-pregnant women. Using an interdisciplinary approach and building on the standard practice from the physiological research field of collecting and publishing normative data, the results from Chapter 6 could form the start of a database of normative data. Sometimes developments such as this can only be made when looking at a discipline from a fresh perspective.

Using the novel peak-valley analysis outlined in section 9.2.1, has allowed the data collected as part of this thesis to be analysed at a deeper and more complex level. The starting point for the analytical approach was to take a hypothesis-driven, mechanistic approach to interrogating the data. In other words, based upon an understanding of how the cardiovascular system is regulated, the analyses sought to identify the 'error signals' for this regulation, which are generated by SDB. This has revealed acute cardiovascular responses previously not observed in the research published to date. Furthermore, combining the novel analysis methods and an interdisciplinary approach, permitted the research to take an evidence driven approach to the design of a SDB intervention for pregnant women. Additionally, the use of patient and public involvement (PPI) in the designing of the clinical study protocol supports a women-centred model of care, and ensures the research is tailored to the outcomes that matter to women. The evidence that will be produced from the trial, when completed, will add further evidence to the normative cardiovascular data of women who develop PIH, which would be unlikely to be produced and published without taking this approach.

Limitations of the thesis

Unfortunately, due to COVID-19 and the pausing of all non-COVID studies in the NHS (National Institute for Health Research (NIHR) 2020), an inevitable limitation of this thesis is that the planned clinical study (Felton et al. 2021), presented in Chapter 8, could not be undertaken. Consequently, it is unknown whether SDB can reduce BP in pregnant women who develop PIH, or whether it is an acceptable potential treatment method for pregnant women. The PPI undertaken indicated that pregnant women were more reluctant to take part in research during the coronavirus pandemic (section 8.4), including not wanting to visit a university campus for an induction meeting. This meant that the protocol was not feasible to re-start as part of the PhD. However, both initial and recent PPI work conducted during the thesis suggests there is an interest in non-pharmacological methods to treat BP and that women would be interested in taking part in the SDB intervention, supporting the need and desire for a study such as this. The PPI has also provided guidance on the processes, support and reassurances needed to re-start the project in a world where coronavirus will undoubtably cause additional worries and restrictions for at least the immediate future. This guidance on re-starting research, directly from pregnant women and new mothers, will support the re-starting of the trial in the future.

Unsurprisingly recruitment for Chapter 6 was most successful when the researcher was able to attend face-to-face events and build a rapport with the pregnant women as

potential participants. Community recruitment was decided upon as a pragmatic approach, rather than recruiting through the local maternity unit, which would require NHS ethical approval. Although social media has been recommended as a means of recruiting women in pregnancy, and although it led to a wide reach of engagements with the research project, less than half of the women who made contact following a Facebook promotion went on to participate in the study. This mirrors previous studies with pregnant women, where only 18% of women who clicked through from a Facebook advertisement consented to participate (Arcia 2013). Therefore, although social media is a good way to promote information, the experiences from recruitment for this thesis suggest that for recruitment purposes face-to-face meetings are still preferable. This is in accordance with methods to increase recruitment for pregnancy trials including building trust by increasing the visibility of the research team (Strömmer et al. 2018). If social media is to be used then Facebook and Instagram, as opposed to Twitter, are the platforms in which pregnant women and new mothers are most active (based on the authors experience of engagement with women and where local maternity groups can be found on social media). Local maternity groups are exclusively on Facebook and while larger organisations have a following on both Facebook and Twitter, engagement and activity is much higher on Facebook. Recruiting women during pregnancy is always a difficult task; up to 71% of women may decline to participate with 40% of those women not providing a reason (van Delft et al. 2013).

An evaluation of the methods utilised in this thesis is required to objectively evaluate the protocols used. Cardiovascular variables by their nature are dynamic and fluctuate both within and between days. There are many external stressors and factors that can influence variables such as caffeine consumption causing an immediate and sustained increase in heart rate and BP. The data for this study were collected in a one-off session and consequently variability between days was not assessed. However, pre-session requirements such as avoiding exercise, caffeine and fasting were used to minimise external influences. Additionally, Elstad (2012) found no difference in heart rate, stroke volume or cardiac output variations between 2 experimental days during spontaneous breathing. As the participants in this study acted as their own controls due to the randomised crossover design, the study design reduces any potential problems with day-to-day variability, as all comparisons are within-participants on the same day.

Additionally, all equipment utilised in this thesis used indirect, non-invasive measures. Consequently, all cardiovascular variables are estimates and calculated values of haemodynamic responses. The Modelflow method used by the Finapres to estimate SV, produces measurements that show excellent agreement with SV measured by Doppler

ultrasound (Van Lieshout et al. 2003) and when blood is withdrawn by phlebotomy (Leonetti et al. 2004). Additionally, BP measures from the Finapres correlate highly with auscultatory BP measurements in normotensive participants (Carlson et al. 2019), but an acknowledgement of the use of indirect measurement in this thesis is required. Additionally, the Finapres has not been fully validated for use in pregnancy, and research shows it may overestimate SBP and underestimate DBP (Hehenkamp et al. 2002; Grindheim et al. 2012). Although individually within each chapter the participants act as their own controls, and therefore consistent over/under-estimations would not cause a problem, this thesis compares healthy non-pregnant women to healthy pregnant women. If the Finapres and associated calculations are not valid during pregnancy and create a bias and difference compared with non-pregnant women, then comparisons between groups should be made with caution.

End-tidal CO_2 was only measured in Chapter 4 and although no significant differences were found between SDB and spontaneous breathing conditions, this was measured in healthy male and non-pregnant female participants. However, during pregnancy PCO_2 increases as a result of an increase in ventilation (Weissgerber and Wolfe 2006), which may influence the response to SDB. Consequently, future studies exploring acute responses to SDB in pregnant women specifically should monitor end-tidal CO_2 to evaluate whether baseline changes in PCO_2 during pregnancy influence end-tidal CO_2 response to SDB.

More as a word of caution, than a direct limitation, the relationships stated between the phenomena observed in this study and underlying physiology associated with the autonomic nervous system are hotly debated topics. Moreover, a point-counterpoint on whether cardiovascular variability is/is not an index of autonomic control of circulation discussed this in detail (Parati et al. 2006). Within this series of articles Taylor and Studinger argued that the quantification of any variability is only truly a measurement of the resulting phenomenon, and not necessarily an analysis of the complex underlying interactions. Although the cardiovascular variability observed in this thesis suggests a degree of autonomic cardiovascular regulation, it does not suggest they can be substituted as a measure of that regulation. The variability measured in this study represents the end response to the complex interactions between sympathetic and parasympathetic activity and should be analysed with caution. However, there is a degree of physiological interpretation that can be applied to relate acute responses to potential clinical applications in the use of SDB to reduce BP, which has been conducted as part of this thesis.

Finally, as it has been argued that menstrual phase and use of oral contraception can influence cardiovascular responses (Minson et al. 2000b, 2000a) and as this was not controlled in any studies that form part of this thesis, this could be considered a limitation. Previous research has recommended that when examining cardiovascular function, data collection with women should be completed during the early follicular phase of the menstrual cycle, or placebo phase of oral contraception use (Minson et al. 2000a; Wallin et al. 2010). However, previous SDB research found no change in cardiovascular responses to SDB related to menstrual cycle or oral contraceptive use (Nili et al. 2017). Accordingly, Chapter 4 did not control for, or measure menstrual phase and/or contraceptive phase in the 6 female participants, and it must be acknowledged that it is unknown whether the cardiovascular responses of the women in this study (Chapter 4) were influenced by these female sex hormones. However, the menstrual phase and oral contraceptive use of the participants in the study reported in Chapter 5 were analysed. No significant difference was found between menstrual phase or contraceptive use and cardiovascular responses to SDB, or between cardiovascular variables at baseline. Therefore, it is reasonable to suggest that the responses of the female participants in the study reported in Chapter 4 were similarly unaffected by menstrual phase or contraceptive use.

9.4 Conclusion and contribution to knowledge

In conclusion, this thesis has contributed extensive new knowledge to the understanding of acute cardiovascular responses to slow and deep breathing, specifically in relation to the increase in amplitude of BP oscillations and their relationship to increased levels of RSA. The data presented in this thesis show the importance of using peak-valley methods of analysis to reveal the true magnitude of the cardiovascular perturbations caused by SDB, which are otherwise overlooked when only mean values of cardiovascular variables are analysed. The thesis also identified an important influence of differences in the kinetics of haemodynamic responses to SDB, which influence the timing of the breath phases, relative to the induced perturbations.

The body's internal monitoring systems, which aim to maintain homeostasis may be given a nudge by SDB to re-set BP at normal levels following daily practice of SDB, and daily exposure to the acute cardiovascular fluctuations. This could be linked to repeated stimulation of the baroreceptors, mirroring mechanisms observed following baroreflex activation therapy. The acute cardiovascular responses were present in all population group studies (men, healthy non-pregnant women, and healthy pregnant women) suggesting that the previous long-term reductions in BP observed in men and mixed-sex

participant groups following SDB, should also be observed in pregnant women. Based on the data analysed, 6 breaths·min^{-1} is recommended as the optimal breathing frequency to use for SDB interventions, as it matches the existing recommended device (RESPeRATE) in terms of acute cardiovascular responses, and may provide a longer exposure to the stimulus provided by cardiovascular perturbations, with a longer duration spent at the most perturbing SDB frequencies (<8 breaths·min^{-1}).

The results from the studies examining acute responses provides evidence to support continuation of this line of research and the development of a SDB intervention designed specifically for pregnant women who develop hypertension during pregnancy (PIH). The thesis shows the importance of undertaking interdisciplinary research to draw on the strengths of both fields. A clinical protocol for the intervention study has been developed, peer-reviewed and published. When completed, this research has the potential to provide an easy to use, inexpensive, intervention that could save lives, improve the health and experiences of women during pregnancy and have long-term consequences for reducing cardiovascular risk later in life.

9.5 Directions for future research

The first direction for future research is to undertake the clinical study outlined in Felton et al. (2021) (presented in Chapter 8). As part of the short-term responses protocol, there is a need to identify any differences in the acute cardiovascular responses to SDB for women with PIH, compared with normotensive pregnant women. Comparisons are important to identify responses that are not associated with the normal adaptations associated with pregnancy and which could be related to the underlying pathophysiology of the cardiovascular disease. This will provide further evidence of the mechanisms by which SDB may reduce long-term BP and allow interventions to be designed around the specific responses of women with PIH. By publishing and analysing cardiovascular variables, which may differ in women with PIH, the data may also reveal potential pathophysiological differences that underpin the development of PIH.

The feasibility section of the clinical research study would be the first step in assessing whether SDB is accepted as a potential treatment method for PIH. Initial PPI supports women's interest in participating in the trial, but recruitment and adherence rates from the completed trial will provide direct evidence of its acceptance. By providing women with a non-pharmacological treatment method, the intervention provides them with greater choice, and greater control over their own pregnancy and maternity care. If a SDB intervention is acceptable to women, then not only might the intervention improve their experiences of pregnancy and their present and future health status, it might reduce the burden on overworked maternity units. In present times during the global pandemic, both underlying health status and demand on healthcare settings are of the upmost importance. As the SDB intervention is managed independently by the women at home, and involves an element of self-monitoring of BP, it provides an easy to prescribe and undertake intervention that could result in less face-to-face contact and attendance at hospital by pregnant women. Furthermore, as the SDB intervention proposed in the protocol is a relatively inexpensive method of delivery it could easily be transferred for clinical use in low- and middle-income countries.

There is much debate on the treatment of mild to moderate hypertension during pregnancy and how or if this should be treated at all (Moser et al. 2012). The decision to treat normally rests on the risk benefit ratio of hypertensive medication. However, as there are no known negative effects of SDB, the risk is low. Consequently, SDB could offer a non-pharmacological treatment method for women with mild levels of hypertension during pregnancy, at a time when clinicians are reluctant to prescribe medication and/or the women is reluctant to take it. Additionally, despite all hypertension

guidelines stating definitive boundaries which define hypertension, it has been suggested that BP should be seen as a continuum with no clear boundary between normal and the point where the health risk becomes unacceptably high (Haase et al. 2019). NHS online guidance to the public (NHS 2019) also suggests that anyone with BP levels above normal, but lower than hypertensive levels (between 120/80 mmHg and 140/90 mmHg), could benefit from lifestyle changes such as changes in diet and exercise. SDB would be classed as an adjunctive therapy, similar to exercise, and therefore this opens up the possibility that SDB could be beneficial at levels of BP lower than the currently accepted definitions of hypertension.

The potential origins of the development of PIH specifically can be seen during the first trimester, prior to diagnosis, as sympathetic overactivity (Pal et al. 2011). Additionally, women classed as pre-hypertensive prior to 20 weeks gestation (120-139 mmHg SBP or 80-89 mmHg DBP) are associated with preterm and small-for-gestation age infants (Nagao et al. 2021), suggesting a health risk for pregnancies and adverse perinatal outcomes in pregnancies classified as being within normal BP ranges. If SDB can be prescribed to women at-risk of developing PIH or at prehypertensive levels, to maintain autonomic balance early in pregnancy, then there is potential that the sympathetic activity could be stopped before it develops into PIH and decrease adverse perinatal outcomes such as still births and neonatal deaths (Ananth and Basso 2010).

Furthermore, although it is outside the scope of this thesis, it should be noted that the acceptable levels of BP that define hypertension have recently been reduced by the American College of Cardiology and American Heart Association (Whelton et al. 2018). The definition of hypertension has now changed from the long-standing threshold of 140/90 mmHg to 130/80mmHg, which increased the prevalence of hypertension by 14% (from 32 to 46%) in the United States of America (Anaheim 2018). Other countries have since assessed whether they should also change their guidance on BP thresholds, and an article reviewing the guidelines in Canada calculated that using the thresholds recommended by the American Heart Association would nearly double the cases of hypertension in Canada and should be considered carefully as most of the individuals re-classified as hypertensive were young and at low to moderate cardiovascular risk (Garies et al. 2019). A recent review questioned whether the strength of evidence was sufficient to support the new BP thresholds and debated the problems with inconsistency of guidelines worldwide (Kaul 2020).

Although the UK and NICE guidelines have not yet changed their hypertension threshold in the general population, or for pregnancy, the new threshold could be implemented in

the future if stronger evidence supports such a move. In fact, in 2017 NICE published an alert which shared the results of a systematic review reviewing BP targets in a total of 55,163 patients (NICE: National Institute for Health Research 2017). They concluded that a target of <130mmHg for SBP was optimal, to have the best balance of efficacy and safety. If BP is viewed as a continuum rather than a boundary for hypertension and healthy/unhealthy levels, then SDB could be beneficial for anyone at the higher end of this spectrum, including pregnant women who have not yet been diagnosed with hypertension but have BP higher than 130/80mmHg. The changing thresholds for diagnosis of hypertension add weight to the need for more normative data on BP during pregnancy. Normative BP data is needed specifically for gestational ages to allow accurate assessment of hypertensive risk in pregnant women. Whichever guidelines and thresholds are used, the importance of reinforcing lifestyle modification is agreed unanimously, as the preferred intervention, rather than simply writing prescriptions for more BP medications (Bakris et al. 2019).

Additionally, women's long term BP, measured postpartum, may be classed as 'normal' in women who have previously been diagnosed with PIH when in fact it is higher than it was prior to pregnancy and experiencing PIH (Davis et al. 2016). Therefore, the women's BP would not be classed as normal for their individual level of baseline BP pre-pregnancy, although using BP thresholds in the current guidance they would not be monitored. Research is currently underway to generate data sets of normal values for BP post-partum in women who were normotensive during pregnancy, to compare against those who had PIH and pre-eclampsia (Davis et al. 2016). SDB could be a valuable intervention in these women. If cardiovascular abnormalities remain following a pregnancy affected by PIH, then SDB may be able to utilise the potential mechanisms outlined earlier in this chapter (9.2.2 and 9.2.3) to return the body's set-point to normal levels, replicating their pre-pregnancy normal levels.

Furthermore, women who have experienced PIH are also at a higher risk of subsequent diagnosis of high BP or pre-eclampsia during pregnancy (NICE: National Institute for Health and Care Excellence 2019b), developing primary hypertension (Stuart et al. 2018) and heart failure later in life (Chen et al. 2018). Women diagnosed with pre-eclampsia have an estimated doubling of risk odds for increased risk of future cardiovascular or cerebrovascular events (Brown et al. 2013). Therefore, as well as the potential for SDB to remove this future risk by minimising effects of hypertension during pregnancy, or stopping its development entirely, SDB could be also used postpartum. SDB may be able to help stop the development of subsequent episodes of hypertension or other cardiovascular disease, whether that is primary hypertension later in life, subsequent

PIH or pre-eclampsia in subsequent pregnancies. In this case, research could investigate whether daily use of SDB is beneficial for women who previously experienced a hypertensive pregnancy, and whether rates of future hypertensive pregnancies and cardiovascular disease are reduced in women who undertake SDB.

As a starting point this thesis has focused on women with PIH as a population group to provide proof of concept for SDB reducing BP during pregnancy. If results from the clinical study (Felton et al. 2021) presented in Chapter 8) reveal beneficial effects, the intervention could be expanded to include pregnant women with chronic hypertension and pre-eclampsia. Although pre-eclampsia is a multi-system disease, SDB has the potential to reduce the hypertensive element of pre-eclampsia and due its potential re-setting of the autonomic nervous system and/or normal BP level SDB has the potential to benefit other elements of the condition.

Based on the findings presented in Chapter 4, regarding the acute cardiovascular responses to RESPeRATE, this thesis also provided generic recommendations for SDB research, i.e. not limited to hypertension during pregnancy. RESPeRATE may not produce low enough breathing frequencies in all individuals to maximise the cardiovascular perturbations caused by SDB. The duration RESPeRATE is used for, and specifically the average breathing frequency across this duration should be carefully considered when designing future interventions utilising SDB.

When using the RESPeRATE device, Gavish (2010) calculated that for significant long-term BP changes to occur a threshold of 180 minutes total SDB time was needed across the intervention period. With an average 8-week intervention in the previous studies, this equates to an average of only 22.5 minutes per week, showing that lower levels of engagement can potentially still produce health benefits compared with the recommended engagement with SDB. On the other hand, a comparison of SDB at 6 breaths·min^{-1} found a similar heart rate variability response across different durations of SDB (5-, 7- and 9-min), but only a reduction in depression score following daily practice for 9-min (Cheng et al. 2019). The authors suggest that SDB duration may impact the shift in autonomic system activation and that longer durations are needed to produce a shift to parasympathetic activation. There is not currently enough evidence to suggest whether the same long-term benefits can be produced by different durations of SDB, but future research should examine this further. Future research should examine long-term BP changes in the context of the specific breathing frequencies experienced by the participants, and the total exposure time to the SDB frequencies that maximised cardiovascular perturbation. The present research suggests this should be examined at

both <10 breaths·min^{-1} and <8 breaths·min^{-1} to explore any differences in long-term BP reductions based on different breathing frequencies.

Finally, maternal health research cannot neglect the impact of any intervention upon the fetus. It is not known whether the maternal cardiovascular response to SDB is mirrored by changes in fetal heart rate. However, fetal heart rate has been observed to increase alongside maternal heart rate increases during aerobic exercise (Hegewald and Crapo 2011), suggesting a possibility of a fetal cardiovascular response to SDB. Synchronisation of maternal and fetal heart rate has not been shown, although occasional coupling does occur in normal breathing conditions (Van Leeuwen et al. 2003), with increasing synchronisation at higher breathing frequencies (Van Leeuwen et al. 2009). SDB at a frequency of 7.5 breaths·min^{-1} has been shown to decrease fetal heart rate (Vasundhara et al. 2018), but feto-placental circulation and fetal cardiac function are not significantly affected by breathing conditions such as obstructive sleep apnoea (Robertson et al. 2020). Consequently, it is unknown whether fetal heart rate and/or placental flow would be influenced by breathing at the frequencies recommended in this thesis. Although the author did not set out to measure fetal movements, anecdotally there were multiple occasions during the data collection when participants indicated that the fetus had become noticeably more active during the episodes of SDB. Nonetheless, there is no empirical evidence to support that increased movement was linked to the SDB. Increased fetal movement could be due to maternal relaxation, the long period of sitting or simply coincidence, which was noticed by the author. Future studies should include fetal ECG or other monitoring devices to observe how the fetus reacts to SDB and investigate synchronisation between fetal and maternal responses to SDB.

References

Aalami, M., Jafarnejad, F. and ModarresGharavi, M., 2016. The effects of progressive muscular relaxation and breathing control technique on blood pressure during pregnancy. *Iranian Journal of Nursing and Midwifery Research*, 21 (3), 331-336.

Abbas, A. E., Lester, S. J. and Connolly, H., 2005. Pregnancy and the cardiovascular system. *International Journal of Cardiology*, 98 (2), 179-189.

Adams, J., Lui, C. W., Sibbritt, D., Broom, A., Wardle, J., Homer, C. and Beck, S., 2009. Women's use of complementary and alternative medicine during pregnancy: a critical review of the literature. *Birth*, 36 (3), 237-245.

Adler, T. E., Coovadia, Y., Cirone, D., Khemakhem, M. L. and Usselman, C. W., 2019. Device-guided slow breathing reduces blood pressure and sympathetic activity in young normotensive individuals of both sexes. *Journal of Applied Physiology*, 127 (4), 1042-1049.

Altena, M. R., Kleefstra, N., Logtenberg, S. J., Groenier, K. H., Houweling, S. T. and Bilo, H. J., 2009. Effect of device-guided breathing exercises on blood pressure in patients with hypertension: a randomized controlled trial. *Blood Pressure*, 18 (5), 273-279.

Anaheim, C. A., 2018. Hypertension Guideline Lowers Threshold to 130/80 mm Hg. A new guideline for preventing, detecting, evaluating, and managing adult hypertension includes more than 100 recommendations regarding blood pressure in American medical practice. *Neurology Reviews,*, 26 (2), 34-35.

Ananth, C. V. and Basso, O., 2010. Impact of pregnancy-induced hypertension on stillbirth and neonatal mortality in first and higher order births: a population-based study. *Epidemiology (Cambridge, Mass.)*, 21 (1), 118-123.

Anderson, D. E., McNeely, J. D. and Windham, B. G., 2009. Device-guided slow-breathing effects on end-tidal CO(2) and heart-rate variability. *Psychology, Health & Medicine*, 14 (6), 667-679.

Anderson, D. E., McNeely, J. D. and Windham, B. G., 2010. Regular slow-breathing exercise effects on blood pressure and breathing patterns at rest. *Journal of Human Hypertension*, 24 (12), 807.

Arcia, A., 2013. Facebook Advertisements for Inexpensive Participant Recruitment Among Women in Early Pregnancy. *Health Education & Behavior*, 41 (3), 237-241.

Bakris, G., Ali, W. and Parati, G., 2019. ACC/AHA Versus ESC/ESH on Hypertension Guidelines: JACC Guideline Comparison. *Journal of the American College of Cardiology*, 73 (23), 3018-3026.

Band, R., Hinton, L., Tucker, K. L., Chappell, L. C., Crawford, C., Franssen, M., Greenfield, S., Hodgkinson, J., McCourt, C. and McManus, R. J., 2019. Intervention planning and modification of the BUMP intervention: a digital intervention for the early detection of raised blood pressure in pregnancy. *Pilot and Feasibility Studies*, 5 (1), 1-12.

Bateman, G. A., Giles, W. and England, S. L., 2004. Renal venous Doppler sonography in preeclampsia. *Journal of Ultrasound in Medicine*, 23 (12), 1607-1611.

Bau, P. F. D., Bau, C. H. D., Naujorks, A. A. and Rosito, G. A., 2005. Early and late effects of alcohol ingestion on blood pressure and endothelial function. *Alcohol*, 37 (1), 53-58.

Bazzini, C., Ferrari, A., Boddi, M., Costanzo, G., Romano, M. S., Massetti, L., Tartarisco, G., Pioggia, G. and Modesti, P. A., 2011. Effects On Baroreflex Sensitivity And Renal Resistive Index Of Daily Sessions Of Music Guided Slow-breathing. *Journal of Hypertension*, 29, e510.

Benson, H., Rosner, B. A., Marzetta, B. R. and Klemchuk, H. M., 1974. Decreased blood-pressure in pharmacologically treated hypertensive patients who regularly elicited the relaxation response. *The Lancet*, 303 (7852), 289-291.

Bernardi, L., Gordin, D., Rosengård-Bärlund, M., Mäkinen, V.-P., Mereu, R., DiToro, A. and Groop, P.-H., 2014. Arterial function can be obtained by noninvasive finger pressure waveform. *International Journal of Cardiology*, 175 (1), 169-171.

Bernardi, L., Porta, C., Spicuzza, L., Bellwon, J., Spadacini, G., Frey, A. W., Yeung, L. Y. C., Sanderson, J. E., Pedretti, R. and Tramarin, R., 2002. Slow Breathing Increases Arterial Baroreflex Sensitivity in Patients With Chronic Heart Failure. *Circulation*, 105, 143-145.

Bernardi, L., Sleight, P., Bandinelli, G., Cencetti, S., Fattorini, L., Wdowczyc-Szulc, J. and Lagi, A., 2001. Effect of rosary prayer and yoga mantras on autonomic cardiovascular rhythms: comparative study. *British Medical Journal*, 323 (7327), 1446-1449.

Bertisch, S. M., Schomer, A., Kelly, E. E., Baloa, L. A., Hueser, L. E., Pittman, S. D. and Malhotra, A., 2011. Device-guided paced respiration as an adjunctive therapy for hypertension in obstructive sleep apnea: a pilot feasibility study. *Applied Psychophysiology and Biofeedback*, 36 (3), 173-179.

Bhatt, D., Kristensen, A. M. D., Pareek, M. and Olsen, M. H., 2019. Baroreflex Activation Therapy for Resistant Hypertension and Heart Failure. *US Cardiology Review*, 12 (2), 83-87.

Bilo, G., Revera, M., Bussotti, M., Bonacina, D., Styczkiewicz, K., Caldara, G., Giglio, A., Faini, A., Giuliano, A., Lombardi, C., Kawecka-Jaszcz, K., Mancia, G., Agostoni, P. and Parati, G., 2012. Effects of Slow Deep Breathing at High Altitude on Oxygen Saturation, Pulmonary and Systemic Hemodynamics. *PLOS ONE*, 7 (11), e49074.

Blake, M. J., Martin, A., Manktelow, B. N., Armstrong, C., Halligan, A. W., Panerai, R. B. and Potter, J. F., 2000. Changes in baroreceptor sensitivity for heart rate during normotensive pregnancy and the puerperium. *Clinical Science*, 98 (3), 259-268.

Bogert, L. W. J. and van Lieshout, J. J., 2005. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Experimental Physiology*, 90 (4), 437-446.

British Hypertension Society, 2012. *Efficacy of the RESPeRATE Device for Lowering Blood Pressure: Statement from the British Hypertension Society* [online]. Available from: https://bihsoc.org/wp-content/uploads/2017/11/Statement-on-RESPeRATE-April-12.pdf [Accessed 25 Feb 2021].

Brook, R. D., Appel, L. J., Rubenfire, M., Ogedegbe, G., Bisognano, J. D., Elliott, W. J., Fuchs, F. D., Hughes, J. W., Lackland, D. T., Staffileno, B. A., Townsend, R. R.

and Rajagopalan, S., 2013. Beyond medications and diet: alternative approaches to lowering blood pressure: a scientific statement from the American Heart Association. *Hypertension*, 61 (6), 1360-1383.

Brooks, V. L., Dampney, R. A. L. and Heesch, C. M., 2010. Pregnancy and the endocrine regulation of the baroreceptor reflex. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 299 (2), R439-R451.

Brown, M. C., Best, K. E., Pearce, M. S., Waugh, J., Robson, S. C. and Bell, R., 2013. Cardiovascular disease risk in women with pre-eclampsia: systematic review and meta-analysis. *European Journal of Epidemiology*, 28 (1), 1-19.

Buchner, T., 2018. A quantitative model of relation between respiratory-related blood pressure fluctuations and the respiratory sinus arrhythmia. *Medical & Biological Engineering & Computing*, 57 (5), 1069-1078.

Busch, V., Magerl, W., Kern, U., Haas, J., Hajak, G. and Eichhammer, P., 2012. The effect of deep and slow breathing on pain perception, autonomic activity, and mood processing—an experimental study. *Pain Medicine*, 13 (2), 215-228.

Calcaterra, V., Vandoni, M., Correale, L., Larizza, D., DeBarbieri, G., Albertini, R., Tinelli, C., Arpesella, M. and Bernardi, L., 2014. Deep breathing acutely improves arterial dysfunction in obese children: Evidence of functional impairment? *Nutrition, Metabolism and Cardiovascular Diseases*, 24 (12), 1301-1309.

Calcaterra, V., Vandoni, M., Debarbieri, G., Larizza, D., Albertini, R., Arpesella, M. and Bernardi, L., 2013. Deep breathing improves blunted baroreflex sensitivity in obese children and adolescents with insulin resistance. *International Journal of Cardiology*, 168 (2), 1614-1615.

Camm, A. J., Malik, M., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., Coumel, P., Fallen, E. L., Kennedy, H. L. and Kleiger, R. E., 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *European Heart Journal*, 17, 354-381.

Carlin, A. and Alfirevic, Z., 2008. Physiological changes of pregnancy and monitoring. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 22 (5), 801-823.

Carlson, D. J., Dieberg, G., McFarlane, J. R. and Smart, N. A., 2019. Blood pressure measurements in research: suitability of auscultatory, beat-to-beat, and ambulatory blood pressure measurements. *Blood Pressure Monitoring*, 24 (1), 18.

Castiglioni, P. and Parati, G., 2011. Present trends and future directions in the analysis of cardiovascular variability. *Journal of Hypertension*, 29 (7), 1285-1288.

Cernes, R. and Zimlichman, R., 2017. Role of Paced Breathing for Treatment of Hypertension. *Current Hypertension Reports*, 19 (6), 45.

Chaddha, A., Modaff, D., Hooper-Lane, C. and Feldstein, D. A., 2019. Device and Non-Device-Guided Slow Breathing to Reduce Blood Pressure: A Systematic Review and Meta-Analysis. *Complementary Therapies in Medicine*, 45, 179-184.

Chalaye, P., Goffaux, P., Lafrenaye, S. and Marchand, S., 2009. Respiratory effects on experimental heat pain and cardiac activity. *Pain Medicine*, 10 (8), 1334-1340.

Chan, E., Leong, P., Malouf, R. and Quigley, M. A., 2016. Long-term cognitive and school outcomes of late-preterm and early-term births: a systematic review. *Child: Care, Health and Development*, 42 (3), 297-312.

Chang, Q., Liu, R. and Shen, Z., 2013. Effects of slow breathing rate on blood pressure and heart rate variabilities. *International Journal of Cardiology*, 169 (1), e6-8.

Chen, S.-N., Cheng, C.-C., Tsui, K.-H., Tang, P.-L., Chern, C.-u., Huang, W.-C. and Lin, L.-T., 2018. Hypertensive disorders of pregnancy and future heart failure risk: A nationwide population-based retrospective cohort study. *Pregnancy Hypertension*, 13, 110-115.

Cheng, K. S., Chang, Y. F., Han, R. P. S. and Lee, P. F., 2017. Enhanced conflict monitoring via a short-duration, video-assisted deep breathing in healthy young adults: an event-related potential approach through the Go/NoGo paradigm. *PeerJ*, 5, e3857.

Cheng, K. S., Croarkin, P. E. and Lee, P. F., 2019. Heart Rate Variability of Various Video-Aided Mindful Deep Breathing Durations and Its Impact on Depression, Anxiety, and Stress Symptom Severity. *Mindfulness*, 10 (10), 2082-2094.

Chung, L.-J., Tsai, P.-S., Liu, B.-Y., Chou, K.-R., Lin, W.-H., Shyu, Y.-K. and Wang, M.-Y., 2010. Home-based deep breathing for depression in patients with coronary heart disease: A randomised controlled trial. *International Journal of Nursing Studies*, 47 (11), 1346-1353.

Cifkova, R., 2011. Why is the treatment of hypertension in pregnancy still so difficult? *Expert Review of Cardiovascular Therapy*, 9 (6), 647-649.

Clark, M. E. and Hirschman, R., 1990. Effects of paced respiration on anxiety reduction in a clinical population. *Biofeedback and Self-regulation*, 15 (3), 273-284.

Clements, K. M., Barfield, W. D., Ayadi, M. F. and Wilber, N., 2007. Preterm birth–associated cost of early intervention services: An analysis by gestational age. *Pediatrics*, 119 (4), e866-e874.

Cluver, C., Novikova, N., Koopmans, C. M. and West, H. M., 2017. Planned early delivery versus expectant management for hypertensive disorders from 34 weeks gestation to term (Review). *The Cochrane Library*.

Coad, J., Pedley, K. and Dunstall, M., 2020. *Anatomy and Physiology of Midwives,* . Fourth Edition edition. Edinburgh: Elsevier.

Convertino, V. A., 2019. Mechanisms of inspiration that modulate cardiovascular control: the other side of breathing. *Journal of Applied Physiology*, 127 (5), 1187-1196.

Cooke, W. H., Cox, J. F., Diedrich, A. M., Taylor, A., Beightol, L. A., Ames IV, J. E., Hoag, J. B., Seidel, H. and Eckberg, D. L., 1998. Controlled breathing protocols probe human autonomic cardiovascular rhythms. *Respiration and Human Autonomic Rhythms*, Special Communication, H709-H718.

Cowan, J., Walker, I. and Redman, C., 2017. *Understanding pre-eclampsia: a guide for parents and health professionals*. Watford: Clearsay Publishing.

Cracchiolo, M., Ottaviani, M. M., Panarese, A., Strauss, I., Vallone, F., Mazzoni, A. and Micera, S., 2021. Bioelectronic medicine for the autonomic nervous system: clinical applications and perspectives. *Journal of Neural Engineering*.

Cramer, H., Lauche, R., Haller, H. and Dobos, G., 2013. A systematic review and meta-analysis of yoga for low back pain. *The Clinical Journal of Pain*, 29 (5), 450-460.

Cruz, M. O., Gao, W. and Hibbard, J. U., 2012. What is the optimal time for delivery in women with gestational hypertension? *American Journal of Obstetrics and Gynecology*, 207 (214), e1-e6.

Cullins, S. W., Gevirtz, R. N., Poeltler, D. M., Cousins, L. M., Harpin, R. E. and Muench, F., 2013. An exploratory analysis of the utility of adding cardiorespiratory biofeedback in the standard care of pregnancy-induced hypertension. *Applied Psychophysiology and Biofeedback*, 38 (3), 161-170.

Curtis, K., Weinrib, A. and Katz, J., 2012. Systematic review of yoga for pregnant women: current status and future directions. *Evidence-Based Complementary and Alternative Medicine*, 2012, Article ID 715942.

da Silva Correa, M., Catai, A. M., Milan-Mattos, J. C., Porta, A. and Driusso, P., 2019. Cardiovascular autonomic modulation and baroreflex control in the second trimester of pregnancy: A cross sectional study. *PLOS ONE*, 14 (5), e0216063.

da Silva, E. G., de Godoy, I., de Oliveira Antunes, L. C., da Silva, E. G. and Peraçoli, J. C., 2010. Respiratory parameters and exercise functional capacity in preeclampsia. *Hypertension in Pregnancy*, 29 (3), 301-309.

da Silva, V. P., de Oliveira, N. A., Silveira, H., Mello, R. G. T. and Deslandes, A. C., 2015. Heart rate variability indexes as a marker of chronic adaptation in athletes: a systematic review. *Annals of Noninvasive Electrocardiology*, 20 (2), 108-118.

Daly, J., Sindone, A. P., Thompson, D. R., Hancock, K., Chang, E. and Davidson, P., 2002. Barriers to participation in and adherence to cardiac rehabilitation programs: a critical literature review. *Progress in cardiovascular nursing*, 17 (1), 8-17.

Davis, G. K., Roberts, L., Mangos, G., Henry, A., Pettit, F., O'Sullivan, A., Homer, C. S. E., Craig, M., Harvey, S. B. and Brown, M. A., 2016. Postpartum physiology, psychology and paediatric follow up study (P4 Study) – Study protocol. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, 6 (4), 374-379.

de Barros, S., da Silva, G. V., de Gusmão, J. L., de Araujo, T. G. and Mion Jr, D., 2014. Reduction of Sympathetic Nervous Activity With Device-Guided Breathing. *The Journal of Clinical Hypertension*, 16 (8), 614-615.

Del Bene, R., Barletta, G., Mello, G., Lazzeri, C., Mecacci, F., Parretti, E., Martini, E., Vecchiarino, S., Franchi, F. and La Villa, G., 2001. Cardiovascular function in pregnancy: effects of posture. *BJOG: An International Journal of Obstetrics & Gynaecology*, 108 (4), 344-352.

DeLucia, C. M., De Asis, R. M. and Bailey, E. F., 2018. Daily inspiratory muscle training lowers blood pressure and vascular resistance in healthy men and women. *Experimental Physiology*, 103 (2), 201-211.

Descilo, T., Vedamurtachar, A., Gerbarg, P. L., Nagaraja, D., Gangadhar, B. N., Damodaran, B., Adelson, B., Braslow, L. H., Marcus, S. and Brown, R. P., 2010. Effects of a yoga breath intervention alone and in combination with an exposure therapy for post-traumatic stress disorder and depression in survivors of the 2004 South-East Asia tsunami. *Acta Psychiatrica Scandinavica*, 121 (4), 289-300.

Dick, T. E., Mims, J. R., Hsieh, Y.-H., Morris, K. F. and Wehrwein, E. A., 2014. Increased cardio-respiratory coupling evoked by slow deep breathing can persist in normal humans. *Respiratory Physiology & Neurobiology*, 204, 99-111.

Dillbeck, M. C., 1977. The effect of the Transcendental Meditation technique on anxiety level. *Journal of Clinical Psychology*, 33 (4), 1076-1078.

DiPietro, J. A., Costigan, K. A., Nelson, P., Gurewitsch, E. D. and Laudenslager, M. L., 2008. Fetal responses to induced maternal relaxation during pregnancy. *Biological Psychology*, 77 (1), 11-19.

DiPietro, J. A., Mendelson, T., Williams, E. L. and Costigan, K. A., 2012. Physiological blunting during pregnancy extends to induced relaxation. *Biological Psychology*, 89 (1), 14-20.

Dudenhausen, J. W. and Travis, S. E., 2014. Maternal disorders in pregnancy: Pregnancy-induced hypertension (PIH), preeclampsia, eclampsia, HELLP syndrome. *Practical Obstetrics.* Berlin/Boston: De Gruyter.

Eckberg, D. L., 2009. Point:Counterpoint: Respiratory sinus arrhythmia is due to a central mechanism vs. respiratory sinus arrhythmia is due to the baroreflex mechanism. *Journal of Applied Physiology*, 106 (5), 1740-1742.

Ekholm, E. M. K., Piha, S. J., Antila, K. J. and Erkkola, R. U., 1993. Cardiovascular autonomic reflexes in mid-pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology*, 100 (2), 177-182.

Ekholm, E. M. K., Tahvanainen, K. U. O. and Metsälä, T., 1997. Heart rate and blood pressure variabilities are increased in pregnancy-induced hypertension. *American Journal of Obstetrics and Gynecology*, 177 (5), 1208-1212.

Elkus, R. and Popovich Jr, Jr., 1992. Respiratory physiology in pregnancy. *Clinics in Chest Medicine*, 13 (4), 555.

Elstad, M., 2012. Respiratory variations in pulmonary and systemic blood flow in healthy humans. *Acta Physiologica*, 205 (3), 341-348.

Elstad, M., O'Callaghan, E. L., Smith, A. J., Ben-Tal, A. and Ramchandra, R., 2018. Cardiorespiratory interactions in humans and animals: rhythms for life. *American Journal of Physiology-Heart and Circulatory Physiology*, 315, H6-H17.

Elstad, M., Toska, K., Chon, K. H., Raeder, E. A. and Cohen, R. J., 2001. Respiratory sinus arrhythmia: opposite effects on systolic and mean arterial pressure in supine humans. *The Journal of Physiology*, 536 (1), 251-259.

Elstad, M. and Walløe, L., 2015. Heart rate variability and stroke volume variability to detect central hypovolemia during spontaneous breathing and supported ventilation in young, healthy volunteers. *Physiological Measurement*, 36 (4), 671.

Elstad, M., Walløe, L., Chon, K. H. and Toska, K., 2011. Low-frequency fluctuations in heart rate, cardiac output and mean arterial pressure in humans: what are the physiological relationships? *Journal of Hypertension*, 29 (7), 1327-1336.

Elstad, M., Walløe, L., Holme, N. L. A., Maes, E. and Thoresen, M., 2015. Respiratory sinus arrhythmia stabilizes mean arterial blood pressure at high-frequency interval in healthy humans. *European Journal of Applied Physiology*, 115 (3), 521-530.

Eneroth-Grimfors, E., Westgren, M., Ericson, M., Ihrman-Sandahl, C. and Lindblad, L. E., 1994. Autonomic cardiovascular control in normal and pre-eclamptic pregnancy. *Acta Obstetricia et Gynecologica Scandinavica*, 12 (5), 527-536.

Esposito, P., Mereu, R., De Barbieri, G., Rampino, T., Di Toro, A., Groop, P.-H., Dal Canton, A. and Bernardi, L., 2016. Trained breathing-induced oxygenation acutely reverses cardiovascular autonomic dysfunction in patients with type 2 diabetes and renal disease. *Acta Diabetologica*, 53 (2), 217-226.

European Society of Hypertension and European Society of Cardiology, 2013. 2013 ESH/ESC Guidelines for the management of arterial hypertension. *European Heart Journal*, 34, 2159-2219.

European Union, 2017. Medical Device Regulations (2017-745).

Facco, F. L., Parker, C. B., Reddy, U. M., Silver, R. M., Koch, M. A., Louis, J. M., Basner, R. C., Chung, J. H., Nhan-Chang, C.-L. and Pien, G. W., 2017. Association between sleep-disordered breathing and hypertensive disorders of pregnancy and gestational diabetes mellitus. *Obstetrics and Gynecology*, 129 (1), 31.

Felton, M., Hundley, V. A., Grigsby, S. and McConnell, A. K., 2021. Effects of slow and deep breathing on reducing obstetric intervention in women with pregnancy-induced hypertension: a feasibility study protocol. *Hypertension in Pregnancy*, 40 (1), 81-87.

Finapres, 2012. *Finapres NOVA Technology* [online]. Available from: www.finapres.com/Products/Finpares-NOVA-Technology [Accessed 22/4/2020].

Fischer, C. and Voss, A., 2014. Three-dimensional segmented Poincaré plot analyses SPPA3 investigates cardiovascular and cardiorespiratory couplings in hypertensive pregnancy disorders. *Frontiers in Bioengineering and Biotechnology*, 2, 51.

Fleming, N., 2020. Are breathing techniques good for your health? *The Observer*, 12/07/2020. Available from: https://www.theguardian.com/lifeandstyle/2020/jul/12/are-breathing-techniques-good-for-your-health [Accessed 17/07/2020].

Fonkoue, I. T., Marvar, P. J., Norrholm, S. D., Kankam, M. L., Li, Y., DaCosta, D., Rothbaum, B. O. and Park, J., 2018. Acute effects of device-guided slow breathing on sympathetic nerve activity and baroreflex sensitivity in posttraumatic stress disorder. *American Journal of Physiology-Heart and Circulatory Physiology*, 315 (1), H141-H149.

Franklin, K. A., Holmgren, P. Å., Jönsson, F., Poromaa, N., Stenlund, H. and Svanborg, E., 2000. Snoring, pregnancy-induced hypertension, and growth retardation of the fetus. *Chest*, 117 (1), 137-141.

Garies, S., Hao, S., McBrien, K., Williamson, T., Peng, M., Khan, N. A., Padwal, R. S., Quan, H. and Leung, A. A., 2019. Prevalence of Hypertension, Treatment, and Blood Pressure Targets in Canada Associated With the 2017 American College of Cardiology and American Heart Association Blood Pressure Guidelines. *JAMA Network Open*, 2 (3), e190406.

Garovic, V. D., White, W. M., Vaughan, L., Saiki, M., Parashuram, S., Garcia-Valencia, O., Weissgerber, T. L., Milic, N., Weaver, A. and Mielke, M. M., 2020. Incidence

and Long-Term Outcomes of Hypertensive Disorders of Pregnancy. *Journal of the American College of Cardiology (JACC)*, 75 (18), 2323-2334.

Gassler, J. P. and Bisognano, J. D., 2014. Baroreflex activation therapy in hypertension. *Journal of Human Hypertension*, 28 (8), 469-474.

Gavin, N. R., Kogutt, B. K., Fletcher, W. and Szymanski, L. M., 2020. Fetal and maternal responses to yoga in the third trimester. *The Journal of Maternal-Fetal & Neonatal Medicine*, 33 (15), 2623-2627.

Gavish, B., 2010. Device-guided breathing in the home setting: technology, performance and clinical outcomes. *Biological Psychology*, 84 (1), 150-156.

Gerritsen, R. J. S. and Band, G. P. H., 2018. Breath of Life: The Respiratory Vagal Stimulation Model of Contemplative Activity. *Frontiers in Human Neuroscience*, 12, Article 397.

Grindheim, G., Estensen, M.-E., Langesaeter, E., Rosseland, L. A. and Toska, K., 2012. Changes in blood pressure during healthy pregnancy: a longitudinal cohort study. *Journal of Hypertension*, 30 (2), 342-350.

Grossman, E., Grossman, A., Schein, M., Zimlichman, R. and Gavish, B., 2001. Breathing-control lowers blood pressure. *Journal of Human Hypertension*, 15 (4), 263-269.

Guzik, P., Piskorski, J., Krauze, T., Schneider, R., Wesseling, K. H., Wykretowicz, A. and Wysocki, H., 2007. Correlations between the Poincare plot and conventional heart rate variability parameters assessed during paced breathing. *The Journal of Physiological Sciences*, 57 (1), 63-71.

Haase, C. B., Gyuricza, J. V. and Brodersen, J., 2019. New hypertension guidance risks overdiagnosis and overtreatment. *BMJ*, 365, 1657.

Hansen, T. W., 2010. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors:'establishing normal and reference values'. *European Heart Journal*, 31 (19), 2338-2350.

Harada, D., Asanoi, H., Takagawa, J., Ishise, H., Ueno, H., Oda, Y., Goso, Y., Joho, S. and Inoue, H., 2014. Slow and deep respiration suppresses steady-state sympathetic nerve activity in patients with chronic heart failure: from modeling to clinical application. *American Journal of Physiology-Heart and Circulatory Physiology*, 307 (8), H1159-H1168.

Harrison, D. C., Goldblatt, A., Braunwald, E., Glick, G. and Mason, D. T., 1963. Studies on cardiac dimensions in intact, unanesthetized man. *Circulation Research*, 13 (5), 448-467.

Hart, E. C., Charkoudian, N., Wallin, B. G., Curry, T. B., Eisenach, J. H. and Joyner, M. J., 2009. Sex differences in sympathetic neural-hemodynamic balance: implications for human blood pressure regulation. *Hypertension*, 53 (3), 571-576.

Hegewald, M. J. and Crapo, R. O., 2011. Respiratory physiology in pregnancy. *Clinics in Chest Medicine*, 32 (1), 1-13.

Hehenkamp, W. J. K., Rang, S., van Goudoever, J., Bos, W. J. W., Wolf, H. and van der Post, J. A. M., 2002. Comparison of Portapres® with standard sphygmomanometry in pregnancy. *Hypertension in Pregnancy*, 21 (1), 65-76.

Heidemann, B. H. and McClure, J. H., 2003. Changes in maternal physiology during pregnancy. *British Journal of Anaesthesia CEPD Reviews*, 3 (3), 65-68.

Herakova, N., Nwobodo, N. H. N., Wang, Y., Chen, F. and Zheng, D., 2017. Effect of respiratory pattern on automated clinical blood pressure measurement: an observational study with normotensive subjects. *Clinical Hypertension*, 23 (1), 15.

Higgins, J. R. and de Swiet, M., 2001. Blood-pressure measurement and classification in pregnancy. *The Lancet*, 357 (9250), 131-135.

Hoppe, U. C., Brandt, M.-C., Wachter, R., Beige, J., Rump, L. C., Kroon, A. A., Cates, A. W., Lovett, E. G. and Haller, H., 2012. Minimally invasive system for baroreflex activation therapy chronically lowers blood pressure with pacemaker-like safety profile: results from the Barostim neo trial. *Journal of the American Society of Hypertension*, 6 (4), 270-276.

Huang, A. J., Grady, D., Mendes, W. B., Hernandez, C., Schembri, M. and Subak, L. L., 2019. A Randomized Controlled Trial of Device Guided, Slow-Paced Respiration in Women with Overactive Bladder Syndrome. *The Journal of Urology*, 202 (4), 787-794.

Huang, A. J., Phillips, S., Schembri, M., Vittinghoff, E. and Grady, D., 2015. Device-guided slow-paced respiration for menopausal hot flushes: a randomized controlled trial. *Obstetrics and Gynecology*, 125 (5), 1130.

Hughey, M. J., McElin, T. W. and Young, T., 1978. Maternal and fetal outcome of Lamaze-prepared patients. *Obstetrics and Gynecology*, 51 (6), 643-647.

Iliescu, R., Tudorancea, I. and Lohmeier, T. E., 2014. Baroreflex Activation: from Mechanisms to Therapy for Cardiovascular Disease. *Current Hypertension Reports*, 16 (8), 453.

International Society for the Advancement in Kinanthropometry, 2011. International Standards for Anthropometric Assessment.

Isaacs, R., Smith, G., Gale-Andrews, L., Wee, M., Van Teijlingen, E., Bick, D. and Hundley, V., 2019. Design errors in vital sign charts used in consultant-led maternity units in the United Kingdom. *International Journal of Obstetric Anesthesia*, 39, 60-67.

James, P. R. and Nelson-Piercy, C., 2004. Management of hypertension before, during, and after pregnancy. *Heart*, 90 (12), 1499-1504.

Jansen, J. R. C., Schreuder, J. J., Mulier, J. P., Smith, N. T., Settels, J. J. and Wesseling, K. H., 2001. A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. *British Journal of Anaesthesia*, 87 (2), 212-222.

Jerath, R., Barnes, V. A. and Fadel, H. E., 2009. Mechanism of development of pre-eclampsia linking breathing disorders to endothelial dysfunction. *Medical Hypotheses*, 73 (2), 163-166.

Jette, E. D., Adler, T. E., Niro, F., Coovadia, Y., Jensen, D. and Usselman, C. W., 2019. Evaluating Cardiorespiratory Responses to Slow Breathing in Young Healthy Individuals: FitBit “Relax Mode” versus RESPeRATE. *The FASEB Journal*, 33 (1_supplement), 533.513-533.513.

Jones, C. U., Sangthong, B. and Pachirat, O., 2010. An inspiratory load enhances the antihypertensive effects of home-based training with slow deep breathing: a randomised trial. *Journal of Physiotherapy*, 56 (3), 179-186.

Jones, M. T., Heiden, E., Fogg, C., Meredith, P., Smith, G., Sayer, N., Toft, L., Williams, E., Williams, M. and Brown, T., 2020. An Evaluation of Agreement of Breathing Rates Measured by a Novel Device, Manual Counting, and Other Techniques Used in Clinical Practice: Protocol for the Observational VENTILATE Study. *JMIR Research Protocols*, 9 (7), e15437.

Joseph, C. N., Porta, C., Casucci, G., Casiraghi, N., Maffeis, M., Rossi, M. and Bernardi, L., 2005. Slow breathing improves arterial baroreflex sensitivity and decreases blood pressure in essential hypertension. *Hypertension*, 46 (4), 714-718.

Julien, C., 2006. The enigma of Mayer waves: facts and models. *Cardiovascular Research*, 70 (1), 12-21.

Julien, C., Parkes, M. J., Tzeng, S. Y. C., Sin, P. Y. W., Ainslie, P. N., Van De Borne, P., Fortrat, J.-O., Custaud, M.-A., Gharib, C. and Porta, A., 2009. Comments on point: counterpoint: respiratory sinus arrhythmia is due to a central mechanism vs. respiratory sinus arrhythmia is due to the baroreflex mechanism. *Journal of Applied Physiology*, 106 (5), 1745-1749.

Kametas, N. A., McAuliffe, F., Krampl, E., Chambers, J. and Nicolaides, K. H., 2003. Maternal cardiac function in twin pregnancy. *Obstetrics & Gynecology*, 102 (4), 806-815.

Karemaker, J. M., 2009a. Counterpoint: respiratory sinus arrhythmia is due to the baroreflex mechanism. *Journal of Applied Physiology*, 106 (5), 1742-1743.

Karemaker, J. M., 2009b. Last word on point: counterpoint: respiratory sinus arrhythmia is due to a central mechanism vs. respiratory sinus arrhythmia is due to the baroreflex mechanism. *Journal of Applied Physiology*, 106 (5), 1750.

Karthiga, K., Pal, G. K., Velkumary, S., Papa, D. and Nanda, N., 2019. Role of Sympathovagal Imbalance in Gestational Hypertension: A Mini-Review. *International Journal of Clinical and Experimental Physiology*, 6 (4), 107-110.

Kaul, S., 2020. Evidence for the Universal Blood Pressure Goal of <130/80 mm Hg Is Strong. *Hypertension*, 76 (5), 1391-1399.

Kaushik, R. M., Kaushik, R., Mahajan, S. K. and Rajesh, V., 2006. Effects of mental relaxation and slow breathing in essential hypertension. *Complementary Therapies in Medicine*, 14 (2), 120-126.

Keerthi, G. S., Bandi, H. K., Suresh, M. and Redd, M., 2013. Effect of slow deep breathing (6 breaths/min) on pulmonary function in healthy volunteers. *International Journal of Medical Research & Health Sciences*, 2 (3), 597-602.

Koh, T. C., 1982. Qigong - Chinese breathing exercise. *The American Journal of Chinese Medicine*, 10 (1-4), 86-91.

Kolovetsiou-Kreiner, V., Moertl, M. G., Papousek, I., Schmid-Zalaudek, K., Lang, U., Schlembach, D., Cervar-Zivkovic, M. and Lackner, H. K., 2018. Maternal cardiovascular and endothelial function from first trimester to postpartum. *PLOS ONE*, 13 (5), e0197748.

Lakhno, I., 2016. Maternal respiratory sinus arrhythmia captures the severity of pre eclampsia. *Archives of Perinatal Medicine*, 22 (2), 14-17.

Landman, G. W., Drion, I., van Hateren, K. J., van Dijk, P. R., Logtenberg, S. J., Lambert, J., Groenier, K. H., Bilo, H. J. and Kleefstra, N., 2013. Device-guided breathing as treatment for hypertension in type 2 diabetes mellitus: a randomized, double-blind, sham-controlled trial. *JAMA Internal Medicine*, 173 (14), 1346-1350.

Landman, G. W., van Hateren, K. J., van Dijk, P. R., Logtenberg, S. J., Houweling, S. T., Groenier, K. H., Bilo, H. J. and Kleefstra, N., 2014. Efficacy of device-guided breathing for hypertension in blinded, randomized, active-controlled trials: a meta-analysis of individual patient data. *JAMA Internal Medicine*, 174 (11), 1815-1821.

Langewouters, G. J., Settels, J. J., Roelandt, R. and Wesseling, K. H., 1998. Why use Finapres or Portapres rather than intraarterial or intermittent non-invasive techniques of blood pressure measurement? *Journal of Medical Engineering & Technology*, 22 (1), 37-43.

Lauder, L., Azizi, M., Kirtane, A. J., Boehm, M. and Mahfoud, F., 2020. Device-based therapies for arterial hypertension. *Nature Reviews Cardiology*, 17 (10), 614-628.

Lavallee, L., Tucker, K. and McManus, R., 2018. Pregnant women are doing it for themselves: Self-monitoring of blood pressure in pregnancy. *British Journal of Midwifery*, 26 (7), 451-454.

Leboyer, F., 1985. *The Art of Breathing*. Element Books.

Leduc, L., Wasserstrum, N., Spillman, T. and Cotton, D. B., 1991. Barorefiex function in normal pregnancy. *American Journal of Obstetrics and Gynecology*, 165 (4, Part 1), 886-890.

Leonetti, P., Audat, F., Girard, A., Laude, D., Lefrère, F. and Elghozi, J.-L., 2004. Stroke volume monitored by modeling flow from finger arterial pressure waves mirrors blood volume withdrawn by phlebotomy. *Clinical Autonomic Research*, 14 (3), 176-181.

Levick, J. R., 2013. *An introduction to cardiovascular physiology*. Fourth Edition edition. Great Britain: Arnold.

Lewis, R., Seo, Y., Hackfort, B. T., Pozehl, B. and Schultz, H. D., 2018. Eight weeks of slow deep breathing training alters cardiorespiratory function and improves functional exercise capacity in chronic heart failure patients. *The FASEB Journal*, 32 (1_supplement), 903-916.

Little, B. C., Benson, P., Beard, R. W., Hayworth, J., Hall, F., Dewhurst, J. and Priest, R. G., 1984. Treatment of hypertension in pregnancy by relaxation and biofeedback. *The Lancet*, 323 (8382), 865-867.

Liu, L., Oza, S., Hogan, D., Chu, Y., Perin, J., Zhu, J., Lawn, J. E., Cousens, S., Mathers, C. and Black, R. E., 2016. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *The Lancet*, 388 (10063), 3027-3035.

Loerup, L., Pullon, R. M., Birks, J., Fleming, S., Mackillop, L. H., Gerry, S. and Watkinson, P. J., 2019. Trends of blood pressure and heart rate in normal pregnancies: a systematic review and meta-analysis. *BMC Medicine*, 17 (1), 167.

LoMauro, A. and Aliverti, A., 2015. Respiratory physiology of pregnancy: Physiology masterclass. *Breathe*, 11 (4), 297-301.

LoMauro, A. and Aliverti, A., 2018. Sex differences in respiratory function. *Breathe*, 14 (2), 131-140.

Lote, C. J., 2012. Principles of Renal Physiology. New York, NY: Springer, 88-91.

Lovallo, W. R., Wilson, M. F., Vincent, A. S., Sung, B. H., McKey, B. S. and Whitsett, T. L., 2004. Blood pressure response to caffeine shows incomplete tolerance after short-term regular consumption. *Hypertension*, 43 (4), 760-765.

Lutsenko, O. I. and Kovalenko, S. O., 2017. Blood pressure and hemodynamics: Mayer waves in different phases of ovarian and menstrual cycle in women. *Physiological Research*, 66 (2), 235.

Madhavi, S., Raju, P. S., Reddy, M. V., Annapurna, N., Sahay, B. K., Kumari, D. G. and Murthy, K. J., 1985. Effect of yogic exercises on lean body mass. *The Journal of the Association of Physicians of India*, 33 (7), 465-466.

Magee, L. A., von Dadelszen, P., Rey, E., Ross, S., Asztalos, E., Murphy, K. E., Menzies, J., Sanchez, J., Singer, J. and Gafni, A., 2015. Less-tight versus tight control of hypertension in pregnancy. *New England Journal of Medicine*, 372 (5), 407-417.

Mahtani, K. R., Nunan, D. and Heneghan, C. J., 2012. Device-guided breathing exercises in the control of human blood pressure: systematic review and meta-analysis. *Journal of Hypertension*, 30 (5), 852-860.

McAuliffe, F., Kametas, N., Costello, J., Rafferty, G. F., Greenough, A. and Nicolaides, K., 2002. Respiratory function in singleton and twin pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology*, 109 (7), 765-769.

McConnell, A., Vargas, P. M. F. and Wegerif, S., 2017. *A device and method for guiding breathing of a user.* UK Patent Application 1717276.8.

McCormack, M. C. and Wise, R. A., 2009. Respiratory physiology in pregnancy. *Pulmonary Problems in Pregnancy.* Springer, 19-26.

Medicines and Healthcare products Regulatory Agency, 2018. *Guidance: Medical device stand-alone software including apps (including IVDMDs)*.

Miles, W. R., 1964. Oxygen consumption during three yoga-type breathing patterns. *Journal of Applied Physiology*, 19 (1), 75-82.

Minson, C. T., Halliwill, J. R., Young, T. M. and Joyner, M. J., 2000a. Influence of the Menstrual Cycle on Sympathetic Activity, Baroreflex Sensitivity, and Vascular Transduction in Young Women. *Circulation*, 101 (8), 862-868.

Minson, C. T., Halliwill, J. R., Young, T. M. and Joyner, M. J., 2000b. Sympathetic activity and baroreflex sensitivity in young women taking oral contraceptives. *Circulation*, 102 (13), 1473-1476.

Miyazato, K. and Matsukawa, K., 2010. Decreased cardiac parasympathetic nerve activity of pregnant women during foot baths. *Japan Journal of Nursing Science*, 7 (1), 65-75.

Modesti, P. A., Ferrari, A., Bazzini, C. and Boddi, M., 2015. Time sequence of autonomic changes induced by daily slow-breathing sessions. *Clinical Autonomic Research : Official Journal of the Clinical Autonomic Research Society*, 25 (2), 95-104.

Monk, C., Fifer, W. P., Myers, M. M., Bagiella, E., Duong, J. K., Chen, I. S. and Leotti, L., 2011. Fetal Heart Rate Reactivity Differs by Women's Psychiatric Status during Psychological Stress, but Not Paced Breathing. *Developmental Psychobiology*, 53 (3), 221.

Mori, H., Yamamoto, H., Kuwashima, M., Saito, S., Ukai, H., Hirao, K., Yamauchi, M. and Umemura, S., 2005. How does deep breathing affect office blood pressure and pulse rate? *Hypertension Research*, 28 (6), 499-504.

Moser, M., Brown, C. M., Rose, C. H. and Garovic, V. D., 2012. Hypertension in pregnancy: is it time for a new approach to treatment? *Journal of Hypertension*, 30 (6), 1092-1100.

Mujika, I. and Padilla, S., 2000. Detraining: loss of training-induced physiological and performance adaptations. Part I. *Sports Medicine*, 30 (2), 79-87.

Muthukrishnan, S., Jain, R., Kohli, S. and Batra, S., 2016. Effect of mindfulness meditation on perceived stress scores and autonomic function tests of pregnant Indian women. *Journal of Clinical and Diagnostic Research*, 10 (4), CC05.

Nafz, B., Stegemann, J., Bestle, M. H., Richter, N., Seeliger, E., Schimke, I., Reinhardt, H. W. and Persson, P. B., 2000. Antihypertensive effect of 0.1-Hz blood pressure oscillations to the kidney. *Circulation*, 101 (5), 553-557.

Nagao, T., Saito, K. and Yamanaka, M., 2021. Prehypertension in early pregnancy is a risk factor for hypertensive disorders during pregnancy: A historical cohort study in Japan. *Hypertension in Pregnancy*, 40 (1), 51-55.

Narendran, S., Nagarathna, R., Narendran, V., Gunasheela, S. and Nagendra, H. R. R., 2005. Efficacy of yoga on pregnancy outcome. *Journal of Alternative & Complementary Medicine*, 11 (2), 237-244.

National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *American Journal of Obstetrics and Gynecology*, 183 (1), s1-s22.

National Horizon Scanning Unit, A. H. T. A., 2004. *Hoizon scanning prioritising summary: RESPeRATE: Self-guided breathing device for the treatment of hypertension in the home*.

National Institute for Health Research (NIHR), 2020. *DHSC issues guidance on the impact of COVID-19 on research funded or supported by NIHR* [online]. Available from: https://www.nihr.ac.uk/news/dhsc-issues-guidance-on-the-impact-on-covid-19-on-research-funded-or-supported-by-nihr/24469 [Accessed 16/03/21].

Ngene, N. C. and Moodley, J., 2017. Physiology of blood pressure relevant to managing hypertension in pregnancy. *The Journal of Maternal-Fetal & Neonatal Medicine*, 32 (8), 1368-1377.

NHS, 2019. *High Blood Pressure (Hypertension)* [online]. Available from: https://www.nhs.uk/conditions/high-blood-pressure-hypertension/ [Accessed 21/02/2021].

NICE: National Institute for Health and Care Excellence, 2019a. *Hypertension in adults: diagnosis and management*. NICE Guidelines (NG136).

NICE: National Institute for Health and Care Excellence, 2019b. *Hypertension in pregnancy: diagnosis and management*.

NICE: National Institute for Health Research, 2017. *Alert: New evidence for lower blood pressure targets* [online]. Available from: https://evidence.nihr.ac.uk/alert/new-evidence-for-lower-blood-pressure-targets/ [Accessed 21/02/2021].

Nili, M., Abidi, S., Serna, S., Kim, S. and Edgell, H., 2017. Influence of sex, menstrual cycle, and oral contraceptives on the cerebrovascular response to paced deep breathing. *Clinical Autonomic Research*, 27 (6), 411-415.

Norwitz, E. R., Edusa, V. and Park, J. S., 2005. Maternal Physiology and Complications of Multiple Pregnancy. *Seminars in Perinatology*, 29 (5), 338-348.

Nuckowska, M. K., Gruszecki, M., Kot, J., Wolf, J., Guminski, W., Frydrychowski, A. F., Wtorek, J., Narkiewicz, K. and Winklewski, P. J., 2019. Impact of slow breathing on the blood pressure and subarachnoid space width oscillations in humans. *Nature Scientific Reports*, 9 (1), 6232.

Okur, M. E., Karantas, I. D., Okur, N. U. and Siafaka, P. I., 2017. Hypertension in 2017: Update in treatment and pharmaceutical innovations. *Current Pharmaceutical Design*, 23 (44), 6795-6814.

Oneda, B., Ortega, K. C., Gusmão, J. L., Araújo, T. G. and Mion Jr, D., 2010. Sympathetic nerve activity is decreased during device-guided slow breathing. *Hypertension Research*, 33, 708.

Pal, G. K., Shyma, P., Habeebullah, S., Pal, P., Nanda, N. and Shyjus, P., 2011. Vagal withdrawal and sympathetic overactivity contribute to the genesis of early-onset pregnancy-induced hypertension. *International Journal of Hypertension*, 2011, 361417.

Parati, G. and Cerretta, R., 2007. Device-guided slow breathing as a non-pharmacological approach to antihypertensive treatment: efficacy, problems and perspectives. *Journal of Hypertension*, 25 (1), 57-61.

Parati, G., Izzo, J. L. and Gavish, B., 2008. Respiration and Blood Pressure. *In:* Izzo, J. L., Sica, D. A. and Black, H. R., eds. *Hypertension Primer: The essentials of high blood pressure. Basic science, population science and clinical management.* Fourth Edition edition.: Lippincott Williams & Wilkins, 136-138.

Parati, G., Mancia, G., Rienzo, M. D. and Castiglioni, P., 2006. Point:Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology*, 101 (2), 676-682.

Parati, G., Ravogli, A., Frattola, A., Groppelli, A., Ulian, L., Santucciu, C. and Mancia, G., 1994. Blood pressure variability: clinical implications and effects of antihypertensive treatment. *Journal of Hypertension*, 12 (5), S35-S40.

Parati, G., Saul, J. P., Di Rienzo, M. and Mancia, G., 1995. Spectral analysis of blood pressure and heart rate variability in evaluating cardiovascular regulation: a critical appraisal. *Hypertension*, 25 (6), 1276-1286.

Parati, G., Ulian, L., Santucciu, C., Tortorici, E., Villani, A., Di Rienzo, M. and Mancia, G., 1997. Clinical value of blood pressure variability. *Blood pressure. Supplement*, 2, 91-96.

Patel, C., 1975. Yoga and biofeedback in the management of hypertension. *Journal of Psychosomatic Research*, 19 (5), 355-360.

Pels, A., Mol Ben Willem, J., Singer, J., Lee, T., von Dadelszen, P., Ganzevoort, W., Asztalos, E., Magee Laura, A., null, n., Gafni, A., Gruslin, A., Helewa, M., Hutton, E., Lee, S., Logan, A., Menzies, J., Moutquin, J.-M., Murphy, K., Rey, E., Ross, S., Sanchez, J., Thornton Jim, G., Welch, R., Hoac, T., Kirton, J., Trigiani, K., Zahid, A., Bracken Michael, B., Crowley, P., Duley, L., Ehrenkranz, R., Thorpe, K., Chan, S., Shi, M., Yu, S., de Lourdes Martin, R., Bassi Maria, F., Caruso Mirta, C., Lagunas, V., Vera, F., Mohedano de Duhalde, M., Roque Alicia, B., Roldan, P., Duhalde Esteban, M., Dip, V., Aguirre Jesus, D., Morales Elba Mirta, A., Abreo Griselda, I., De Sagastizabal, T., Gomez, C., Rizzi, N., Arias, C., Bruno Ricardo, A., Mahomed, K., Drew, A., Green, A., Hoare, J., Hague, B., Coat, S., Crowther, C., Muller, P., Trenowden, S., Walters, B., Parker, C., Graham, D., Pennell, C., Sung, E., Makris, A., Lee, G., Thornton, C., Hennessy, A., Farrell, L., Sass, N., Korkes, H., Ferreira Dayana, C., Moreira de Sa Renato, A., Abreu Monique Schmidt, M., Bornia Rita, G., da Silva Nancy, R., Cardoso Fernanda Freitas, O., Marques Caio, C., Hornos, J., Davdt Ricardo, L., Paula Letícia, G., Zanella Pedro, L., Inglis, G., Dillon, R., Docherty, A., Hutfield, A., Still, K., Lalji, S., Van Tent, T., Hotz, C., Messmer, T., Ray Joel, G., Berger, H., De Souza, L., Lausman, A., Freire-Lizama, T., Besel, K., Gibson, P., Ellsworth, G., Miller, L., Hawkins, T. L.-A., Hladunewich, M., Rogowsky, A., Hui, D., Collins, V., Delisle, I., Fanning, C., Demianczuk, N., Khurana, R., Sia, W., Marnoch, C., Young, C., Lux, C., Perreault, S., Tremblay, V., Desindes, S., Côté, A.-M., Dagenais, V., Clark, H., O'Shea, E., White Ruth, R., Gandhi, S., Martin, M.-J., Brush, C., Seaward, G., Newstead-Angel, J., Brandt, J., Martel, J., Mytopher, K., Buschau, E., Keely, E., Waddell, P., Shachkina, S., Karovitch, A., Anderson, R., Koenig, N., Yong, T., Vasiliou, M., Johnson, P., Allan, B., Natale, R., Kennedy, L., Opatrny, L., Lavigne, L., Carson, G., Kelly, S., Crane, J., Hutchens, D., Kusanovic Juan, P., Figueroa, C., Neculman Karla, S., Ortiz Juan, A., Vargas, P., Ferrand, P., Carrillo, J., Borrero Rodrigo, C., Gallo Dahiana, M., Moreno Luisa, F., Kirss, F., Rull, K., Kirss, A., Major, T., Fodor, A., Bartha, T., Hallak, M., Aslih, N., Anabousi-Murra, S., Pri-Or, E., Harel, L., Siev, S., Hakim, M., Khoury Christina, S., Hamati, N., El-Zibdeh, M., Yousef, L., Hughes, R., Leishman, D., Pullar, B., Farrant, M., Swiatkowska-Freund, M., Preis, K., Traczyk-Los Anette, A., Partyka, A., Preis-Orlikowska, J., Lukaszuk, M., Krasomski, G., Krekora, M., Kedzierska-Markowicz, A., Zych-Krekora, K., Breborowicz Grzegorz, H., Dera-Szymanowska, A., Bakker, J., Akkermans, J., van den Akker, E., Logtenberg, S., Koenen, S., de Reus, M., Borman, D., Oudijk Martijn, A., Bolte, A., Verfaille, V., Graaf, B., Porath, M., Verhoeven, C., Franssen Maureen, T. M., Ulkeman, L., Hamming, I., Keurentjes Jose, H. M., van der Wal, I., Bijvank, S. W. A. N., Lutjes, A. A., Visser, H., Scheepers Hubertina Catharina, J., van Beek, E., van Dam, C., van den Berg-Swart, K., Pernet, P., van der Goes, B., Schuitemaker, N., Kleiverda, G., van Alphen, M., Rosman, A., Gaugler-Senden, I., Linders, M., Nelson-Piercy, C., Briley, A., Soh May, C., Harding, K., Tarft, H., Churchill, D., Cheshire, K., Icke, J., Ghosh, M., Thornton, J., Toomassi, Y., Barker, K., Fisher, J., Grace, N., Green, A., Gower, J., Molnar, A., Parameshwaran, S., Simm, A., Bugg, G., Davis, Y., Desphande, R., Gunn, Y., Houda, M., Jones, N., Waugh, J., Allan, C., Waring, G., Walkinshaw Steve, A., Pascall, A., Clement-Jones, M., Dower, M., Houghton, G., Longworth, H., Purewal, T., Tuffnell, D., Farrar, D., Syson, J., Butterfield, G., Jones, V., Palethorpe, R., Germaine, T., Habiba, M., Lee, D., Eniola, O., Blake, L., Khan, J., Cameron Helen, M., Hinshaw, K., Bargh, A., Walton, E., Sorinola, O., Guy, A., D'Souza, Z., Gabriel, R., Williams, J.,

Hollands, H., Jibodu, O., Collier, S., Tottie, P., Oxby, C., Dwyer, J., Majoko, F., Goldring, H., Jones, S., Cresswell, J., Underwood, L., Kelly-Baxter, M., Robinson, R., Anumba, D., Chamberlain, A., Pye, C., Tower, C., Woods, S., Horrocks, L., Prichard, F., Moorhead, L., Lee, S., Stephens, L., Taylor, C., Thomas, S., Whitworth, M., Myers, J., Knox, E., Freitas, K., Kilby, M., Cotterill, A., Abdo, K., Rigby, K., Butler, J., Crosfill, F., Hughes, S., Prashar, S., Soydemir, F., Ashworth, J., Mycock, L., Smith, J., Ikomi, A., Goodsell, K., Byrne, J., Masuku, M., Pilcher, A., Khandelwal, M., Simpkins, G., Iavicoli, M., Kim Yon, S., Fischer, R., Perry, R., Chang Eugene, Y., Saunders Tamara, D., Oswald Betty, W., Zaks Kristin, D., Rana, S., McCullough, D., Sfakianaki, A., Danton, C., Kustan, E., Coraluzzi, L., How, H., Waldon, C., Livingston, J., Jackson, S., Greene, L., Shah, D., Tolosa Jorge, E., Rincon, M., Pereira, L., Lawrence Amy, E., Snyder Janice, E., Armstrong, D. M., Blue, T., Hester, A. and Salisbury, K., 2018. Influence of Gestational Age at Initiation of Antihypertensive Therapy. *Hypertension*, 71 (6), 1170-1177.

Peňáz, J., 1973. Photoelectric measurement of blood pressure, volume and flow in the finger. *Digest of the 10th International Conference on Medical and Biological Engineering*, 104.

Pinna, G. D., Maestri, R., La Rovere, M. T., Gobbi, E. and Fanfulla, F., 2006. Effect of paced breathing on ventilatory and cardiovascular variability parameters during short-term investigations of autonomic function. *American Journal of Physiology Heart and Circulation Physiology*, 290 (1), H424-H433.

Pramanik, T., Sharma, H. O., Mishra, S., Mishra, A., Prajapati, R. and Singh, S., 2009. Immediate effect of slow pace bhastrika pranayama on blood pressure and heart rate. *The Journal of Alternative and Complementary Medicine*, 15 (3), 293-295.

Quinlan, P., Lane, J. and Aspinall, L., 1997. Effects of hot tea, coffee and water ingestion on physiological responses and mood: the role of caffeine, water and beverage type. *Psychopharmacology*, 134 (2), 164-173.

Rakhshani, A., Nagarathna, R., Mhaskar, R., Mhaskar, A., Thomas, A. and Gunasheela, S., 2012. The effects of yoga in prevention of pregnancy complications in high-risk pregnancies: a randomized controlled trial. *Preventive Medicine*, 55 (4), 333-340.

Rampalliwar, S., Rajak, C., Arjariya, R., Poonia, M. and Bajpai, R., 2013. The effect of bhramari pranayama on pregnant women having cardiovascular hyper-reactivity to cold pressor. *National Journal of Physiology, Pharmacy & Pharmacology*, 3 (2), 137-141.

Rankin, J., 2020. *Myles Midwifery Anatomy & Physiology Workbook*. Second edition edition. Edinburgh: Elsevier.

Reed, S. F., Porges, S. W. and Newlin, D. B., 1999. Effect of alcohol on vagal regulation of cardiovascular function: contributions of the polyvagal theory to the psychophysiology of alcohol. *Experimental and Clinical Psychopharmacology*, 7 (4), 484-492.

Roberts, C. L., Algert, C. S., Morris, J. M. and Ford, J. B., 2015. Increased planned delivery contributes to declining rates of pregnancy hypertension in Australia: a population-based record linkage study. *BMJ Open*, 5 (10), e009313.

Roberts, L. M., Davis, G. K. and Homer, C. S. E., 2017. Pregnancy with gestational hypertension or preeclampsia: A qualitative exploration of women's experiences. *Midwifery*, 46, 17-23.

Roberts, L. M., Davis, G. K. and Homer, C. S. E., 2019. Depression, anxiety and postttraumatic stress disorder following a hypertensive pregnancy: a narrative literature review. *Frontiers in Cardiovascular Medicine*, 6, 147.

Robertson, N., Okano, S. and Kumar, S., 2020. Feto-placental Dopplers are not altered in women with obstructive sleep apnoea symptoms. *The Australian & New Zealand Journal of Obstetrics & Gynaecology*, 60 (6), 877-883.

Roeth, N. A., Ball, T. R., Culp, W. C., Todd Bohannon, W., Atkins, M. D. and Johnston, W. E., 2014. Effect of Increasing Heart Rate and Tidal Volume on Stroke Volume Variability in Vascular Surgery Patients. *Journal of Cardiothoracic and Vascular Anesthesia*, 28 (6), 1516-1520.

Rosenthal, T., Alter, A., Peleg, E. and Gavish, B., 2000. B006: Device-guided breathing exercises reduce blood pressure-ambulatory and home measurements. *American Journal of Hypertension*, 13 (S2), 56A.

Ross, A. and Thomas, S., 2010. The health benefits of yoga and exercise: a review of comparison studies. *The Journal of Alternative and Complementary Medicine*, 16 (1), 3-12.

Royal College of Midwives, 2014. *High Quality Midwifery Care.*

Royal College of Obstetricians and Gynaecologists, 2019. *New NICE guideline update on Hypertension in pregnancy* [online]. Available from: https://www.rcog.org.uk/en/about-us/nga/nga-news/nice-guideline-hypertension-pregnancy-update/ [Accessed 30.06.21].

Russo, M. A., Santarelli, D. M. and O'Rourke, D., 2017. The physiological effects of slow breathing in the healthy human. *Breathe*, 13 (4), 298-309.

Salles, G. F., Schlüssel, M. M., Farias, D. R., Franco-Sena, A. B., Rebelo, F., Lacerda, E. M. A. and Kac, G., 2015. Blood pressure in healthy pregnancy and factors associated with no mid-trimester blood pressure drop: a prospective cohort study. *American Journal of Hypertension*, 28 (5), 680-689.

Sanghavi, M. and Rutherford, J. D., 2014. Cardiovascular physiology of pregnancy. *Circulation*, 130 (12), 1003-1008.

Santino, T. A., Chaves, G. S. S., Freitas, D. A., Fregonezi, G. A. F. and Mendonça, K., 2020. Breathing exercises for adults with asthma. *Cochrane Database of Systematic Reviews*, (3).

Satyapriya, M., Nagendra, H. R., Nagarathna, R. and Padmalatha, V., 2009. Effect of integrated yoga on stress and heart rate variability in pregnant women. *International Journal of Gynecology & Obstetrics*, 104 (3), 218-222.

Say, L., Chou, D., Gemmill, A., Tunçalp, Ö., Moller, A.-B., Daniels, J., Gülmezoglu, A. M., Temmerman, M. and Alkema, L., 2014. Global causes of maternal death: a WHO systematic analysis. *The Lancet Global Health*, 2 (6), e323-e333.

Scantlebury, D. C., Schwartz, G. L., Acquah, L. A., White, W. M., Moser, M. and Garovic, V. D., 2013. The treatment of hypertension during pregnancy: when should blood pressure medications be started? *Current Cardiology Reports*, 15 (11), 412.

Schram, B., Hing, W. and Climstein, M., 2016. The physiological, musculoskeletal and psychological effects of stand up paddle boarding. *BMC Sports Science, Medicine and Rehabilitation*, 8 (1), 32.

Seals, D. R., Suwarno, N. O., Joyner, M. J., Iber, C., Copeland, J. G. and Dempsey, J. A., 1993. Respiratory modulation of muscle sympathetic nerve activity in intact and lung denervated humans. *Circulation Research*, 72 (2), 440-454.

Shaffer, F. and Ginsberg, J. P., 2017. An overview of heart rate variability metrics and norms. *Frontiers in Public Health*, 5, Article 258.

Sharma, M., Frishman, W. H. and Gandhi, K., 2011. RESPeRATE: nonpharmacological treatment of hypertension. *Cardiology in Review*, 19 (2), 47-51.

Sheel, A. W., Dominelli, P. B. and Molgat-Seon, Y., 2016. Revisiting dysanapsis: sex-based differences in airways and the mechanics of breathing during exercise. *Experimental Physiology*, 101 (2), 213-218.

Sica, D. A., 2011. Device-guided breathing and hypertension: a yet to be determined positioning. *Cardiology in Review*, 19 (2), 45-46.

Sidery, M. B. and Macdonald, I. A., 1994. The effect of meal size on the cardiovascular responses to food ingestion. *British Journal of Nutrition*, 71 (6), 835-848.

Silverberg, D. S., 1990. Non-pharmacological treatment of hypertension. *Journal of Hypertension*, 8 (4), S21-26.

Singh, J. P., Larson, M. G., Tsuji, H., Evans, J. C., O'Donnell, C. J. and Levy, D., 1998. Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. *Hypertension*, 32 (2), 293-297.

Smith, G. B., Isaacs, R., Andrews, L., Wee, M. Y. K., Van Teijlingen, E., Bick, D. E., Hundley, V. and Research, M. O. E. W. S. M., 2017. Vital signs and other observations used to detect deterioration in pregnant women: an analysis of vital sign charts in consultant-led UK maternity units. *International journal of obstetric anesthesia*, 30, 44-51.

Smits, P., Thien, T. H. and Van't Laar, A., 1985. The cardiovascular effects of regular and decaffeinated coffee. *British Journal of Clinical Pharmacology*, 19 (6), 852-854.

Soma-Pillay, P., Catherine, N.-P., Tolppanen, H., Mebazaa, A., Tolppanen, H. and Mebazaa, A., 2016. Physiological changes in pregnancy. *Cardiovascular Journal of Africa*, 27 (2), 89-94.

Spaak, J., Merlocco, A. C., Soleas, G. J., Tomlinson, G., Morris, B. L., Picton, P., Notarius, C. F., Chan, C. T. and Floras, J. S., 2008. Dose-related effects of red wine and alcohol on hemodynamics, sympathetic nerve activity, and arterial diameter. *American Journal of Physiology-Heart and Circulatory Physiology*, 294 (2), H605-H612.

Spicuzza, L., Gabutti, A., Porta, C., Montano, N. and Bernardi, L., 2000. Yoga and chemoreflex response to hypoxia and hypercapnia. *The Lancet*, 356 (9240), 1495-1496.

Spradley, F. T., 2018. Sympathetic nervous system control of vascular function and blood pressure during pregnancy and preeclampsia. *Journal of Hypertension*, 36, 000-000.

Strömmer, S., Lawrence, W., Rose, T., Vogel, C., Watson, D., Bottell, J. N., Parmenter, J., Harvey, N. C., Cooper, C., Inskip, H., Baird, J. and Barker, M., 2018. Improving recruitment to clinical trials during pregnancy: A mixed methods investigation. *Social Science & Medicine*, 200, 73-82.

Stuart, J. J., Tanz, L. J., Missmer, S. A., Rimm, E. B., Spiegelman, D., James-Todd, T. M. and Rich-Edwards, J. W., 2018. Hypertensive disorders of pregnancy and maternal cardiovascular disease risk factor development: an observational cohort study. *Annals of Internal Medicine*, 169 (4), 224-232.

Tanaka, K., Kamihira, K., Minoura, F., Watanabe, M., Fujiyoshi, E., Nakamura, K. and Katafuchi, T., 2015. Postprandial decrease in vascular resistance correlated with change in second derivative of finger plethysmogram in young subjects. *Vasa: Zeitschrift für Gefaesskrankheiten*, 44 (1), 43.

Taylor, J. A. and Eckberg, D. L., 1996. Fundamental relations between short-term RR interval and arterial pressure oscillations in humans. *Circulation*, 93 (8), 1527-1532.

Terathongkum, S. and Pickler, R. H., 2004. Relationships among heart rate variability, hypertension, and relaxation techniques. *Journal of Vascular Nursing*, 22 (3), 78-82.

Toska, K. and Eriksen, M., 1993. Respiration-synchronous fluctuations in stroke volume, heart rate and arterial pressure in humans. *The Journal of Physiology*, 472 (1), 501-512.

Tranquilli, A. L., Dekker, G., Magee, L., Roberts, J., Sibai, B. M., Steyn, W., Zeeman, G. G. and Brown, M. A., 2014. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, 4 (2), 97-104.

Twigg, M. J., Lupattelli, A. and Nordeng, H., 2016. Women's beliefs about medication use during their pregnancy: a UK perspective. *International Journal of Clinical Pharmacy*, 38 (4), 968-976.

Ubolsakka-Jones, C., Sangthong, B., Khrisanapant, W. and Jones, D. A., 2017. The effect of slow-loaded breathing training on the blood pressure response to handgrip exercise in patients with isolated systolic hypertension. *Hypertension Research*, 40 (10), 885-891.

Ubolsakka-Jones, C., Tongdee, P., Pachirat, O. and Jones, D. A., 2018. Slow loaded breathing training improves blood pressure, lung capacity and arm exercise endurance for older people with treated and stable isolated systolic hypertension. *Experimental Gerontology*, 108, 48-53.

Ubolsakka-Jones, C., Tongdee, P. and Jones, D. A., 2019. The effects of slow loaded breathing training on exercise blood pressure in isolated systolic hypertension. *Physiotherapy Research International*, 24 (4), e1785.

van Delft, K., Schwertner-Tiepelmann, N., Thakar, R. and Sultan, A. H., 2013. Recruitment of pregnant women in research. *Journal of Obstetrics and Gynaecology*, 33 (5), 442-446.

van Hateren, K. J., Landman, G. W. and Kleefstra, N., 2015. Re: "RESPeRATE: the role of paced breathing in hypertension treatment". *Journal of the American Society of Hypertension*, 9 (8), 656-657.

Van Leeuwen, P., Geue, D., Lange, S., Cysarz, D., Bettermann, H. and Grönemeyer, D. H., 2003. Is there evidence of fetal-maternal heart rate synchronization? *BMC Physiology*, 3 (1), 2.

Van Leeuwen, P., Geue, D., Thiel, M., Cysarz, D., Lange, S., Romano, M. C., Wessel, N., Kurths, J. and Grönemeyer, D. H., 2009. Influence of paced maternal breathing on fetal–maternal heart rate coordination. *Proceedings of the National Academy of Sciences*, 106 (33), 13661-13666.

Van Lieshout, J. J., Toska, K., van Lieshout, E. J., Eriksen, M., Walløe, L. and Wesseling, K. H., 2003. Beat-to-beat noninvasive stroke volume from arterial pressure and Doppler ultrasound. *European Journal of Applied Physiology*, 90, 131-137.

Vargas, P. M. F., 2017. *Acute Cardiovascular Responses to Slow and Deep Breathing.* Thesis (PhD).Brunel University.

Vaschillo, E. G., Vaschillo, B. and Lehrer, P. M., 2006. Characteristics of resonance in heart rate variability stimulated by biofeedback. *Applied Psychophysiology and Biofeedback*, 31 (2), 129-142.

Vasundhara, V. R., Bhavanani, A. B., Ramanathan, M., Ghose, S. and Dayanidy, G., 2018. Immediate effect of Sukha Pranayama: A slow and deep breathing technique on maternal and fetal cardiovascular parameters. *Yoga Mimamsa*, 50 (2), 49.

Vemulapalli, M., Jones, T. N., Fonkoue, I. T., Kankam, M. L. and Park, J., 2019. Sympathetic and Cardiovascular Response to Device Guided Slow Breathing Acutely Depends on Post-Traumatic Stress Disorder (PTSD) Severity. *The FASEB Journal*, 33 (1_supplement), 562.510-562.510.

Viskoper, R., Shapira, I., Priluck, R., Mindlin, R., Chornia, L., Laszt, A., Dicker, D., Gavish, B. and Alter, A., 2003. Nonpharmacologic treatment of resistant hypertensives by device-guided slow breathing exercises. *American Journal of Hypertension*, 16 (6), 484-487.

Voss, A., Malberg, H., Schumann, A., Wessel, N., Walther, T., Stepan, H. and Faber, R., 2000. Baroreflex sensitivity, heart rate, and blood pressure variability in normal pregnancy. *American Journal of Hypertension*, 13 (11), 1218-1225.

Vranish, J. R. and Bailey, E. F., 2015. Daily respiratory training with large intrathoracic pressures, but not large lung volumes, lowers blood pressure in normotensive adults. *Respiratory Physiology & Neurobiology*, 216, 63-69.

Waaler, B. A. and Eriksen, M., 1992. Post-prandial cardiovascular responses in man after ingestion of carbohydrate, protein or fat. *Acta Physiologica Scandinavica*, 146 (3), 321-327.

Waaler, B. A., Eriksen, M. and Toska, K., 1991. The effect of meal size on postprandial increase in cardiac output. *Acta Physiologica Scandinavica*, 142 (1), 33-39.

Wallbach, M., Born, E., Kämpfer, D., Lüders, S., Müller, G. A., Wachter, R. and Koziolek, M. J., 2020. Long-term effects of baroreflex activation therapy: 2-year follow-up data of the BAT Neo system. *Clinical Research in Cardiology*, 109 (4), 513-522.

Wallbach, M. and Koziolek, M. J., 2018. Baroreceptors in the carotid and hypertension—systematic review and meta-analysis of the effects of baroreflex activation therapy on blood pressure. *Nephrology Dialysis Transplantation*, 33 (9), 1485-1493.

Wallin, B. G., Hart, E. C., Wehrwein, E. A., Charkoudian, N. and Joyner, M. J., 2010. Relationship between breathing and cardiovascular function at rest: sex-related differences. *Acta Physiologica*, 200 (2), 193-200.

Weissgerber, T. L. and Wolfe, L. A., 2006. Physiological adaptation in early human pregnancy: adaptation to balance maternal-fetal demands. *Applied Physiology, Nutrition, and Metabolism*, 31 (1), 1-11.

Wesseling, K. H., Jansen, J. R., Settels, J. J. and Schreuder, J. J., 1993. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *Journal of Applied Physiology*, 74 (5), 2566-2573.

Whelton, P. K., Carey, R. M., Aronow, W. S., Casey, D. E. J., Collins, K. J., Dennison Himmelfarb, C., DePalma, S. M., Gidding, S., Jamerson, K. A., Jones, D. W., MacLaughlin, E. J., Muntner, P., Ovbiagele, B., Smith, S. C. J., Spencer, C. C., Stafford, R. S., Taler, S. J., Thomas, R. J., Williams, K. A. S., Williamson, J. D. and Wright, J. T. J., 2018. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension*, 71, e13-e115.

World Health Organization, 2006. *Reproductive Health Indicators* [online]. Available from: http://www.ossyr.org.ar/pdf/bibliografia/2.22.pdf [Accessed 15/11/18].

World Health Organization, 2016. *WHO recommendations on antenatatl care for a positive pregnancy experience*.

Yepryntseva, I. A. and Shekh, V. E., 2019. Is the effect of slow breathing on blood pressure a function of prehypertension and body weight? *The FASEB Journal*, 33 (1_supplement), 533.538-533.538.

Young, Y., Abdolhosseini, P., Brown, F., Faulkner, J., Lambrick, D., Williams, M. A. and Stoner, L., 2015. Reliability of oscillometric central blood pressure and wave reflection readings: effects of posture and fasting. *Journal of Hypertension*, 33 (8), 1588-1593.

Zhang, Z., Wang, W., Wang, B., Wu, H., Ang, Q., Liu, H. and Zhang, Y., 2009. Cardiovascular variability analysis under gradually guided breathing protocol (pp. 1-4): IEEE.

Zimlichman, R., 2017. Response to the Letter from Landman et al. *Current Hypertension Reports*, 19 (8), 66.

Zou, Y., Zhao, X., Hou, Y.-Y., Liu, T., Wu, Q., Huang, Y.-H. and Wang, X.-H., 2017. Meta-Analysis of Effects of Voluntary Slow Breathing Exercises for Control of Heart Rate and Blood Pressure in Patients With Cardiovascular Diseases. *The American Journal of Cardiology*, 120 (1), 148-153.

Appendices

Appendix I: Research Outputs

Appendix Ia: Bournemouth University Doctoral College Live Exhibition (2018)

The Doctoral College Live Exhibition was an event at Bournemouth University (BU) to allow postgraduate researchers the opportunity to disseminate their research in new, creative and interactive ways to a wide audience.

The abstract below was associated with a live demonstration of the Brythm app and acute cardiovascular blood pressure responses using the Finapres machine.

Abstract

Acute Cardiovascular Responses to Slow and Deep Breathing in Healthy Females using BU's Brythm App

A breathing technique known as slow and deep breathing (SDB) has been shown to reduce high blood pressure through daily practice. The mechanisms by which SDB reduces blood pressure are not fully understood and the acute cardiovascular responses to SDB require further exploration. Potential mechanisms for decreasing blood pressure may be the within-breath changes in cardiovascular variables such as stroke volume and heart rate. Using an external pacing device to guide SDB is the most robust method of delivery, and previous research suggests that the optimal breathing frequency for induction of cardiovascular perturbation may vary between individuals. BU's Brythm App has a patent-pending algorithm that drives breathing frequency to a personalised optimum.

In this study, female participants breathed at different breathing frequencies that have been found to span the optimal frequency in men. In addition to exploring any gender differences, the study compared the relative magnitudes of the cardiovascular responses to different breathing frequencies, including the Brythm algorithm.

Appendix Ib: Physiology 2019 Conference

Abstract

Acute cardiovascular responses to slow and deep breathing in healthy women

M.L. Felton[1], V. Hundley[1], A.K. McConnell[1]

1. Faculty of Health & Social Sciences, Bournemouth University, Bournemouth, United Kingdom.

Background: Daily practice of device-guided slow breathing (DSB) has been shown to decrease blood pressure (Chaddha et al., 2019). However, there is still a lack of understanding of the mechanisms underpinning the improvements in blood pressure. Relatively few studies have characterised the acute cardiovascular responses to DSB, which hold the key to the mechanisms by which DSB might lower blood pressure. This study characterised the acute cardiovascular responses to different DSB protocols. **Methods:** Eighteen healthy, normotensive women completed five 5-minute protocols in randomised order: spontaneous breathing (SfR), fixed breathing frequencies of 4, 6 and 8 breaths.min-1 (4fR, 6fR, 8fR) and a dynamic breathing frequency (DfR) determined by an algorithm designed to maximise respiratory sinus arrhythmia (RSA). Cardiovascular variables and respiratory airflow were monitored continuously and non-invasively. Data are mean±SD, compared by repeated measures ANOVA with planned pairwise comparisons. **Results:** Average breathing frequency for DfR was 6.3±1.1 breaths.min-1. Mean heart rate was not significantly different between breathing protocols, but RSA increased significantly between SfR and all DSB protocols (SfR 0.12±0.05, 4fR 0.25±0.10, 6fR 0.25±0.08, 8fR 0.21±0.07, DfR 0.25±0.09 sec; $p<0.001$). The 'peak-valley' amplitude of intra-breath phase (inhalation vs exhalation) fluctuations of mean arterial pressure (MAP) were significantly different between 4fR and SfR, 6fR, 8fR and DfR (SfR 5.1±1.87, 4fR 7.8±11.81, 6fR 6.8±10.81, 8fR 9.1±2.38, DfR 4.8±11.30 mmHg; $p<0.001$). Peak MAP occurred during expiration for all protocols except 4fR. Intra-breath phase fluctuations also increased during DSB for stroke volume, cardiac output, systolic and diastolic blood pressure, and pulse wave velocity ($p<0.001$). **Conclusions:** DSB induces significant increases in intra-breath phase fluctuations of haemodynamic variables. It is conceivable that these acute haemodynamic perturbations generate error signal(s) for chronic regulation of blood pressure. Further research is required to understand the acute responses to DSB in people who have hypertension.

Acute Cardiovascular Responses to Slow and Deep Breathing in Healthy Women

***Malika Felton**, Vanora Hundley and Alison K. McConnell*
Faculty of Health & Social Sciences, Bournemouth University

Introduction

A breathing technique known as slow and deep breathing (SDB) has been shown to reduce high blood pressure through daily practice. The mechanisms by which SDB reduces blood pressure are not fully understood and the acute cardiovascular responses to SDB require further exploration.

Methodology

Eighteen females (age 30.1±8.8 years; stature 166±5.4cm; mass 65.6±10.3kg) participated in a SDB protocol consisting of 5 five-minute breathing protocols. Protocols were performed in a randomised order with five-minute recovery periods between each protocol (Figure 1). Arterial blood pressure, heart rate and respiratory airflow were monitored continuously and non-invasively (Figure 2).

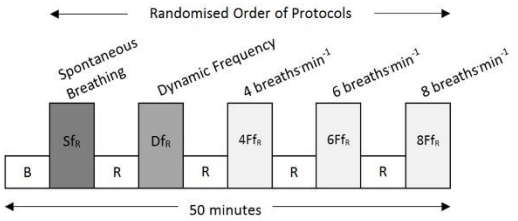


Figure 1: Schematic of protocol.
Spontaneous baseline breathing (B), recovery periods (R), uncontrolled spontaneous breathing (Sf_R), dynamic breathing frequency (Df_R), fixed breathing frequency of 4 ($4Ff_R$), 6 ($6Ff_R$) and 8 breaths·min^{-1} ($8Ff_R$).

Slow and Deep Breathing

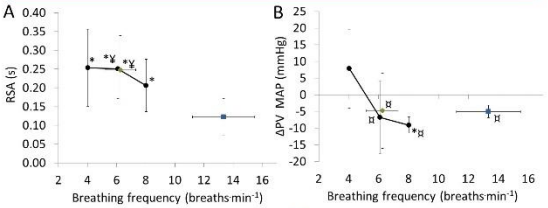
The SDB was delivered using a bespoke app, which provides visual feedback to guide breathing frequency. Participants inhale when the dome graphic rises and exhale when the dome falls (Figure 3). The app uses a patent-pending algorithm (Df_R) designed to maximise respiratory sinus arrhythmia (RSA), and can also deliver fixed breathing frequencies (Ff_R).

Figure 2: Equipment set up measuring arterial blood pressure, heart rate and respiratory airflow.

Figure 3: Screenshots of app feedback display
N.B. Arrows shown for illustrative purposes only and do not appear on app

Results

- RSA was significantly different from spontaneous breathing for all protocols ($p<0.001$) and between $8Ff_R$ and $6f_R$ and Df_R ($p<0.05$; Figure 4A).
- 'Peak-valley' amplitude of intra-breath (inhalation *vs.* exhalation) fluctuations of mean arterial pressure (MAP) was significantly different between protocols ($p<0.001$; Figure 4B).
- Within-breath variations increased significantly in a number of variables including stroke volume and systolic blood pressure for both inspiration and expiration ($p<0.05$; Figure 4C & 4D).



N.B. A&B; Spontaneous breathing (■), fixed breathing frequencies (●), dynamic frequency (♦); respiratory sinus arrhythmia (RSA), peak valley intra-breath mean arterial pressure variation (ΔPV MAP). Significant difference from Sf_R (*), $8Ff_R$ (¥), $4Ff_R$ (¤).

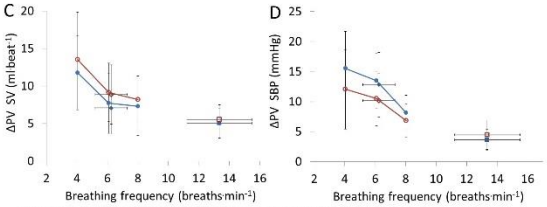


Figure 4: Cardiovascular response to SDB.
N.B. C&D: Inspiration (blue filled); expiration (red outline); within-breath peak-valley stroke volume variation (ΔPV SV), within-breath peak valley systolic blood pressure variation (ΔPV SBP).

Conclusion

SDB induces significant increases in intra-breath fluctuations of haemodynamic variables. It is conceivable that these acute haemodynamic perturbations generate error signal(s) for chronic regulation of blood pressure.

Contact Details

The Physiological Society

@MalikaFelton
mfelton@bournemouth.ac.uk

Appendix Ic: Bournemouth University Doctoral College Conference (2019)

The following abstract was presented as an oral presentation at Bournemouth University's 2019 Postgraduate Researcher Conference

Abstract

Cardiovascular Responses to Slow and Deep Breathing in Healthy Pregnant and Non-pregnant Women

Slow and deep breathing (SDB) causes immediate changes to cardiovascular variables (heart rate and blood pressure) but these are not fully understood. To understand how SDB can reduce blood pressure long-term through daily practice, we need to first understand the short-term responses. Pregnancy induces physiological changes that may affect how women respond to SDB and therefore it is important to include both pregnant and non-pregnant women.

Continuous heart rate and blood pressure were measured while women conducted a series of breathing exercises. SDB causes greater within-breath cardiovascular changes than breathing at a normal frequency (spontaneous breathing), e.g. increasing respiratory sinus arrhythmia. Responses were similar in both groups, but respiratory sinus arrhythmia was lower in pregnant women, with SDB increasing respiratory sinus arrhythmia to non-pregnant spontaneous breathing levels. Understanding the within-breath cardiovascular changes during SDB can be used to enhance clinical interventions to lower blood pressure in pregnant women.

Appendix Id: Bournemouth University Doctoral College Conference (2020)

The following abstract was presented as an oral presentation at Bournemouth University's 2020 Postgraduate Researcher Conference, which was presented virtually on Zoom due to the coronavirus pandemic.

Abstract

Adapting postgraduate research in the context of the coronavirus pandemic

Undertaking a PhD is an independent journey and no two people will have the same experience, although everyone will face both ups and downs. However, in 2020 we all share a common struggle, completing our research during a global pandemic. While the exact barriers coronavirus caused will differ between projects, we have all adapted and changed how we work, and even what we are researching. This presentation will discuss how I coped with the changes to my PhD, including the stopping of all clinical studies in the NHS. I will discuss the skills and experience I gained, while sharing my coping strategies. Ultimately, I would not have got to this stage without sharing my highs and lows with my peers so I hope this presentation shows that although a PhD can feel like a lonely journey that there is shared experience with others, and we can get through it together.

Appendix II: Participant information sheet

Appendix IIa: Chapter 4 participant information sheet

Participant Information Sheet

'Device guided slow breathing for the treatment of hypertension: Comparison of BU's Brythm App with an NHS approved device'

We are inviting BU staff and students to take part in a research project and you have been selected for invitation because you are part of the HSS Faculty and may have an interest in health research. Before you decide, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. The following people are involved in this study:

Contact	Position	Phone	Email
Professor Alison McConnell	Professor of Sport/Heath Science	01202 962313	amcconnell@bournemouth.ac.uk
Pedro Vargas	Research Project Manager	---	pedrovargas@sapo.pt
Malika Felton	PhD Student	01202 961845	mfelton@bournemouth.ac.uk

Study Background

Breathing exercises have long been used as part of yoga and meditation, claiming to aid relaxation. Research has more recently investigated the use of a breathing technique that employs a pacing device to guide slow and deep breathing (SDB) to test its effect on blood pressure.

Based on their research into the short-term effects of slow and deep breathing, researchers from FHSS have developed an App (Brythm), which guides breathing frequency to produce a personalised optimum (typically around 6 breaths per minute). The Brythm App now needs to be validated against an existing device that is already approved by the NHS and FDA.

Study Purpose

The purpose of this study is to investigate the acute physiological response to slow breathing delivered using the BU Brythm App against that of an NHS/FDA approved device (RESPeRATE®).

Key Requirements Summary

The key requirements to the study are outlined overleaf. If you are eligible, and after reading the key requirements are interested in finding out more about participating, you can find a more detailed protocol description in the 'Study Design' section below and on pages 5-6.

- You must be a non-smoker to participate in the study;
- If you have a previous medical history of any the following conditions you will not be able to participate in the study;
    - Cardiovascular or respiratory disease (e.g. asthma, bronchitis, COPD (chronic obstructive pulmonary disease));
    - An allergy or reaction to the conducting gel used for the ECG or for the ultrasound;
- The entire experiment will require a single visit of approximately 1 ½ - 2 hours;
- During the testing session we will collect some non-invasive cardiovascular and respiratory measurements, and you will learn how to control your breathing at a specific rhythm and depth, using a visual feedback device and an auditory feedback device;
- You will be asked to refrain from eating for 2 hours and from caffeine, strenuous exercise and alcohol for 12 hours prior to testing.

Study Design

If you choose to participate in this study you will be required to attend the BU Cardiorespiratory Research Laboratory (Bournemouth House) on one occasion for 1 ½ to 2 hours. Approximately 1 hour and 20 minutes of the whole session will be used for the data collection and will involve using the Brythm App and RESPeRATE® to breathe at different frequencies, in addition to a spontaneous breathing condition. The spontaneous breathing condition will involve you breathing normally with no restrictions, while we collect data for 10 minutes. The App will be installed on an iPad and you will not be required to use your own device. See Figure 1 for an example equipment set up from a previous study involving pregnant women.

Figure 1: Participant set up with equipment

Two of the slow and deep breathing conditions will be delivered using Brythm, the App developed at BU, and you will be asked to follow the visual feedback for 10 minutes for each condition. When using the App you will be instructed to inhale when the dome graphic rises and exhale when the dome falls (see Figure 2). You will be wearing a finger sensor throughout the testing that connects to the headphone socket of the tablet on which the App is installed (see Figure 3). You will be asked to follow a set breathing frequency of 6 breaths per minute, and a dynamic frequency determined by the App (this will likely fall within 4-8 breaths per minute).

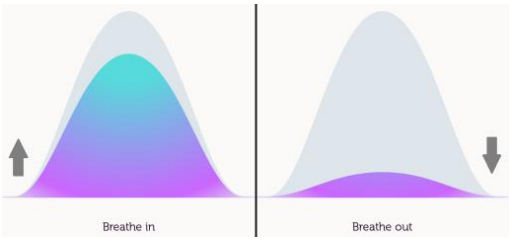


Figure 2: Screenshot of Brythm App Feedback Display.Figure 3: Brythm App Finger Sensor
N.B. Arrows shown for illustrative purposes only and do not appear on App

The final condition will be 10-minutes of controlled breathing using the NHS/FDA approved device, RESPeRATE®, which guides your breathing frequency using an auditory tone (Figure 4). You will be asked to wear a breathing sensor either across your chest, or your abdomen, depending on how you breathe (Figure 4 shows abdomen position). You will wear headphones and be asked to breathe in time with the auditory tones; a short high note is heard for start of inhalation and a short low note is heard for start of exhalation. RESPeRATE® aims to lower your breathing frequency to the 'Therapeutic Breathing Zone' of less than 10 breaths per minute.

Figure 4: RESPeRATE® device

There will be a 10-minute break between each breathing condition, where you can breathe normally, and take off the mask if you wish. Your normal breathing frequency is around 12 breaths per minute, so you will be asked to breathe at around half the normal rate. This is not as difficult as you might think, because the reduction in breathing rate is compensated by increasing breathing depth, which your body will do automatically. You will be given an opportunity to practice this before the study begins. A visual overview of the protocol is provided in Figure 5.

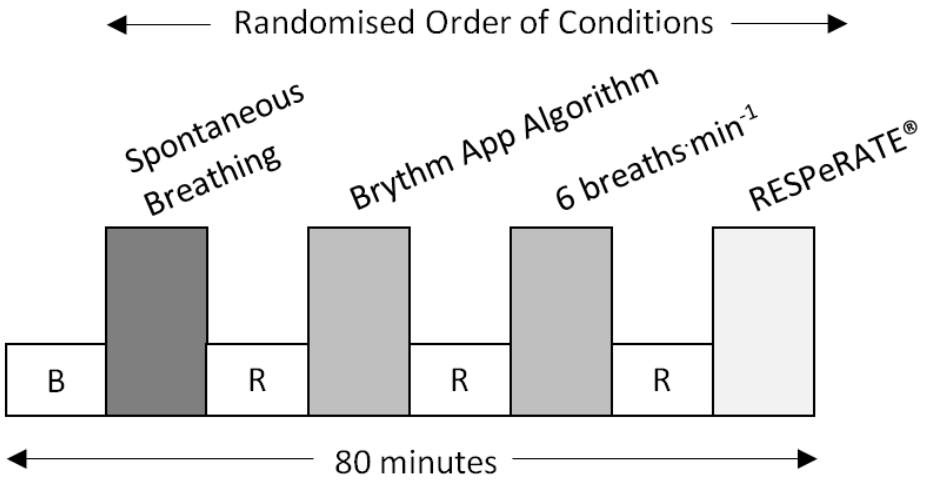


Figure 5: Schematic of breathing protocol

Each block is 10-minutes in duration, including spontaneous baseline breathing (*B*) and all rest periods (*R*) of unrestricted breathing between each condition.

You will be randomly assigned the order of breathing frequency conditions; Spontaneous Breathing, Brythm App Algorithm, Brythm App 6 breaths per minute and RESPeRATE®.

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

Your participation in this research is entirely voluntary and it is up to you to decide whether or not to take part. You will be given this information sheet to keep and if you do decide to take part, you will be asked to sign a participant agreement form. You can withdraw at any time, up to the point where the data are processed and become anonymous, so your identity cannot be determined, without it affecting any benefits that you are entitled to in any way. You do not have to give a reason. Deciding to take part or not will not impact upon/adversely affect your employment or education/studies (or that of others).

What are the possible disadvantages and risks of taking part?

Since you will be breathing at a lower breathing frequency than your usual spontaneous breathing frequency, you may experience sensations of heat, sweating and 'flushing' and a strong urge to breath more. This is quite normal and does not present any known risk to your safety. In the unlikely event that you feel too uncomfortable, you are free to interrupt the procedure and remove the mask. The sensations subside quickly once spontaneous breathing is resumed.

What will I get in return?

You will get information about your current blood pressure. You will also be provided with a report of your test results at the end of the study and learn more about how breathing affects your blood pressure. No financial compensation will be given for participating in this study.

What will happen to me if I take part?

As stated in the 'Study Design' section you will perform a total of 40 minutes of controlled breathing (including the 10 minutes of unrestricted spontaneous breathing, see Figure 5), during which we will carry out a number of physiological measurements. These measurements are described below.

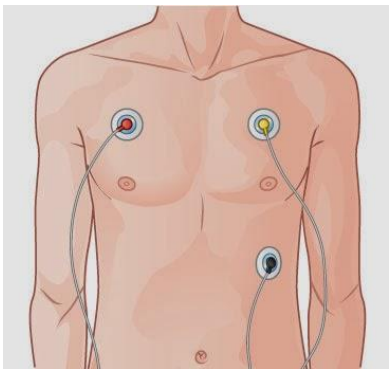
Pre-test requirements

Upon arrival, you will first need to complete a health questionnaire and sign a consent form to confirm that you're healthy and able to participate in the study. Your height and weight will also be measured at this stage; this will not require you to remove any clothes except your shoes.

Physiological measurement

During the controlled breathing you will be asked to wear a mask to measure breathing patterns, which covers your mouth and nose but allows you to breathe normally through both (see Figure 1). 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 (see Figure 6). The silver box is secured on the wrist, but does not take any measurements from your wrist. You will also have a finger sensor attached on the same hand (green sensor in Figure 6).

Figure 6 (left): The finger cuff used for the continuous measurement of arterial blood pressure



Heart rate will be measured continuously using a 3-lead electrocardiogram (ECG). Sensors will be placed on your chest; 2 sensors just below each of your clavicles and 1 sensor placed on the lower ribs (see Figure 7). These will be placed under your clothing and you will not need to remove any items of clothing for attach

Figure 7 (above): Placement sites for the 3-lead ECG electrodes

Will my taking part in this study be kept confidential and what will happen to the results of the research study?

All the information we collect about you during the course of the research will be kept strictly in accordance with current Data Protection Regulations. 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 kept for 5 years after publication on a BU password protected secure network and will not be released without written permission from yourself or unless required by law. The information collected about you may be used in an anonymous form to support other research projects in the future and access to it in this form will not be restricted. It will not be possible for you to be identified from this data.

What type of information will be sought from me and why is the collection of this information relevant for achieving the research project's objectives?

Prior to taking part in the study you will be required to fill in a health check questionnaire. This information is paramount to our research as your health status might show that you meet one of our exclusion criteria and cannot participate in this study. Also, a number of individual characteristics (age, ethnicity, current fitness level, medication, etc.), as well as some health conditions are known to impact several cardiovascular variables being measured in this study.

Who is organising and funding the research?

The research is being organised by the Faculty of Health and Social Sciences at Bournemouth University as part of pump-priming funding.

What if something goes wrong?

The procedures involved in this study are extremely low risk, requiring participants to do nothing more than to sit, and to breathe slowly and deeply, in a relaxed way. Nonetheless, an emergency name and contact telephone number must be provided by all participants in the health check questionnaire. **You can find the researchers' contact details at the beginning of this participant information sheet.**

In case of complaints you can contact the Deputy Dean of Research and Professional Practice, Professor Vanora Hundley, as an independent member of BU Staff, by email at researchgovernance@bournemouth.ac.uk.

If you have any questions regarding this research project, please contact:

Malika Felton
Faculty of Health and Social Sciences
Bournemouth University
Room 305, Royal London House
Christchurch Road
Bournemouth, BH1 3LT
Phone: 01202 961845
Email: mfelton@bournemouth.ac.uk

Thank you for considering taking part in this research project.

Appendix IIb: Chapter 5 participant information sheet

Participant Information Sheet

'Acute effects of slow and deep breathing upon healthy young women'

We are inviting women of reproductive age (18-49 years) to take part in a research project and you have been selected for invitation because you are part of the HSS Faculty and may have an interest in health research. Before you decide, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. The following people are involved in this study:

Contact	Position	Phone	Email
Malika Felton	PhD Student	01202 961845	mfelton@bournemouth.ac.uk
Professor Alison McConnell	Professor of Sport/Heath Science	01202 962313	amcconnell@bournemouth.ac.uk
Professor Vanora Hundley	Deputy Dean of Research And Professional Practice, Health and Social Sciences	01202 965206	vhundley@bournemouth.ac.uk
Warren Foster	Deputy Director of AECC University College's School of Medical Ultrasound	01202 436504	wfoster@aecc.ac.uk

This research investigation has been reviewed in line with Bournemouth University's Research Ethics Code of Practice. Approval #: *Ethics ID 19148.*

Study Background

Breathing exercises have long been used as part of yoga and meditation, claiming to aid relaxation. Research has more recently investigated the use of a breathing technique that employs a pacing device to guide slow and deep breathing (SDB) to test its effect on blood pressure. SDB has an immediate impact on the cardiovascular system, such as blood pressure and heart rate. However, a complete understanding of the way in which SDB affects long term blood pressure has not been achieved and further research is needed.

Using an external pacing device to guide SDB is the most robust method of delivery, and normally involves reducing your breathing to approximately 6 breaths per minute, which is around half normal breathing frequency. However, recent research found that the optimal breathing frequency to produce the maximum immediate changes in

cardiovascular variables is unique to each person. This research was undertaken with healthy young men, and the next stage of the research is to test the responses to SDB of healthy young women.

Study Purpose

The ultimate aim of this programme of research is to assess the effects of daily SDB exercises upon long term blood pressure in pregnant women who have developed pregnancy-induced high blood pressure. The first step towards achieving this aim is to test the immediate effects of different SDB frequencies with healthy young women. This will allow us to identify the most effective breathing method(s), before moving on to test the effects in pregnant women.

Key Requirements Summary

The key requirements to the study are outlined below. If you are eligible, and after reading the key requirements are interested in finding out more about participating, you can find a more detailed protocol description in the 'Study Design' section below and on pages 5-6.

- You must be a female non-smoker of reproductive age (18-49 years) and not currently pregnant to participate in the study;
- If you have a previous medical history of any the following conditions you will not be able to participate in the study;
    - Cardiovascular or respiratory disease (e.g. asthma, bronchitis, COPD (chronic obstructive pulmonary disease));
    - An allergy or reaction to the conducting gel used for the ECG or for the ultrasound;
    - Spontaneous collapsed lung (pneumothorax), or a recent traumatic pneumothorax;
    - Known or suspected eardrum rupture, or other middle ear conditions;
    - Current sinus infection (participation is allowed once this condition has been resolved).
- The entire experiment will require a single visit of approximately 1 ½ hours;
- During the testing session we will collect some non-invasive cardiovascular and respiratory measurements, and you will learn how to control your breathing at a specific rhythm and depth, using a visual feedback device;
- You will be asked to refrain from eating for 2 hours and from caffeine, strenuous exercise and alcohol for 12 hours prior to testing;
- We ask that you attend the session in appropriate clothing that allows access to the abdominal region for the ultrasound measurements (see Figure 8). You will only need to roll up your top and will not need to remove any clothing for any measurements.

Study Design

If you choose to participate in this study you will be required to attend the BU Cardiorespiratory Research Laboratory (Bournemouth House) on one occasion. Approximately 1 hour of the whole 1 ½ session will be used for the data collection and

will involve using an App to breathe at different frequencies, in addition to a spontaneous breathing condition. The spontaneous breathing condition will involve you breathing normally with no restrictions, while we collect data. The App will be installed on an iPad and you will not be required to use your own device. See Figure 1 for an example equipment set up from a previous study involving pregnant women.

Figure 1: Participant set up with equipment

The slow and deep breathing will be delivered using an App developed at BU and you will be asked to follow the visual feedback for 5 different conditions, for 5 minutes each. There will be a 5-minute break between each breathing condition, where you can breathe normally, and take off the mask if you wish. When using the App you will be instructed to inhale when the dome graphic rises and exhale when the dome falls (see Figure 2). You will be wearing a finger sensor throughout the testing that connects to the headphone socket of the tablet on which the App is installed (see Figure 3).

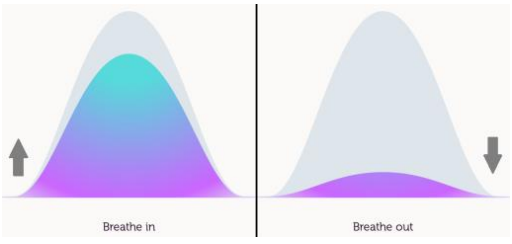


Figure 2: Screenshot of Brythm App Feedback Display. Figure 3: Brythm App Finger Sensor

N.B. Arrows shown for illustrative purposes only and do not appear on App

You will be asked to follow breathing frequencies of 4, 6 and 8 breaths per minute and a dynamic frequency determined by the App (this will likely fall within the range of breathing frequencies above). The final condition will be 6 breaths per minute with a small

resistance during inhalation (roughly equivalent to breathing through one nostril). This resistance will be provided by a medical device (POWERbreathe Medic; see Figure 4).

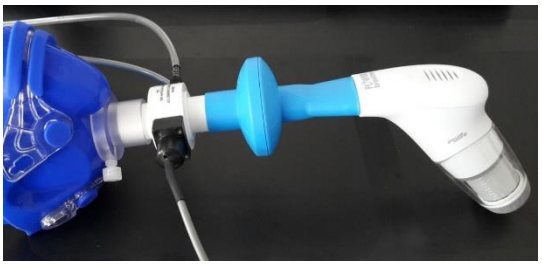


Figure 4: Inspiratory Resistance added to mouthpiece using POWERbreathe Medic

Your normal breathing frequency is around 12 breaths per minute, so you will be asked to breathe at around half the normal rate. This is not as difficult as you might think, because the reduction in breathing rate is compensated by increasing breathing depth, which your body will do automatically. You will be given an opportunity to practice this before the study begins. A visual overview of the protocol is provided in Figure 5.

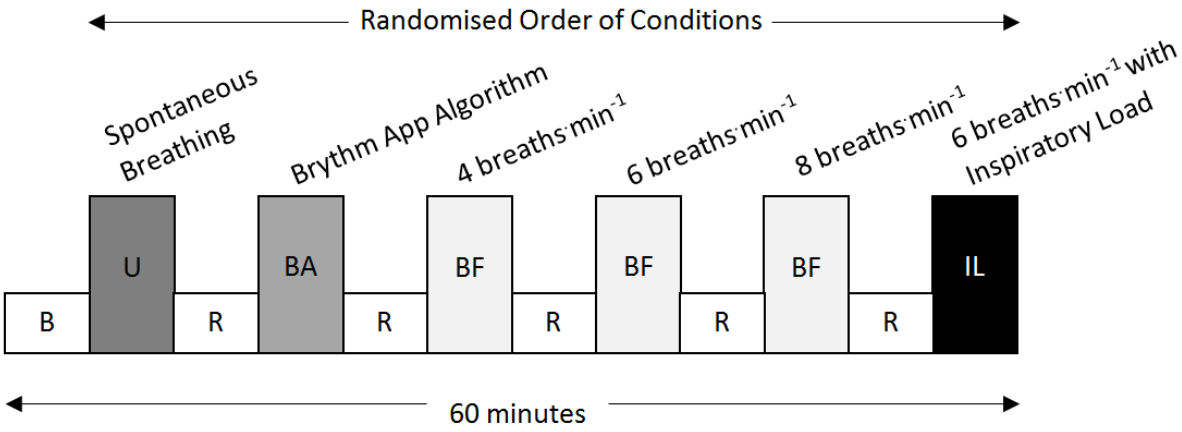


Figure 5: Visual overview of the protocol for breathing frequencies

Each block is 5-minutes in duration, including spontaneous baseline breathing (*B*) and all rest periods (*R*) of unrestricted breathing between each condition.
The order of breathing conditions will be assigned randomly at the start of the testing session;
U – Uncontrolled spontaneous breathing; *BA* – App Algorithm; *BF* – Fixed Breathing Frequency; *IL* – Inspiratory Load

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

Your participation in this research is entirely voluntary and it is up to you to decide whether or not to take part. You will be given this information sheet to keep and if you do decide to take part, you will be asked to sign a participant agreement form. You can withdraw at any time, up to the point where the data are processed and become anonymous, so your identity cannot be determined, without it affecting any benefits that you are entitled to in any way. You do not have to give a reason. Deciding to take part or not will not impact upon/adversely affect your employment or education/studies (or that of others).

What are the possible disadvantages and risks of taking part?

Since you will be breathing at a lower breathing frequency than your usual spontaneous breathing frequency, you may experience sensations of heat, sweating and 'flushing' and a strong urge to breath more. This is quite normal and does not present any known risk

to your safety. In the unlikely event that you feel too uncomfortable, you are free to interrupt the procedure and remove the mask. The sensations subside quickly once spontaneous breathing is resumed.

What will I get in return?

You will get information about your current blood pressure. You will also be provided with a report of your test results at the end of the study and learn more about how breathing affects your blood pressure. No financial compensation will be given for participating in this study.

What will happen to me if I take part?

As stated in the 'Study Design' section you will perform a total of 30 minutes of controlled breathing (including the 5 minutes of unrestricted spontaneous breathing, see Figure 5), during which we will carry out a number of physiological measurements. These measurements are described below:

Pre-test requirements

Upon arrival, you will first need to complete a health questionnaire and sign a consent form to confirm that you're healthy and able to participate in the study. Your height and weight will also be measured at this stage; this will not require you to remove any clothes except your shoes. Finally, you will be asked to tell us about your menstrual cycle stage and whether you are taking oral contraceptives, as these factors may influence the test results.

Physiological measurement

During the controlled breathing you will be asked to wear a mask to measure breathing patterns, which covers your mouth and nose but allows you to breathe normally through both (see Figure 1). 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 (see Figure 6). The silver box is secured on the wrist, but does not take any measurements from your wrist. You will also have a finger sensor attached on the same hand (green sensor in Figure 6).

Figure 6: The finger cuff used for the continuous measurement of arterial blood pressure

Heart rate will be measured continuously using a 3-lead electrocardiogram (ECG). Sensors will be placed on your chest; 2 sensors just below each of your clavicles and 1 sensor placed on the lower ribs (see Figure 7). These will be placed under your clothing and you will not need to remove any items of clothing for attachment.

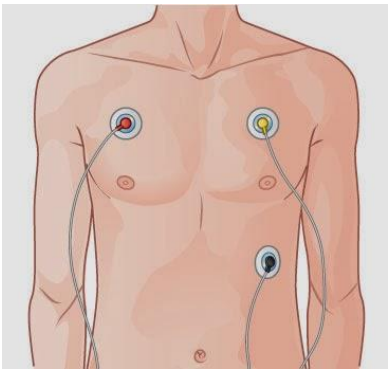


Figure 7: Placement sites for the 3-lead ECG electrodes

Following the completion of each 5-minute breathing condition we will perform an ultrasound measurement of your kidney blood flow (see Figure 8), which will involve seconds only.

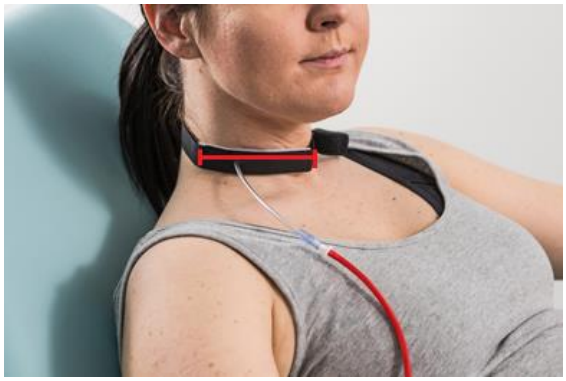


Figure 8 (right): Ultrasound probe position for kidney blood flow measurement

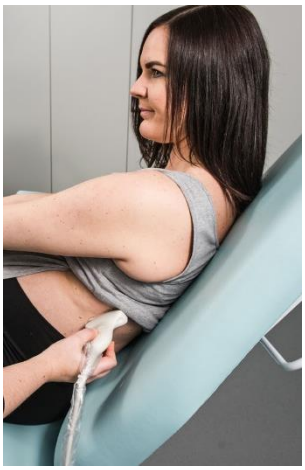


Figure 9 (left): Neck cuff positioning and inflation bladder length (show in red)

An additional measure of blood pressure will also be made using the arm cuff and additional cuffs placed on your upper leg and neck (see Figure 9). The neck cuff is placed loosely around the neck, with a 1 finger gap between the cuff and the neck, and therefore is not tight. The air bladder only inflates for the length shown in red in Figure 9, i.e. not around the whole neck.

Will my taking part in this study be kept confidential and what will happen to the results of the research study?

All the information we collect about you during the course of the research will be kept strictly in accordance with current Data Protection Regulations. 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 kept for 5 years either from the date of publication or after the award of the PhD, whichever is later, on a BU password protected secure network and will not be released without written permission from yourself or unless required by law. The information collected about you may be used in an anonymous form to support other research projects in the future and access to it in this form will not be restricted. It will not be possible for you to be identified from this data.

What type of information will be sought from me and why is the collection of this information relevant for achieving the research project's objectives?

Prior to taking part in the study you will be required to fill in a health check questionnaire, and you will be asked to tell us about your menstrual cycle stage and whether you are taking oral contraceptives. This information is paramount to our research as your health status might show that you meet one of our exclusion criteria and cannot participate in this study.

Also, a number of individual characteristics (age, menstrual cycle, ethnicity, current fitness level, medication, etc.), as well as some health conditions are known to impact several cardiovascular variables being measured in this study.

Who is organising and funding the research?

The research is being organised by the Faculty of Health and Social Sciences at Bournemouth University as part of the PhD doctoral studies of Malika Felton.

What if something goes wrong?

The procedures involved in this study are extremely low risk, requiring participants to do nothing more than to sit, and to breathe slowly and deeply, in a relaxed way. Nonetheless, an emergency name and contact telephone number must be provided by all participants in the health check questionnaire. **You can find the researchers' contact details at the beginning of this participant information sheet.**

In case of complaints you can contact the Acting Dean of the Faculty of Health & Social Sciences, Professor Elizabeth Rosser, as an independent member of BU Staff, by email at researchgovernance@bournemouth.ac.uk.

If you have any questions regarding this research project, please contact:

Malika Felton
Faculty of Health and Social Sciences
Bournemouth University
Room 305, Royal London House
Christchurch Road
Bournemouth, BH1 3LT
Phone: 01202 961845
Email: mfelton@bournemouth.ac.uk

Thank you for considering taking part in this research project.

Appendix IIc: Chapter 6 participant information sheet

Participant Information Sheet

'Acute effects of slow and deep breathing upon pregnant women'

We are inviting women who are over 20 weeks pregnant with their first pregnancy to take part in a research study. The study is part of a larger project to test the effect of slow and deep breathing upon blood pressure with pregnant women. Before you decide, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

This research investigation has been reviewed in line with Bournemouth University's Research Ethics Code of Practice (*Ethics ID 22930).*

Study Background & Purpose

Breathing exercises have long been used as part of yoga and meditation, claiming to aid relaxation, and are often encouraged during pregnancy. Research has recently investigated the use of a breathing pacing device to test the effect of slow and deep breathing (SDB) upon blood pressure. SDB has an immediate impact on the cardiovascular system, such as blood pressure and heart rate, but the responses have not been studied with pregnant women.

The ultimate aim of this programme of research is to assess the effects of daily SDB exercises upon long term blood pressure in pregnant women who have developed pregnancy-induced high blood pressure. The first step towards achieving this aim is to test the immediate effects of different SDB frequencies with pregnant women who do not have hypertension (high blood pressure). This will allow us to identify the most effective breathing method(s), before moving on to test the effects with women who have developed pregnancy-induced hypertension.

Key Requirements Summary

The key requirements to the study are outlined below. If you are eligible, and after reading the key requirements are interested in finding out more about participating, you can find a more detailed protocol description in the 'Study Design' section below.

- You must be currently pregnant with your first pregnancy and be over 20 weeks gestation;
- You must be carrying a singleton pregnancy (**not** twins, triplets, etc.);
- You must be aged 18 or over and a non-smoker;
- If you have a current medical diagnosis of any of the following conditions you will not be able to participate in the study;
    - Hypertension, pregnancy-induced hypertension or preeclampsia;
    - Cardiovascular or respiratory disease (e.g. asthma, bronchitis, COPD [chronic obstructive pulmonary disease]);
    - An allergy or reaction to the conducting gel used for the ECG;

- The project will require a single visit to Bournemouth University of approximately 1 ½ hours;
- During the data collection session we will collect some non-invasive cardiovascular and respiratory measurements, and you will learn how to control your breathing at a specific rhythm and depth, using a visual feedback device;
- After participation in the data collection session you will be asked to submit your blood pressure measurements taken at your antenatal sessions;
- You will be asked to refrain from eating for 2 hours and from caffeine, strenuous exercise and alcohol for 12 hours prior to the data collection session. You are not required to refrain from drinking during this time;
- We ask that you attend the session in appropriate clothing that allows access to the abdominal region for the ECG attachment (see Figure 6).

Study Design

If you choose to participate in this study you will be required to attend the Bournemouth University (BU) Cardiorespiratory Research Laboratory (Bournemouth House, Lansdowne Campus) on one occasion. Approximately 50 minutes of the whole 1 ½ hour session will be used for the data collection and will involve using an App to guide your breathing at different frequencies, as well as monitoring you during spontaneous breathing. You will be given a break at the mid-way point of the data collection to allow a bathroom break or for you to get up and move around. The spontaneous breathing protocol will involve you breathing normally with no pacing, while we collect data. The App will be installed on a laboratory iPad and you will not be required to use your own device. See Figure 1 for example equipment set up.

Figure 1: Participant set up with equipment

The slow and deep breathing will be delivered using an App developed at BU and you will be asked to follow the visual feedback for 4 different breathing protocols, for 5 minutes each. There will be a 5-minute break between each breathing protocol, where you can breathe normally, and take off the mask if you wish. When using the App you will be instructed to inhale when the dome graphic rises and exhale when the dome falls (see Figure 2). You will be wearing a finger sensor throughout that connects to the headphone socket of the tablet on which the App is installed (see Figure 3).

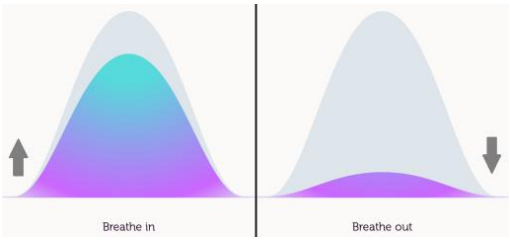


Figure 2: Screenshot of Brythm App Feedback Display Figure 3: Brythm App Finger Sensor
N.B. Arrows shown for illustrative purposes only and do not appear on App

You will be asked to follow breathing frequencies of 4, 6 and 8 breaths per minute and a dynamic frequency determined by the App (this will likely fall within the range of breathing frequencies above and for non-pregnant females was on average 6.2 breaths per minute). Normal breathing frequency is around 12 breaths per minute, so you will be asked to breathe at around half the normal rate. This is not as difficult as you might think, because the reduction in breathing rate is compensated by increasing breathing depth, which your body will do automatically. You will be given an opportunity to practice this before the study begins. A visual overview of the protocol is provided in Figure 4.

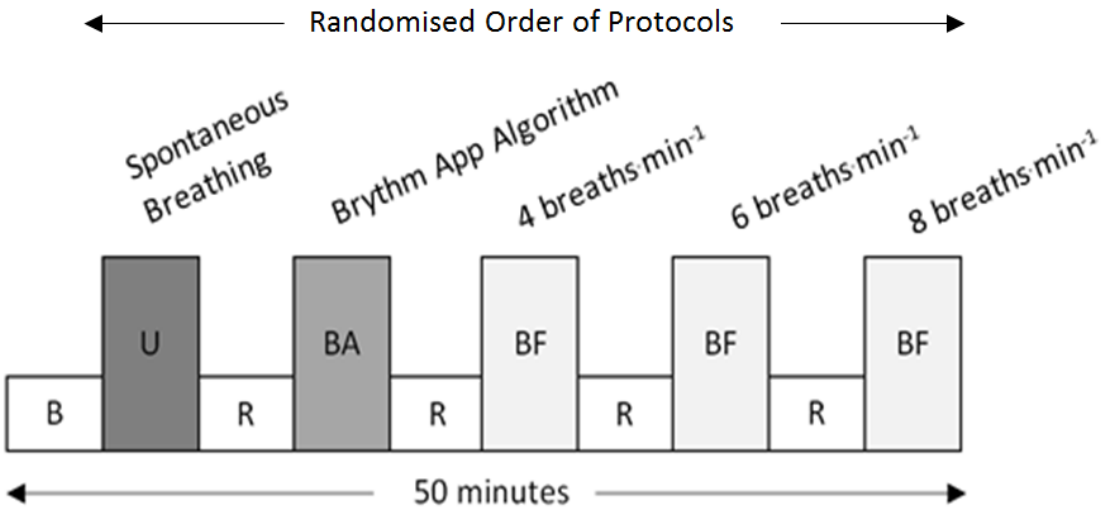


Figure 4: Visual overview of the protocol for breathing frequencies

Each block is 5-minutes in duration, including spontaneous baseline breathing (*B*) and all rest periods (*R*) of unrestricted breathing between each protocol.
The order of breathing protocols will be assigned randomly at the start of the data collection session and you will be informed of the order before you start.
U – Uncontrolled spontaneous breathing; *BA* – Brythm App Algorithm; *BF* – Fixed Breathing Frequency.

What will happen to me if I take part?

You will perform a total of 25 minutes of breathing protocols (including the 5 minutes of unrestricted spontaneous breathing, see Figure 4), during which we will carry out a number of physiological measurements. These measurements are described below:

Pre-data collection requirements

Upon arrival, you will first need to complete a health questionnaire and sign a consent form to confirm that you're healthy and able to participate in the study. You will complete a form with your contact details and contact preference to allow us to contact you after the data collection session (see post-data collection section below). Your height and weight will also be measured at this stage; this will not require you to remove any clothing except your shoes.

Physiological measurement

During the breathing protocols you will be asked to wear a mask to measure breathing patterns, which covers your mouth and nose but allows you to breathe normally through both (see Figure 1). The mask can be taken off between breathing protocols in the 5-minute rest periods. 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 (see Figure 5). The silver box is secured on the wrist, but does not take any measurements from your wrist. You will also have a finger sensor attached on the same hand (green sensor in Figure 5).

Figure 5: The finger cuff used for the continuous measurement of arterial blood pressure

Heart rate will be measured continuously using a 3-lead electrocardiogram (ECG). Sensors will be placed on your chest; 2 sensors just below each of your clavicles and 1 sensor placed on the lower ribs (see Figure 6). These will be placed under your clothing and you will not need to remove any items of clothing for attachment. ECG monitoring is safe to have while pregnant, with no known risks.

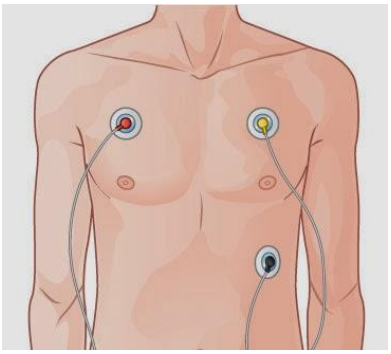


Figure 6: Placement sites for the 3-lead ECG electrodes

Post Data Collection

After participating in the data collection session, you will be asked to submit your blood pressure measurements that are taken during your antenatal appointments, as recorded in your maternity record notes. You will be provided with a link to a secure online form to

submit these measures. Alternatively, you can request to be sent a paper template and send this back to us in the stamped envelope provided. You will be contacted at 29 and 36 weeks and after your due date to remind you to complete the form. You will have a choice on how to be contacted, either by phone, text, e-mail or post and can indicate your preference on the contact form before participating in the study. We ask for this blood pressure information so we can monitor your blood pressure throughout your pregnancy.

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

Your participation in this research is entirely voluntary and it is up to you to decide whether or not to take part. You will be given this information sheet to keep and if you do decide to take part, you will be asked to sign a participant agreement form. You can withdraw from participation during the data collection session at any time and without giving a reason., If you decided to withdraw we will usually remove any data collected about you from the study. Once the data collection session has finished you may still be able to withdraw your data up to the point where the data is analysed and incorporated into the research findings or outputs. At this point your data will usually become anonymous, so your identity cannot be determined, and it may not be possible to identify your data within the anonymous dataset. Withdrawing your data at this point may also adversely affect the validity and integrity of the research. You can withdraw from being contacted regarding your ongoing blood pressure measurements by contacting an investigator. Deciding to take part or not will not impact upon/adversely affect your treatment, care or access to other services.

What are the possible disadvantages and risks of taking part?

Since you will be breathing at a lower breathing frequency than your usual spontaneous breathing frequency, you may experience sensations of heat, sweating and 'flushing' and a strong urge to breath more. This is uncommon but a normal response to slow and deep breathing, and does not present any known risk to your safety or the pregnancy. In the unlikely event that you feel uncomfortable, you are free to interrupt the protocol and remove the mask. The sensations subside quickly once spontaneous breathing is resumed.

What will I get in return?

You will get information about your current blood pressure, in the form of graphical representation of your antenatal blood pressure measurements. You will be assisting in a research project which is looking for a potential alternative treatment method for high blood pressure during pregnancy. No financial compensation will be given for participating in this study.

How will my information be kept?

All the information we collect about you during the course of the research will be kept strictly in accordance with current data protection legislation. Research is a task that we perform in the public interest, as part of our core function as a university. Bournemouth University (BU) is a Data Controller of your information which means that we are responsible for looking after your information and using it appropriately. BU's

Research Participant Privacy Notice sets out more information about how we fulfil our responsibilities as a data controller and about your rights as an individual under the data protection legislation. We ask you to read this Notice so that you can fully understand the basis on which we will process your information (www.bournemouth.ac.uk/about/governance/access-information/data-protection-privacy/research-participant-privacy-notice).

Publication
You will not be able to be identified in any external reports or publications about the research without your specific consent. Otherwise your information will only be included in these materials in an anonymous form, i.e. you will not be identifiable.

Security and access controls
BU will hold the information we collect about you in hard copy in a secure location and on a BU password protected secure network where held electronically. Except where it has been anonymised your personal information will be accessed and used only by appropriate, authorised individuals and when this is necessary for the purposes of the research or another purpose identified in the Privacy Notice. This may include giving access to BU staff or others responsible for monitoring and/or audit of the study, who need to ensure that the research is complying with applicable regulations.

Sharing and further use of your personal information
The information collected about you may be used in an anonymous form to support other research projects in the future and access to it in this form will not be restricted. It will not be possible for you to be identified from this data. Anonymised data will be added to BU's Data Repository (a central location where data is stored) and which will be publicly available.

Retention of your data
All personal data collected for the purposes of this study will be held for 5 years either from the date of publication of the research or after the award of the PhD, whichever is later. Although published research outputs are anonymised, we need to retain underlying data collected for the study in a non-anonymised form for a certain period to enable the research to be audited and/or to enable the research findings to be verified.

What type of information will be sought from me and why is the collection of this information relevant for achieving the research project's objectives?

Prior to taking part in the study you will be required to fill in a health check questionnaire. This information is paramount to our research as your health status might show that you meet one of our exclusion criteria and cannot participate in this study. We ask for your due date so that we know when to contact you to remind you to submit your blood pressure measurements. We need to monitor your blood pressure to ensure that all participants in the study maintain normal blood pressure levels throughout their pregnancy.

Physiological changes caused by pregnancy-induced hypertension (PIH) and preeclampsia could affect the study results, as we do not yet know if these conditions affect the responses to slow and deep breathing. If you are diagnosed with PIH or

preeclampsia then your results will be removed from the main analysis but your data may still be used as part of a small subsection data analysis.

Who is organising and funding the research?

The research is being organised by the Faculty of Health and Social Sciences at Bournemouth University as part of the PhD doctoral studies of Malika Felton.

What if something goes wrong?

The procedures involved in this study are extremely low risk, requiring participants to do nothing more than to sit, and to breathe slowly and deeply, in a relaxed way. Nonetheless, an emergency name and contact telephone number must be provided by all participants in the health check questionnaire. **You can contact any of the research team regarding any queries using the contact details below.**

In case of complaints you can contact the Executive Dean of the Faculty of Health & Social Sciences, Professor Stephen Tee, as an independent member of BU Staff, by email at researchgovernance@bournemouth.ac.uk.

If you have any questions or would like further information regarding this research project, please contact the main investigator:

Malika Felton
Bournemouth University, Room 305, Royal London House, Christchurch Road, Bournemouth, BH1 3LT
Phone: 01202 961845
Email: mfelton@bournemouth.ac.uk

Contact	Position	Phone	Email
Professor Alison McConnell	Professor of Sport/Heath Science	01202 962313	amcconnell@bournemouth.ac.uk
Professor Vanora Hundley	Deputy Dean of Research And Professional Practice, Health and Social Sciences	01202 965206	vhundley@bournemouth.ac.uk

Thank you for considering taking part in this research project.

Appendix IId: Chapter 8 participant information sheet

Participant Information Sheet (PIS)

Effects of slow and deep breathing (SDB) on reducing obstetric intervention in women with pregnancy-induced hypertension (PIH): A feasibility study

Introduction

We are inviting pregnant women with high blood pressure (pregnancy-induced hypertension; PIH) to take part in our research study. Before you decide, it is important you understand why the research is being completed and what it will involve. Please read this information carefully and discuss it with others. Ask us if there is anything that is unclear or if you would like more information. Take time to decide whether or not you wish to take part.

Study Background & Purpose

Breathing exercises have long been used as part of yoga and meditation to aid relaxation during pregnancy. Slow and deep breathing immediately changes blood pressure and heart rate. It can also reduce blood pressure in the long-term when practiced daily. Using a video graphic is the easiest way to guide breathing to a slower rate, but to date this hasn't been used with pregnant women.

Many women do not want to take medications during pregnancy. This means there is an urgent need for new and alternative ways of treating PIH (high blood pressure during pregnancy). Slow and deep breathing has produced promising but mixed results in other groups with high blood pressure. However, we believe that slow and deep breathing may be more effective on PIH than other types of high blood pressure. This is because pregnancy affects women's breathing, which may be a reason for PIH.

This study is the first stage of a project using slow and deep breathing with pregnant women. We will be looking at how the study processes work in practice. We will assess whether we could use them in a future larger trial. We will look at the number of women taking part in the study and how often they complete the breathing exercises. The purpose of this study is to look at whether using slow and deep breathing with pregnant women with PIH is successful. The study will also look at the short term responses to slow and deep breathing to help our understanding of how blood pressure is reduced in the long-term.

Participant Criteria

- You must have been diagnosed with high blood pressure that has developed after 20 weeks gestation, which you did not have before the pregnancy. **OR** diagnosed during pregnancy with one-off new high blood pressure but at risk of developing PIH;
- You must be carrying a single pregnancy (**not** twins, triplets, etc.);
- You must be aged 18 or over and a non-smoker;

- You must be under the care of a midwife for your pregnancy rather than being placed under the care of a doctor/obstetrician;
- If you have a **current** medical diagnosis of asthma, bronchitis or COPD (chronic obstructive pulmonary disease) you will not be able to take part. An older (not current) diagnosis would not exclude you from taking part i.e. childhood asthma;
- We will conduct an ECG (see page 6 for more details). If you have an allergy or previous reaction to the conducting gel used for the ECG you will not be able to take part.

Key details to know

The list below provides a summary of the key details for the study. If you are interested in taking part, then you can find more details in the 'what would taking part involve' section below:

- You will be asked to complete a 10-min slow and deep breathing exercise every day until birth;
- You will receive guidance on the breathing exercise and be given a blood pressure monitor to take home if you do not already have one;
- We will ask you to measure your blood pressure daily using an automated blood pressure monitor. You will be asked to record the blood pressure results and how often you complete the breathing exercise on a daily record sheet;
- You will attend 1 session at Bournemouth University (BU) lasting approximately 1 ½ hours. We will collect some non-invasive measurements such as heart rate and blood pressure while you complete different breathing exercises;
- You will be asked to refrain from eating for 2 hours before the session and for 12 hours before, have no caffeine or alcohol and no strenuous exercise. You can drink water or other liquids during this time;
- At 36 weeks gestation you will be asked to complete an online survey on your experiences of taking part in the study.

What would taking part involve?

You will come to Bournemouth University (BU) once at the start of the study. You can bring a partner, friend or family member with you if you wish. Children under 16 are not permitted to attend the sessions. The session will be arranged at a time convenient to you. Free visitor parking will be available.

During this session you will be given the instructions and equipment needed to complete your daily breathing exercise and blood pressure measurements. We will also look at your responses to the slow and deep breathing exercises during a short protocol. At 36 weeks gestation, you will be invited to complete an online survey. You can find more information about each of the 3 study sections in the next 3 pages.

At BU, we will talk through what will be involved in taking part in the study. You will have the opportunity to ask any questions before consenting to take part in the study. You will also complete a health survey and a contact details form so we can get in touch with you during the study. The health survey will ask for your age, estimated date of delivery, gestational age, and other medical conditions.

1. Daily breathing exercise overview

For the main study you will be asked to perform a 10-minute breathing exercise every day until you give birth. You can complete the breathing exercise at any time of day. The breathing exercise will be guided by a video. You will be asked to follow the visual graphic displayed; breathing in when the dome graphic rises and breathing out when the dome falls (see Figure 1).

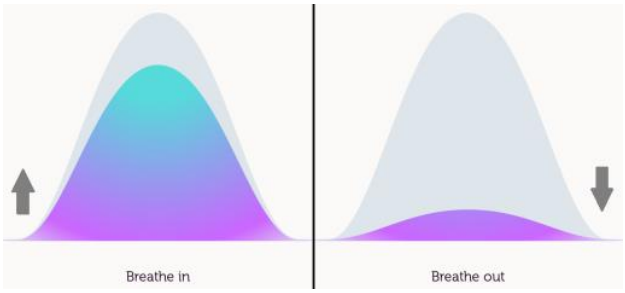


Figure 1: Screenshots of video graphic
Note: Arrows do not appear on video. They are shown here to display the direction of movement

When you breathe in time with the video graphic your breathing rate will be lowered to 6 breaths per minute. Your normal breathing rate is around 14 breaths per minute. So you will be asked to breathe at around half the normal rate. This is not as difficult as you might think. The body automatically increases how deeply you breathe. This means that you still receive enough oxygen for both you and your baby. You will be given the chance to practice breathing with the video during the BU session. Potential feelings of light-headedness are rare. Most people report feeling relaxed during the breathing exercise. The video is hosted on the Panopto website and will be accessed via a link e-mailed to you. More details about how you will view the video are provided in Appendix A at the end of the document.

In addition to the breathing exercise you will be asked to measure your blood pressure daily. You may already have been given an automated blood pressure monitor from Poole Hospital. If you have, then you can submit these readings. You do not have to take extra readings. If you haven't been given a monitor, we will provide one for you to take home. You will be shown how to use it during the BU session.

We will ask you to keep a record of your blood pressure results and how often you complete the breathing exercise on a daily record. You will complete the time of the breathing exercise session, duration and your blood pressure readings. You can do this online, via an e-mail link, or using a paper template that we provide. If you are placed under obstetric-led care (consultant/ doctor) during the study you may continue with the breathing exercise. You will be asked to make a note of this, including the date and medication type (if applicable) in your daily record. Finally, you will be asked to record your delivery date and mode of delivery after you have given birth.

If you have any problems viewing the video or using the blood pressure monitor please contact Malika Felton for help (contact details at end of document). The blood pressure monitor we supply can be returned at the end of the study to Poole Hospital labelled c/o Steph Grigsby, Lead Research Midwife, or to Studland House reception desk at Bournemouth University c/o of Malika Felton.

This study does not replace standard care. You should continue to attend your appointments and take any regular or new medication as directed by your obstetrician or midwife.

2. Breathing responses protocol overview

The short-term responses section of the research study will last approximately 45 minutes of the BU session. This section will include the video used in the daily breathing exercise (6 breaths per minute), as well as 2 other videos. The 2 other videos will guide your breathing rate to 4 and 8 breaths per minute. We will also measure your responses during your normal breathing. The reason we ask you to breathe at 4 and 8 breaths per minute is to ensure we are using the best rate for women with PIH.

We will monitor your blood pressure, heart rate and breathing rate using the equipment shown in Figure 2. More details are provided on the next page. You can have a break halfway through the data collection if you want to get up and move around or for a bathroom break. Before we start we will measure your height and weight. This will not require you to remove any clothing except your shoes.

Figure 2: Equipment set up

The slow and deep breathing videos will be viewed on a BU iPad. You will not need to use your own device for this part of the study. The only difference between the 3 breathing exercises (4, 6 and 8 breaths per minute) will be the speed of the video graphic. For the 'normal' breathing you will breathe normally with no pacing or guidance.

Each breathing exercise will be performed for 5 minutes. There will be a 5-minute break between each exercise. During the break you can breathe normally and take off the mask if you wish.

You will complete 15 minutes of fixed breathing (following the video graphic), 5 minutes of normal breathing and 20 minutes of normal breathing while data is not collected (during the breaks). An overview of the protocol is shown below (Figure 3).

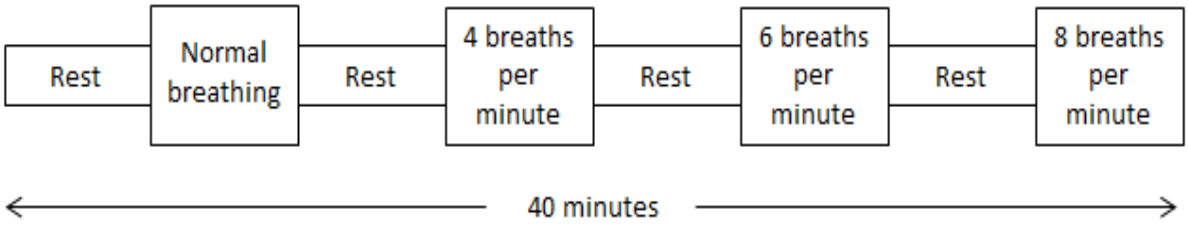


Figure 3: Overview of the protocol for short-term responses to slow and deep breathing

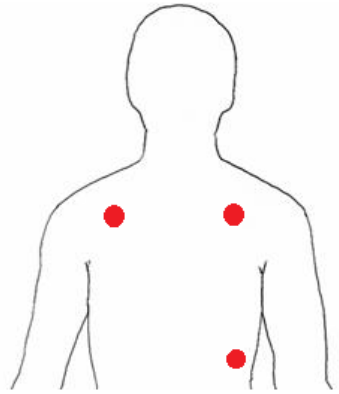
Each block is 5-minutes long. The order of breathing exercises will be randomly selected before the session. You will be told the order before you start.

Measurements of respiration, blood pressure and heart rate

During the breathing exercises you will be asked to wear a mask to measure your breathing. The mask covers your mouth and nose but allows you to breathe normally through both (see Figure 2). The mask can be taken off between breathing exercises in the 5-minute rest periods.

Figure 4: The finger cuff used for the continuous measurement of blood pressure

We will measure your blood pressure and estimate the amount of blood that is pumped out of your heart using two cuffs. One cuff placed on the upper arm and one on the middle finger (see Figure 4). The silver box is just secured at the wrist. It does not take any measurements. You will also have a finger sensor attached on the same hand (green sensor in Figure 4).



Heart rate will be measured continuously using a 3-lead electrocardiogram (ECG). Sensors will be placed on your chest. 2 sensors just below your collarbone on each side and 1 sensor placed on the lower ribs just above your baby bump (see Figure 5 for example). These will be placed under your clothing but you will not need to remove any items. ECG monitoring is safe to have while pregnant, with no known risks.

Figure 5: Placement sites for the 3-lead ECG electrodes

3. Survey overview

At 36 weeks gestation we will invite you to complete an online survey. If you give birth before 36 weeks you will be invited to complete the survey as soon as appropriate within 3 weeks. The survey will ask about your experiences of completing the daily breathing

exercise and blood pressure measurements. This could be any problems you had or anything that stopped you completing the sessions. These data will help shape the design of future research studies.

What are the possible benefits of taking part?

You will have the opportunity to monitor your blood pressure every day. You will receive a graph to show your blood pressure changes over your pregnancy. This study is looking at the impact of slow and deep breathing so we cannot say whether it will have a beneficial effect on your blood pressure.

You will continue to receive standard care. This will not be affected by taking part in the study. This research is not intended to replace any clinical treatments. Taking part in the short term responses section may add to the understanding of PIH and the potential benefits that slow and deep breathing could have as an alternative treatment method. No financial compensation will be given for taking part in this study but visitor parking will be provided during your BU session.

What are the possible disadvantages and risks of taking part?

There are no known risks to pregnancy, but as you will be breathing at a lower breathing rate than normal, you may have feelings of sweating, heat, 'flushing' and a strong urge to breath more. This is **uncommon** but a normal response to slow and deep breathing. It does not present any known risk to your safety or the pregnancy. In the unlikely event that you feel uncomfortable, you can stop the breathing exercise and return to breathing normally. The feelings decrease quickly once normal breathing is resumed. In a past research study with pregnant women breathing at these rates, none of the women felt uncomfortable during the short-term responses protocol. In the unlikely event that this occurs at home, this should be reported to Malika Felton using the contact details provided at the end of the document.

Only Malika Felton and Steph Grigsby, Lead Research Midwife, within the research team will have access to information that could identify you, including your contact details. All other data will be anonymised before other members of the team view it.

Further information

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

Taking part in this research is voluntary. It is up to you to decide whether or not to take part. This study is outside of standard care but it will not affect the standard care you receive for your pregnancy or any other health matters. Deciding to take part or not won't affect your treatment, quality of care or access to other services.

You will be given this information sheet to keep. If you decide to take part, you will be asked to sign a consent form. You can withdraw from taking part at any time and without giving a reason. If you wish we can remove any previously collected data from you up until the point that data are analysed and included in research findings. At this point your data will become anonymous, so your identity cannot be determined. It may not be possible to identify your data within the anonymous dataset.

Informing Healthcare Staff

We will include this participant information sheet in your maternity records so that your obstetrician and midwife are aware you are taking part. We will also write to your GP to inform them.

How will my information be kept confidential?

In this research study we will use information from you. We will only use information that we need for the research study. We will let very few people know your name or contact details, and only if they really need it for this study. Everyone involved in this study will keep your data safe and secure. We will also follow all privacy rules. At the end of the study we will save some of the data in case we need to check it and for future research. This does not include identifiable data. We will make sure no-one can work out who you are from the reports we write. The 'GDPR information' pack tells you more about this.

Who is organising and funding the research?

The research is being organised by the Faculty of Health and Social Sciences at Bournemouth University as part of the PhD doctoral studies of Malika Felton.

What will happen to the results of this study?

The results of this study will be used as part of Malika Felton's doctoral thesis. It is expected that the results will be published in peer-reviewed scientific journals and presented at conferences. All data will be anonymised at publication. You will not be identifiable in any way. The results could also be used to support future funding bids for a larger trial. You will be able to access a summary report of the results at www.bournemouth.ac.uk/research/projects/using-deep-breathing-lower-blood-pressure-pregnant-women.

How have patients and the public been involved in this study?

Pregnant women/ mothers and antenatal class teachers were involved in reviewing the Participant Information Sheet. In designing this study, we have considered pregnant women's opinions on the breathing rate they would feel most comfortable completing daily.

Who has reviewed this study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Hampshire B Research Ethics Committee.

What if something goes wrong?

The procedures involved in this study are extremely low risk, with women having to do nothing more than to sit, and to breathe slowly and deeply, in a relaxed way. In case of an adverse event during the BU session, an emergency name and contact telephone number must be provided by all women in the health survey. Bournemouth University

holds Public Liability and Professional Indemnity insurance to cover the legal liability of the University involved in research and for its employees in the case of harm to a research participant arising from the management, design or conduct of the research by the University.

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (contact details below). If you remain unhappy and wish to complain formally, you can do this by contacting the Executive Dean of the Faculty of Health & Social Sciences, Professor Stephen Tee, as an independent member of BU Staff, by email at researchgovernance@bournemouth.ac.uk. You can also contact Poole Hospital PALS (Patient Advice and Liaison Service) on 01202 448499 or patientexperienceteam@poole.nhs.uk for confidential support.

Thank you for reading this information and for considering taking part in this research.

Contact Details

If you have any questions or would like further information regarding this research project, please contact the principal investigator:

Malika Felton
Bournemouth University, Room R305, Royal London House, Christchurch Road, Bournemouth, BH1 3LT

Phone: 01202 961845
Email: mfelton@bournemouth.ac.uk

Appendix 1: Accessing the breathing aid video

The video will be viewed on a webpage hosted by Panopto, an online video platform site. You will be shown how to access this in your BU session. The video can be accessed on any computer, tablet or phone with an internet connection. You will be e-mailed a link to access the video. We recommend watching the video while connected to a Wi-Fi internet connection. Watching the full 10-minute video could use approx. 200MB each time. Please note we cannot guarantee exact data usage. If you do not have access to Wi-Fi then you can request to download the file directly to your device. Please speak to Malika Felton.

The video does not have to be watched on the same device for each session. We recommend bookmarking the webpage for easy access. We can show you how to do this during your BU session. Panopto records the following when you watch the video; number of views, time of day of each view, and duration of each view. This is linked only to the e-mail address to which the link is sent. No other personal data is collected from you or the device you use to access the video. BU will be able to access this information. Panopto's privacy notice can be viewed at www.panopto.com/privacy/.

Appendix III: Consent form

Appendix IIIa: Chapter 4 consent form

Participant Id No: _____

Participant Agreement Form

'Device guided slow breathing for the treatment of hypertension: Comparison of BU's Brythm App with an NHS approved device'

Contact	Position	Phone	Email
Professor Alison McConnell	Professor of Sport/Heath Science	01202 962313	amcconnell@bournemouth.ac.uk
Pedro Vargas	Research Project Manager	---	pedrovargas@sapo.pt
Malika Felton	PhD Student	01202 961845	mfelton@bournemouth.ac.uk

	Please Initial Here
I have read and understood the participant information sheet for the above research project.	
I confirm that I have had the opportunity to ask questions.	
I understand that my participation is voluntary.	
I understand that I am free to withdraw up to the point where the data are processed and become anonymous, so my identity cannot be determined.	
During the task or experiment, I am free to withdraw without giving reason and without there being any negative consequences.	
Should I not wish to answer any particular question(s) or complete a test, I am free to decline.	
I give permission for members of the research team to have access to my anonymised information for the purposes of this research project. I understand that my name will not be linked with the research materials, and I will not be identified or identifiable in the outputs that result from the research.	
I agree to take part in the above research project.	
I understand that the anonymised data I provide may be used by the research team to support other research projects in the future, including future publications, reports or presentations.	

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature

This form should be signed and dated by all parties after the participant receives a copy of the participant information sheet and any other written information provided to the participants. A copy of the signed and dated participant agreement form should be kept with the project's main documents which must be kept in a secure location.

Appendix IIIb: Chapter 5 consent form

Participant Id No: _____

Participant Agreement Form

'Acute effects of slow and deep breathing upon healthy young women'

Contact	Position	Phone	Email
Malika Felton	PhD Student	01202 961845	mfelton@bournemouth.ac.uk
Professor Alison McConnell	Professor of Sport/Heath Science	01202 962313	amcconnell@bournemouth.ac.uk

	Please Initial Here
I have read and understood the participant information sheet for the above research project.	
I confirm that I have had the opportunity to ask questions.	
I understand that my participation is voluntary.	
I understand that I am free to withdraw up to the point where the data are processed and become anonymous, so my identity cannot be determined.	
During the task or experiment, I am free to withdraw without giving reason and without there being any negative consequences.	
Should I not wish to answer any particular question(s) or complete a test, I am free to decline.	
I give permission for members of the research team to have access to my anonymised information for the purposes of this research project. I understand that my name will not be linked with the research materials, and I will not be identified or identifiable in the outputs that result from the research.	
I agree to take part in the above research project.	
Use of the information I provide beyond this project:	---
I agree for the anonymised data I provide to be archived at BU's Online Research Data Repository.	
I understand that the anonymised data I provide may be used by the research team to support other research projects in the future, including future publications, reports or presentations.	

_____ _____ _____

Name of Participant Date Signature

_____ _____ _____

Name of Researcher Date Signature

This form should be signed and dated by all parties after the participant receives a copy of the participant information sheet and any other written information provided to the participants. A copy of the signed and dated participant agreement form should be kept with the project's main documents which must be kept in a secure location.

Appendix IIIc: Chapter 6 consent form

Participant Id No: _____

Participant Agreement Form

'Acute effects of slow and deep breathing upon pregnant women'

Contact	Position	Phone	Email
Malika Felton	PhD Student	01202 961845	mfelton@bournemouth.ac.uk
Professor Alison McConnell	Professor of Sport/Heath Science	01202 962313	amcconnell@bournemouth.ac.uk

In this Form we ask you to confirm whether you agree to take part in the Project. You should only agree to take part in the Project if you understand what this will mean for you. If you complete the rest of this Form, you will be confirming to us that:

- You have read and understood the Project Participant Information Sheet [V1] and have been given access the BU Research Participant Privacy Notice which sets out how we collect and use personal information (https://www1.bournemouth.ac.uk/about/governance/access-information/data-protection-privacy);
- You have had the opportunity to ask questions;
- You understand that:
  - Taking part in the research will include a data collection session where we will collect non-invasive cardiovascular and respiratory measurements, while you breathe at a specific rhythm and depth, using a visual feedback device.
  - Taking part in the research will include submitting your blood pressure measurements taking during your antenatal appointments either via an online or paper form.
  - Your participation is voluntary. You can stop participating in research activities at any time without giving a reason, and you are free to decline to answer any particular question(s).
  - If you withdraw from participating in the Project, you may not always be able to withdraw all of your data from further use within the Project, particularly once we have anonymised your data and we can no longer identify you.
  - Data you provide may be included in an anonymised form within a dataset to be archived at BU's Online Research Data Repository.
  - Data you provide may be used in an anonymised form by the research team to support other research projects in the future, including future publications, reports or presentations.

Consent to take part in the Project	***Yes***	***No***
I agree to take part in the Project on the basis set out above		

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature

Appendix IIId: Chapter 8 consent form

Official Researcher Use Only	
IRAS ID	**Participant Identification Number for this trial**
251062	

Bournemouth University

CONSENT FORM

Title of Project: Effects of slow and deep breathing (SDB) on reducing obstetric intervention in women with pregnancy-induced hypertension (PIH): A feasibility study

Name of Researcher: Malika Felton

Please initial box

1. I confirm that I have read the participant information sheet dated 10/03/2020 (version 3) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. If I withdraw from participating in the project, I understand I may not always be able to withdraw all of my data from further use within the project, particularly once my data has been anonymised and I can no longer be identified. ☐
3. I understand that relevant sections of my medical notes, may be looked at by individuals from Bournemouth University (the Sponsor), regulatory authorities or from Poole Hospital NHS Foundation Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐
5. I agree to my General Practitioner being informed of my participation in the study. ☐
6. I understand that the information held and maintained by Bournemouth University and Poole Hospital NHS Foundation Trust may be used to help contact me or provide information about my health status. Your medical notes will not be accessed for this project. ☐
7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

Participant ID:

Appendix IV: Health questionnaire

Appendix IVa: Chapter 4 health questionnaire

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

'Device guided slow breathing for the treatment of hypertension: Comparison of BU's Brythm App with an NHS approved device'

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 recommended only medically supervised exercise?	☐	☐
2. Do you suffer from chest pains, heart palpitations, arrhythmia or tightness of the chest?	☐	☐
3. Do you have known high blood pressure? If yes, please give details below (i.e. medication).	☐	☐
4. Do you suffer from any lung/chest problem? e.g., asthma, bronchitis, emphysema? If yes, please give details below.	☐	☐
5. Do you suffer from epilepsy? If yes, when was the last episode?	☐	☐
6. Are you a smoker? If yes, please give number of cigarettes per week.	☐	☐
7. Do you have any known allergy to conductive gel? (i.e. the gel used with ECG)	☐	☐

Please give details if you answered yes to any of the above questions (number each answer in relation to the question above):

_____

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 this study 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: _____

Witness name & signature:_____ Date: _____

Participant ID:

Appendix IVb: Chapter 5 health questionnaire

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

'Acute effects of slow and deep breathing upon healthy young women'

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 recommended only medically supervised exercise?	☐	☐
2. Do you suffer from chest pains, heart palpitations, arrhythmia or tightness of the chest?	☐	☐
3. Do you have known high blood pressure? If yes, please give details below (i.e. medication).	☐	☐
4. Do you suffer from any lung/chest problem? e.g., asthma, bronchitis, emphysema? If yes, please give details below.	☐	☐
5. Do you suffer from epilepsy? If yes, when was the last episode?	☐	☐
6. Are you a smoker? If yes, please give number of cigarettes per week.	☐	☐
7. Do you have any known allergy to conductive gel? (i.e. the gel used with ECG, ultrasounds, etc.)	☐	☐
8. Have you ever been diagnosed with a spontaneous pneumothorax (collapsed lung) or a recent traumatic pneumothorax? If yes, please give details below.	☐	☐
9. Do you suffer from an eardrum rupture, or other middle ear condition?	☐	☐
10. Are you currently (or have you ever) been prescribed the oral contraceptive pill? If yes, please give details (i.e. pill type, years of use)	☐	☐
11. Are you currently pregnant?	☐	☐
12. How many days are you into your current menstrual cycle? First day of bleeding is Day 1. Day _____	☐	☐

Please give details if you answered yes to any of the above questions (number each answer in relation to the question above):

_____

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.

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 this study 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: _____

Witness name & signature:_____ Date: _____

Appendix IVc: Chapter 6 health questionnaire

Participant ID:

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

'Acute effects of slow and deep breathing upon pregnant women'

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: _____ **Age:** _____

Emergency Contact Name: _____ **Emergency Contact Tel:** _____

Please answer the following questions:

		YES	NO
1.	Has your doctor ever diagnosed a heart condition or recommended only medically supervised exercise?	☐	☐
2.	Do you suffer from chest pains, heart palpitations, arrhythmia or tightness of the chest?	☐	☐
3.	Do you have known high blood pressure? If yes, please give details below (i.e. medication).	☐	☐
4.	Do you suffer from any lung/chest problem? e.g., asthma, bronchitis, emphysema? If yes, please give details below.	☐	☐
5.	Do you suffer from epilepsy? If yes, when was the last episode?	☐	☐
6.	Are you a smoker? If yes, please give number of cigarettes per week.	☐	☐
7.	Do you have any known allergy to conductive gel? (i.e. the gel used with ECG, ultrasounds, etc.)	☐	☐
8.	How many weeks pregnant are you? Week_____		
9.	Are you carrying a multiple pregnancy? (i.e. twins, triplets, etc.)	☐	☐
10.	Have you previously been pregnant?	☐	☐

Please give details if you answered yes to any of the above questions (number each answer in relation to the question above):

_____

If you feel at all unwell as a result of a temporary illness (cold or fever) please inform the investigator.

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 this study and I am fit and fully able to volunteer for this investigation. I understand I will be taking part at my own risk.

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Witness	Date	Signature

Appendix IVd: Chapter 8 health questionnaire

Official Researcher Use Only	
IRAS ID	**Participant Identification Number for this trial**
251062	

Bournemouth University

PRE-PARTICIPATION HEALTH QUESTIONNAIRE

Effects of slow and deep breathing (SDB) on reducing obstetric intervention in women with pregnancy-induced hypertension (PIH): A feasibility study

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 research procedures. Additionally, the following questions are designed to check that you are suited to take part in this study. Your responses to one or more of the questions may mean that you are ineligible to participate in this study.

Participant name: _____ **Date of birth:** _____

Emergency Contact Name: _____ **Emergency Contact Tel:** _____

Please answer the following questions:	**YES**	**NO**
1. Do you have high blood pressure that has developed during pregnancy?	☐	☐
2. Were you told that you had one-off high blood pressure ☐ or pregnancy-induced hypertension? ☐		
3. How many weeks pregnant were you when you were diagnosed with high blood pressure?	Week_____	
4. Are you on any medication for your high blood pressure? Please provide details _____	☐	☐
5. Are you carrying a multiple pregnancy? (i.e. twins, triplets, etc.)	☐	☐
6. Have you previously been pregnant and given birth? If yes, how many times (including this pregnancy)? _____		
7. Did you experience pre-eclampsia or pregnancy-induced hypertension during any of your previous pregnancies? Please provide details _____		
8. Do you suffer from chest pains, heart palpitations, arrhythmia or tightness of the chest?	☐	☐
9. Do you currently suffer from any lung/chest problem? e.g., asthma, bronchitis, emphysema, COPD? If yes, please provide details _____	☐	☐
10. Are you a smoker? (either cigarettes, electronic cigarettes or vaping)	☐	☐
11. Do you have any known allergy to conductive gel? (i.e. the gel used with ECG)	☐	☐
12. How many weeks pregnant are you?	Week_____	
13. When is your estimated date of delivery?	___/____/20__	

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 this study and I am fit and fully able to volunteer. I understand the implications and procedures involved in this research project.

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Witness	Date	Signature

Appendix V: Post-intervention questionnaire (copy of OnlineSurveys)

Intro page: Welcome to the slow and deep breathing feasibility questionnaire

You have been invited to take part in this questionnaire as part of your participation in the slow and deep breathing research study (Full Title: Effects of slow and deep breathing on reducing obstetric intervention in women with pregnancy-induced hypertension: A feasibility study).

The questionnaire will ask about your experiences of taking part in the intervention and completing the daily breathing exercises. Your feedback will have a direct influence on future studies looking to use this intervention in a larger group of women.

If you have any questions regarding the study you can contact the principal investigator Malika on mfelton@bournemouth.ac.uk or 01202 961845.

The questionnaire should take approximately 10 minutes to complete. If viewing on a phone, for questions requiring statements of how strongly you agree you can zoom out to see all the answers together on the screen.

By clicking next you are consenting to take part in the questionnaire. If you do want to take part or change your mind part way through the questionnaire please close the browser and the answers will not be sent to us.

Thank you again for your continued participation in this research project.

New page (pg.2): Research motivations and initial meeting

1. What **initially** motivated you to take part in the research study? Please tick all which apply.
    - I am keen to participate in research ☐
    - I am interested in alternative treatment methods (drug-free) ☐
    - I wanted to feel in control of treating my high blood pressure ☐
    - I hoped to gain health benefits ☐
    - I wanted to help develop a new way of reducing blood pressure ☐
    - I thought it sounded interesting ☐
    - I wanted to help the heath of future pregnant women ☐
    - Other ☐

If you selected Other, please specify:

2. What motivated you to **continue** with the research study and breathing exercise intervention once you had started? Please tick all which apply.
    - I was committed to complete the research ☐
    - I found it relaxing ☐
    - I enjoyed taking time out of my day to complete the breathing exercise ☐
    - I thought it was helping my blood pressure ☐
    - I liked helping develop a better treatment for high blood pressure ☐
    - Other ☐

If you selected Other, please specify:

3. Please indicate your agreement with the following statements regarding the initial meeting at Bournemouth University:

(Table with Likert scale; strongly agree, agree, uncertain, disagree, strongly disagree)

The meeting contained enough detail to understand the study
It was clear how to access the video aid for breathing exercise
It was clear how to use the blood pressure monitor

New page (pg.3): Experiences of completing the breathing exercise

4. How many weeks pregnant were you when you started the intervention (breathing exercise)? ____ weeks

5. Please indicate your agreement with the following statements regarding the breathing video aid:

(Table with Likert scale; strongly agree, agree, uncertain, disagree, strongly disagree)

The video graphic's appearance encouraged me to complete the breathing exercise
It was easy to breathe in time with the breathing graphic
The breathing graphic felt too slow to breathe in time with
The breathing graphic felt too fast to breathe in time with

6. Did you experience any issues with accessing the video aid? *(feeder question)*

Yes ☐ No ☐

6a. Please provide details of any issues that you experienced while accessing the video aid, even if these were resolved.
(Only asked if 'yes' to Q6)

Please provide details:

6b. Which of the following did you use to resolve the issues? Please tick all which apply.
(Only asked if 'yes' to Q6)

Referred to the invitation email ☐
Contacted the study team ☐
Spoke with my midwife/GP ☐
Searched the Internet (i.e. Google) ☐
Other ☐

If you selected Other, please specify:

7. Please tell us of any other issues which may have prevented you from completing the **breathing exercise** during the course of the study. You should answer this specifically about the breathing exercise, and not the blood pressure measurements. We will ask about this later. Please tick all which apply.

I struggled to remember each day	☐
I was too busy to find time each day	☐
I went away/on holiday	☐
It was inconvenient	☐
It took too much time	☐
Having to access Wi-Fi made completing the breathing exercises more difficult to complete	☐
Other	☐

If you selected Other, please specify:

New page (pg.4): Experiences of using the blood pressure monitor

8. Did you use a Bournemouth University or Poole Hospital NHS Foundation Trust blood pressure monitor for this research study?

   Bournemouth University ☐ Poole Hospital ☐ Both ☐ Neither (own monitor) ☐

9. Please indicate your agreement with the following statements regarding the blood pressure monitor:
   (Table with Likert scale; strongly agree, agree, uncertain, disagree, strongly disagree)

It was straightforward to use
It was easy to put the cuff on
I had time to complete the blood pressure measurements as directed
It was easy to add the blood pressure readings to the website

10. Did you experience any issues with using the blood pressure monitor? *(feeder question)*

    Yes ☐ No ☐

10a. Please tell us of any issues you experienced while using the blood pressure monitor, even if these were resolved. Please tick all which apply. *(Only asked if 'yes' to Q10)*
Four columns and tick in appropriate column; problem with Bournemouth University monitor, problem with Poole Hospital, problem with both monitors and no problem

Putting the cuff on	☐
Getting a valid reading	☐
Other	☐

If you selected Other, please specify (and include whether this was a problem with a Bournemouth University or Poole Hospital monitor):

10b. If yes, which of the following did you use to resolve the issues? Please tick all which apply.

(Only asked if 'yes' to Q10)

- Referred to the product blood pressure monitor user instructions ☐
- Referred to the BU blood pressure monitor user guide ☐
- Contacted the study team ☐
- Spoke with my midwife/GP ☐
- Searched the Internet (i.e. Google) ☐
- Other ☐

If you selected Other, please specify:

11. Please tell us of any other issues which may have prevented you from regularly measuring and recording your blood pressure during the course of the study. Please tick all which apply.

- I struggled to remember each day ☐
- I was too busy to find time each day ☐
- I went away/on holiday ☐
- It was inconvenient ☐
- It took too much time ☐
- I could not remember how to record blood pressure on the website ☐
- Other ☐

If you selected Other, please specify:

New page (pg.5): Please tell us a bit about how the study fitted into your day

12. Where did you **mainly** undertake the breathing exercise? *(feeder question)*

- At home ☐
- At work ☐
- During my morning commute ☐
- During my afternoon commute ☐
- It varied depending on circumstances each day ☐
- Other ☐

If you selected Other, please specify:

12b. If it varied depending on circumstances each day please tick all places where you undertook the breathing exercise.

(Only asked if 'if varied depending…' selected in Q12)

- At home ☐
- At work ☐
- During my morning commute ☐
- During my afternoon commute ☐
- It varied depending on circumstances each day ☐
- Other ☐

If you selected Other, please specify:

13. What device did you **mainly** used to watch the breathing video?

- Phone ☐
- Tablet ☐
- Laptop ☐
- Desktop computer ☐

Other ☐

If you selected Other, please specify:

14. Please indicate your agreement with the following statements regarding the research study:
(Table with Likert scale; strongly agree, agree, uncertain, disagree, strongly disagree)

The daily breathing exercise was easy to incorporate into my daily life
The blood pressure measurement was easy to incorporate into my daily life
It was easy to complete the breathing exercise daily for the full duration of the study
I would recommend the breathing exercise to other pregnant women with high blood pressure

15. Did you experience any additional barriers to incorporating the **breathing exercise** into your day that you haven't already mentioned? You should answer this specifically about the breathing exercise, and not the blood pressure measurements. We will ask about this in the next question. *(feeder question)*
Yes ☐ No ☐

15a. Please describe the additional barriers you experienced:
(Only asked if 'yes' to Q15)

16. Did you experience any additional barriers to incorporating the **blood pressure measurement** into your day that you haven't already mentioned? *(feeder question)*
Yes ☐ No ☐

16a. Please describe the additional barriers you experienced:
(Only asked if 'yes' to Q16)

New page (pg. 6): Future studies

17. As you know this research study is a feasibility study being used to help plan for future larger-scale trials. To help us understand women's views about a future study we would be grateful if you would answer the following questions as if you were considering taking part in this future study. Please tick all that apply.

I would be willing to be randomised i.e. you would be randomised (like flipping a coin) into an intervention group (slow and deep breathing) or a placebo group (another breathing rate) ☐
I would prefer to access the video aid as a file that is available to download onto my device, rather than accessing online ☐
I would prefer to submit my blood pressure measurements online ☐
I would prefer to submit my blood pressure measurements on a paper daily record ☐
I would prefer to submit my blood pressure readings using an app ☐
I would be willing to downloading and use an app to deliver the slow and deep breathing ☐

Note: To improve our future research studies we welcome your feedback so please comment freely in the following questions, including any information you haven't been able to convey above, so that we can improve for future studies.

18. From a participant's point of view, is there anything that could be improved in the process of undertaking the study? (i.e. the initial meeting, information provided)

19. Is there anything that could be improved for the breathing graphic or video? Please describe what you would change.

20. Is there anything you particularly like about completing the breathing exercise?

21. Is there anything you particularly dislike about completing the breathing exercise?

22. Finally, if you were asked to provide helpful top tips of best practice for completing the breathing exercise for future participants what would they be?

Final page (pg. 7)

Thank you for taking part in this questionnaire.

Your answers will be used to help design future larger trials involving slow and deep breathing to lower blood pressure in pregnant women.

If you have any questions please contact Malika Felton (mfelton@bournemouth.ac.uk) or 01202 961845.

Appendix VI: Ethical approval

Appendix VIa: Chapter 4 BU ethical approval

Bournemouth University

Research Ethics Checklist

About Your Checklist	
Reference Id	20679
Status	Approved
Date Approved	26/03/2018 10:27:49
Date Submitted	22/03/2018 15:55:25

Researcher Details	
Name	Alison McConnell
Faculty	Faculty of Health & Social Sciences
Status	Staff
Course	Staff - HSS
Is This External Funding?	No
Please list any persons or institutions that you will be conducting joint research with, both internal to BU as well as external collaborators.	Dr Pedro Vargas (external consultant and form BU PDRF), Ms Malika Felton (FHSS PhD student)

Project Details	
Title	Device guided slow breathing for the treatment of hypertension: Comparison of BU's Brythm App with an NHS approved device
End Date of Project	28/06/2018
Proposed Start Date of Data Collection	28/05/2018
Supervisor	
Approver	Research Ethics Panel

Summary - no more than 500 words (including detail on background methodology, sample, outcomes, etc.)

Background: Slow and deep breathing (SDB) has been used for many decades within yoga and meditation and shows an ability to lower blood pressure (Benson et al. 1974; Rampalliwar et al. 2013). The most robust method to deliver SDB is using device-guided breathing (DGB; (Rosenthal et al. 2001; Cernes and Zimlichman 2015)), which employs biofeedback to lower breathing frequency to around 6 breaths.min-1 (around half normal frequency). The applicant and colleagues have developed an algorithm that drives breathing frequency to a personalised optimum, via an App (Brythm), which was developed at BU using HEIF 5 funding. The proposed project seeks to test the Brythm App against a device already approved by the NHS and FDA to provide slow and deep breathing (RESPeRATE®). The project addresses feedback received from external funders about the use of the Brythm App in clinical trials, with the aim of improving the success of future bids.Aim: The purpose of this study is to investigate the acute physiological response to SDB delivered using the BU Brythm App against that of an NHS/FDA approved device (RESPeRATE®).Methods: Twelve healthy men and women will be recruited to participate in the study. Recruitment will be aimed at BU staff and students using the BU research blog and paper notices (see attached). Each participant will be required to attend the laboratory on a single occasion for approximately 1½ - 2 hours. Participants will first complete a health check questionnaire that will allow any exclusion criteria to be identified (e.g., an existing diagnosis of any cardiovascular and/or respiratory disease/conditions). During testing, a total of three controlled breathing conditions will be implemented, two using the Brythm App and one using RESPeRATE®. Specifically, the Brythm App will guide participants to breathe at a fixed breathing frequency of 6 breaths.min-1, as well as in response to the Brythm App algorithm, which guides breathing

Appendix VIb: Chapter 5 BU ethical approval

Bournemouth University

Research Ethics Checklist

Reference Id	19148
Status	Approved
Date Approved	06/03/2018

Researcher Details

Name	Malika Felton
Faculty	Faculty of Health & Social Sciences
Status	Postgraduate Research (MRes, MPhil, PhD, DProf, DEng)
Course	Postgraduate Research - HSC
Have you received external funding to support this research project?	No
Please list any persons or institutions that you will be conducting joint research with, both internal to BU as well as external collaborators.	Warren Foster, Deputy Director of the School of Medical Ultrasound, AECC University College

Project Details

Title	Acute effects of slow and deep breathing upon healthy young women
Proposed Start Date of Data Collection	14/02/2018
Proposed End Date of Project	18/09/2020
Original Supervisor	Alison McConnell
Approver	Research Ethics Panel

Printed On 22/03/2018 14:02:47

Appendix VIc: Chapter 6 BU ethical approval

Bournemouth University

Research Ethics Checklist

About Your Checklist	
Reference Id	22930
Date Created	24/10/2018 15:29:01
Status	Approved
Date Approved	24/01/2019 16:30:36
Date Submitted	23/01/2019 18:33:53

Researcher Details	
Name	Malika Felton
Faculty	Faculty of Health & Social Sciences
Status	Postgraduate Research (MRes, MPhil, PhD, DProf, EngD, EdD)
Course	Postgraduate Research - HSC
Have you received external funding to support this research project?	No
Please list any persons or institutions that you will be conducting joint research with, both internal to BU as well as external collaborators.	Warren Foster, AECC University College

Project Details	
Title	Acute effects of slow and deep breathing upon pregnant women
Start Date of Project	18/09/2017
End Date of Project	17/09/2020
Proposed Start Date of Data Collection	04/02/2019
Original Supervisor	Alison McConnell
Approver	Research Ethics Panel

Summary - no more than 500 words (including detail on background methodology, sample, outcomes, etc.)

Background: Slow and deep breathing (SDB) has been shown to lower blood pressure when practiced daily. The most robust method of delivering SDB is via biofeedback, which paces breathing using visual and/or auditory cues. SDB is typically undertaken at a frequency of ~6 breaths.min-1 (approximately half normal breathing frequency). SDB is a promising treatment for hypertension, which has yet to be tested with pregnant women. The main aim of this PhD project is to address this deficit. Yoga, which incorporates SDB, is practiced widely by pregnant women, and possesses no known risks (DiPeitro et al., 2008; Curtis et al., 2012; Rampalliwar et al., 2012). BU has developed an App (Brythm) to deliver SDB, and as a first step towards achieving the main study aim, the acute responses SDB have been characterised in non-pregnant females (BU ethics ID 19148).

Aim: The purpose of the proposed project is to compare the acute cardiovascular responses to SDB of pregnant women with those observed previously in non-pregnant women.

Appendix VId: Chapter 8 HRA & REC ethical approval

NHS
Health Research Authority
South Central - Hampshire B Research Ethics Committee
Level 3 Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Telephone: 0207 104 8012

19 December 2019

Professor Vanora Hundley
Bournemouth University
R716, Royal London House, Christchurch Road
Bournemouth
BH1 3LT

Dear Professor Hundley

Study title:	**Effects of slow and deep breathing (SDB) on reducing obstetric intervention in women with pregnancy-induced hypertension (PIH): A feasibility study**
REC reference:	**19/SC/0510**
Protocol number:	**N/A**
IRAS project ID:	**251062**

Thank you for your submission responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice Chair, Professor Colburn and Ms Brinton.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Confirmation of Capacity and Capability (in England, Northern Ireland and Wales) or NHS management permission (in Scotland) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Professor Vanora Hundley
Bournemouth University
R716, Royal London House, Christchurch Road
Bournemouth
BH1 3LT

Email: hra.approval@nhs.net
HCRW.approvals@wales.nhs.uk

19 December 2019

Dear Professor Hundley

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Effects of slow and deep breathing (SDB) on reducing obstetric intervention in women with pregnancy-induced hypertension (PIH): A feasibility study**
IRAS project ID:	**251062**
Protocol number:	**N/A**
REC reference:	**19/SC/0510**
Sponsor	**Bournemouth University**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.