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) 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) 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 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 2019). 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), 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.

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

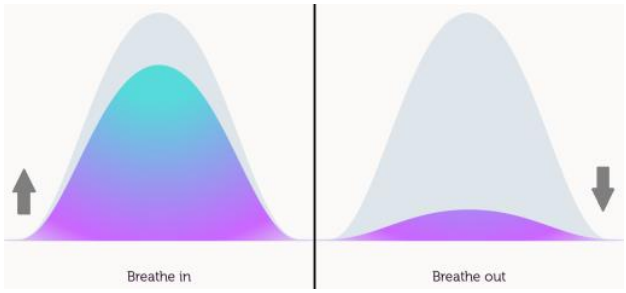

Figure Error! No text of specified style in document.**-1 Screenshots of Brythm graphic**
N.B: Arrows do not appear on app but are shown here to display the direction of graphic movement.

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 **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.

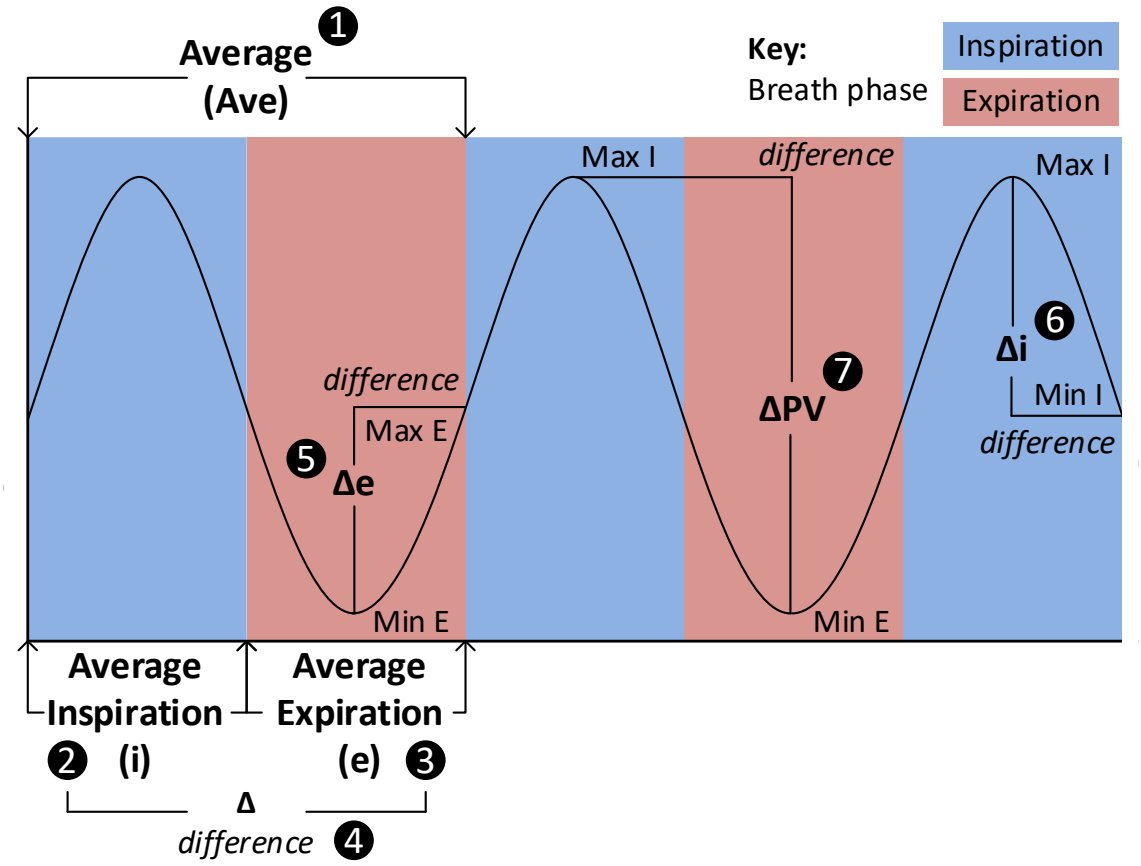

Figure Error! No text of specified style in document.**-2 Calculations for example cardiovascular variable plot**
1) Ave = average of whole breath. 2) i = Average inspiration. 3) e = Average expiration. 4) Δ = i – e (average inspiration – average expiration). 5) Δe = Max E – Min E. 6) Δi = Max I – Min 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 **Error! No text of specified style in document.**-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 Error! No text of specified style in document.**-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 Error**! No text of specified style in document.**-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 Error! No text of specified style in document.**-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 6F*f$_r$* 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 Error! No text of specified style in document.**-3 Peak-valley differences (±SD)**

		Sf$_r$	**8Ff$_r$**	**Df$_r$**	**6Ff$_r$**	**4Ff$_r$**	**Effect of condition *P* value**	**Group difference P value**
SBP	*NP*	3.6 ± 1.7¥¤†§	8.2 ± 2.9*¤†§	12.8 ± 5.4*¥	13.5 ± 4.6*¥	15.5 ± 6.1*¥	<0.001	
Δi	*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	*NP*	4.5 ± 2.5¥¤†§	6.9 ± 2.7*¤†§	10.2 ± 4.6*¥	10.5 ± 4.6*¥	12.1 ± 6.6*¥	<0.001	
Δe	*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	*NP*	-8.6 ± 3.6	-13.1 ± 7.0§	-11.5 ± 13.6§	-15.3 ± 9.5§	2.3 ± 18.4¥¤†	0.001	
ΔPV	*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	*NP*	15.0 ± 6.1	17.5 ± 5.6	19.1 ± 6.3	19.0 ± 5.1	17.4 ± 6.9	0.014	
ΔPV_Ind	*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	*NP*	2.5 ± 1.2¥¤†§	5.2 ± 1.8*¤†§	8.8 ± 2.9*¥	9.3 ± 3.1*¥	10.0 ± 3.0*¥	<0.001	
Δi	*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	*NP*	3.2 ± 1.6¥¤†§	5.6 ± 2.1*¤†§	7.6 ± 3.1*¥	8.2 ± 3.2*¥	8.8 ± 3.1*¥	<0.001	
Δe	*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	*NP*	-4.0 ± 2.2¥§	-7.6 ± 2.0*§	-1.9 ± 11.2§	-3.4 ± 11.1§	11.6 ± 7.3*¥¤†	<0.001	
ΔPV	*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	*NP*	9.9 ± 4.4	10.6 ± 2.9	12.1 ± 3.3	12.1 ± 2.5	12.7 ± 3.3	0.014	
ΔPV_Ind	*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*

for blood pressure variables (mmHg)

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 (∆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 (*), $8Ff_r$ (¥), 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 **Error! No text of specified style in document.**-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.

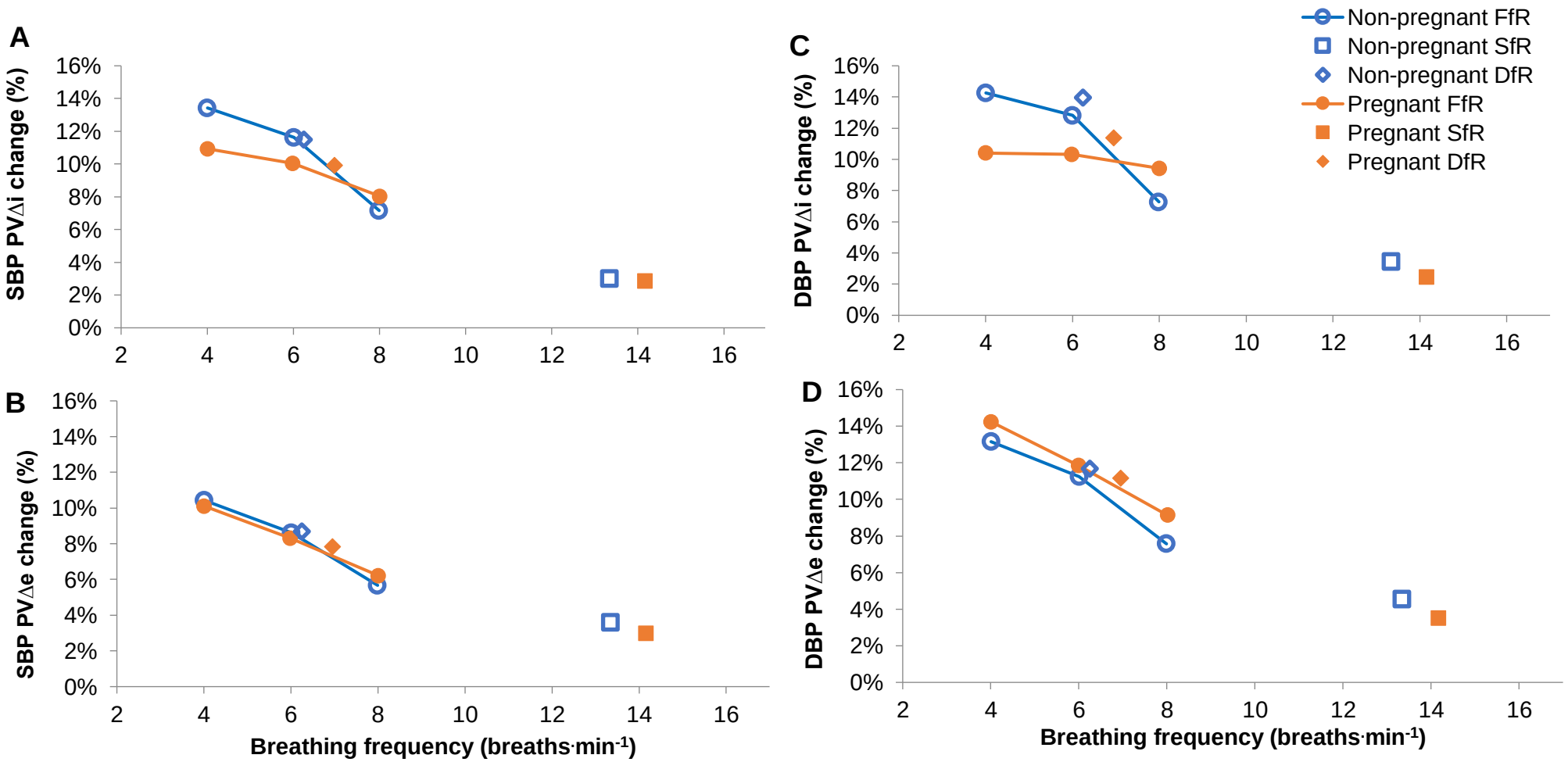

Figure Error! No text of specified style in document.**-3 Blood pressure oscillations: Relative change for systolic blood pressure of Δi (A), Δe (B) and diastolic blood pressure of Δi (C), Δi (D)**

Systolic blood pressure (SBP), diastolic blood pressure (DBP); *within expiration difference (∆i); within expiration difference (∆e); Fixed breathing frequency (Ff_r) Spontaneous breathing (Sf_r), optimisation algorithm dynamic breathing frequency (Df_r). Variable calculated as SBP∆i 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 Error**! No text of specified style in document.**-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 Error**! No text of specified style in document.**-4).

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

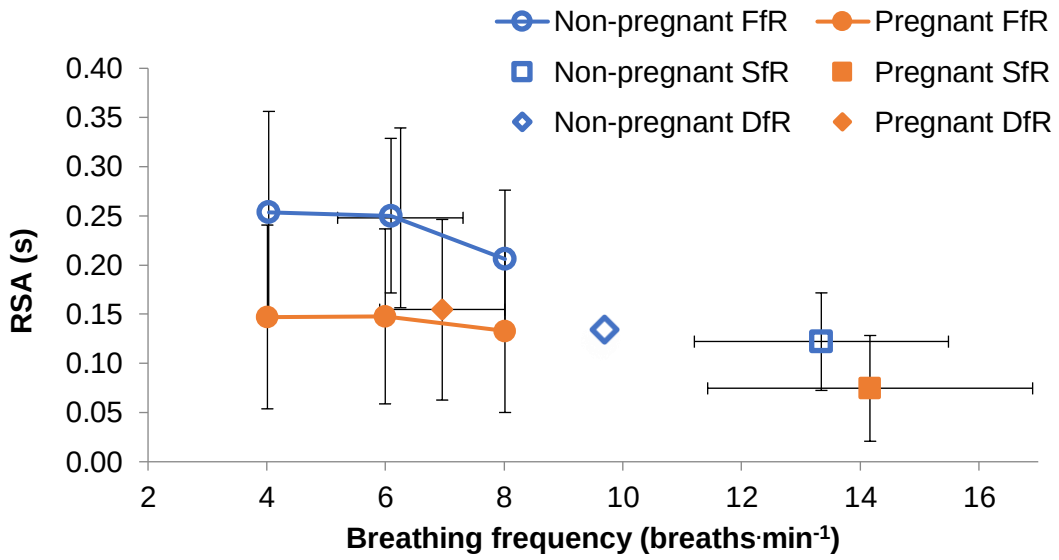

Figure Error! No text of specified style in document.**-4 Respiratory sinus arrythmia (RSA) response to slow and deep breathing**
Values are mean ± SD; Respiratory sinus arrhythmia (RSA); Fixed breathing frequency (Ff$_r$) Spontaneous breathing (Sf$_r$), optimisation algorithm dynamic breathing frequency (Df$_r$).

Stroke volume and cardiac output

Mean stroke volume (SV) and cardiac output (Q̇) were significantly higher for pregnant participants than non-pregnant participants (supplementary data). Peak-valley amplitude for SV and Q̇ during inspiration and expiration (SVΔi, SVΔe, Q̇Δi, Q̇Δe) were significantly different during all SDB conditions compared with Sf_r for non-pregnant and pregnant participants (Table Error**! No text of specified style in document.**-5). The only exception was SVΔi, 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 Q̇, compared with peak-valley values linked with breath phases for both non-pregnant and pregnant women.

Table Error! No text of specified style in document.**-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*
Q̇Δi	*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*
Q̇Δe	*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*
Q̇ΔPV _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 **Error! No text of specified style in document.**-6 shows a significant increase in TPRΔi and TPRΔe during SDB compared with Sf_r for both pregnant and non-pregnant women.

Table Error! No text of specified style in document.**-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) 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 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 **Error! No text of specified style in document.**-3). This supports the data from Felton et al. (2021 – in preparation), 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 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}.

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