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 **Error! No text of specified style in document.**-1).

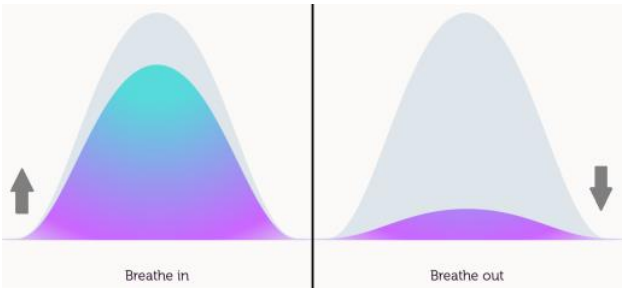

Figure Error! No text of specified style in document.**-1 Screenshots of Brythm graphic**
N.B: Arrows do not appear on app but are shown here to display the direction of graphic movement.

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_cΔ); 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 **Error! No text of specified style in document.**-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 **Error! No text of specified style in document.**-2).

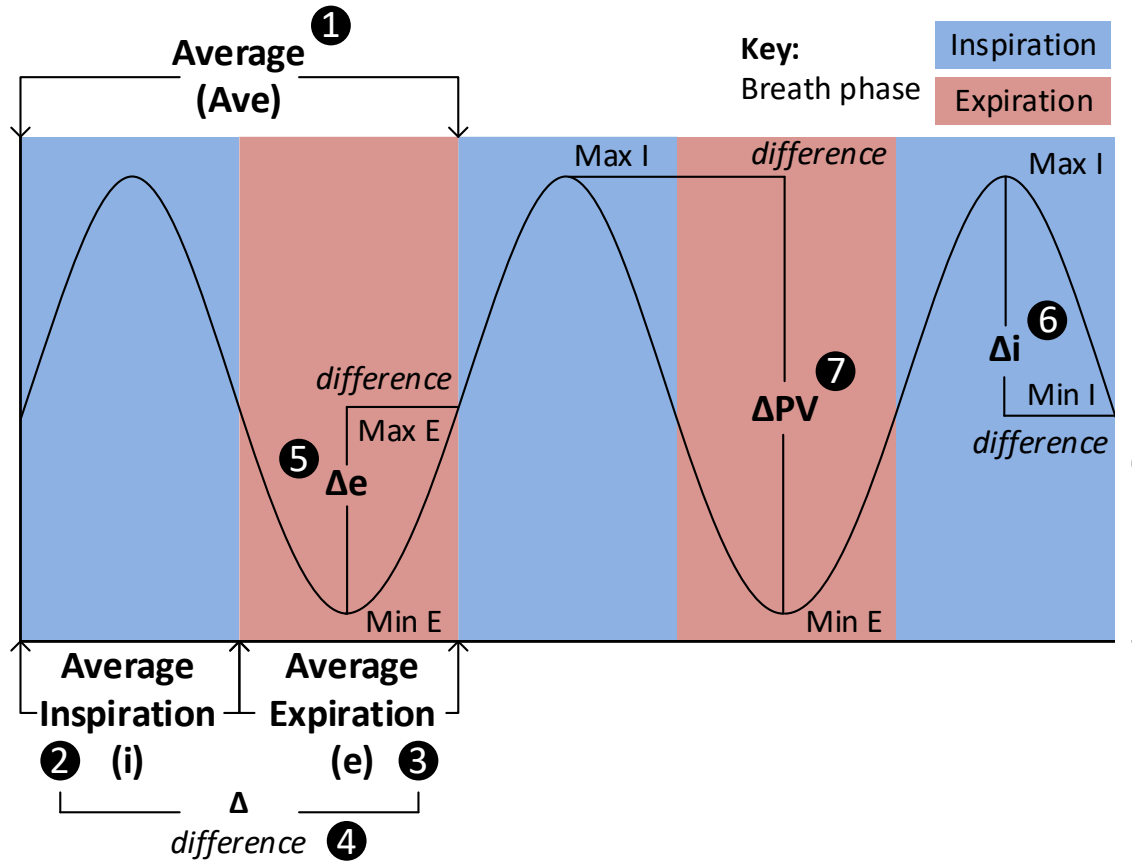

Figure Error! No text of specified style in document.**-2 Calculations for example cardiovascular variable plot**
1) Ave = average of whole breath. 2) i = Average inspiration. 3) e = Average expiration. 4) Δ = i minus e (average inspiration minus average expiration). 5) Δe = Max E minus Min E. 6) Δi = 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 **Error! No text of specified style in document.**-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 **Error! No text of specified style in document.**-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 Error! No text of specified style in document.**-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 **Error! No text of specified style in document.**-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 **Error! No text of specified style in document.**-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 Error! No text of specified style in document.**-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.

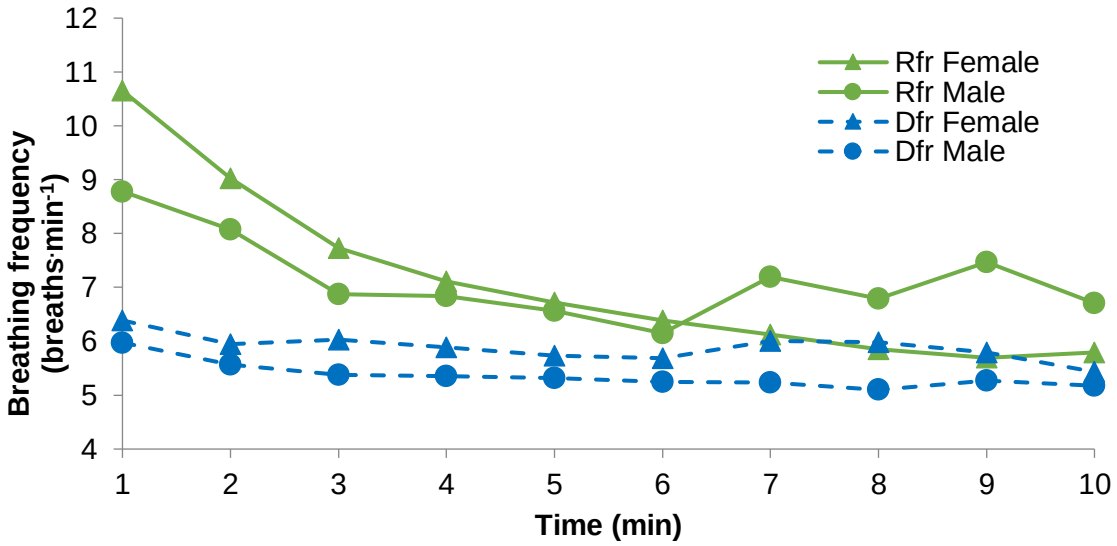

Figure Error! No text of specified style in document.**-3 Breathing frequency during RESPeRATE (Rf_r) and dynamic breathing frequency (Df_r) conditions**
Solid line RESPeRATE (Rf_r), dashed line dynamic algorithm (Df_r); Circle data points - male; Triangle data points - female; Data points represent 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 **Error! No text of specified style in document.**-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 Error! No text of specified style in document.**-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 (∆i), within expiration difference (∆e), inter-breath phase peak-valley difference (∆PV), breath phase independent peak-valley difference (∆PV_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.

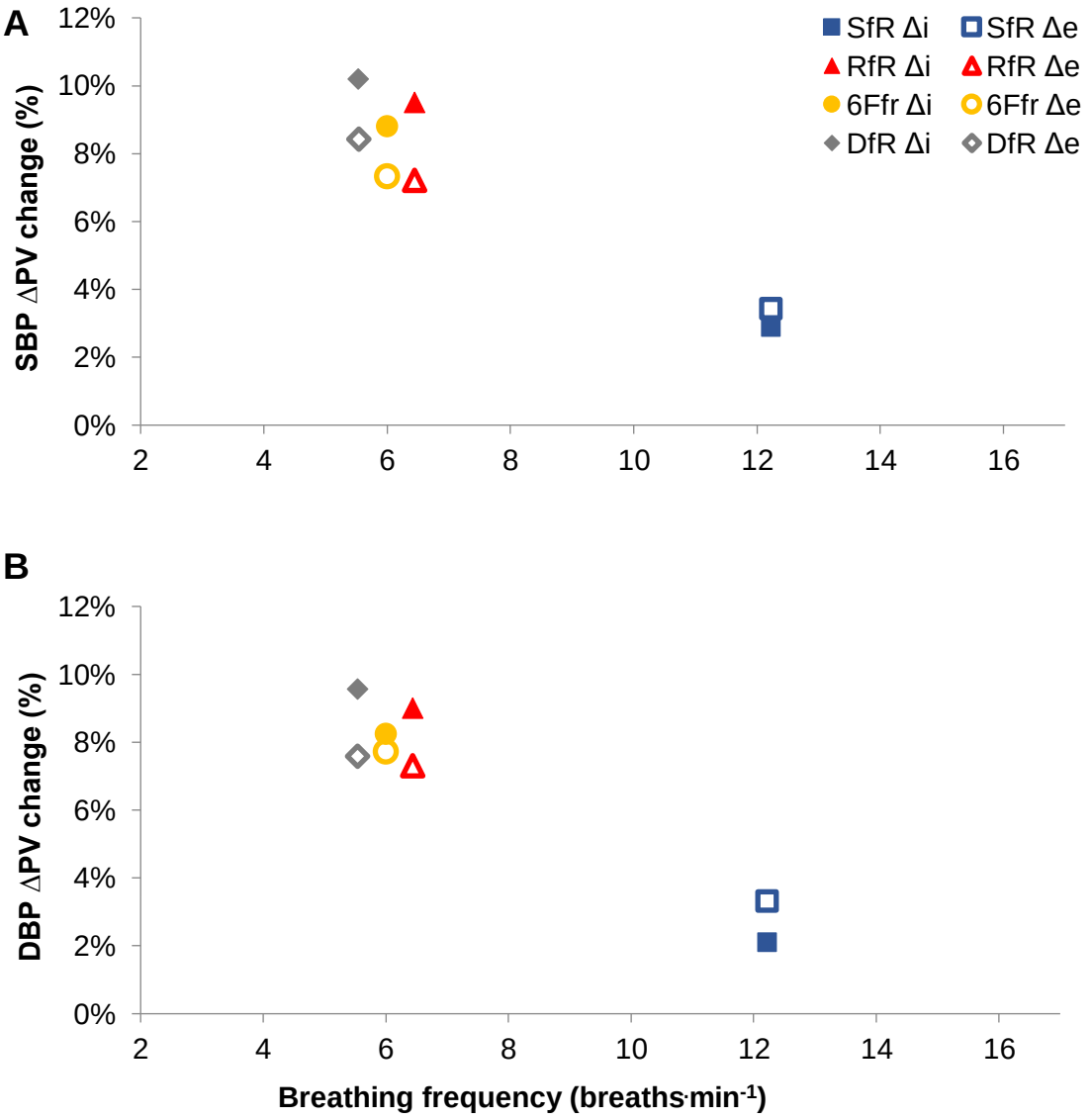

Figure Error! No text of specified style in document.**-4 Blood pressure oscillations: Relative change of ΔI and ΔE for systolic blood pressure (A) and diastolic blood pressure (B)**

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 **Error! No text of specified style in document.**-5).

Table Error! No text of specified style in document.**-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_cΔi	*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_cΔe	*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_cΔPV	*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
RSA (s)	*F*	0.09 ± 0.04	0.16 ± 0.05	0.14 ± 0.08	0.15 ± 0.04		
	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 (∆i), within expiration difference (∆e), inter-breath phase peak-valley difference (∆PV); Significantly different from Sf_r (*), Rf_r (¥), 6Ff_r (†), Df_r (¤); P<0.05.

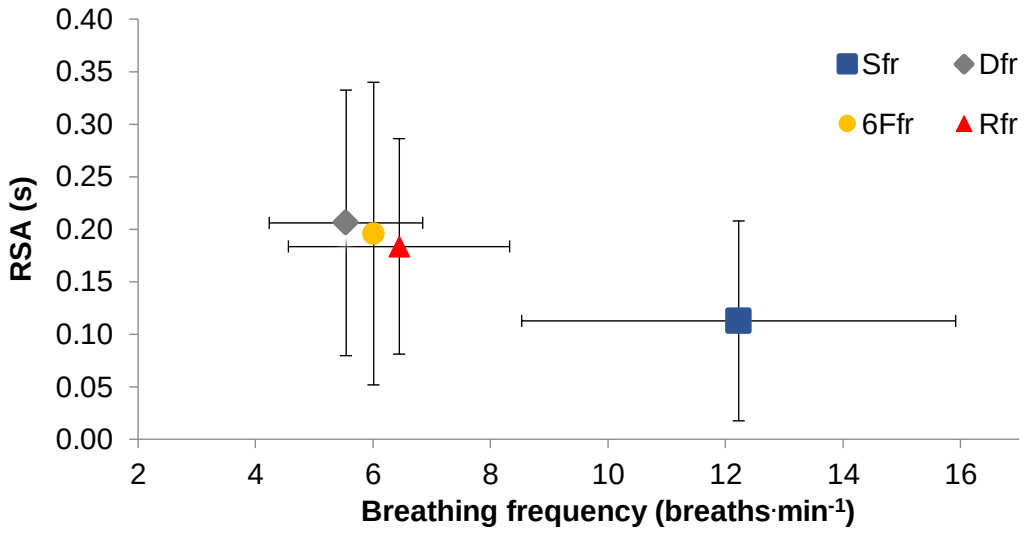

Figure Error! No text of specified style in document.**-5 Respiratory sinus arrythmia (RSA) response to slow and deep breathing**

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 SV∆i and SV∆e, but paired comparisons revealed no significant differences between breathing conditions (Table **Error! No text of specified style in document.**-5). Intra-breath phase cardiac output ($\dot{Q}$) increased during SDB significantly and was significantly different from Sf_r for $6Ff_r$ for ∆i and ∆e, and for Df_r for ∆i.

Table Error! No text of specified style in document.**-5 Mean (±SD) peak-valley differences for stroke volume (SV) and cardiac output (Q̇)**

		Sf_r	**Rf_r**	**6Ff_r**	**Df_r**	**Effect of condition *P* value**	**Sex x Condition *P* value**
	F	5.3 ± 2.5	8.5 ± 3.8	9.5 ± 5.4	10.3 ± 6.2		
SVΔi	*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
	F	6.7 ± 3.0	9.9 ± 3.9	9.3 ± 1.1	11.1 ± 5.0		
SVΔe	*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
	F	-10.4 ± 3.3	-13.7 ± 3.3	-8.5 ± 12.4	-14.2 ± 4.5		
SVΔPV	*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
	F	11.2 ± 2.7	11.1 ± 2.6	12.1 ± 3.3	13.0 ± 4.8		
SVΔPV_Ind	*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
	F	363.0 ± 301.2	878.2 ± 463.5	943.6 ± 474.3	1042.4 ± 694.5		
Q̇Δi	*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
	F	517.8 ± 452.7	821.2 ± 485.3	860.1 ± 363.3	760.8 ± 449.2		
Q̇Δe	*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
	F	-751.2 ± 337.6	719.2 ± 1015.5	281.3 ± 1187.0	486.6 ± 1200.3		
Q̇ΔPV	*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
	F	842.6 ± 344.6	1034.6 ± 560.9	1086.2 ± 474.4	941.9 ± 584.6		
Q̇ΔPV_Ind	*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 (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 (*); 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 Error! No text of specified style in document.**-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 **Error! No text of specified style in document.**-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 **Error! No text of specified style in document.**-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).

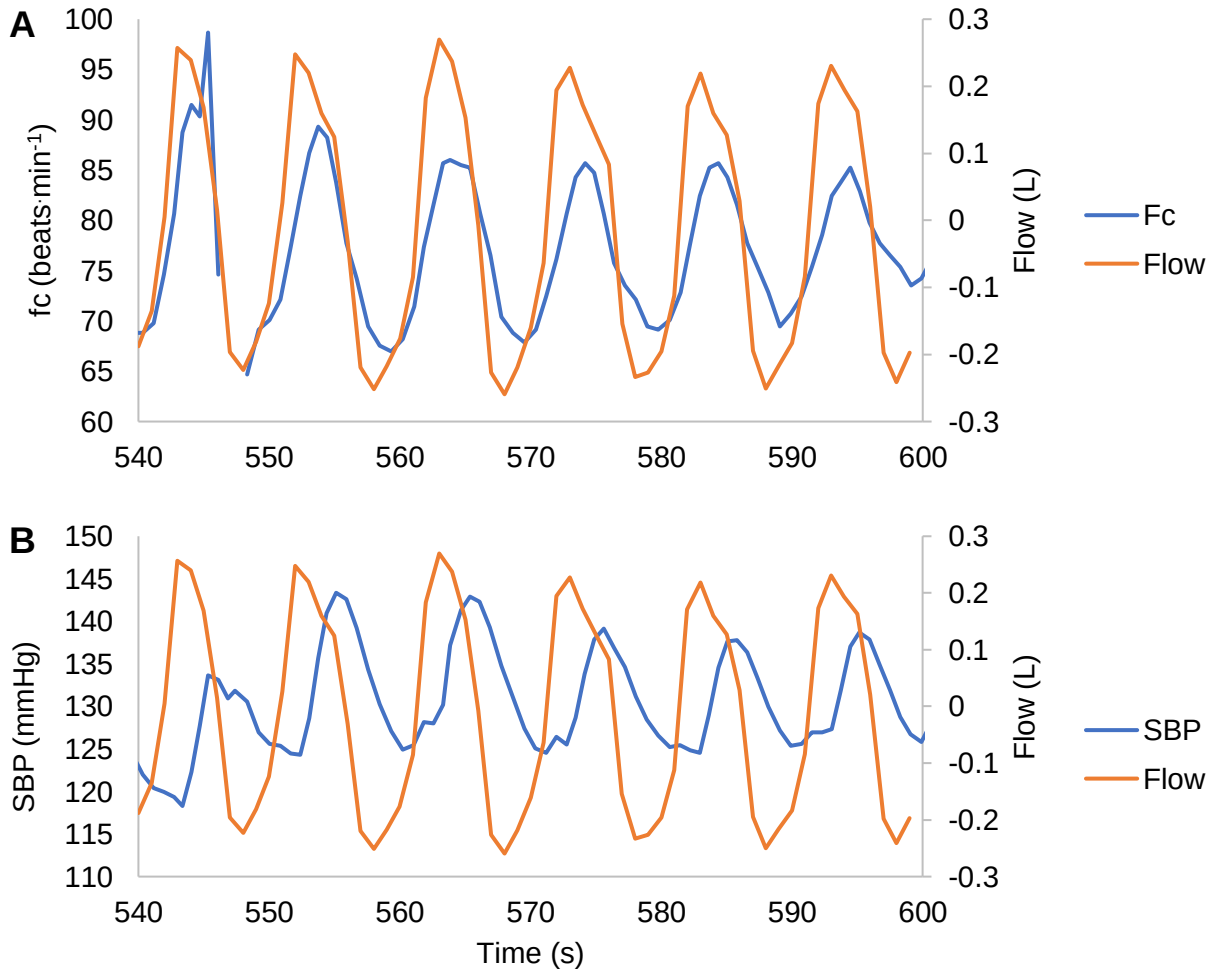

Figure Error! No text of specified style in document.**-6 Respiratory synchronisation of heart rate (fc) (A) and systolic blood pressure (SBP) (B)**
Heart rate (f_c; beats·min^{-1}), systolic blood pressure (SBP; mmHg), inspiratory flow (L: 1 second average). Data for 1 participant during last minute 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 **Error! No text of specified style in document.**-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 **Error! No text of specified style in document.**-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.

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