UNIVERSITY OF BIRMINGHAM

Is early evidence of cardiovascular and respiratory disease already present in young healthy South Asians or White Europeans: could respiratory training lead to improvement?

By

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Abstract

Background: Hypertension is well-known as the forerunner of most cardiovascular diseases. The prevalence of hypertension, coronary artery diseases, and other cardiovascular diseases has been reported to be higher among men and women of South Asian ethnicity than White Europeans. Further, South Asians have lower lung function than White Europeans, and poor lung function has been reported as a risk for developing future hypertension and cardiovascular disease.

Objective: The overall objective of this PhD project was to investigate whether young healthy normotensive South Asian adults show early markers of autonomic dysfunction at rest and in response to mental stress that may lead to future hypertension and to assess whether acute or long-term slow breathing may have beneficial effects on autonomic regulation of arterial blood pressure (ABP), especially in South Asians.

Methods: Three studies were conducted to test the hypothesises. The first compared haemodynamic variables, heart rate variability (HRV) which largely reflects vagal influences on HR and baroreceptor reflex sensitivity (BRS) between young normotensive South Asian and White European adults at rest, in response to acute slow breathing at 6 breaths/ minute and acute mental stress. The second tested whether there were sex-dependent differences in these responses within and between ethnicities. Thirdly, a systematic review was performed to evaluate the effects of acute slow breathing and slow breathing training on autonomic regulation of ABP in healthy, normotensive South Asians

Results and Conclusions: Young South Asians showed a pronounced pressor response to mental stress associated with greater peripheral vasoconstriction than occurred in White Europeans, vasoconstriction being most prevalent in South Asian women. These novel findings are consistent with mental stress facilitating development of hypertension in South Asians. Further, White Europeans showed the characteristic inhibition of the vagal and sympathetic

component of BRS in mental stress, but both components of BRS to falls in ABP were preserved in South Asians, possibly protecting them against vasovagal syncope but facilitating their risk of hypertension. By contrast, acute slow breathing decreased ABP and increased HRV in both young South Asians and White Europeans but decreased tonic peripheral vasoconstriction in WEs only. These new findings suggest that South Asians may be more resistant to the beneficial effects of slow breathing on sympathetic activity. However, the systematic review and meta-analysis showed that both acute slow breathing and four weeks of slow breathing training were able to decrease ABP and increase HRV in healthy normotensive South Asians. Thus, these outcomes are consistent with our hypotheses. Importantly, considered together with our experimental findings they suggest that a study should be performed to evaluate whether slow breathing can provide a beneficial therapeutic intervention for young normotensive South Asians and reduce their risk of future hypertension and cardiovascular disease.

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List of abbreviations

ABP	Arterial Blood Pressure
ACLS	Advanced Cardiac Life Support
ANB	Alternative Nostril Breathing
ANOVA	Analysis of Variance
BLS	Basic Life Support
BMI	Body Mass Index
BP	Blood Pressure
BRS	Baroreceptor Reflex Sensitivity
CAD	Coronary Artery Disease
CI	Confidence Interval
CO	Cardiac Output
COPD	Chronic Obstructive Pulmonary Disease
CVD	Cardiovascular Disease
CVS	Cardiovascular System
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
ES	Effect Size
ETCO2	End Tidal CO2
FEV1	Predicted Forced Expiratory Volume in the first second
FVC	Forced Vital Capacity
FVR	Forearm Vascular Resistance
HCU	Height Correction Unit
HF	High Frequency

HR	Heart Rate
HRV	Heart Rate Variability
HUT	Head-Up Tilting
ICU	Intensive Care Unit
IPAQ	International Physical Activity Questionnaire
IQR	Interquartile Range
LF	Low Frequency
M	Mean
mABP	Mean Arterial Blood Pressure
MAP	Mean Arterial Pressure
MI	Myocardial Infarction
MS	Mental Stress
MSNA	Muscle Sympathetic Nerve Activity
NIBP	Non-Invasive Blood Pressure
NICE	National Institute for Health and Care Excellence
NIH	National Institute of Health
NO	Nitric Oxide
NTS	Nucleus Tractus Solitarius
PE	Phenylephrine
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PIS	Participant Information Sheet
RCT	Relevant Randomised Control Trial
RMSSD	Root Mean Square of Successive Differences between normal heart beats
ROB	Cochrane Risk of Bias
ROBINS-I	Cochrane Risk Of Bias In Non-randomized Studies - of Interventions

RR	Respiratory Rate
RRI	R-R Interval
RSA	Respiratory Sinus Arrhythmia
SA	South Asians
SB	Slow Breathing
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDNN	Standard Deviation of the R-R intervals of Normal sinus beats
SEes	Standard Error of effect size
SEM	Standard Error of Mean
SNP	Sodium Nitroprusside
SV	Stroke Volume
TPR	Total Peripheral Resistance
UK	United Kingdom
ULF	Ultra Low Frequency
USA	United States of America
VLF	Very Low Frequency
WE	White Europeans
WHO	World Health Organisation

Chapter 1: General introduction

1.1 Overview

Essential hypertension, which is the forerunner of much CVD, is characterized by chronic elevation of the arterial blood pressure (ABP) with no primary underlying medical cause. As classified by the new National Institute for Health and Care Excellence (NICE) guidelines, the definition value of hypertension is 140/85 mmHg [(1)]. In 2010, 31% of the world's adult population, about 1.39 billion individuals, had hypertension [(2)]. Hypertension prevalence increased globally by 5.2% between 2000 and 2010 [(2)]. The World Health Organisation (WHO) reported that around 46% of individuals are unaware of having hypertension, and of those, only around 42% were diagnosed and treated [(3)]. Clinically, the high and rising essential hypertension prevalence is important due to the increased risk of developing further cardiovascular disease (CVD) that result from untreated hypertension [(3)].

Amongst ethnic groups, it is recognized that the risk of developing CVD, including essential hypertension, is greater in South Asians (SAs), with ethnic roots originating from India, Pakistan, Bangladesh, Sri Lanka, or Nepal than in White Europeans (WEs) [(4)]. Additionally, SAs are more affected by coronary artery disease (CAD) than WEs, with SA women presenting earlier and with greater severity than WE women [(5-8)]. Thus, SAs have a higher mortality rate related to essential hypertension and CAD compared to other ethnic groups [(9)]. There is also evidence that lung function is depressed in SAs relative to WEs [(10-12)], that this is associated with future hypertension and CVD [(13)] and that in general across ethnicities, CVD and respiratory disease are associated [(14, 15)]. The mechanisms underlying this association are not clear. Chronic systemic inflammation may be an underlying pathophysiological link [(14)], but the possibility is raised that autonomic regulation of the cardiovascular system is disturbed in SAs given that changes in ventilation affect both sympathetic and parasympathetic control of the cardiovascular system (CVS) [(16)].

Given this background the general aims of this PhD project were to compare autonomic regulation of ABP in SAs and WEs and the influences of changes in respiration on a range of systemic cardiovascular variables. Subsequent studies were planned to investigate the mechanisms underlying any differences that emerged. In addition, the plan was to test the effect of inspiratory muscle training on respiratory and cardiovascular functioning in SAs and WEs. However, as a consequence of the Covid-19 pandemic, laboratory-based experimental work was curtailed after the first study and could not be resumed within the period of financial support for the PhD. Thus, a systematic review was performed to evaluate published studies on the effects of slow breathing on cardiovascular function in SAs. The sections below, cover the background to the project in more detail and explain the methodological approaches used.

1.2 Prevalence of hypertension and CVD in SAs

The high risk of developing CVD is greater in SAs living in the United Kingdom (UK) and western countries. Thus, comparing SAs living in the UK and other western countries including the United States of America (USA) and Canada with WEs, the risk of developing CVD including hypertension, stroke, and CAD is 2-3-fold higher in SAs than WEs [17, 18]. It has also been reported that SAs have a 10.7% prevalence of CVD, compared to 5.4% and 2.4% in WEs and Chinese, respectively [19]. Moreover, in comparison to WEs, the burden and mortality of CVD are significantly higher among SAs. For example, SAs had a CAD mortality rate 40-50% higher than the general population in England and Wales in 1991 [20]. An analysis over a 15-year period of age-standardized CAD also showed that the proportional mortality rate from CAD is greater for Canadian men and women of SA descent (42% and 29%, respectively) than for those of European (29 % and 19%, respectively) or Chinese descent (18% and 11%, respectively) [21]. At the time of their first myocardial infarction (MI) or development of heart failure, SAs tend to have larger MIs and more severe CAD as seen during angiography

[22]. Further, several studies have shown that SA living in the UK and other countries worldwide had 3-5-fold higher risk of CVD and MI death compared to other ethnic populations [23-26]. Interestingly, South Asian women are particularly at high risk of CAD: they present earlier and with greater severity than women of other ethnicities [5, 8].

There are studies showing that the risk of stroke and CAD are associated with hypertension in SAs living in Western countries as in WEs [27]. The prevalence of hypertension has been previously reported to be higher in SAs living in the UK [28]. Some previous studies have reported greater prevalence of hypertension in SA men than WE men [29-32], while others have found that hypertension prevalence is higher in SA women compared to WE women [33]. In addition to this, it has been reported that SA men [29, 34, 35] and women [34] show higher resting blood pressure (BP) than WE men and women, respectively. However, it should be noted that some studies have reported lower or similar hypertension prevalence in SA men [33, 36] and women [30, 32, 37], and lower BP levels among SA men and women [33, 38].

Importantly, Battu, Bhopal [39] who conducted a study to compare young SAs with WEs showed that SA children (~15 years old) have higher BP than WE children and the discrepancy increased between 1999 and 2004 suggesting the risks are increasing for SAs. This group also demonstrated that hypertension prevalence is greater in Pakistanis and Bangladeshis than Indians which explains the discrepancies between studies as to whether hypertension does have higher prevalence in SAs than WEs. This study certainly gives good reason for looking at young adults.

There are also studies showing that BP variability in hypertensives is associated with increased risk of CAD and stroke in WEs and African Americans, although this has not been tested in SAs [40, 41]. Theses authors suggested that the increased variability may be related to changes in the elastic properties of blood vessels and aortic distensibility and/or disturbed

baroreflex function leading to exaggerated pressor response to physical and emotional stimuli as well as social and lifestyle factors [42]. Thus, it is important to test baroreceptor reflex sensitivity (BRS) and effects of mental stress in young SA men and women (see sections 1.5 and 1.8).

Taken together, these findings suggest that SA ethnicity is a risk factor for CVD and points the attention to the possibility that young normotensive SA adults may be at a high risk of developing CVD. Thus, it is important to investigate the early markers of hypertension and CVD in young men and women of this ethnic group further.

1.3 Respiratory function

SAs have reduced lung function compared to WEs as assessed by % predicted forced expiratory volume in 1 second (FEV1) [10-12], and FEV_1 is ranked second as a predictor of cardiovascular mortality [43]. Moreover, SAs also have lower forced vital capacity (FVC) compared to WEs [10-12]. Importantly, in the present context it was shown that decline in FVC even within the normal range over 10-20 years from young adulthood (i.e. 18-30 years old) predicts future hypertension [44]. These authors suggested that the relationship might be explained by parallel reduction in elasticity of lung tissue and arterial walls, or to low levels of inflammation that affect both lung tissue and vascular endothelium. Further, the CARDIA study by Cuttica *et al* [13] showed that baseline FEV_1 and FVC in young adults predicted heart failure and stroke, but not CAD in middle age, independently of traditional CVD risk factors. Since there was no association between lung function and markers of atherosclerosis it was again suggested that coincident reduction in lung and arterial compliance may underlie the development of both CVD and lung disease [13]. To date, no studies have apparently been performed to compare the predictive value of lung function for prevalence of CVD or acute cardiovascular events in SAs and WEs. However, it should be noted that changes in ventilation

affect cardiovascular and ABP regulation and heart rate variability (HRV) via central neural mechanisms and several different autonomic reflexes (see section 1.6). The relevance of these mechanisms to the link between respiratory and CVD in SAs has received little attention and is good reason to study these issues in young normotensive SAs relative to WEs.

1.4 Autonomic function in hypertension

Autonomic influences are crucial in the homeostatic regulation of the cardiovascular system and in controlling ABP, and of particular relevance to the present study, autonomic dysfunction has been shown to help predict hypertension [45]. Indeed, one of the most widely accepted and tested hypotheses in cardiovascular research is that essential hypertension is induced by an autonomic dysfunction and imbalance of sympathovagal control of the cardiovascular system [46-49]. Pharmacological evidence demonstrated that this dysfunction is characterised by a decrease in vagal (parasympathetic) activity to the heart and increase in sympathetic nerve activity, resulting in elevated heart rate (HR) and vasoconstriction, leading to raised vascular resistance and elevated ABP [49, 50]. Autonomic function was shown to be more impaired in those with uncontrolled hypertension than those who have controlled hypertension [51].

Pharmacological evidence of imbalanced sympathetic and vagal influences on HR in individuals with hypertension is one indicator of altered sympathovagal balance and autonomic dysfunction. Another indicator of autonomic imbalance and sympathetic hyperactivity is increased plasma norepinephrine [49]. Hypertensives, particularly who were aged <40 years, show increased plasma norepinephrine relative to normotensive controls, indicating increased sympathetic nerve activity [52]. Further, in hypertensive animals, both raised sympathetic activity and reduced vagal activity are linked with and responsible for the development and maintenance of elevated BP, leading to hypertension-related complications [53-55].

Similar observations of imbalanced sympathovagal activity has been reported in several studies which demonstrated that young hypertensives, those in the early stages of hypertension, borderline hypertensives, and offspring of hypertensive parents also show increased sympathetic activity and decreased cardiac vagal tone [56-59]. Furthermore, increased activity in both central and peripheral components of the sympathetic nervous system was found in individuals in early stages of hypertension, leading to persistent sympathetic hyperactivation [49]. As a result, it appears that this imbalance may the forerunner of hypertension in early and borderline hypertension, as well as contributing to the maintenance of persistent hypertension [46].

In borderline hypertensives who have significantly higher resting ABP and HR than normotensives [60], propranolol injection to block beta-adrenoreceptors caused a larger reduction in HR than in normotensives, while subsequent muscarinic receptor inhibition with atropine produced a smaller elevation in HR in the borderline hypertensive individuals, leading to similar HR values in both groups [60]. These results showed that the elevation in HR in borderline hypertensive individuals is due to greater sympathetic and less vagal inhibitory effects on the heart [49, 60]. In addition, it is interesting to note that further investigations also demonstrated that atropine caused a smaller reduction in glandular secretions in borderline hypertensives than in normotensives, indicating that the vagal activity in early hypertension is impaired in all systematic vagally dependant functions, rather than just in the cardiovascular system [49, 61].

Long term excitation of the sympathetic system and hypertension leads to left ventricular remodelling involving hypertrophy, which is at least in part attributable to the trophic influences of catecholamines and angiotensin [62], and to vascular remodelling,

involving thickening of the walls of the resistance arteries and arterioles and hyperresponsiveness to vasoconstrictor stimuli [62]. Muscle sympathetic nerve activity (MSNA) which is an indicator of vasoconstrictor nerve traffic to the blood vessels [63] is greater in hypertensives than in normotensives, according to recordings of sympathetic nerve traffic [64]. It has been shown that there is a positive correlation between MSNA and ABP as shown in Figure 1.1, which indicates the activity in the sympathetic fibres progressively rises with the severity of hypertension [49, 65]. Individuals with established essential hypertension also show raised total peripheral resistance (TPR), while stroke volume (SV) and cardiac output (CO) are maintained normal, or decreased [66]. Further, essential hypertension is associated with reduced BRS [65, 67] and HRV [68] (see section 1.5 and 1.6). Taken together, these findings indicate autonomic imbalance in essential hypertension as reflected by raised sympathetic and decreased vagal influences on the heart and increased sympathetic drive to the vasculature.

Interestingly, SA adolescents were found to have higher BP than matched WEs [39], and it was also reported that resting HR was higher in SA than WE children (aged 10-11 years) [69]. These findings indicate that ethnic differences are already present even at young age between SAs and WEs. Further, there is evidence of a positive correlation between HR and BP at all ages and in both sexes [46, 70], and it has been established that increased HR can predict future hypertension [71, 72]. Possibly the most significant finding is the positive correlation between higher HR and future CVD mortality [73, 74], and overall mortality [75]. Thus, these findings suggest that sympathovagal imbalance may be present in young SAs and may increase their risk of future hypertension. Little research has been conducted to elucidate sympathetic/vagal balance in young normotensive SAs adults. Indeed, such studies would be valuable.

These links between autonomic dysfunction and hypertension, which is a forerunner of most CVD raise a question of whether certain individuals are predisposed to the risk factors of

developing CVD, or if certain risk factors predispose to CVD. Whichever is the case, autonomic dysfunction and early markers of hypertension might be expected in young normotensives who are at high risk of developing hypertension, and this could include SAs.

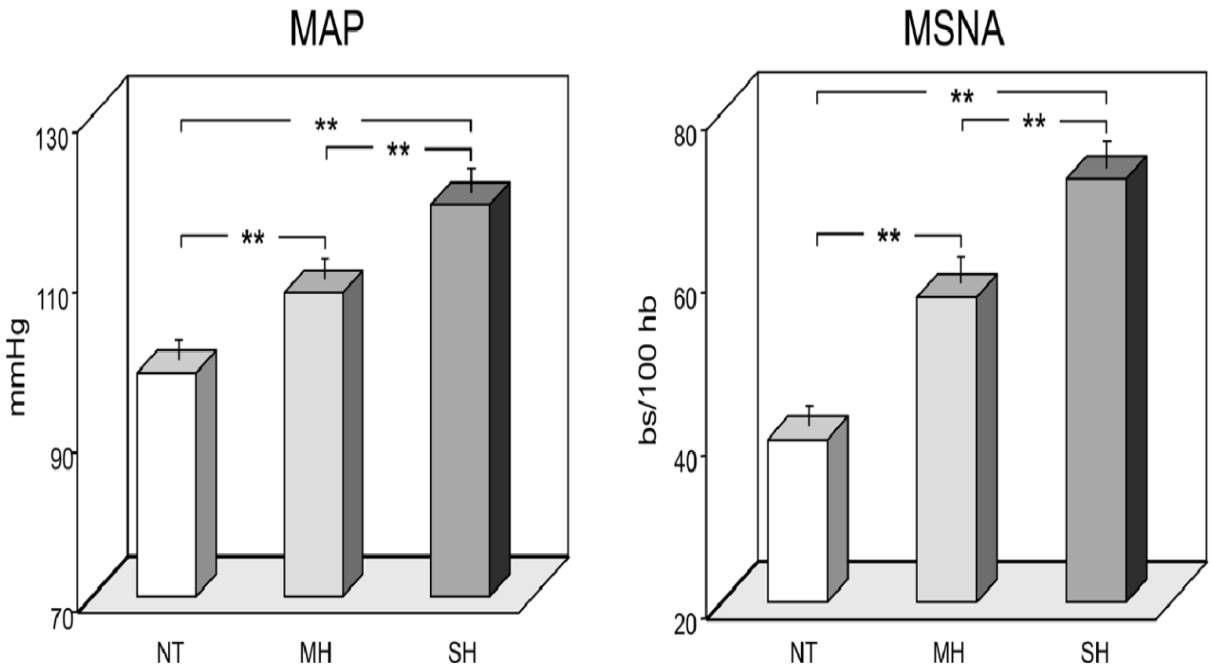


Figure 1.1 A comparison showing the association between MSNA and MAP in normotensives, individuals with moderate essential hypertension, and individuals with severe essential hypertension.

NT: normotensives; MH: moderate essential hypertension; SH: more severe essential hypertension; MAP: mean arterial pressure; MSNA: muscle sympathetic nerve activity.
Obtained from Mancia *et al* [49]

1.5 Baroreceptor reflex

Arterial baroreceptors are stretch receptors located in the walls of the aortic arch and carotid sinus: the baroreceptor reflex regulates ABP [16]. Baroreceptors are very sensitive and respond rapidly to increases or decreases in ABP. A decrease in ABP causes a decrease in stretch of the baroreceptors and decreases the afferent activity into nucleus tractus solitarius (NTS). This, in turn, evokes a reflex decrease in parasympathetic activity and an increase in sympathetic activity to the heart which increases HR, while the increase in cardiac sympathetic activity also causes an increase in ventricular contractility, so improving SV. In addition, there is an increase in sympathetic activity to the vasculature which causes arteriolar and venous constriction, so increasing TPR and improving ventricular filling, end diastolic volume and SV, respectively. Taken together, these reflex changes increase CO and TPR and increase ABP towards normal. An increase in ABP causes exactly the opposite set of reflex changes [16]. Importantly, the reflex change in TPR makes a much bigger contribution to the change in ABP than the change in CO (80% vs 20%, respectively) [16]. By helping to stabilize ABP at near normal levels the baroreceptor reflex helps to maintain blood perfusion to the brain [49, 76]. The baroreceptor reflex therefore play an essential role in the autonomic regulation of cardiovascular function in healthy individuals [16].

The properties of the baroreceptor reflex can be shown by plotting ABP against the reflex change in a controlled variable, such as HR, or MSNA: the input-output relationship. Experimentally, this can be done by changing ABP over a range of ABP values (input) and recording the evoked changes in the variable under investigation (output) [16]. The input-output relationship can be represented as a sigmoidal curve where the slope of the curve shows the gain or sensitivity of the baroreceptor reflex (BRS) [49].

As indicated above, one of the major risk factors in the development of essential hypertension is sympathetic overdrive [49]: the increase in baseline ABP in established hypertension reflects sympathetic overdrive [49, 77-79] and blunted excitability of cardiac vagal neurones [49]. This raises an obvious question of whether the baroreceptor reflex is disturbed in hypertension, and indeed, whether the baroreceptor reflex is disturbed in those who are at higher risk of hypertension or of other CVDs. Not surprisingly, several different methods have been developed to test BRS. These are dealt with below, together with their advantages and disadvantages and emphasising the methods that were used in the present studies.

1.5.1 Methods of testing baroreflex sensitivity (BRS):

1.5.1.1 Injection of vasoactive drugs

For many years one of the standard ways of testing BRS in human subjects was to give intravenous bolus injections of pressor and depressor agents, for example, phenylephrine and sodium nitroprusside respectively [80], often known as the "Oxford Method". Injection of a pressor agent, for example phenylephrine (PE) evokes a rise in systolic blood pressure (SBP) and reduces HR via the baroreflex, and *vice versa* for injection of a depressor agent for example sodium nitroprusside (SNP). The change in HR or pulse interval (R-R Interval taken from a standard ECG), usually with a delay of one beat, is plotted against the evoked SBP over a range of concentrations of the two agents to give a regression line above and below the resting SBP, which is taken as the measure of BRS [80, 81]. This method provides only a measure of the cardiac component of BRS and the changes in HR are mainly due to change in vagal activity, given they are almost entirely blocked by atropine [81]. The disadvantage of this method is that it is invasive and is technically complicated requiring cannulation and use of infusion pumps.

1.5.1.2 Neck Chamber

This method involves the use of a specially constructed box which is sealed around the neck of the individual, around the location of the carotid baroreceptors in the carotid sinus. Tubing is connected to a pressure device such that pressure within the chamber can be reduced in a graded fashion to produce graded stimulation of the baroreceptors or increased, so as to produce graded unloading of the baroreceptors [82]. This technique holds the advantage that it can be used to assess the vascular as well as the cardiac component of the baroreceptor reflex. Further, it allows changes in BRS to be assessed during longer lasting changes in baroreceptor activity whereas this is not possible with bolus injections of vasoactive drugs. On the other hand, it is a cumbersome device which makes it difficult to use for assessing BRS during changes in activity, for example exercise and mental stress. Further, the changes in chamber pressure affect only the carotid baroreceptors and so the evoked reflex changes are buffered by the aortic baroreceptors [81].

1.5.1.3 Head-Up Tilting

A further test that is used to assess the BRS is head-up tilting (HUT). Changing body position from horizontal to HUT leads to a decrease cardiac output and reduction ABP, which causes a reflex changes including an increase in TPR and HR, so restoring ABP [83]. This method has the advantage that it is relatively simple, and it mimics the effects of standing up from supine and can be used in a graded manner to evoke graded reflex changes. Also, it allows the cardiac and vascular components of the reflex responses to be assessed by measuring changes in cardiac function, regional vascular resistances and sympathetic nerve activity [81]. However, HUT also produces deactivation of cardiopulmonary receptors and affects the vestibular apparatus, which means the test is not specific to the baroreceptor reflex. [81].

1.5.1.4 Spontaneous BRS assessment: the sequence method

The sequence method relies on calculating BRS during spontaneous changes in arterial pressure, specifically spontaneous changes in SBP. The definition of BRS is the slope of the regression line between changes in SBP and R-R interval, where R-R interval is taken from a standard ECG [84]. On the basis of analyses performed on cats and humans and comparisons between estimates of BRS made with the Oxford method and sequence method, the sequence method is generally based on identifying spontaneously occurring sequences of four or more consecutive beats in which there is either a progressive rise in systolic blood pressure and lengthening of the R-R interval (+RR/+SBP sequences) or a progressive decrease in systolic blood pressure and a shortening of the R-R interval (-RR/-SBP sequences) [81, 85] (see figure 1.2). The choice of 4 beats was selected on the basis that the BRS showed a small decrease when the number of beats was increased from 3-5, possibly reflecting central neural influences or loss of sensitivity of the baroreceptors (acute re-setting) [85]. For practical reasons, it is considered that that the sequential changes in SBP and R-R intervals should be equal to, or greater than 1 mmHg and 5 ms, respectively [81, 85]. Furthermore, it has been shown that the average duration of BRS assessment during the sequence method should be at least 10 to 15 mins in order for the BRS to be reliable and highly reproducible [86]. It can be assumed that baroreflex regulation of cardiac vagal activity is demonstrated by the sequence method, because the changes in R-R interval nearly disappear after injection of atropine [87].

The sequence technique has the advantage that it allows the reflex effects of spontaneous baroreceptor stimulation and deactivation to be assessed separately. It also allows BRS to be readily assessed in any situation in which ABP can be continuously recorded including exercise, mental stress and sleep. A disadvantage of the sequence technique relative

to the Oxford method is that it only assesses BRS over small changes in SBP and not over the full range as is allowed by higher concentrations of vasoactive agonists [81, 85].

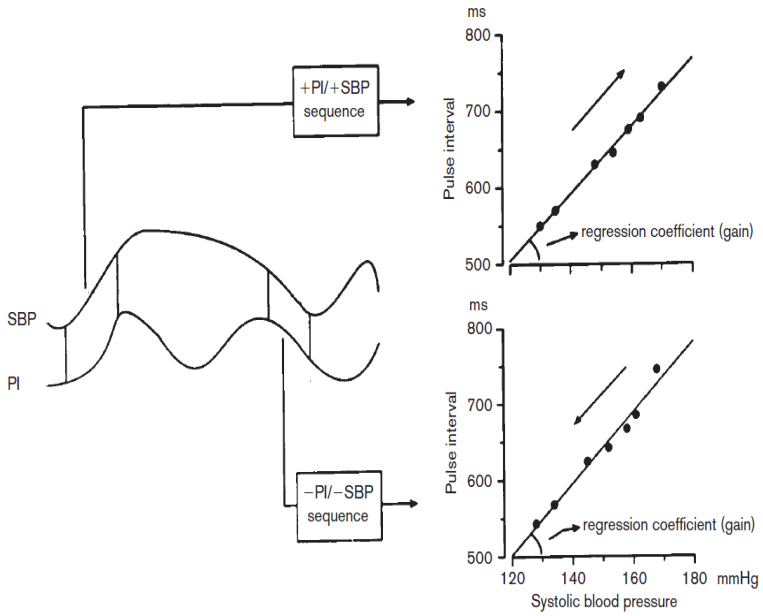


Figure 1.2 Spontaneous BRS assessment by the sequence method.

Adapted from Parati *et al* [81]

1.5.1.5 Squatting and standing

Another test which allows assessment of BRS over more dramatic changes in ABP is the squatting test. This is a non-invasive manoeuvre [88], which can be used to assess the sympathetic and parasympathetic components of the baroreceptor reflex in humans who are physically capable of squatting and standing [89, 90]. Changing position from standing to squatting causes an immediate, substantial increase in ABP, by increasing venous return and CO and physically increasing TPR, and is quickly followed by reflex bradycardia [88]. In contrast, changing posture from squatting to standing is associated with rapid reduction in BP due to venous pooling, followed by a reflex increase in HR and in TPR, and eventual restoration of ABP [88]. The reflex bradycardia evoked by the increase in ABP which occurs on squatting

was abolished by atropine and was therefore attributed to an increase in cardiac vagal activity [72]. On the other hand, the transition from squatting to standing causes a fall in ABP over ~10s and is closely followed by a reflex increase in HR which has been attributed predominantly to an increase in cardiac sympathetic activity [88]. In fact, the R-R intervals during squat-to-stand can be plotted against the SBP to give a slope which represents BRS [88] (see Figure 1.3) [71]. This reflex tachycardia was virtually abolished by the beta-adrenoreceptor antagonist propranolol indicating it was sympathetically mediated [89] [72]. Furthermore, during squat-to-stand, HRV assessed in the frequency domain by spectral analysis (see section 1.6 below) showed an increase in relative power in the low frequency band 0.05-0.1 Hz consistent with an increase in cardiac sympathetic activity [91, 92] . These findings indicate that the squat-to-stand test is a useful non-invasive way of testing the cardiac sympathetic component of BRS and suggest it may prove an appropriate way of comparing this aspect of BRS in young SAs and WEs.

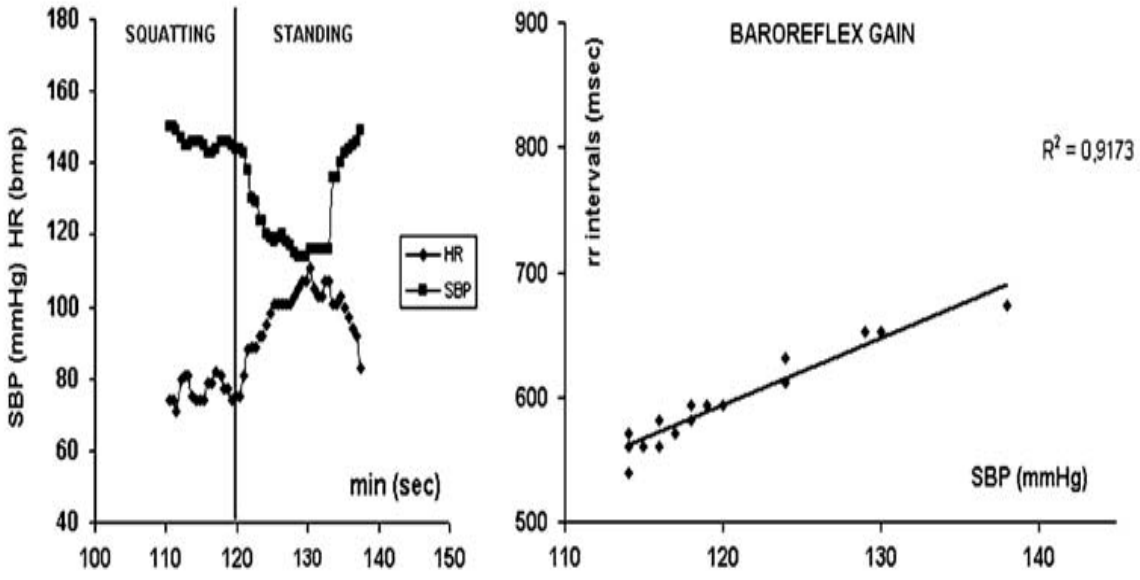


Figure 1.3 BRS gain calculation during squat-to-stand test. Changes in HR and SBP during posture change from squatting to standing position

Adapted from Scheen *et al* [88]

1.5.2 Baroreceptor reflex in hypertension and CVD

As indicated above in section 1.4, hypertension is associated with a resting increase in sympathetic drive to the vasculature which is graded with the severity of the hypertension and an associated reduction in cardiac vagal tone. Experiments performed using the Oxford technique showed that BRS for the relationship between MSNA and mean arterial pressure (MAP) was unchanged in moderate and severe hypertensives relative to control normotensive subjects, but the curves were displaced towards higher levels of MAP, i.e. there was re-setting of the baroreceptor reflex [65]. By contrast, the cardiac component of the baroreceptor reflex was found to be impaired in hypertension: the BRS for the relationship between HR and MAP was progressively reduced in moderate and severe hypertensives relative to normotensives, coincident with the increase in resting MAP [49, 65] and consistent with reduced excitability of cardiac vagal neurons in established hypertension [49]. Similarly, Ormezzano, Cracowski [93] found when using the sequence method to assess the BRS to spontaneous rises and falls in SBP in a group of untreated mild-moderate hypertensives, that the cardiac component of the baroreceptor reflex was depressed relative to that of normotensives (mean age 55 years and 47 years, respectively).

In relation to the present project, important questions are whether BRS is changed in those at higher risk of hypertension or CVD and whether any changes in BRS may precede development of disease, occur simultaneously with it, or afterwards. These issues have been investigated by several groups. For example, it was shown that BRS assessed as the bradycardia evoked by graded bolus injections of PE was depressed in young, pre-hypertensives relative to normotensives (mean age 19 vs 18 years, respectively) [94]. Further, it was shown that the cardiac component of BRS as assessed by PE, or by the sequence method to spontaneous rises

in SBP, was depressed in borderline hypertensives relative to normotensives (mean age 35 vs 32 years, respectively [95].

As far as studies on BRS in South Asians are concerned, there seem to have been few studies to date. Bathula, Francis [96] conducted a study on 84 SA and 83 WE men (aged 45-85 years; mean age - 60 and 65 years respectively), living in London, UK and recruited from the general population. As expected from the higher risk of CVD in SAs, higher proportions of the SAs in this study had diabetes and/or were being treated for hypertension. They found that the SAs had higher HR (shorter R-R intervals) which was associated with higher glucose in the SAs than WEs, but not with insulin resistance or hyperlipidaemia [96]. However, they could find no difference between ethnicities in the cardiac component of BRS as judged by the sequence method[21]. Nevertheless, in a subsequent study [97] on larger groups of SAs and WEs (151 and 149 respectively, including men aged 35-75 and women aged 55-75 years), HR was again higher in SAs than WEs and in this study BRS, as assessed by sequence analysis, was lower in SAs than WEs and this was associated with hyperglycaemia. The fact that these studies were performed on early middle-aged to elderly people means that it is impossible to deduce the extent to which age may have affected autonomic function in either ethnicity. However, the finding that BRS became significantly lower in SAs than WEs when women were included in the study raises the question of whether BRS might be lower in SA women. There appears to have been no study comparing BRS in young WE and SA men and women.

1.6 Heart rate variability (HRV)

HR is a measure of the number of heart beats/min. HRV is a measure of the variability of the intervals between successive heart beats, or the variability of the R-R intervals. HRV is typically measured over short-term (~ 5 min) periods, over 24 hr, and also over "ultra-short-term" periods (<5 min). In the short- and ultra-short term, it is recognised that HRV is affected

by parasympathetic and sympathetic influences on the sino-atrial node, and by the influence of reflexes such as the baroreceptor reflex on the parasympathetic and sympathetic activity [92]. HRV is also influenced by respiration, via the cyclical inhibitory influences that central respiratory drive and reflexes arising from respiratory movements have on cardiac vagal motor neurone activity and which are collectively responsible for respiratory sinus arrhythmia (RSA) [98, 99]. Over 24 hours, HRV is additionally influenced by circadian rhythms, core body temperature, the renin-angiotensin system and the sleep wake cycle [92]. Although 5 min recordings of HRV data are generally regarded as the gold standard for analysis of HRV, periods of <2 min are more efficient and practical in many experimental and clinical situations. Importantly, direct comparisons between analyses made over 5 min and periods ranging from 10 s to 5 min, indicate that periods of 2 min are reliable for healthy subjects providing an experienced individual removes artefacts such as ectopic beats [92]. Essentially, assessment of HRV has become important experimentally and clinically for assessing autonomic influences on the heart, particularly vagal influences, assessing cardiovascular health, and predicting cardiovascular morbidity and mortality [92].

Not surprisingly, several different methods have been developed for analysis of HRV of which the most commonly used are time-domain and frequency domain analysis. In order to standardise the methodology and allow meaningful comparison between results obtained by different groups around the world, A Task Force created by the European Society of Cardiology and the North American Society of Pacing and Electrophysiology devised and published a report which standardises the nomenclature, specifies standard methods of measurement and defines the physiological and pathological correlates of each of the measured indices on the basis of the available evidence [100]. This Task Force report and the review by Shaffer & Ginsberg [92] formed the basis of the description of the main methods of analysing HRV provided below.

1.6.1 Methods of analysing HRV

1.6.1.1 Time-domain measurements

These are the simplest ways of analysing HRV. Several indices have been described which quantify the amount of variability in measurements of R-R intervals obtained from a continuous recording of the ECG, expressed as original units or as the natural logarithm, to give a more normal distribution. SDNN is the standard deviation of the R-R intervals of "normal" sinus beats in ms, "normal" indicating that abnormal beats such as ectopic beats have been removed: SDNN is generally preferred to SDRR which includes the abnormal beats [92]. Standard deviation (SD) is the square root of variance and it is mathematically equal to the total power calculated by spectral analysis (see below). Thus, SDNN reflects all of the factors that contribute to HRV over the recording period although in the short term ($\leq$ 5 min) this largely reflect vagally-mediated influences [92, 100]. Another commonly used index is RMSSD which is the root mean square of successive differences between normal heart beats: each successive R-R interval is calculated first, then each value is squared and the result is averaged before the square of the total is calculated. RMSSD reflects beat-to-beat variance in HR and it is considered to be the time-domain index that provides the best estimate of vagally-mediated changes in HRV. Importantly, it is correlated with high frequency (HF) power estimated by frequency-domain analysis (see below), but it has been shown to be less affected by changes in respiratory rate (RR) than other indices of HRV[101], even when slow breathing is used to reduce RR to 6 breaths/min. The other commonly used time-domain index is pNN50 which is the percentage of adjacent NN intervals which differ from each other by > 50 ms: it also provides an assessment of vagal influences on HRV [92, 100].

1.6.1.2 Frequency-domain measurements

These measurements essentially provide information on how the power, ie the variance of HRV distributes as a function of frequency. The variance is split into its components – ultra low frequency (ULF), very low frequency (VLF), low frequency (LF), and high frequency (HF) by using an algorithm – usually Fast Fourier Transformation. The process is analogous to using a prism to split light into its wavelengths [92]. The ULF band (<0.0003 Hz) can only be discerned with 24-hour recordings and it probably reflects circadian rhythms [92]. The VLF band (0.0033–0.04 Hz) requires a recording period of at least 5 min. The physiological mechanisms contributing to it are not clear, but it seems to be fundamental to cardiovascular health and low power in this region is a strong predictor of all-cause mortality [92]. Thus, the two bands that are most useful in terms of evaluating autonomic influences on HRV in different conditions are LF and HF.

The LF band (0.04-0.15 Hz) can be recorded over 2 min. It is widely considered to largely reflect sympathetic activity, but this is questioned by many, for example, because LF does not disappear in the presences of sympathetic blockade and because vagally-mediated influences which are considered to dominate the HF band can cross over into the LF band when RR is reduced below ~8 breaths/min [92, 102]. Nevertheless, it is accepted that the HF band (0.15-0.4 Hz) does largely reflect parasympathetic influences on HRV, including influences of respiration, i.e. the cyclical changes with the inspiratory/expiratory cycle - RSA. Indeed, cholinergic receptor blockade virtually eliminates HF oscillations, but as implied above LF oscillations are also reduced [103]. However, the fact that at low RR values, the respiratory-related influences on HRV shift into the LF band, mean that HF power is not a simple index of vagal influences. On the assumption that LF has a dominant sympathetic component and HF mainly reflects parasympathetic vagal activity, the ratio LF/HF was put forward as a measure

of sympathovagal balance [104]. Although this measure has gained widespread acceptance and is very widely used, this has also been challenged for the reasons discussed above and more [92, 102].

On balance, mainly because one of the aims of the present study was to compare the effects of slow breathing at 6 breaths/min on HRV and other cardiovascular variables in WEs and SAs, the decision was taken to perform analysis of HRV only in the time domain, although data obtained in published studies by using frequency domain analysis are referred to when appropriate.

1.6.2 HRV in health and cardiovascular disease (CVD)

It was recognised in 1963, that low HRV is a marker of fetal distress before any changes in HR occur [105]. Since then, low HRV has been shown in multiple studies to predict autonomic dysfunction in diabetic patients and to be a strong predictor of CVD and all-cause mortality [103]. But, HRV is also known to decline with healthy ageing [106]. On the other hand, HRV has also been shown to increase with exercise training in young healthy individuals, in athletes and during cardiac rehabilitation, in association with an increase in vagal tone [107].

In the context of the present study, 2 large community-based studies on men and women aged 45-65 years which included African Americans as well as WEs, showed that those with hypertension had reduced HRV relative to those who were normotensive. However, low HRV amongst the normotensives predicted greater risk of hypertension in the 4 and 9-year follow-up periods [108, 109]. These results indicate that changes in the autonomic regulation of the heart and reduced HRV precede the development of hypertension in both African American and WE men and women. Similarly, it was demonstrated in large population studies on men and women that CAD is associated with low HRV and that low HRV in those with no evidence of CAD

was an independent predictor of CAD and myocardial ischaemia over a 4-year follow-up period [110, 111]. Thus, it seems that low HRV also predicts future CAD. Interestingly, a recent study performed in the UK indicated that low HRV is present in SA children relative to WE children (mean age 8±1 years) [112].

On the basis of the evidence discussed above, it seems easonable to propose that young normotensive SA adults have lower HRV than matched WE adults.

1.7 Slow breathing and effects on autonomic regulation of the cardiovascular system

As described above, SAs have a greater risk of developing hypertension and other CVD compared to WEs, while hypertension and other CVD are associated with autonomic dysfunction and reduced HRV and BRS (see sections 1.4, 1.5.2, and 1.6.2). Accordingly, people with established essential hypertension were shown to have increased resting respiratory rate and resting hypoventilation and hypocapnia relative to normotensives [67], differences which were associated with reduced HRV [68] and reduced BRS in the hypertensives [65, 67]. Further, a short 2-minute period of slow breathing at 6 breaths/minute reduced SBP and diastolic blood pressure (DBP) in the hypertensives but not normotensives, while BRS assessed by sequence analysis was increased in both groups but without inducing hypoventilation in either group, apparently because both groups showed an increase in tidal volume (Figure 1.4) [67]. It was suggested these findings reflected a reduction in the respiratory influence on sympathetic activity especially in the hypertensives [67], but given BRS assessed by spontaneous changes in SBP is predominantly a measure of vagal activity [49], they also suggested that slow breathing enhances cardiac vagal activity in normotensives as well as hypertensives.

It has since been shown that the resting hyperventilation and hypocapnia, and raised sympathetic activity in untreated hypertensives was partly due to increased tonic peripheral chemoreceptor activity [113], and that acute slow breathing does indeed reduce MSNA in recently diagnosed, untreated hypertensives [114]. Further, slow breathing for 4-5 minutes reduced the raised MSNA and improved BRS in chronic obstructive pulmonary disease (COPD) patients [115].

Turning to slow breathing training, usually at 6 breaths/minute, like Yoga breathing, for short periods on several days/ week for several weeks, this has been shown to reduce ABP [116, 117] in hypertensives, as well as improve BRS [118] and HRV [68, 119]. Whether slow breathing training for several weeks has greater, or longer-lasting effects in SAs than WEs has received no attention, but ABP was reduced in young SA subjects living in India [120, 121], and in middle aged pre-hypertensives in the United States whose ethnicity was not mentioned [122]. HRV was also increased in young normotensive SAs after several weeks of slow breathing training [120]. Furthermore, several studies have reported that long-term regular practise of device-guided slow breathing has an antihypertensive impact and lowers resting blood pressure in hypertensives [123-125].

Given the benefits of slow breathing mentioned above, as well as the fact that SAs are at higher risk of developing hypertension and CVD, this raises the possibility that slow breathing training in young normotensive SAs may offer a non-pharmaceutical therapeutic intervention that could reduce the risk of hypertension and CVD, as well as the morbidity and mortality associated with these diseases.

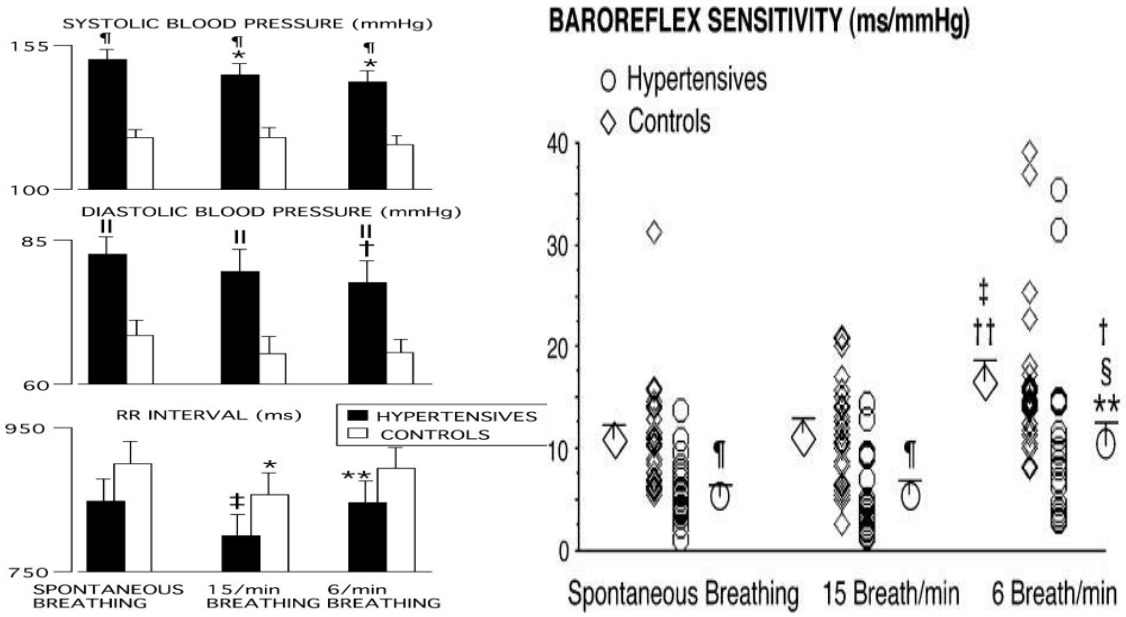


Figure 1.4 Effect of respiratory rate on R-R interval, SBP, BRS, and DBP during spontaneous breathing and slow breathing (6 breaths/min).

Obtained from Joseph *et al* [67]

1.8 Stress and cardiovascular disease

There is substantial evidence that chronic mental stress contributes causally to the development of hypertension and that the magnitude of the pressor response to acute mental stress predicts the risk of hypertension [126-130]. Notably, in the CARDIA study on >4000 American African and White men and women, those who showed the largest pressor response to mental stress tests (video game and star tracing) were more likely to develop hypertension over the 13-year follow-up period, the onset being earlier in those who showed the largest increases in SBP, and the largest increases in DBP occurred in men suggesting larger increases in TPR, with no difference between ethnicities [130]. Further, a meta-analysis of 32 different studies revealed that the magnitude of SBP, DBP and HR responses to laboratory stress tests including mental arithmetic and the colour-word interference task (Stroop test) were strong predictors of CVD risk status, particularly hypertension, that the relationship was stronger in

men than women, and was greatest if they were tested at a younger age (≤ 18 years old) and followed up for longer ≤ 3 years and up to 20 years [129]. Slower recovery of HR and SBP recovery from stress tests was also predictive of future hypertension [129]. Ethnicity was not taken into consideration in this meta-analysis and it seems there have been no longitudinal studies on the association between pressor reactivity to stressors and hypertension or CVD in SAs.

It is recognized that the pattern of cardiovascular response evoked by mental stress which is often referred to as the alerting or defence response, generally comprises a rise in ABP, accompanied by increased HR, sympathetically-mediated vasoconstriction in splanchnic, renal and cutaneous circulations, but vasodilatation in limb muscle [127, 131, 132]. This pattern is accompanied by an increase in respiration that mainly reflects increased respiratory frequency [132, 133]. This pattern is seen as one that is graded with the stimulus strength and ultimately prepares the individual for fighting or running away, i.e. exercise, in situations which are life threatening [132]. However, the muscle vasodilatation is the least stable component of the response. Paradoxically, it is generally accompanied by an increase in MSNA [134] rather than a decrease in MSNA [135] and may be accompanied by muscle vasoconstriction in calf, but vasodilatation in forearm [136]. In fact, the muscle vasodilatation has been mainly attributed to circulating adrenaline and nitric oxide (NO) [135, 137]. It is blunted in African Americans relative to whites and depressed in those with established hypertension [138-140]. Further, young men with borderline hypertension and young men who were normotensive but have hypertensive parents, who are at high risk of developing hypertension showed greater pressor responses, increases in MSNA and smaller forearm vasodilator responses to mental stress than young normotensive men with normotensive parents [127, 141].

This variability in the vascular response in muscle is of particular interest because it

was shown that the magnitude of the pressor response to mental stress was dependent on the increase in TPR rather than the increase in CO and the change in TPR was, in turn, dependent on the size of the muscle vasodilatation [131]. Consistent with this, our research group recently found that young normotensive SA men generally showed forearm vasoconstriction whereas WE men showed forearm vasodilatation to repeated sound stimuli. Moreover, the pressor responses were exaggerated in SAs relative to young WE men and they were directly correlated with the change in forearm vascular resistance in both ethnicities, i.e. when the increase in forearm vascular resistance contributed to the increase in TPR, the increase in ABP was greater [142]. To date there has been no similar study in young SA women. This is of interest because our group recently reported that both young SA men and SA women have blunted endothelium-dependent dilator responses relative to young WE men and women [143, 144], respectively, and in SA women, NO-dependent endothelium-dependent dilator responses were blunted, especially in those with hypertensive parents [144].

The increase in HR that occurs in mental stress has been attributed to increased sympathetic and reduced vagal activity as shown in a systematic review of 12 studies of the effects of mental stress tests in normotensive subjects [145], by considering time domain and frequency domain analyses of HRV (section 1.6.1). Importantly, because changes in respiration affect analysis of HRV in the frequency domain [92, 102], and because respiration increases in mental stress [132, 133], several studies have compared the effect of mental stress on HRV in the time domain and frequency domain, but with respiratory modulation of HRV taken into account. For example, one study revealed that mental arithmetic evoked a decrease in SDNN and RMSD indices of vagal activity in the time domain, even though the decrease in the HF component which is considered to represent the vagal component (section 1.6.1.2) was partly attributable to the increase in respiratory frequency [133]. A study performed in India on young men and women who were presumably SAs, similarly showed a decrease in the vagal

components and an increase in the sympathetic components of HRV during the Stroop test whether they were assessed in the time or frequency domains, but this study also showed that the increase in the sympathetic component was prolonged in recovery and the changes were greater in men than women [146].

Considering those at higher risk of hypertension and CVD in a study on normotensive and borderline hypertensive men, a computer-based stress task increased the LF and reduced the HF component of HRV in both groups, but the increase in low frequency component was larger in the borderline hypertensives [147], suggesting greater sympathetic activation. Further, in a study performed in India on young men and women who were presumably SAs, the decrease in the HF component and increase in LF/HF ratio evoked by mental arithmetic, were greater in those with hypertensive parents than in those with normotensive parents [148]. Finally, in a study on normotensive and untreated hypertensive men aged 19-42 years, the reduction in the HF, and increase in the LF components were greater in the hypertensives *during* mental arithmetic, the increase in LF lasting for at least 3 min after the test [149].

On the basis of these results and given SAs have a higher risk of hypertension and CVD (section 1.2), a greater decrease in the vagal component and increase in the sympathetic component of HRV which is more prolonged might be expected during mental stress in young normotensive SAs relative to WEs. As far as we are aware no such comparisons between young normotensive SA and WE men and women have yet been made.

The fact that ABP rises together with HR during the response to mental stressors has raised the question of whether the ability of the baroreceptor reflex to correct blood pressure is impaired during mental stress. In line with this idea, it was shown by using the Oxford method that the cardiac component of the BRS was reduced in normotensive men and women during mental stress evoked by mental arithmetic [150]. Similarly, the cardiac component of BRS

assessed by the sequence method was reduced during mental arithmetic in young women and was still reduced but to a lesser extent, 3 min after the test [151]. The vascular component of BRS is more difficult to investigate but, although under resting conditions, infusion of PE increased ABP and caused reflex bradycardia and a decrease in MSNA as expected, when mental arithmetic was performed *during* PE infusion, there was a substantial *increase* in ABP, HR and MSNA, suggesting that the sympathetic vascular component as well as the cardiac component of the baroreceptor reflex were inhibited during mental stress [152]. More recently, it was shown by using the sequence method for the relationship between DBP and MSNA, that the sympathetic component of BRS was depressed in the initial 2 minutes of the pressor response to a 5 minutes period of mental arithmetic, but not during the final 3 minutes when ABP plateaued [153].

Interestingly, young men who were offspring of parents with hypertension showed greater reduction in the cardiac component of BRS assessed by the sequence method during mental arithmetic than offspring of normotensives suggesting a depressed ability to reduce ABP in the offspring of the normotensives that would be expected to contribute to their risk of developing hypertension [154]. There seem to have been no other studies on changes in BRS during mental stress in those who are at high risk of hypertension and CVD. Given the focus of the present PhD project, performing such a study to compare young normotensive SA and WE men and women was of obvious interest.

1.9 The aims of the project

Putting these findings together, the initial aims of the present project were to test the hypotheses that:

Chapter 3

- Young normotensive SAs who are non-smokers show reduced baroreceptor reflex regulation of ABP as assessed by BRS influences on vagal and sympathetic control of HR and show reduced HRV with reduced vagal and increased sympathetic influences relative to matched WEs.
- SAs show exaggerated pressor responsiveness to mental stress relative to WEs, reflecting more pronounced increases in HR, CO and TPR and SAs show more marked blunting of the BRS during and after mental stress than WEs.
- Acutely, slow breathing increases BRS and HRV, particularly in young SAs.

Chapter 4:

• SA women show blunted BRS and reduced HRV at rest compared to SA men and WE women.

• SA women show exaggerated pressor responsiveness to mental stress compared to SA men and WE women.

• Slow breathing will improve BRS and HRV in WE men and women, but to a lesser extent in SA women than in SA men.

Chapter 5:

- A Systematic review of published studies will show that slow breathing training causes a reduction in resting ABP in SAs and that this is accompanied by long-lasting increases in HRV and vagal influence on HR.

Chapter 2: General materials and methods

2.1 Study Design

These studies were conducted using a non-randomised control trial design to investigate the ethnic differences in cardiovascular regulation between young healthy SAs and WEs. Specifically, to assess the effect of mental stress and slow breathing on ABP, BRS, and HRV and investigate if young non-smoker normotensive SAs show reduced baroreceptor reflex regulation of HR, CO and TPR and reduced HRV, compared to a matched group of WEs. In addition, to investigate sex differences in BRS, HRV, response to mental stress and slow breathing between SA and WE men and women.

2.2 Ethical Considerations

The experimental studies were approved by the University of Birmingham Ethics Committee (Application number ERN 17-1821) and were conducted in accordance with the Helsinki Declaration [155]. As indicated above, all participants were provided with a copy of the Participant Information Sheet outlining the study details and explaining the procedures in detail. In addition, all participants completed the Participant Questionnaire (see Appendices 2.1 & 2.2). All participants also signed an Informed Consent Form consenting to each protocol component (see Appendix 2.3). All participants were informed that they have the right to withdraw from the study at any time without giving a reason (see Appendix 2.1).

All data were processed and stored according to the Data Protection Act 1998. Upon entry into the study, each participant was assigned a unique identification study code. The key to this code was stored in a password-protected file on a laboratory computer, as was any data that can be used to trace an individual. All identifying characteristics were removed from published data. Data was stored on a secure computer in the University of Birmingham, College of Medical & Dental Sciences. The computers are password protected. The file containing the

key to the study code was also password-protected. Members of the research group have access to the coded data, although only those directly involved in the study have access to the code. The data will be stored for ten years from the completion of the study, at which time it will be automatically erased.

2.3 Study Participants

Forty-two young normotensive WE and SA individuals were recruited to take part in the current studies; 21 WEs and 21 SAs aged 18-25 years with a Body Mass Index (BMI) of 18-29 kg/m^2. The participants were divided into two groups based on their self-reported ethnicity. Approximately equal numbers of men and women were tested in each group (WEs: 9 men/12 women; SAs: 10 men/11 women). The sample size was determined based on the variance of similar data and effect sizes of >10% in previous studies conducted by our research group [142-144].

2.3.1 Recruitment

The participants were recruited as volunteers from undergraduate and postgraduate students and staff at the University of Birmingham. Posters, word of mouth, e-mails and flyers were used to recruit participants.

2.3.2 Eligibility criteria

Young healthy normotensive WE and SA men and women aged 18-25 years with parents from the same ethnicity, either WE or SA, were eligible to participate in these studies. South Asians are defined as those with ethnic roots in India, Pakistan, Bangladesh, Nepal, and Sri Lanka [4].

Participants with a history of cardiovascular, respiratory, kidney, liver and/or metabolic disease were excluded from the study. In addition, mixed racial ancestry, pregnancy, resting ABP > 140/90mmHg, smoking, recreational drugs, alcohol consumption over 21 units/week, BMI over 29 kg/m^2, and medications were considered exclusion criteria.

2.3.3 Risks

There were no obvious risks involved in these experiments. All participants were informed that should any abnormal values be observed, they would be advised to visit their GP. The participants were also told that there was a low risk of feeling faint or experiencing some dizziness when standing up from squatting.

The Queen Elizabeth Hospital is located within 800 meters of the laboratory in case of a serious emergency. The researchers performing these studies were medically trained and fully qualified clinical respiratory therapists who had completed basic life support (BLS), advanced cardiac life support (ACLS) and airway management training and were very experienced working in clinical situations, predominantly in intensive care units (ICUs). There were also several staff qualified in First Aid who work in the department and are accessible by phone, and the phone numbers are listed in the laboratory.

2.4 General experimental condition

Each potential participant attended a familiarisation visit followed by an experimental visit. On the first visit to the laboratory (the familiarisation visit), the participant was familiarised with the methodology and experimental equipment that was going to be used in the experiment and was given a chance to ask questions to make sure they were fully aware of the procedures of the experiment. In addition, each subject was provided with a copy of the participant information sheet (PIS) which provides all the information about the experiment

(see Appendix 2.1). All participants were also asked to complete the participant questionnaire, including the inclusion and exclusion criteria (Appendix 2.2). The questionnaire included their medical history, parental history of cardiovascular disease, respiratory disease, and diabetes. Their dietary intake of vitamins, fresh fruit/vegetables, and oily foods information were also obtained from the questionnaire. Their level of physical activity and sedentary behaviour were also measured by the short version of the international physical activity questionnaire (IPAQ) (see Appendix 2.2). In addition, each participant was given a copy of the Informed Consent Form and asked to sign it if they agreed to participate in these experiments (Appendix 2.3).

Then, height and weight were measured to calculate the BMI and waist and hip circumferences were measured to calculate the waist to hip ratio. Resting ABP and HR were also obtained from each participant by recording three values using a standard automated sphygmomanometer. This was used to verify that they were normotensive.

All participants were asked not to consume non-steroidal anti-inflammatory drugs (NSAIDs) for a week before the experimental visit. They were asked to refrain from alcohol, strenuous exercise, heavy meals, and caffeinated drinks for 12 hours prior to the experimental visit. Females were asked to attend the experimental sessions within seven days of menstruation onset, during the low female hormones phase, to minimise the effects of elevated progesterone and oestrogen, which are known to influence cardiovascular regulation [(156)].

The second visit was the experimental visit. All experiments were conducted in a quiet room (Human Physiology Laboratory) at a room temperature of approximately 23^{o}C. Subjects reclined on a couch at an approximately 45-degree angle with both arms supported and kept at approximately the heart level at all times unless otherwise stated. The equipment was organised to ensure the participants could not see recording equipment and recording monitors, and the noise was kept to the minimum to avoid visual and auditory distraction. In addition, the

participants were instructed to keep minimal movements and talking unless otherwise stated to prevent effects on the recordings.

2.5 Measurements

2.5.1 Blood pressure monitoring

Upon arrival of each participant and before the start of the experiment, a standard sphygmomanometer (Microlife, BP3BXO, Windnau, Switzerland) was used to obtain the mean of 3 recordings of resting SBP and DBP via an upper arm cuff before the start of the continuous automatic photoplethysmography recordings (see below) so as to ensure that these recordings reflected the actual blood pressure values. It was also used to confirm that each subject had a normal blood pressure and was eligible to take part in the study.

The Human Non-Invasive Blood Pressure (NIBP) Nano monitoring system (Human NIBP Nano System, AD instruments, USA) was used to continuously record blood pressure throughout the experiments. The Human NIBP system records and measures continuous blood pressure signal using a non-invasive finger cuff system (Human NIBP Nano Wrist Unit; manufactured by Finapres Medical Systems). The Wrist Unit consists of a photo electric (infra-red) plethysmograph inflatable finger cuff attached to a small controlling unit fastened on the back of the non-dominant hand and via a digital cable and a vacuum line. After choosing an appropriately sized finger cuff (small, medium, or large), the finger cuff was fastened around the middle phalanx of the middle finger of the non-dominant hand which was held at heart level throughout the experiments. The Human NIBP system also contains a height correction unit (HCU), which is used to adjust the recorded blood pressure if the hand is moved above or below heart level. The wrist unit and HCU were attached to the Human NIBP Nano Interface,

which provides the units with power and facilitates data transmissions to LabChart software via USB).

Mean arterial blood pressure (mABP), SBP, DBP, HR, and interbeat interval (R-R interval) were automatically computed and continuously recorded by the Human NIBP system at rest, slow breathing, and mental stress via the finger cuff, which was kept at the heart level throughout the experiments. The R-R interval and SBP recordings made via the Human NIBP allowed BRS calculation by the sequence method (section 2.4.5).

SV, CO, and TPR were computed off-line from the blood pressure trace obtained by the Human NIBP monitoring system using the non-invasive cardiac output module on Labchart software which uses Windkessel three-element model, in which age and sex are considered [157]. The three-element model uses an algorithm based on mathematical analysis of blood pressure waveforms to estimate SV, CO, and TPR. It uses the arterial impedance input to describe the relationship between aortic pressure and flow. Once the model parameters have been determined, stimulating the model then allows the flow to be computed from the measured pressure. This flow measurement can then be used to calculate a continuous estimated measure of CO. It gives an estimated measure of SV when integrated over one heartbeat, and CO when integrated over one minute [157].

2.5.2 Respiration

Respiratory rate was recorded by an inductive respiratory belt fastened around the rib cage (MLTII33 Piezo Respiratory Belt Transducer, AD Instruments, USA), which monitored the respiratory rate (RR) by chest expansion throughout the experiments. The respiratory belt was connected to a PowerLab unit (AD Instruments, USA) which transmitted the recordings to LabChart software (Version 8, AD Instruments).

2.5.3 Electrocardiogram

Electrocardiography (ECG) (Dual Bio Amp, Model FE135, AD Instruments, USA) was used to continuously record HR and R-R intervals via three electrodes arranged in the Lead I configuration. The positive lead was attached to the left shoulder, the negative lead was attached to the right shoulder, and the indifferent lead attached to the right ankle, or right lower trunk when appropriate, to ensure less noisy ECG recordings especially during squatting test. The ECG leads were connected to the ECG amplifier mentioned above, and connected to the PowerLab unit (AD Instruments, USA) to transmit the recordings digitally to LabChart version 8 software (AD Instruments, USA). The ECG was also used to derive HR and this was recorded on another channel to ensure the consistency and accuracy of the HR recorded by the Human NIBP. The ECG recordings allowed assessment of HRV at rest, slow breathing, and mental stress (see section 2.4.4).

2.5.4 Heart rate variability

As described in Chapter 1 (section 1.6), HRV is considered to provide an assessment of autonomic function, in particular sympathetic and vagal influences on HR [92, 100] . Time domain analyses of HRV were assessed from the R-R recordings by using the HRV analysis module in LabChart software (Version 8, AD instruments). The values recorded included the standard deviation of the R-R intervals of "normal" sinus beats (SDNN), the root mean square of successive R-R interval differences (RMSSD), the percentage of adjacent NN intervals which differ from each other by > 50 ms (pNN50%); SDNN reflects sympathetic and vagal influences on HRV, while RMSSD and pNN50 mainly reflect vagal influences on HRV [92]. Assessment of HRV was made during a resting period (baseline), slow breathing, and mental stress (see section 2.7).

2.5.5 Calculation of baroreceptor reflex sensitivity

The R-R interval and SBP recordings via the Human NIBP system allowed the calculation of BRS by the sequence method described in Chapter 1 (see section 1.5.1.4). The relationship between SBP and R-R interval was recorded as BRS during up-sequence (+R-R interval/+SBP) for 3-5 successive beats with progressive rise in SBP and lengthening of the R-R intervals, and down-sequence (-R-R interval/-SBP) for a minimum of 3 successive beats with progressive falls in SBP and shortening of the R-R intervals at rest, during slow breathing, and during mental stress. Up- and down-BRS values were produced by collecting and averaging the up- and down-sequences separately. BRS was also assessed as the relationship between the fall in SBP and R-R intervals during the squat to stand test (-RR/-SBP) before and following mental stress (see section 1.5.1.4). The consecutive changes considered in SBP and R-R intervals were equal to or greater than 1 mmHg and 5 ms, respectively [81].

To calculate BRS, as shown in Figure 2.1, a minimum of 3 consecutive rises or falls in SBP were plotted against subsequent lengthening or shortening of the R-R intervals, respectively, using the regression function in Microsoft Excel software as shown in Figure 2.1: the slope represents the BRS index [81, 84]. The BRS index was considered for further analysis when the correlation (R^2) was found to be > 0.80 [158].

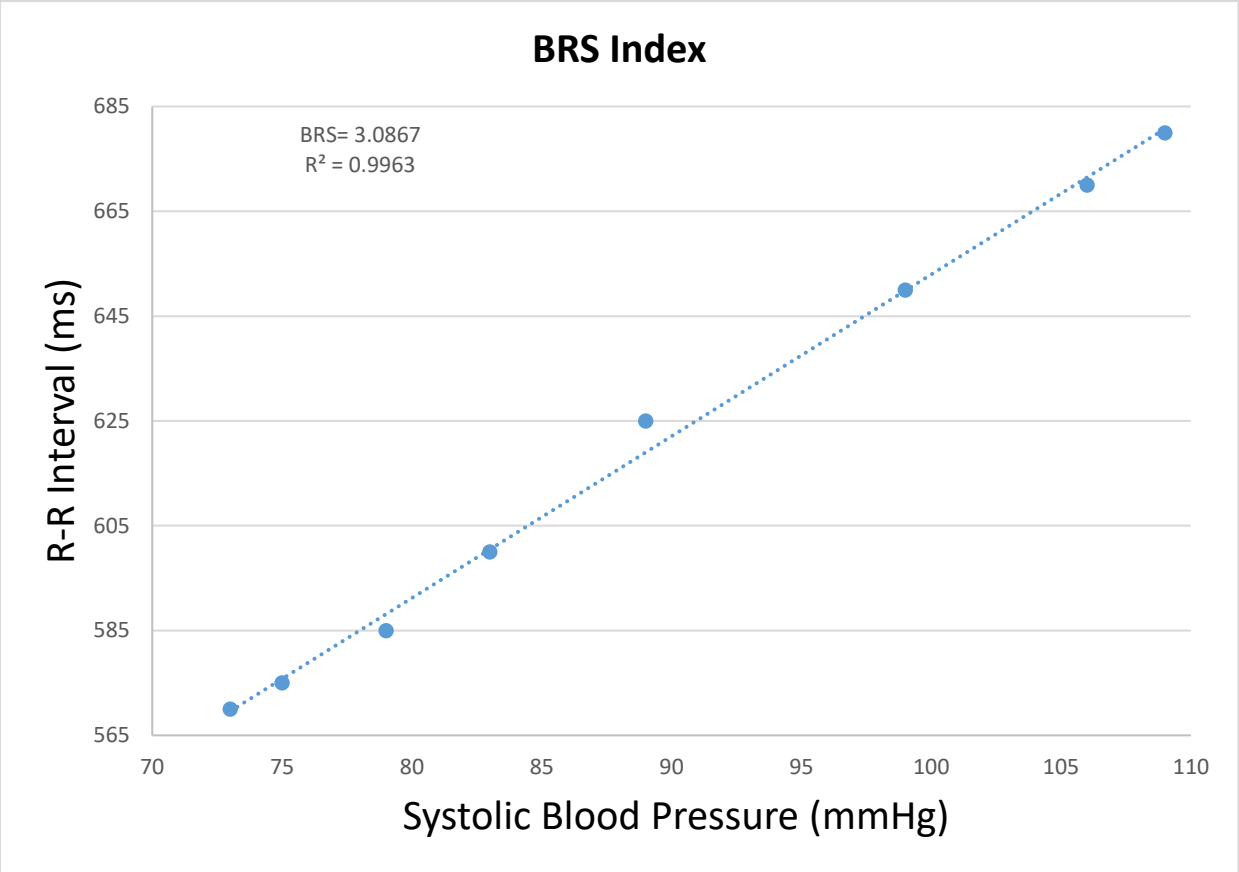


Figure 2.1 An example of an original BRS regression made in an individual subject.

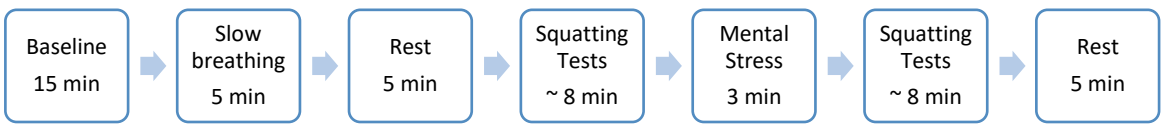
The graph shows the R-R interval plotted against SBP for the changes recorded when the subject moved from a crouch to a standing posture. The relationship has a slope of 3.0867 ms/mmHg (BRS index) and correlation of 0.99.

2.6 Data acquisition

All variables mentioned above were recorded digitally on a laboratory desktop computer via two PowerLab units (PowerLab 4/30, Model: ML866 & PowerLab 4/35, Model: PL3504; AD Instruments, USA) and the Human NIBP monitoring system (Human NIBP Nano System, AD instruments, USA) using LabChart software version 8 (AD Instruments, USA). Nine LabChart channels were used to provide data recordings. Three of these channels displayed the data of the ECG (ECG waveforms) and respiratory belt (Respiratory waveforms and Respiratory Rate in breath/min) translated from the LabChart units. The remaining channels were obtained from the Human NIBP monitoring system: pulsatile ABP (mmHg), mABP (mmHg), SBP (mmHg), DBP (mmHg), HR (BPM), R-R intervals (ms). SV (mL/beat), CO (L/min), and TPR (mmHg.s/mL) were computed off-line from the pulse contour obtained from the Human NIBP monitoring system (see section 2.4.1). The ECG recording allowed HR and R-R intervals to be computed off-line. The HRV analysis module available in LabChart was used off-line to compute time domain indices of HRV (SDNN, ms, RMSSD, ms; pNN50, %) from the ECG recording.

2.7 Experimental Protocol

The Figure below shows the order and duration of the different steps in the protocol:



2.7.1 Baseline recordings

Initially, the participant was asked to recline on the couch, as explained above in section 2.3. Before beginning the continuous ABP recordings, a sphygmomanometer (Microlife, BP3BXO, Windnau, Switzerland) was used to obtain the mean of three records of resting SBP

and DBP to ensure the recordings obtained by the Human NIBP system reflect the actual blood pressure values. Then, continuous baseline recordings started. Resting blood pressure, heart rate, respiratory rate, and ECG were recorded continuously for 15 min to allow calculation and assessment of mABP, SBP, DBP, SV, CO, and TPR, RR, and BRS, HRV, at rest.

2.7.2 Slow breathing

Following the 15 min of baseline recordings, a slow breathing exercise started. The participant was asked to relax and to breathe slowly (6 breaths/min) for ~5 min while resting on the couch, with the aid of a sound signal guiding inspiration and expiration (Breathing Zone Application, version 4.7). The application was set up so that the inspiration time was approximately 5 s, and the expiration time was approximately 5 s. Meanwhile, ABP, HR, R-R intervals and respiration were continuously recorded to allow calculations of BRS, HRV, mABP, SV, CO, and TPR during this part of the protocol. Then, while recordings continued, the participant was instructed to rest for 5 min to eliminate the effect of slow breathing on the cardiovascular system during mental stress.

2.7.3 Mental stress and squatting test

Approximately 5 min later, the participant was asked to stand by the couch for approximately 1.5 min to perform the squatting test: changing position from squatting to standing leads to fast substantial changes in ABP and HR [88] (see Figure 2.2). This allows the baroreflex sensitivity to be assessed using methodology similar to the sequence method described above (see section 2.4.5). Thus, when the recordings had settled while the participant was standing, he/she was asked to squat to the floor for 1 min with the support of a small table to help them maintain their position. After about 1 min, the participant was asked to stand up

and to remain still for ~ 1.5 min, while recordings continued. This squat to stand procedure was repeated three times before the mental stress test.

The participant was then asked to do a mental stress test - the Stroop Colour and Word Test for ~3 min (Figure 2.3). This test was performed using a 3-minute video made by one of our research group, following the standard design [159]. The video comprised a black background and eight different coloured words each describing a colour which appeared on the computer monitor in random order (64 combinations of word and colour) at intervals of ~1.1 s. Each word was one of the eight colours and was coloured either within the same colour, or in a different one (for example: the word green was shown in orange, or yellow was shown in blue) (Figure 2.4). The participant was asked to say the colour of each word that appeared on the screen [159, 160]. Continuous recordings were obtained during the mental stress so that mABP, HR, R-R interval, SV, CO, TPR could be recorded and BRS, HRV could be calculated.

Immediately after the Stroop test, the participant was asked to do the squatting test 3 times, as described above, at ~ 2.5 min intervals. Finally, after the squatting test, the participant was asked to recline on the couch and resting ABP, HR, R-R interval, and respiratory rate were recorded during spontaneous breathing.

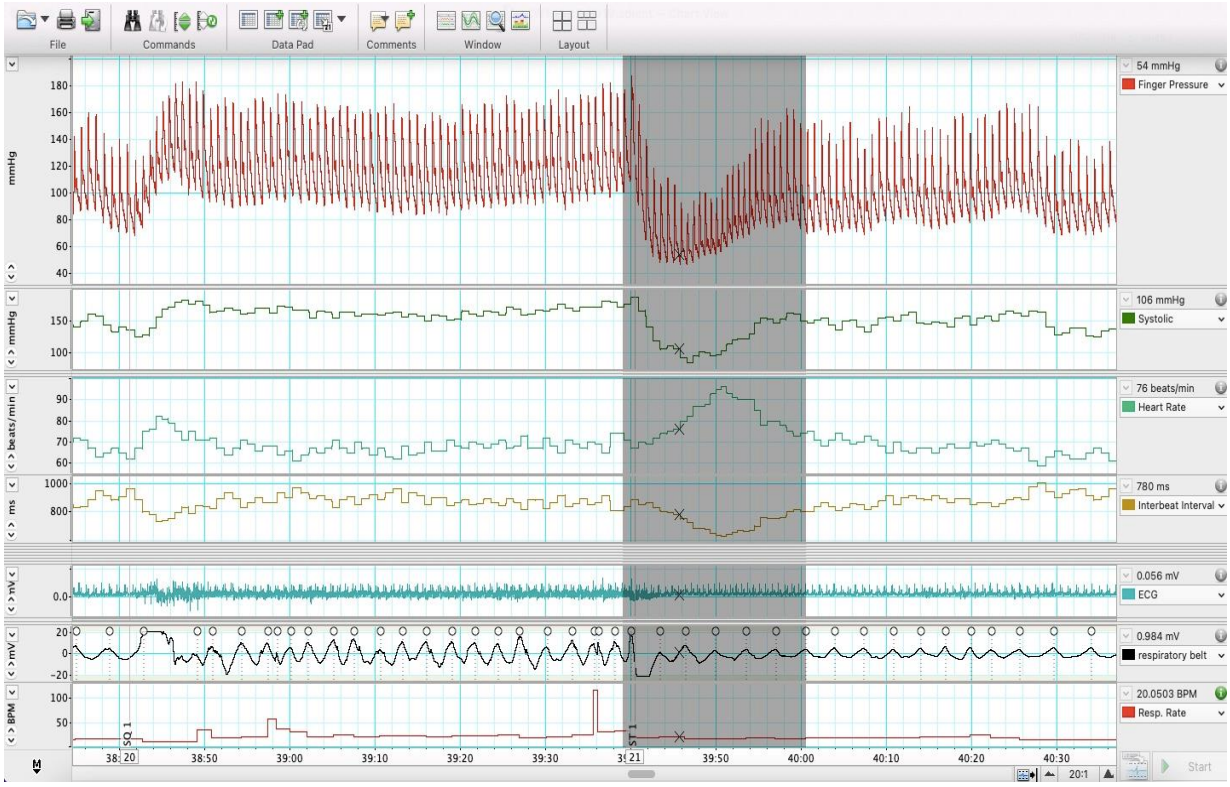


Figure 2.2 Original recordings taken from a participant who changed position from crouch to stand (Squatting test) at the grey area.

Recordings from top – down are pulsatile ABP in mmHg, Systolic pressure in mmHg, Heart rate in beats/min, R-R interval in ms, ECG, respiratory movement and respiratory rate in breath/min. It shows the immediate response of the heart rate to the fall in systolic blood pressure. The fall in SBP and R-R intervals (-R-R interval/-SBP) were used to calculate BRS index by sequence method.

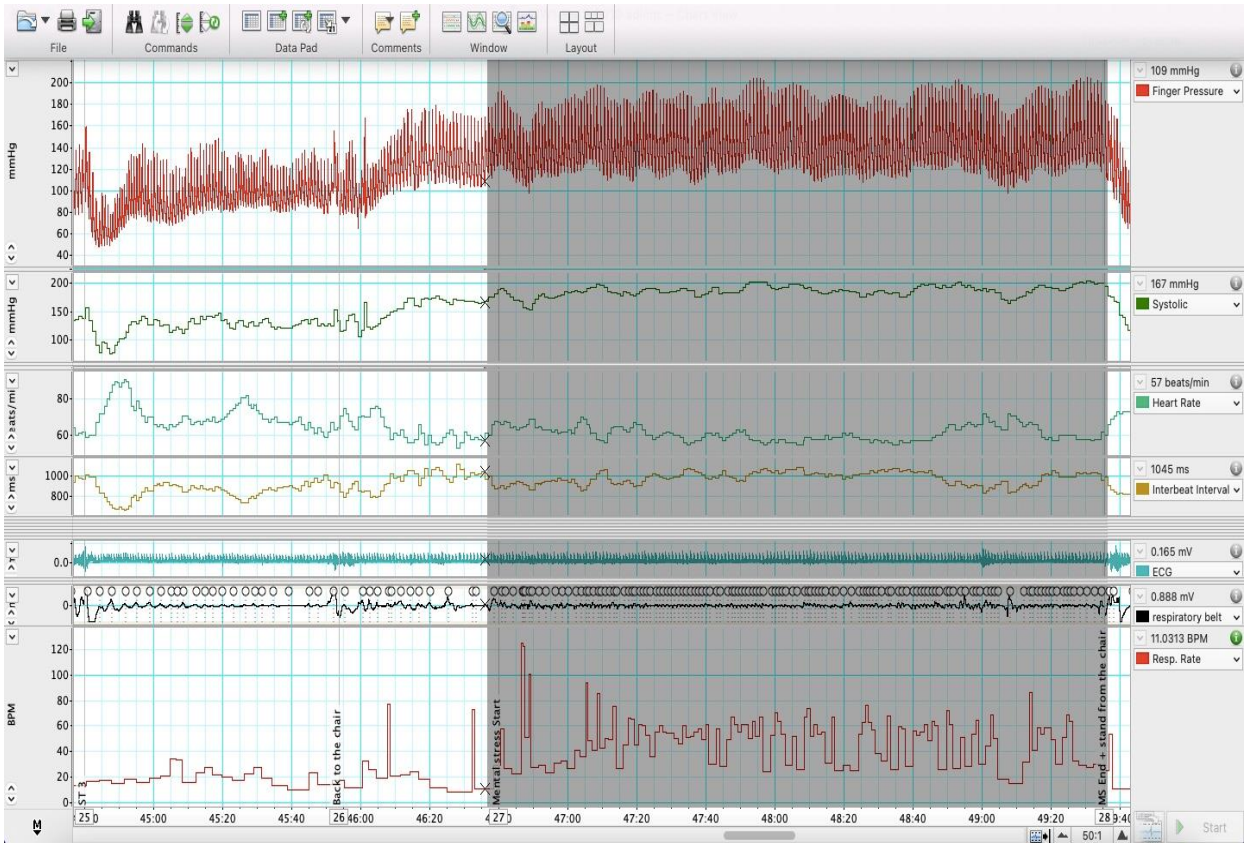


Figure 2.3 An original recording made from a participant during the Stroop test at the grey area.

Recordings from top – downwards as in Figure 2.2. These recordings show the immediate effect evoked by mental stress started at the arrow - SBP and HR increased.

Figure 2.4 An example of a word and colour conflict (Stroop test) that was used to induce mental stress.

Participants were asked to state the colour of the word.

2.8 Data and statistical analysis

After data were transmitted from the two PowerLab units and the Human NIBP system to computer via LabChart version 8 (AD Instruments), all data were then extracted from the Data Pad of the LabChart to Microsoft Excel spread sheet (Version 2019) and GraphPad Prism (version 9.0) software for calculations and analysis. Microsoft Excel was used to calculate the BRS by the sequence method at rest, during slow breathing, during mental stress, and during squatting test. It was also used to organise the data extracted from the LabChart and conduct the analysis for chapter 3. Data was then exported to GraphPad Prism for further analysis for chapter 4. GraphPad Prism was also used to draw and present all the results figures for chapters 3 and 4.

Data were expressed as Mean ± Standard Error of Mean (SEM). Student's Paired t-test, Student's Unpaired t-test, and Two-Way Analysis of Variance (ANOVA) were used to assess and compare the effects of slow breathing and mental stress on the outcomes of interest within and between groups as appropriate: details are provided in the relevant chapter. Outliers were detected using 3 Z scores, and data with Z score of -3 or 3 were removed. A P value of < 0.05 assumed statistical significance.

Chapter 3: Cardiovascular-respiratory interactions during slow breathing and mental stress in young normotensive South Asians relative to young normotensive White Europeans

This study has been partly presented and published in abstract form at several conferences. First, it was presented at the British Irish Hypertension Society (BIHS 2019) conference and published in the Journal of Human Hypertension, entitled **"Baroreflex regulation of arterial blood pressure is disturbed in young South Asians relative to White Europeans, particularly during mental stress"** [161] (See Appendix 3.1). It was also presented at the Physiology Society Symposium 2019, a Symposium for Early Career Scientists, with the title **"Comparisons between young South Asians and White Europeans in the effects of acute mental stress and slow breathing on baroreflex sensitivity and heart rate variability"** [162] (See Appendix 3.2). Following additional data extraction and analysis, it was then presented orally at the Experimental Biology international conference 2021 and published in FASEB Journal, entitled **"Evidence that young normotensive South Asians show early signs of disturbed arterial blood pressure regulation in acute mental stress relative to White Europeans"** [163] (See Appendix 3.3).

3.1 Introduction

As described in the General Introduction, Chapter 1 (Section 1.2), the prevalence of CVD including essential hypertension, stroke, and CAD is greater among SAs living in the UK and other Western countries compared to WEs [164, 165]. In addition, population studies have shown that the risk of developing essential hypertension which is the forerunner of much CVD is higher in SAs than WEs [166]. In fact, SAs develop essential hypertension and other CVD at a younger age than WEs [167-169]. Further, essential hypertension is considered to be caused by autonomic dysfunction and an imbalance of sympathovagal regulation [46-48] see General Introduction (section 1.4). Individuals with established essential hypertension have higher resting heart rate and respiratory rate, which is associated with decreased HRV and reduced BRS [46-48, 67]. Moreover, there is evidence that healthy SAs have lower lung function relative to young WEs and this is associated with higher risk of hypertension and CVD in SAs (Chapter 1, sections 1.2 & 1.3). However, whether disturbed autonomic control of the cardiovascular system or respiratory-cardiovascular interactions are present in young adult SAs has not been tested.

As discussed in the General Introduction (section 1.8), mental stress has been causally linked to several CVDs including essential hypertension [126-128]. It is well-established that mental stress is associated with increased HR and RR, elevated ABP, and decreased HRV [127, 170, 171]. It is also generally accepted that the pressor response to mental stress is accompanied by a reduced ability of the baroreceptor reflex to correct ABP [132, 172]. However, there is evidence that hypertensives, pre-hypertensives, and offspring of hypertensive parents show exaggerated pressor responses to mental stress [127, 141, 173-176]. Importantly, a recent study conducted by our research group found that young SA men show exaggerated forearm vasoconstrictor and pressor responses to mental stress compared to matched WEs [177]. This is

of significance because exaggerated pressor responses to mental stress are associated with future hypertension in young individuals [170, 173, 175, 176]. In fact, studies have shown that the risk of future hypertension can be predicted by the magnitude of the pressor responses to environmental or laboratory stressors [129].

On the other hand, as described in the General Introduction chapter (See section 1.7), it has been shown that slow breathing can decrease resting ABP and increase BRS in hypertensives [67]. Moreover, the reduction in ABP and improvement of BRS were associated with improved sympathovagal balance [67]. In addition, several minutes of slow breathing increased BRS and decreased ABP in young and middle-aged normotensive subjects [178]. Further, there is evidence that slow breathing training reduced ABP in middle-aged pre-hypertensive individuals in the US [122] and that HRV was increased and ABP reduced in young SAs (living in India) after several sessions of slow breathing training [120].

Considering the points made above raises the question of whether the autonomic control of the cardiovascular system is impaired and/or the autonomic responsiveness to mental stress is altered in early adulthood in SAs and whether this might provide early evidence of high risk of developing hypertension. Whether the BRS is depressed during mental stress in SAs, and/or regular slow breathing might reduce ABP and improve BRS in young SAs is also not known. To the best of our knowledge, no study has been conducted to assess the effect of mental stress and slow breathing exercises on autonomic regulation of the cardiovascular system in young normotensive SA adults relative to matched WEs.

3.1.1 The aims of the study

Putting these findings together, this study aims to test the hypotheses that:

- Young normotensive SAs who are non-smokers will show reduced baroreceptor reflex regulation of HR *and* vascular resistance and reduced HRV, relative to matched WEs.
- A single session of slow breathing will increase BRS and HRV, particularly in young SAs.
- Young normotensive SA will show exaggerated pressor responsiveness to mental stress relative to matched WEs and will show more marked blunting of the BRS during and after mental stress than WEs.

3.2 Methods

The methods used in the present study are described in detail in the General Materials and Methods chapter (Chapter 2). This section provides only a brief account. As mentioned above in section (2.2), the current study was approved by the University of Birmingham Ethics Committee (ERN 17-1821) and was conducted in accordance with the Helsinki Declaration [155].

3.2.1 Participants

The study was performed on 40 mixed-sex young normotensive adults, 20 WEs aged 21.9±0.4 years (9M/11F), and 20 SAs aged 20.6±0.4 years (10M/10F) after excluding one WE female before the experimental session due to a history of smoking which was an exclusion criterion, and one SA female due to malfunctioning of the auto-correction unit of the photoplethysmography used for ABP recording. All participants were provided with a participant information sheet. Each participant completed a participant questionnaire and signed a participant consent form.

3.2.2 Experimental procedure and protocol

Briefly, as described in chapter 2, the participant was asked to recline on a laboratory couch in a semi-inclined position unless otherwise stated. Thereafter, a mean of three measurements of resting ABP was obtained via a sphygmomanometer. Subsequently, pulsatile ABP and HR were continuously recorded via a photoplethysmography, an Electrocardiogram (ECG) was recorded and RR was recorded via a respiratory belt. After a resting period of 15 min, the participant was asked to perform slow breathing (SB) at 6 breaths/min for 5 min with the aid of regular sounds generated by software, while resting on the couch. Following slow breathing, the participant was asked to rest for 5 min while the recordings continued.

Thereafter, with recordings continuing, the participant was asked to perform a squatting test. Simply, he/she was asked to stand by the couch for approximately 1.5 min. The participant was then asked to squat for about 1 min, before standing up again and remaining standing for about 1.5 min. When the participant was standing, he/she was asked to remain still. This test was repeated 3 times at approximately 2.5 min intervals. Following this, the participant was asked to return to the couch and to perform the mental stress (Stroop Colour and Word Test) test (MS) for approximately 3 min while continuous recordings were obtained. Immediately after the mental stress test, the participant repeated the squat to stand manoeuvres 3 times as described above. Finally, the participant was asked to recline on the couch after the squat-to-stand test and rest for about 5 min.

3.2.3 Measurements

As described in Chapter 2 (section 2.4.5), up and down BRS sequences were calculated using the sequence method from the relationship between SBP and R-R interval at rest, during slow breathing and during mental stress. BRS was also calculated and assessed as the relationship between the fall in SBP and R-R interval during squat to stand. SV, CO, and TPR were computed off-line from the pulse contour (See section 2.4.1). HRV was assessed by time-domain analysis at rest, during slow breathing and mental stress as described in section 2.4.4.

3.2.4 Analysis of results

Results are expressed as Mean ± SEM. Anthropometric measurements and cardiovascular baseline variables were compared between SAs and WEs groups using the Student's Unpaired t-test. All between groups comparisons (WEs vs SAs) were also made by the Student's Unpaired t-test. Comparisons within groups were conducted by Student's Paired t-test (control vs SB, control vs MS, and squat to stand pre vs post MS). Statistical significance

was assumed with a P value of < 0.05. Microsoft Excel (Version 2019) was used to manipulate and analyse data. Three Z score was conducted to detect outliers and values with a Z score ≤ -3 or ≥ 3 standard deviations from the mean were excluded. GraphPad Prism was used to draw and present the figures and perform statistical analyses.

3.3 Results

3.3.1 Anthropometric and baseline Characteristics

40 young WEs and SAs adults were tested in this study. All participants were normotensive, non-smokers and had no history of cardiovascular, metabolic or respiratory disease. Participants' anthropometric data are shown in Table 3.1. WEs subjects were older than SAs (21.9±0.4 vs 20.6±0.4* years, WEs vs SA; *:P=0.03), otherwise, there were no significant differences between the two ethnicities in anthropometric characterises. In addition, the mean of three sphygmomanometer measurements of resting ABP obtained before the beginning of the protocol revealed similar ABP baseline values between WEs and SAs.

Table 3.1 Participants Anthropometric measurements at rest before the start of continuous recordings

Index	**WEs (n=20)**	**SAs (n=20)**	**P Value**
Age (years)	21.9±0.4	20.6±0.4	0.03 #
BMI (29kg/m^2)	23.1±0.6	24.1±1.0	0.36
WHR	0.8±0.0	0.8±0.0	0.90
Weekly Physical Activity Level	Moderate	Moderate	-
HR (beats/min)	71.1±2.5	76.3±3.7	0.25
SBP (mmHg)	117.3±.8	116.1±2.4	0.69
DBP mmHg	71.7±1.5	73.0±2.0	0.60
mABP (mmHg)	86.9±4.2	87.4±1.8	0.84

Values are shown as Mean ± SEM. WEs: White Europeans; SAs: South Asians; WHR: Waist to Hip Ratio; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; mABP: mean arterial pressure; HR: heart rate. # P<0.05, WEs vs SAs.

3.3.2 Baseline measurements

Results of the continuous recordings made at rest during spontaneous breathing are shown in Table 3.2. WEs and SAs had similar values for mABP, SBP, DBP, HR, CO, TPR, and SV. However, SAs had a higher RR than WEs at rest (16.7± 0.5 vs 14.8±0.7* breath/min; *: P= 0.02, SAs vs WEs; see Table 3.2). Visual observations suggested that up and down-sequences in SP over 3-4 heart beats were less common in SAs than WEs. However, BRS during up-sequences and down-sequences were similar in WEs and SAs. Time domain indices of HRV were also similar in WEs and SAs at rest.

Table 3.2 Baseline cardiovascular variables comparison between WEs and SAs at rest (spontaneous breathing)

	WEs (n=20)	**SAs (n=20)**	
Index	**BL**	**BL**	**P value**
SBP (mmHg)	122.1±2.6	116.2±2.7	0.13
DBP (mmHg)	64.1±1.7	63.7±1.5	0.85
mABP (mmHg)	82.1 ± 1.8	80.4 ± 1.8	0.51
HR (beat/min)	64.6±2.4	66.5±2.7	0.60
RRI (ms)	934.5±44.4	929.7±37.3	0.95
RR (breaths/min)	14.8±0.7	16.7±0.5	0.02#
CO (L/min)	4.8±0.2	5.2 ± 0.2	0.21
TPR (mmHg.s/mL)	1.14±0.06	1.02 ± 0.04	0.10
SV (ml/beat)	76.5±3.9	81.5±3.1	0.32
Log Up-Sequence BRS (ms/mmHg)	1.2±0.1	1.0±0.1	0.20
Log Down-Sequence BRS (ms/mmHg)	1.2±0.0	1.2±0.1	0.93
SDNN (ms)	83.2±7.0	75.5±6.2	0.42
RMSSD (ms)	66.2±7.8	69.0±9.1	0.82
pNN50 (%)	32.0±4.6	39.1±5.5	0.33

Values are in Mean ± SEM. WEs: white Europeans; SAs: South Asians; BL: baseline; SBP: systolic blood pressure; DBP: diastolic blood pressure; mABP: mean arterial pressure; HR: heart rate; RRI: R-R interval; RR: respiratory rate; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume; BRS: baroreceptor reflex sensitivity; SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. (#): significant difference between WEs and SAs ($p<0.05$).

3.3.3 Slow Breathing

The effects of slow breathing are summarized in Table 3.3 and illustrated in Figures 3.1-3.3. During slow breathing, as expected RR decreased in both groups. Concomitantly, SBP was lower in SAs compared to WEs (123.0±3.3 vs 113.5±2.9* mmHg; *: P= 0.03, WEs vs SAs; see Table 3.3), but it was not significantly different in either ethnic group compared to baseline values. There were no other significant differences between the two ethnic groups during slow breathing for the mABP, DBP, HR, CO, TPR, or SV values. The mABP decreased significantly from baseline (from 82.1±1.8 to 79.2±2.1* mmHg) in WEs (P=0.005), and in SAs (from 80.4±1.8 to 79.2±2.1* mmHg; P=0.04) (see Figure 3.1). DBP was also decreased during slow breathing in WEs only (P=0.002) and tended to decrease in SAs (P=0.07). CO, TPR and SV showed qualitatively similar changes in both ethnicities, but there was a significant increase in CO (from 4.8±0.2 to 5.1±0.2* L/min; P=0.006), and in SV (from 76.5±3.9 to 81.0±4.6* ml/beat; P=0.01) in WEs only (Figure 3.1). In addition, TPR decreased from 1.14±0.06 to 0.98±0.04* mmHg.s/mL (P<0.001) during slow breathing in WEs only. No significant changes occurred in CO, TPR or SV in SAs during slow breathing relative to baseline values (See Figure 3.1).

Time domain indices of HRV were similar in both ethnicities during slow breathing (Table 3.3). However, SDNN increased in both groups during slow breathing (WEs: from 83.2±7.0 to 118.3±11.2 ***** ms, P<0.001; SAs: from 75.5±6.2 to 120.9± 8.7***** ms, P<0.001), as did RMSSD from 66.2±7.8 to 91.1±10.9***** ms in WEs (P<0.01), and from 69.0±9.1 to 88.5±9.0***** ms in SAs (P=0.01). There was no significant change in pNN50 in either ethnic group during slow breathing compared to baseline values (Figure 3.3).

BRS during up-sequences was increased significantly during slow breathing in both WEs and SAs (P=0.001 and P=0.05, respectively), but there was no change in BRS during

down sequences in either ethnicity. There were no statistically significant differences between the two ethnicities for BRS during slow breathing (Figure 3.2).

Table 3.3 Effects of slow breathing on cardiovascular variables compared to spontaneous breathing at rest in WEs and SAs

	WEs (n=20)		**SAs (n=20)**	
Index	**BL**	**SB**	**BL**	**SB**
SBP (mmHg)	122.1±2.6	123.0±3.3	116.2±2.7	113.5±2.9#
DBP (mmHg)	64.1±1.7	61.3±2.0*****	63.7±1.5	61.7±1.8
mABP (mmHg)	82.1±1.8	79.2±2.1*****	80.4±1.8	77.9±2.0*****
HR (beats/min)	64.6±2.4	66.2±2.2	66.5±2.7	68.2±2.1
RRI (ms)	934.5 ± 44.4	911.6±40.4	929.7±37.3	908.9±30.2
RR (breaths/min)	14.8±0.7	6.5±0.1*****	16.7±0.5#	6.7±0.2*****
CO (L/min)	4.8±0.2	5.1±0.2*****	5.2±0.2	5.2±0.2
TPR (mmHg.s/mL)	1.14±0.06	0.98±0.04*****	1.02±0.04	1.04±0.05
SV (ml/beat)	76.5±3.9	81.0±4.6*****	81.5±3.1	81.8±3.1
Log Up-Sequence BRS (ms/mmHg)	1.2±0.1	1.3±0.1 *****	1.0±0.1	1.2 ± 0.1*****
Log Down-Sequence BRS (ms/mmHg)	1.2±0.0	1.2±0.0	1.2±0.1	1.2±0.0
SDNN (ms)	83.2±7.0	118.3±11.2 *****	75.5±6.2	120.9± 8.7*****
RMSSD (ms)	66.2±7.8	91.1±10.9*****	69.0±9.1	88.5±9.0*****
pNN50 (%)	32.0±4.6	37.4±3.5	39.1±5.5	39.8±3.3

Values are in Mean ± SEM. WEs: white Europeans; SAs: South Asians; BL: baseline; SBP: systolic blood pressure; DBP: diastolic blood pressure; mABP: mean arterial pressure; HR: heart rate; RRI: R-R interval; RR: respiratory rate; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume; BRS: baroreceptor reflex sensitivity; SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. (#): significant difference between WEs and SAs ($p<0.05$), and (*): significant changes within each group ($p<0.05$).

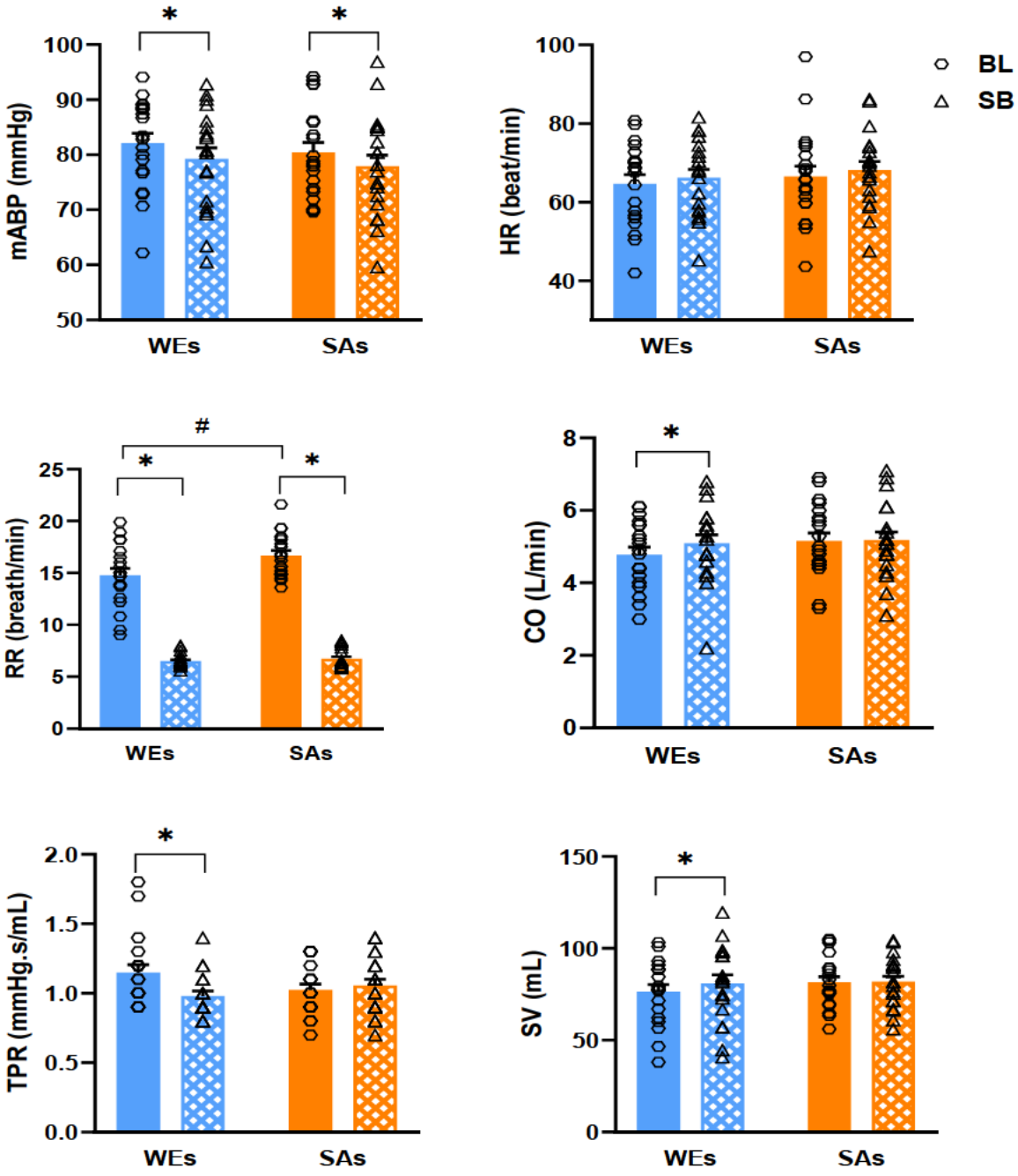


Figure 3.1 Hemodynamic changes in response to 5 minutes of slow breathing in young normotensive WEs and SAs adults

WEs: White Europeans (blue); SAs: South Asians (orange); BL: baseline (filled); SB: slow breathing (hashed); mABP: mean arterial blood pressure. HR: heart rate; RR: respiratory rate; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume. (#): significant difference between WEs and SAs ($p<0.05$), and (*): significant changes within each group ($p<0.05$).

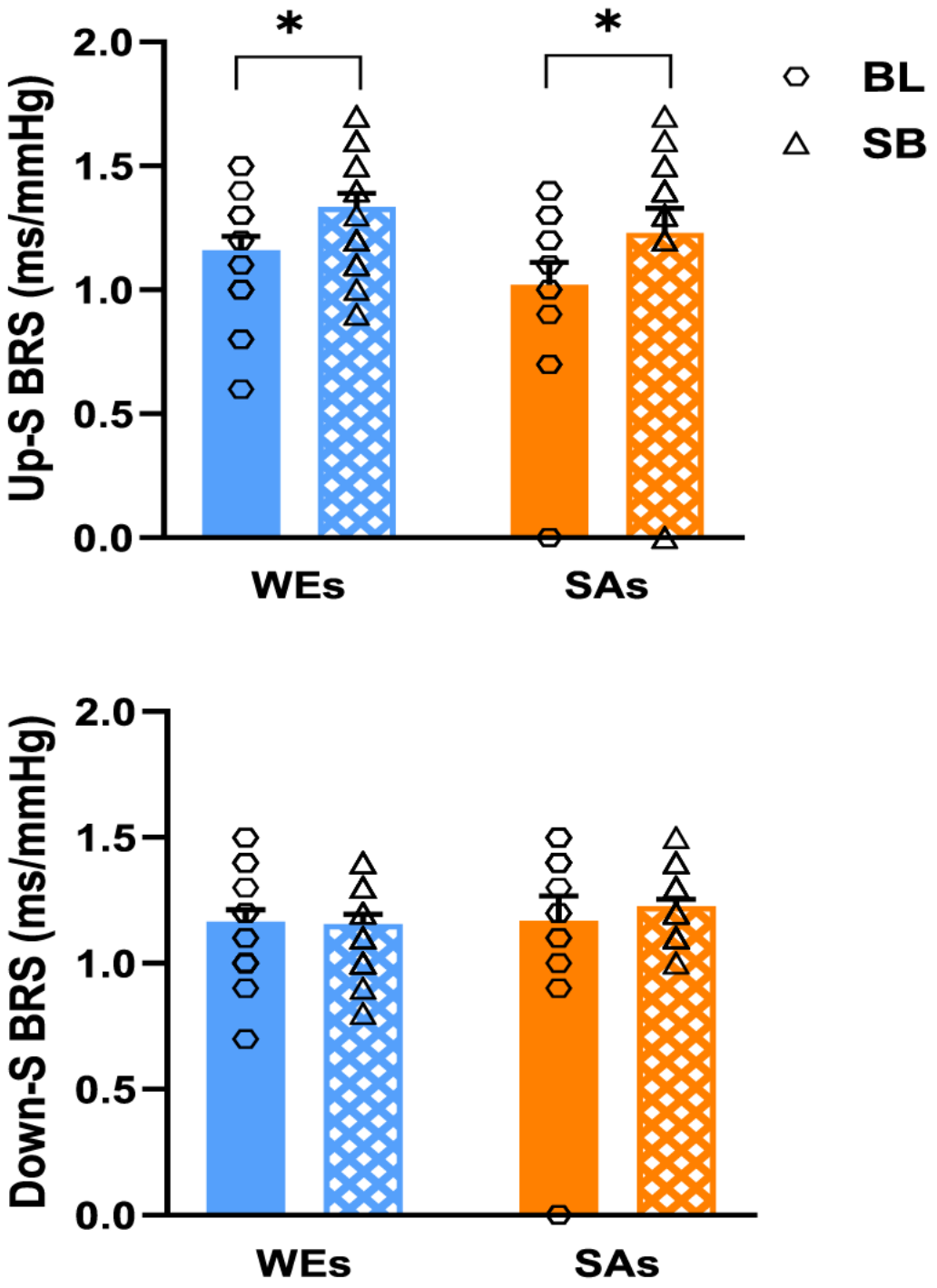


Figure 3.2 Effects of 5 minutes of slow breathing on BRS in young normotensive WEs and SAs adults.

WEs: White Europeans (blue); SAs: South Asians (orange); BL: baseline (filled); SB: slow breathing (hashed); Up-S BRS: baroreceptor reflex sensitivity during up sequence; Down-S BRS: baroreceptor reflex sensitivity during down sequence. (*): significant changes within each group ($p<0.05$).

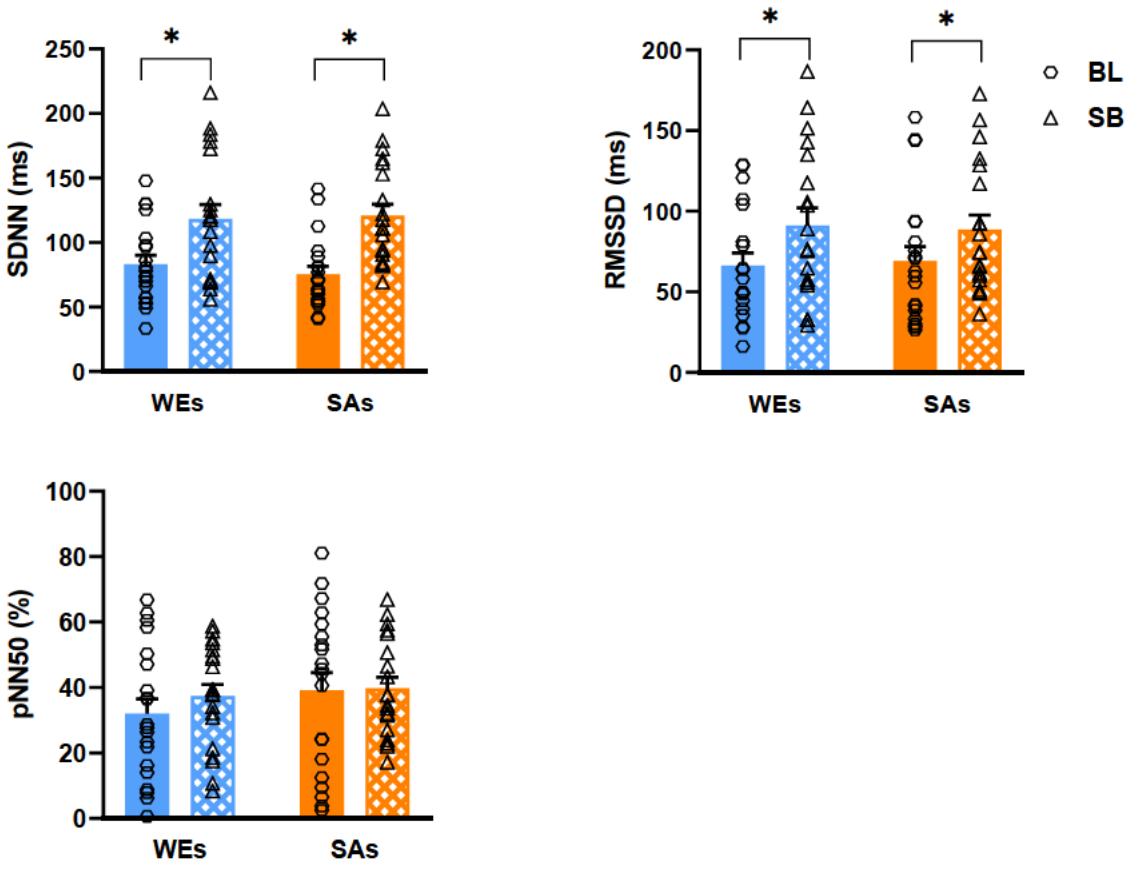


Figure 3.3 Effects of slow breathing on SDNN, RMSSD and pRR50 components of heart rate variability in young WEs and SAs adults.

WEs: White Europeans (blue); SAs: South Asians (orange); BL: baseline (filled); SB: slow breathing (hashed); SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. (*): significant changes within each group ($p<0.05$).

3.3.4 Mental Stress and Squat-Stand test

A summary of the results of the mental stress test is shown in Table 3.4. Mental stress evoked similar significant increases in mABP, SBP, and DBP in both ethnic groups (P<0.05; Figure 3.4), these values reached a plateau within ~30 s of the onset of the test and remained at these levels until its cessation (Figure 3.4). HR and respiratory rate also increased significantly in both ethnicities during mental stress as shown in Figures 3.4, while R-R intervals decreased in both WEs and SAs. Mental stress increased CO from 4.8±0.2 to 5.9±0.4***** L/min (P=0.008) in the WEs group only (Figure 3.4). There was no change in TPR or SV in WEs during mental stress. However, TPR increased significantly in SAs during mental stress compared to baseline values (from 1.02±0.04to 1.29±0.12* mmHg.s/mL; *: P=0.01, baseline vs mental stress), while SV decreased to 68.8±4.2***** ml/beat (P=0.002) in SAs only, as shown in Figure 3.4.

Meanwhile, in both WEs and SAs, SDNN was decreased (to 66.6±5.1***** and 63.5±5.6***** ms respectively *: P<0.05, baseline vs mental stress) compared to baseline values, and RMSSD was decreased (from 66.2±7.8 to 46.4±6.2*, and from 75.5±6.2 to 63.5±5.6* ms, respectively; P<0.05). However, pNN50 was decreased in SAs only, from 39.1±5.5 to 22.7±4.0* % (P<0.001) as shown in Figure 3.6.

During mental stress, BRS was decreased during up-sequences in both groups, but BRS was depressed during down-sequences in WEs only such that it became significantly lower in WEs than SAs (to 0.6±0.1 vs 1.0±0.1* ms/mmHg, *: P<0.01, WE vs SA; Figure 3.5).

During squat-to-stand test, mean log BRS values before mental stress for WEs and SAs were 0.5±0.0 vs 0.5±0.1 ms/mmHg. Immediately following mental stress, BRS during the squat-stand test was depressed in WEs, but not SAs (0.5±0.0 to 0.2±0.1*, 0.2±0.1*, and 0.4±0.1

ms/mmHg) following the 1st and 2nd stand, but not the 3rd stand in WEs vs 0.5±0.1 to 0.6±0.1, 0.5±0.1 and 0.5±0.1 ms/mmHg in the 1st, 2nd and 3rd stand respectively in SAs. BRS was significantly lower in WEs than SAs during the 1st and 2nd stand (P<0.005 and P=0.02, respectively; WEs vs SAs). The mean values for all 3 squat-to-stand manoeuvres were also lower in WEs than SAs (0.3±0.0 vs 0.5±0.1* ms/mmHg; P<0.01) as shown in Figure 3.7.

Table 3.4 Effects evoked by mental stress on cardiovascular variables compared to baseline values in WEs and SAs

	WEs (n=20)		**SAs (n=20)**	
Index	**BL**	**MS**	**BL**	**MS**
SBP (mmHg)	122.1±2.6	155.7±4.3*****	116.2±2.7	140.3±4.4 *****
DBP (mmHg)	64.1±1.7	88.9±3.8*****	63.7±1.5	85.4±2.8*****
mABP (mmHg)	82.1±1.8	108.7±4.0*****	80.4±1.8	103.0± 3.2*****
HR (beats/min)	64.6±2.4	77.8±2.9 *****	66.5±2.7	79.1±3.5*****
RRI (ms)	934.5 ± 44.4	768.3±44.0 *****	929.7±37.3	785.9±32.5*****
RR (breaths/min)	14.8±0.7	30.5±1.8*****	16.7±0.5#	26.6±2.1*****
CO (L/min)	4.8±0.2	5.9±0.4*****	5.2±0.2	5.4±0.3
TPR (mmHg.s/mL)	1.14±0.06	1.33±0.14	1.02±0.04	1.29±0.12*****
SV (ml/beat)	76.5±3.9	70.3 ± 6.0	81.5±3.1	68.8±4.2*****
Log Up-Sequence BRS (ms/mmHg)	1.2±0.1	0.8±0.1*****	1.0±0.1	0.8 ± 0.1*****
Log Down-Sequence BRS (ms/mmHg)	1.2±0.0	0.6±0.1*****	1.2±0.1	1.0±0.1#
SDNN (ms)	83.2±7.0	66.6±5.1*****	75.5±6.2	63.5±5.6*****
RMSSD (ms)	66.2±7.8	46.4±6.2*	69.0±9.1	46.1±5.2*****
pNN50 (%)	32.0±4.6	24.7±4.7	39.1±5.5	22.7 ±4.0*****

Values are in Mean ± SEM. WEs: white Europeans; SAs: South Asians; BL: baseline; MS: mental stress; SBP: systolic blood pressure; DBP: diastolic blood pressure; mABP: mean arterial pressure; HR: heart rate; RRI: R-R interval; RR: respiratory rate; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume; BRS: baroreceptor reflex sensitivity; SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. (#): significant difference between WEs and SAs (p<0.05), and (*): significant changes within each group (p<0.05).

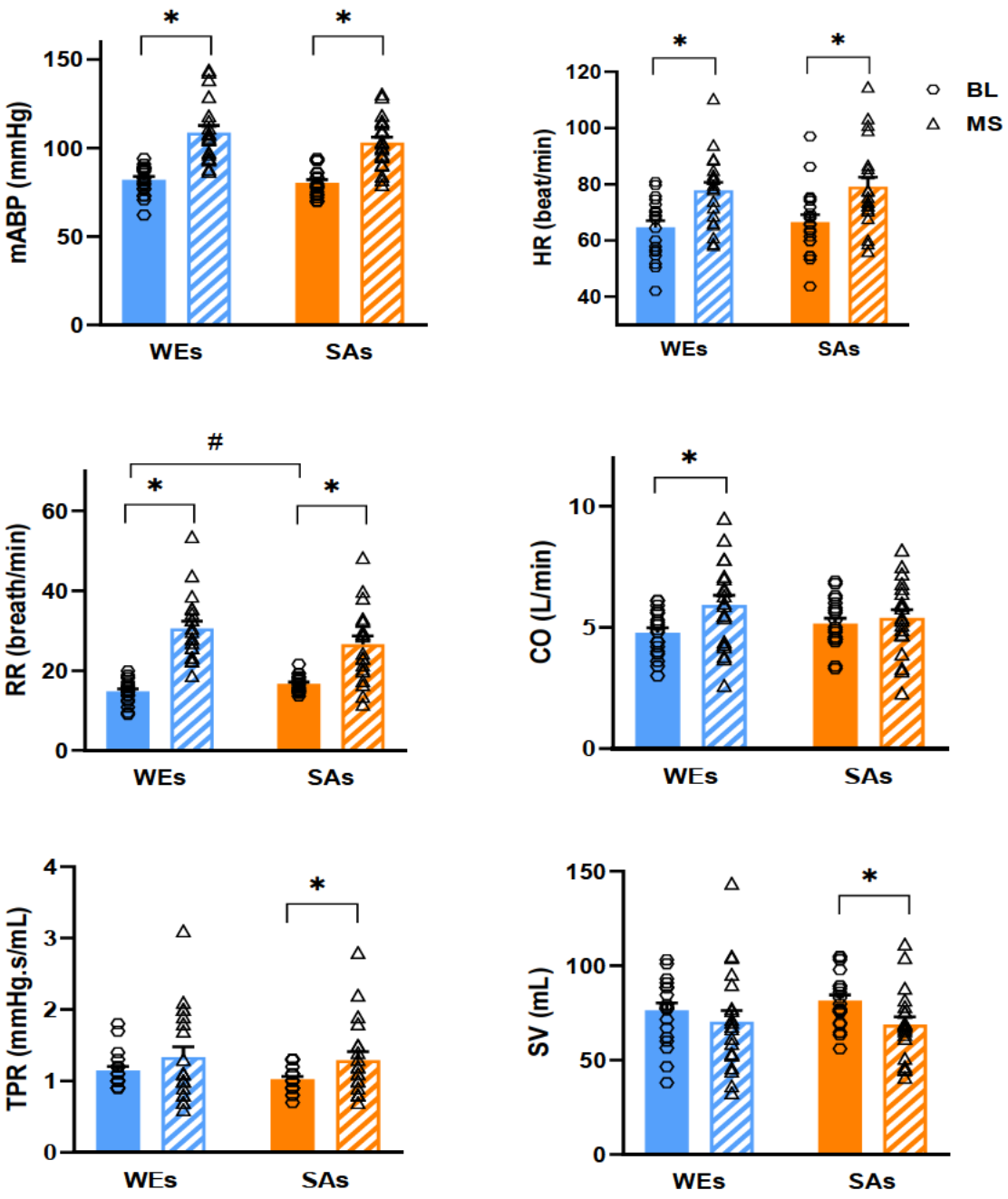


Figure 3.4 Hemodynamic changes evoked by mental stress in young normotensive WEs and SAs adults.

WEs: White Europeans (blue); SAs: South Asians (orang); BL: baseline; MS: mental stress; mABP: mean arterial blood pressure. HR: heart rate; RR: respiratory rate; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume. (#): significant difference between WEs and SAs ($p<0.05$), and (*): significant changes within each group ($p<0.05$).

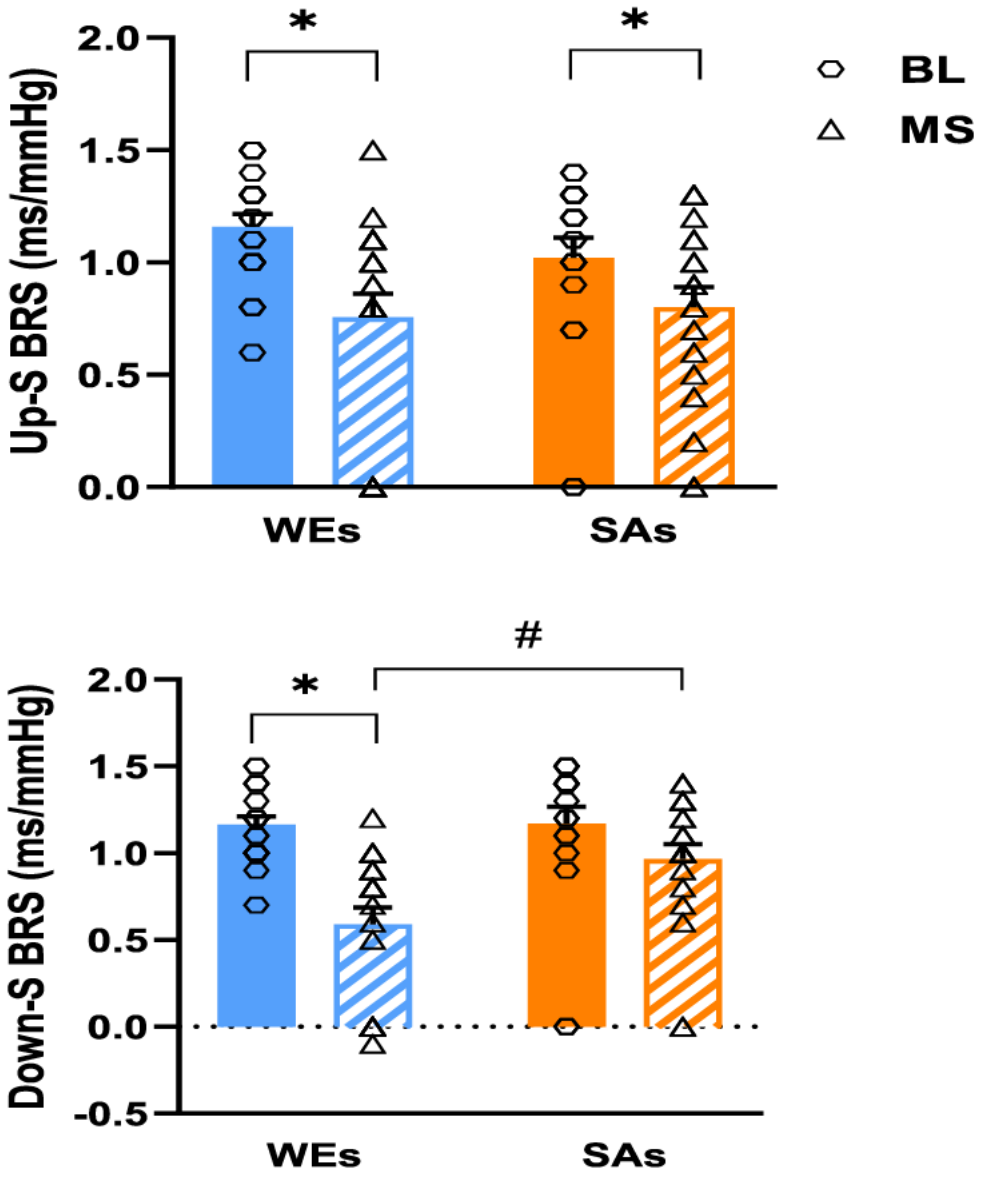


Figure 3.5 Effects evoked by mental stress on BRS in young normotensive WEs and SAs adults.

WEs: White Europeans; SAs: South Asians; BL: baseline; MS: mental stress; Up-S BRS: baroreceptor reflex sensitivity during up sequence; Down-S BRS: baroreceptor reflex sensitivity during down sequence. (#): significant difference between WEs and SAs ($p<0.05$), and (*): significant changes within each group ($p<0.05$).

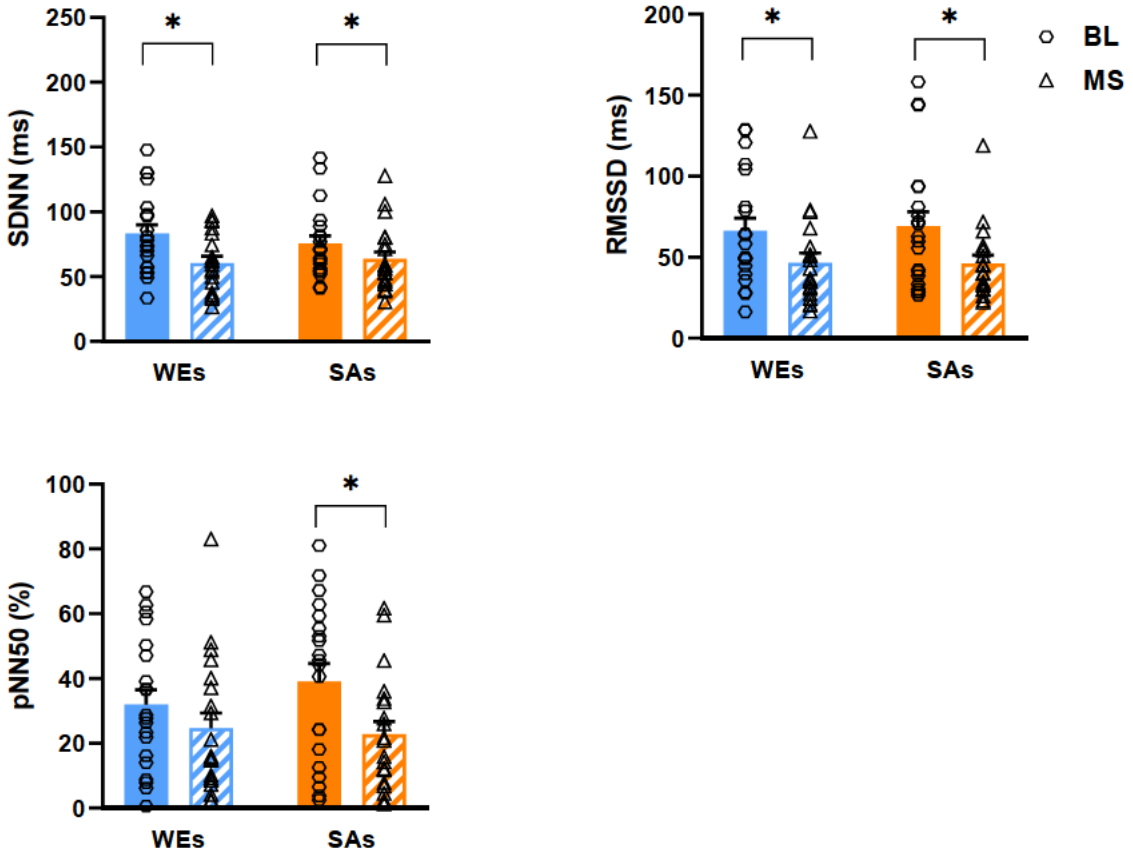


Figure 3.6 Effects of mental stress on time domain indices of heart rate variability in young normotensive WEs and SAs adults.

WEs: white Europeans; SAs: South Asians; BL: baseline; MS: mental stress; SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. (*): significant changes within each group ($p<0.05$).

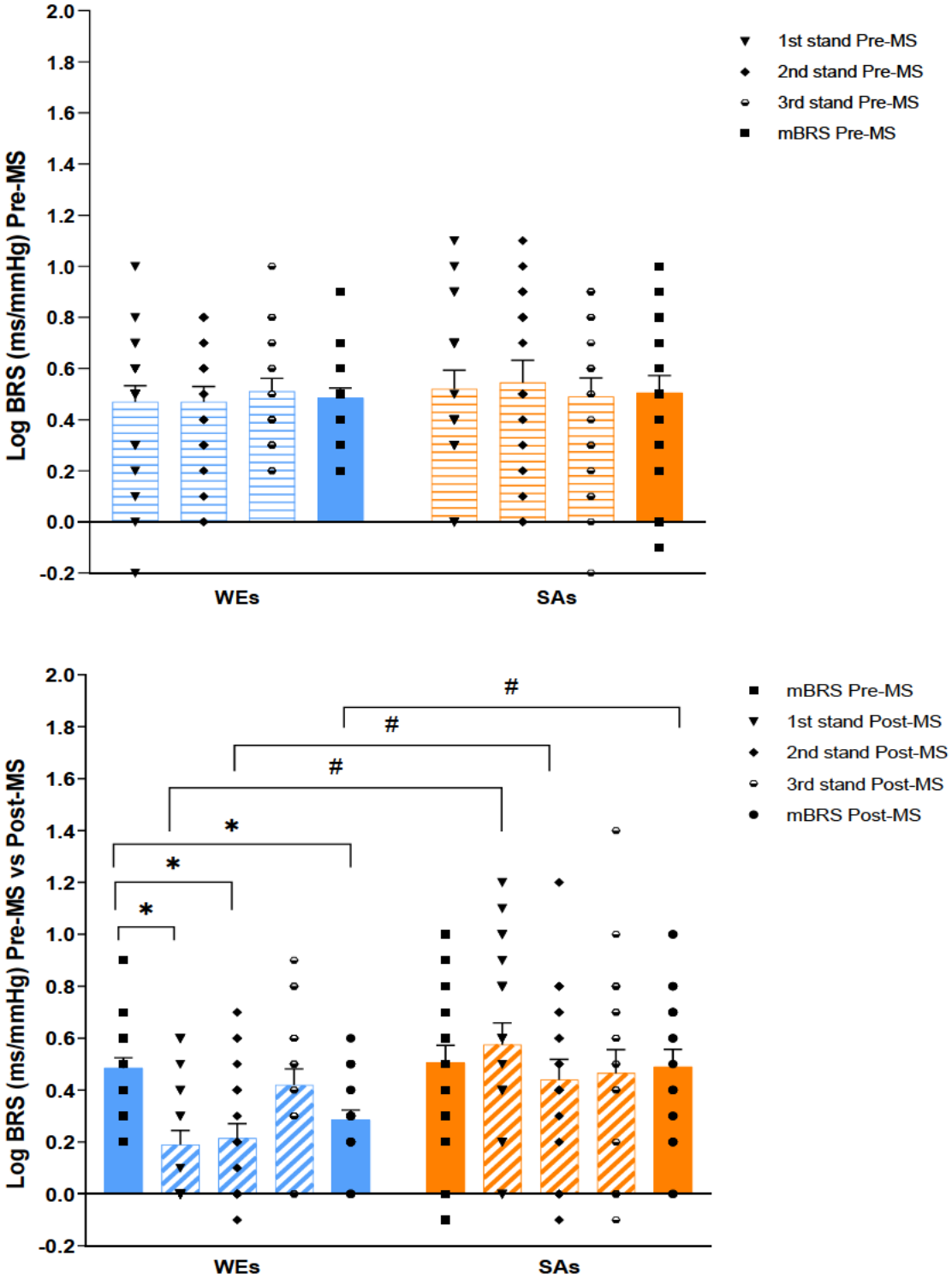


Figure 3.7 Change in BRS before and after mental stress in WEs and SAs (WEs vs SAs).

WEs: White Europeans; SAs: South Asians; BL: baseline; MS: mental stress; BRS: baroreceptor reflex sensitivity; mBRS: mean baroreceptor reflex sensitivity. (#): significant difference between WEs and SAs ($p<0.05$), and (*): significant changes within each group ($p<0.05$).

3.4 Discussion

The main goal of the present study was to assess the autonomic regulation of ABP and baroreceptor reflex regulation of ABP and HRV of young, healthy and non-smoking SAs at rest, during slow breathing and during and after mental stress, compared with a matched group of WEs.

The present results showed that under resting conditions, the systemic cardiovascular variables ABP, HR, CO, SV and TPR were not different between SAs and WEs, although RR was higher in SAs than WEs. Further, under resting conditions BRS and HRV were not different between the two ethnicities. Slow breathing at 6 breaths/min caused a significant fall in ABP in WEs, accompanied by no change in HR, an increase in SV and CO, but a fall in TPR. By contrast, ABP decreased during slow breathing in SAs but any changes in HR, CO, SV and TPR were not statistically significant. In both WEs and SAs, time domain indices of HRV were increased during slow breathing and BRS increased during up-sequences, but not down sequences. On the other hand, mental stress increased ABP, HR and RR in both WEs and SAs, but was associated with an increase in CO in WEs, but not SAs, and an increase in TPR in SAs, but not WEs. In both WEs and SAs, time domain indices of HRV decreased during mental stress and BRS decreased during up-sequences in both WEs and SAs, but BRS decreased during down-sequences in WEs only. Further, for 5-6 min following mental stress, BRS was decreased during squat-stand in WEs, but not in SAs.

3.4.1 Baseline values

Considering these findings in more detail, the present study showed no differences in ABP and HR between the present mixed sex groups of young normotensive SAs and WEs at rest before the beginning of the experimental protocol. These findings are not surprising, in

that a recent systematic review and meta-analysis showed considerable heterogeneity between different SA subgroups (Indian, Bangladeshi, Pakistan) as to whether ABP is higher or lower in SA and WEs across the adult age range although SA adolescents generally had higher ABP than WE adolescents [39]. The number of individuals involved in the present study was not sufficient to be able to make comparisons between WEs and different subgroups of SAs. The analyses of Chapter 4 test whether there were any differences between genders in resting ABP values within or between WEs and SAs.

The present results also showed that at rest, there were no significant differences in SV, CO and TPR, or HRV between SAs and WEs. It is difficult to compare these data with those reported in published studies because as far as we are aware, no previous study has made a comprehensive analysis of all of these variables within the same individuals. Further, there were no differences between SAs and WEs in BRS during up and down-sequences at rest. However, as indicated in the results, it appeared that BRS during up and down-sequences in SP over 3-4 heart beats were less common in SAs than in WEs. Therefore, in future studies it would be interesting to test this possibility by assessing the number of sequences which provides an estimate of the frequency of up and down-sequences and to make direct quantitative comparisons between SAs and WEs. Indeed, this form of analysis should be performed not only for baseline BRS but also for BRS during slow breathing and mental stress.

Sex-related differences in SV, CO, TPR, HRV and BRS at rest within and between WEs and SAs are examined in Chapter 4. Nevertheless, taken together, the results of the present Chapter suggest the contrary to our first hypothesis that young normotensive SA men and women do not show increased ABP and TPR, or decreased HRV and BRS at rest compared to matched WEs.

On the other hand, RR was higher under resting conditions in the SAs than WEs. This may be related to the evidence that SAs have lower lung function compared with WEs (chapter 1, section 1.3). In order to establish whether this the case in future studies, it would be important to measure tidal volume and also end tidal CO_2 ($ETCO_2$). Importantly, it has been reported that RR is increased in hypertensives, with no change in $ETCO_2$, and this was attributed to raised peripheral chemoreceptor activity [67]. In fact, juvenile spontaneously hypertensive rats also have increased peripheral chemoreceptor activity relative to normotensive rats, before they develop hypertension [179]. Thus, the question arises as whether young SAs also have raised peripheral chemoreceptor activity and whether this is associated with their risk of hypertension. Both the resting hyperventilation and the increased sympathetic drive in hypertensives have been attributed at least in part to peripheral chemoreceptor stimulation [113, 180].

3.4.2 Effects of Slow breathing

3.4.2.1 Effects of slow breathing on hemodynamic variables

During slow breathing, the present study showed that mABP decreased from baseline in both WEs and SAs. The present findings on WEs are consistent with previous reports that slow breathing at ~6 breaths /min reduced ABP in WEs [158, 178, 181]. In one previous study, on young SAs in India, there was no significant change in ABP (mABP: from 71.45±12.4 to 71.97±12.6 mmHg) during a yogic breathing exercise called Alternative Nostril Breathing (ANB), rather than a simple slow breathing [182]. However, other studies on normotensive SAs aged 26±4 years, showed that SBP decreased significantly (from 138±20 to 126±24* mmHg) during 5 minutes of slow breathing [183], and a single session of slow breathing also reduced SBP (from 115.4±10.8 to 108±9.8* mmHg) in young and middle-aged healthy SAs [184]. Thus, our findings add to the evidence that in young SAs an acute period of slow breathing is effective in reducing ABP.

In WEs, the decrease in ABP induced by slow breathing was accompanied by an increase in CO and SV, but a fall in TPR. By contrast, there were no significant changes in CO, SV or TPR in SAs. The increase in SV and CO in WEs can be attributed to an increase in cardiac filling secondary to the slower RR and consequent increase in lung inflation [185]. The decrease in TPR seems most likely to be explained by a decrease in sympathetic nerve activity to the vasculature, for slow breathing at 6 breath/min decreased MSNA in normotensive and early hypertensive subjects [186-188]. The mechanisms underlying this decrease in MSNA may include the reflex inhibitory effects of pulmonary stretch receptor stimulation on MSNA [186], the effects of hypocapnia [189], and/or a reduction in the influence of central respiratory neurones on the pre-motor sympathetic outflow [190]. Investigating which of these might be important in reducing TPR in the present study, would require further studies to be performed in which tidal volume, and $ETCO_2$ are recorded during slow breathing. Nevertheless, on the basis of the present findings it can be argued that whatever the mechanisms for reducing sympathetic outflow and TPR during acute slow breathing in young WEs, these mechanisms are blunted or deficient in young normotensive SAs. This outcome is consistent with our hypothesis that young healthy normotensive SAs would show early evidence of blunted respiratory-cardiovascular interactions, at least, on vascular sympathetic activity.

3.4.2.2 Effects of slow breathing on HRV

Concomitant with the effect of slow breathing at 6 breaths/min on ABP, there was an increase in the time domain indices of HRV – RMSSD and SDNN in both WEs and SAs, but not pNN50 in either ethnic group. These indices represent changes in HRV that are caused primarily by vagal influences on the heart, i.e. respiratory sinus arrhythmia (RSA) which is the main contributor to HRV [92, 185]. Of the three indices, RMSSD is considered to be most strongly influenced by parasympathetic nerve activity [92]. Thus, the present findings indicate that slow

breathing increases vagal activity to the heart in *both* WEs and SAs. These findings are consistent with previous reports on both WEs and SAs [158, 183, 184, 191]. Thus, even though we have evidence that respiratory influences on vascular sympathetic outflow are depressed during slow breathing in SAs (see above), we have no reason to think this is the case for respiratory influences on parasympathetic vagal outflow to the heart.

3.4.2.3 Effects of slow breathing on BRS

The present study also showed that there was a significant increase in up-sequence BRS, *during* slow breathing in both WEs and SAs compared to baseline values. The findings in WEs are consistent with the observations of Paprika *et al* [158] that a single session of slow breathing at 6 breaths/min evoked a significant decrease in mABP (from 82±11 to 80±12* mmHg) and increased BRS (Up-BRS: from 12±7 to 17±10* ms/mmHg) and HRV in 27 healthy adults in a study performed in Hungary. Since the ethnicity of these subjects was not mentioned, it seems most likely they were WEs. To our knowledge the finding that up-sequence BRS is also increased by acute slow breathing in young normotensive SAs is novel.

Up sequences in SBP evoke baroreceptor stimulation and cause reflex increase in cardiac vagal activity and reflex bradycardia, whereas down-sequences evoke reflex decreases in cardiac vagal activity and tachycardia due to baroreceptor unloading [81, 85, 192, 193]. Thus, the finding that only the up-sequence BRS was increased in both ethnicities suggests that the increase in vagal neuron activity caused by slow breathing, as reflected by the increase in HRV, facilitates further excitation of these neurons but has no effect on the ability of baroreceptor input to inhibit them. In practice, the effect of slow breathing was that both WEs and SAs became better at buffering rises in ABP by reflex effects on the heart. Thus, it can be proposed that in both ethnicities, acute slow breathing has anti-hypertensive effects by facilitating cardiac vagal BRS.

3.4.3 Pattern of response evoked by Mental stress (Stroop test)

3.4.3.1 Effects of mental stress on hemodynamic variables

The pattern of response evoked by environmental and mental stressors is described as the alerting or defence response: it includes an increase ABP an increase in CO, HR and RR, vasodilatation in limb muscles, but vasoconstriction in renal and splanchnic circulations [132, 172], (Chapter 1 Section 1.9). However, it is also recognised that the muscle vasodilatation is labile, readily habituates and may be blunted or absent in those at higher risk of hypertension and CVD (Chapter 1 Section 1.9). Several previous reports have shown that exaggerated pressor responses to laboratory stressors including cold pressor and mental stress tests are associated with the future development of essential hypertension [129, 194-196]. This association is particularly strong in young individuals who are known to be at high risk of developing essential hypertension because they have hypertensive parents, or are already borderline hypertensive [170, 173, 175, 176].

In the present study, a recognised mental stress test – the Stroop test - evoked a significant increase in ABP, tachycardia and an increase in RR in both WEs and SAs, with no differences between the ethnic groups consistent with the typical alerting response [132, 172]. However, the associated increase in CO achieved statistical significance only in the WEs and was attributable to the tachycardia for there was no significant change in SV. On the other hand, TPR increased during mental stress in the SAs only, and this contributed to the increase in ABP. An increase in TPR suggests that the muscle vasodilatation of the alerting response was blunted or reversed to vasoconstriction in the SAs, for the direction of the change in TPR during mental stress depends on the magnitude of the change in limb vascular resistance [131, 197]. Thus, the present findings are consistent with a recent study performed in our laboratory which showed that the majority of young, normotensive SA men consistently showed

vasoconstriction in forearm in response to a repeated sound stressor, whereas most young WE men consistently showed forearm vasodilatation and in both ethnicities, vasoconstrictor responses were associated with greater pressor responses [142]. Since there is also evidence that young SA men have blunted endothelium-dependent dilatation [143], and the muscle vasodilatation of the response to mental stress is partly endothelium dependent [135, 198], Ormshaw *et al* [142] proposed that in young SA men, the sympathetic vasoconstriction of mental stress overwhelms the blunted endothelium dependent dilatation in limb muscles. Whether there are sex-related differences in the systemic haemodynamic pattern of response evoked by mental stress within and between WEs and SAs is explored in Chapter 4. Nevertheless, on the basis of the present results on mixed sex groups, it can be argued that a larger sympathetically-evoked increase in TPR during mental stress in SAs would be expected to present a greater risk of hypertension given evidence that frequent stress-induced sympathetically-evoked vasoconstriction leads to vascular hypertrophy, and a progressive increase in peripheral resistance [199].

3.4.3.2 Effects of mental stress on HRV

Another important aspect of the response evoked by mental stress in the present study is that the major vagal indices of HRV decreased in young SAs and WEs – SDNN, RMSSD, while pNN50 decreased as well in the SAs. These findings are consistent with substantial evidence that cardiac vagal activity and HRV is reduced during mental stress as discussed in systematic reviews [145, 200], including studies performed on WEs and specifically, on young SA men and women [146]. The reduction in vagal activity to the heart during mental stress occurs together with an increase in cardiac sympathetic activity [52, 132, 145], the combination explaining the increase in HR. The reduction in HRV can be attributed to the influence of the mental stress-induced increase in RR on cardiac vagal neurones [92, 133].

3.4.3.3 Effects of mental stress on BRS

In the present study, on young WEs, BRS assessed by the sequence method was depressed during up- and down-sequences during mental stress relative to BRS recorded at rest. This finding is consistent with those of Conway *et al* [150] who used the Oxford method on a group of men and women and injected phenylephrine to raise ABP during mental arithmetic, and those of Steptoe *et al* [151] who used the sequence method for up- and down-sequences in young women during mental arithmetic. The ethnicity of the subjects was not mentioned in either report, but as the studies were performed in Oxford and London, UK respectively, it seems likely they were conducted on WEs. That the cardiac vagal component of the baroreceptor reflex was depressed during mental stress in young WEs is well established [132] and can be attributed to inhibition of cardiac vagal neurons that is caused by the mental stress-induced increase in respiratory drive [92, 133] and by direct inhibition of the baroreceptor reflex at the NTS via a pathway from the hypothalamic and amygdala defence areas [201-204].

In some contrast, in SAs, mental stress also reduced BRS during up-sequences, but there was no effect on BRS during down-sequences, such that BRS was higher during down-sequences in mental stress in SAs than WEs. Since the ability of the increased respiratory drive caused by mental stress to *inhibit* cardiac vagal neurons was just as great in SAs as WEs as judged by the reduction in HRV (see above), this would explain why the ability of baroreceptor stimulation (in up-sequences) to cause *excitation* of cardiac vagal neurons and evoke bradycardia was similarly depressed in the two ethnicities. On the other hand, the finding that the ability of baroreceptor unloading (down-sequences) to *inhibit* cardiac vagal neurons and evoke tachycardia was less depressed during mental stress in SAs than WEs, might be explained if the inhibitory influence of defence area activation on the baroreceptor reflex pathway [201] is weaker in SAs than WEs.

Turning to the BRS values assessed as the change in R-R interval evoked by the fall in SBP during squat-stand: under resting conditions, the values obtained for BRS were similar in young WEs and SAs (log BRS: 0.49± and 0.52± ms/mmHg, respectively) and these values were comparable to those recorded in healthy men and women [88]. They were also lower than those we and others recorded with the sequence method [81, 193]. This may reflect differences in the sympathetic and vagal components of the cardiac arm of the baroreceptor reflex, for pharmacological evidence indicates that the squat to stand BRS largely reflects the sympathetic component [88] (Chapter 1, section 1.5.1.5), whereas the BRS obtained with the sequence and Oxford methods are attributable to the vagal component [81] (Chapter 1, section 1.5.1.4).

Assuming the BRS recorded during squat to stand is, indeed, sympathetically-mediated, then the finding that in young WEs, the BRS recorded by squat to stand was reduced *immediately after* mental stress and recovered towards the control level over the following 5-6 minutes, suggests that the cardiac *sympathetic* component of the baroreceptor reflex was probably inhibited *during* mental stress, but then gradually recovered when the mental stress ceased. This would be consistent with evidence that the vagal component of the baroreceptor reflex is inhibited not only *during*, but also in the recovery period *after* mental stress [151], and it would be consistent with defence area inhibition of the baroreceptor reflex at the NTS that is present *during* mental stress but wanes afterwards [201].

By contrast, in the young SAs, the BRS tested during squat-stand immediately after mental stress was fully comparable to that recorded under control conditions and there was no change in BRS during the following 6-10 minutes. Thus, there is no reason to suppose that the cardiac *sympathetic* component of the baroreceptor reflex to a fall in ABP was depressed during, or after mental stress in SAs. Indeed, the disparity between the SAs and WEs in the effects of mental stress on the BRS evoked by squat to stand could be explained if the ability

of defence area activation to inhibit the baroreceptor reflex at the NTS and so inhibit the cardiac sympathetic component of the baroreceptor reflex is blunted in SAs relative to WEs, just as suggested for the vagal component (see above).

If this proposal is correct, then it would be expected that the cardiac *and* vascular sympathetic components of the baroreceptor reflex are also be inhibited *during* mental stress in young WEs, but not SAs. It has already been reported that the vascular component of the baroreceptor reflex was inhibited during mental arithmetic as assessed using the sequence method on the relationship between DBP and MSNA in young men and women [(153)]. The ethnicity of the participants was not mentioned suggesting they were probably white Americans of WE heritage. In light of the present results, it would be interesting to make comparisons between WEs and SAs for BRS during up- and down-sequences of DBP while recording MSNA before, during and after mental stress.

The present evidence that the cardiac vagal and sympathetic components of the baroreceptor reflex is blunted during and after mental stress in young WEs, but not young SAs directly contrasts with our hypothesis that SAs would show *greater* blunting of the baroreceptor reflex during mental stress than WEs. As discussed above, our finding that young SAs did not show greater pressor responses to mental stress than WEs, also contrasted with our working hypothesis. Indeed, the only aspect of the cardio-respiratory response to mental stress which was consistent with our hypotheses was that SAs showed more pronounced peripheral vasoconstriction (increase in TPR) than WEs.

3.4.4 Study limitations

Several limitations of the present study should be taken into consideration. The study was conducted on only 21 young SAs and 20 young WEs with ages ranging from 18-25 years and similar numbers of men and women in each group. They were well matched for height weight, BMI, waist to hip ratio and levels of physical activity. However, larger scale studies will be necessary to establish whether the present findings can be extrapolated to a wider population of young adults in the UK, particularly as all the participants of the present study were undergraduate or postgraduate University students and thus, likely to be of similar socioeconomic status. Further, blood samples were not collected and tested in the present study for markers of oxidative stress, lipid profile, and/or insulin resistance. This would have provided indicators of whether even young normotensive SAs have higher risk factors for CVD and diabetes than WEs as might be expected from large population, and pre-clinical studies [164, 205].

In the present study, slow breathing was performed only at one fixed frequency – 6 breaths/min. There is evidence that the resonance frequency at which HR and RR are in phase varies between individuals, from 4.5 and 6.5 breaths/min, is the RR at which HRV is maximal and BRS for vagal control of HR is also maximal [185]. Further, MSNA as well as ABP were shown to decrease when young normotensive men of unspecified ethnicity performed slow breathing at their own resonance frequency [206]. Thus, the question arises as to whether the resonance frequency varies between WEs and SAs and whether asking the participants to breathe at their own resonance frequency would have changed the outcomes for SAs or WEs and specifically, would have resulted in a significant decrease in TPR in SAs. It was also a limitation that $ETCO_2$ was not monitored in the present study, for differences between $ETCO_2$ between WEs and SAs at resting conditions and/or during slow breathing or mental stress could

have affected the evoked cardiovascular responses. This was a particularly important issue given resting RR was higher in SAs than WEs and it was possible they were hypocapnic.

The fact that we used the Stroop test as a mental stressor was beneficial because the predictive value of the pressor response to stressors for future CVD is considered to be better for tests involving cognition, rather than physical stress such as the cold pressor test [129]. However, the fact that the participants had to vocalise their decision on whether the colour match was correct or not was a disadvantage because speaking sometimes produced artefacts on the recording of RR via a respiratory belt. This was important given we were assessing interactions between respiratory and cardiovascular responses; this could be avoided in future studies by asking the participant to press one of two different keys on a laptop.

Finally, it was a limitation that we tested BRS by applying the sequence method to spontaneous increases and decreases in SBP at rest, during slow breathing and during mental stress, but used the squat-stand test to assess the BRS in the period immediately *following* mental stress. We followed this protocol because time domain analysis of BRS using the sequence method gives a reliable assessment of the vagal component of the baroreceptor reflex even when RR is changed, whereas frequency domain analysis of BRS using the spectral method is affected by the movement of the dominant HR rhythm between the HF and LF bands [81, 92]. On the other hand, the BRS assessed from the squat-stand test has been attributed mainly to the cardiac sympathetic component of the baroreceptor reflex, and so together, they gave us an indication of the effects of mental stress on both components of the baroreceptor reflex effects on HR. However, it would have been practically difficult for participants to perform squat to stand *during* the mental stress test, and we therefore have no assessment of cardiac sympathetic BRS during mental stress. We are also reliant on the study of Marfella *et al* [89] which was performed on young normotensive men and women for pharmacological evidence

that the increase in HR evoked by the fall in ABP that occurs during squat to stand was blocked by propranolol, but unaffected by atropine. It would have been good to re-assess this finding in young WEs and SAs. A further limitation is that the performance of 3 squat to stand tests following mental stress meant that it was impossible to assess the vagal component of BRS during this time by using the sequence method on spontaneous changes in SBP. A repeat of the protocol omitting squat-to-stand in which BRS is assessed by the sequence method would allow direct comparisons to be made between effects on the vagal and sympathetic component of the cardiac baroreceptor reflex during recovery from mental stress. But, as far as we are aware there is no simple way of assessing the cardiac sympathetic component of BRS during mental stress. As indicated above (see section 3.4.3.3) the vascular sympathetic component of BRS could be compared between WEs and SAs during mental stress by using the sequence method on spontaneous changes in DBP and reflex changes in MSNA.

3.5 Conclusions

In the present study, young normotensive SA men and women showed similar increases in ABP, HR, and RR and similar reductions in vagal indices of HRV in response to an established mental stress test as matched WEs, but the SAs showed a greater sympathetically-mediated increase in TPR. Further, SAs did not show the inhibition of the cardiac vagal and sympathetic components of the baroreceptor reflex during and following mental stress that occurred in the WEs and which characterise the response to mental stress. These novel findings suggest that young SAs may be better than WEs at buffering changes in ABP during mental stress, but raise the possibility that exaggerated sympathetic vasoconstrictor responses to stressors may facilitate future hypertension in SAs. On the other hand, although resting RR was slightly higher in SAs than WEs, slow breathing at 6 breaths/min induced a similar reduction in ABP, increase in HRV and increase in BRS during spontaneous increases in SBP in both SAs and WEs, but reduced TPR in WEs, not SAs. These findings indicate that ability of slow breathing to increase vagal activity and reveal respiratory-dependent changes in vagal activity are similar in WEs and SAs, but the ability of reduced respiratory drive to reduce sympathetic vasoconstriction is weaker in SAs than WEs.

Taken together, these results raise the question of whether the responses evoked by acute mental stress and slow breathing are similar in men and women. This is dealt with in Chapter 4 which considers sex-dependent analyses of the laboratory data. They also raise the question of whether slow breathing training might be effective in reducing resting ABP and improving BRS and sympathovagal balance in SAs. These issues are addressed in Chapter 5 which presents a systematic review and meta-analysis of the relevant literature.

Chapter 4: Sex-dependent differences within and between young normotensive White European and South Asian adults in response to acute mental stress and slow breathing

4.1 Introduction

As described in the General Introduction chapter (section 1.2), several previous studies have shown that the prevalence of hypertension and CVD is higher in the population of SA ethnicity living in the UK compared to WEs [6, 7, 26, 31, 33]. Importantly, SA women have a greater prevalence of CAD relative to WE women, and they present earlier and with greater severity than women of other ethnicities [5, 8]. Furthermore, an age-standardized CAD analysis conducted over a 15-year period revealed that the proportional mortality rate from CAD is higher for Canadian men and women of SA ethnicity (42% and 29%, respectively) than for those of WE ethnicity (29% and 19%, respectively) [21]. Therefore, the mortality rate related to CAD and essential hypertension is higher in SAs compared to other ethnicities [9].

As discussed in chapter 1 (section 1.5.2), a previous study on 84 SA and 83 WE men (aged 59.5 ± 8.6 64.5 ± 8.1 years, respectively) who were living in the UK showed a greater percentage of SAs had diabetes and/or were receiving treatment for hypertension, as would be predicted given the higher risk of CVD in SAs. The SAs also had higher HR than the WEs and this was associated with higher glucose levels in the SAs, but not with insulin resistance or hyperlipidaemia [96]. However, using the sequencing method, no difference was revealed between SAs and WEs in the cardiac component of BRS. In contrast with these findings, in a subsequent study on larger groups of 151 SAs and 149 WEs (including both men aged 35-75 *and* women aged 55-75 years), HR was again greater in SAs than WEs, but BRS, as measured by sequence method, was lower in SAs than WEs, and associated with hyperglycaemia [97]. It is likely that ageing may have impacted autonomic function in both ethnicities since both these investigations were conducted in early middle-aged to elderly individuals. However, when women were included, BRS became significantly lower in SAs than WEs, raising the

possibility that SA women may have lower BRS than WE women [97]. As far as we are aware, this has not been tested in young WEs and SAs.

As also described in Chapter 1 (section 1.8), there is evidence that mental stress is associated with hypertension and that the amplitude of the pressor response to acute mental stress predicts hypertension risk [129, 130]. Notably, a meta-analysis of 32 studies demonstrated that the magnitude of SBP, DBP, and HR responses to laboratory stress tests, such as the Stroop test and mental arithmetic, were found to be strong predictors of CVD risk status, particularly hypertension [129]. Interestingly, the relationship was stronger in men than in women, and it was greatest when the subjects were younger (under or 18 years old) and followed up for 3 years and longer (up to 20 years) [129]. Future hypertension was also predicted by slower HR and SBP recovery after stress tests [129]. This meta-analysis did not take ethnicity into consideration, and it seems that no longitudinal studies have been done on the relationship between exaggerated pressor response to stress and hypertension or CVD in SA men and women.

Because it has been demonstrated that the magnitude of the pressor response to mental stress is dependent on the change in TPR rather than the change in CO, and that the change in TPR is dependent on the size of the muscle vasodilatation, variability in the vascular response in muscle is of particular interest [131]. Our research group's recent findings are consistent with these relationships for young normotensive SA men mainly showed forearm vasoconstriction whereas WE men showed vasodilatation in response to repeated sound stimuli [144]. Furthermore, the young SA men showed exaggerated pressor responses compared to the WE men, and they were directly correlated with changes in forearm vascular resistance in both ethnic groups, meaning that when an increase in forearm vascular resistance contributed to a rise in TPR, the increase in ABP was greater [142]. Young SA women have not been tested in a similar study. This is of interest because other recent studies conducted by our research group

showed that both young SA men and women have blunted endothelium-dependent dilator responses compared to young WE men and women respectively [144, 207], but NO-, endothelium-dependent dilator responses are particularly blunted in young SA women with hypertensive parents [144]. The forearm vasodilatation evoked by mental stress is NO-dependent [135, 208], and is blunted in those with hypertension and in Black Africans who have blunted endothelium-dependent dilatation and high risk of CVD [138-140]. Thus, it is a reasonable proposition that SA women show greater increases in TPR and ABP in response to mental stress than SA men or WE women.

There seem to have been no published studies on whether there are sex-dependent differences in the effects of slow breathing on the systemic hemodynamic variables, nor on HRV and BRS. The study described in Chapter 3, showed that slow breathing induced a fall in ABP in mixed-sex groups of WEs and SAs, but only in the WEs was there an accompanying decrease in TPR. On the other hand, slow breathing induced comparable increases in vagal indices of HRV and in vagal BRS in the mixed-sex groups of WEs and SAs. Given the evidence that SA women are at greater risk of hypertension and CAD than WE women and SA men [5, 8, 33], a question arises as to whether the ability of slow breathing to reduce sympathetically-mediated vasoconstriction and thereby reduce TPR might be especially blunted in SA women relative to SA men or WE women and men.

4.1.1 The aims of the study

The aim of this study is to test the following hypotheses:

- SA women show blunted BRS and reduced HRV at rest compared to SA men and WE women.
- SA women show exaggerated pressor responsiveness to mental stress compared to SA men and WE women.
- Slow breathing will improve BRS and HRV in WE men and women, but to a lesser extent in SA women than in SA men.

4.2 Methods

Study methods have been explained in detail in the general material and methods chapter (see chapter 2) and are described in brief here. This study was approved by the University of Birmingham Ethics Committee with application number ERN 17-1821(see section 2.2), and was conducted in accordance with the Helsinki Declaration [155].

4.2.1 Participants

Data from the same participants as those was used in Chapter 3 was used in the present study to test sex- and ethnicity-related differences in cardiovascular responses evoked by mental stress and slow breathing. Forty-two young normotensive WE and SA adults (9 WE males, 12 WE females, 10 SA males, and 11 SA females; aged 18-25 years) were recruited to take part in the study. One WE female was excluded before the experimental session due to a history of smoking which was an exclusion criterion, and one SA female was excluded due to malfunctioning of the auto-correction unit of the photoplethysmography used for ABP recording. As described in Chapter 2, a participant information sheet was given to each participant. Each participant filled out a participant questionnaire and signed a consent form.

4.2.2 Experimental procedure and protocol

The full protocol of the study was described in Chapter 2 (See section 2.6). To summarise, each participant was asked to recline on a couch in a semi-inclined position unless stated otherwise. Then, using a sphygmomanometer, the mean of three measures of resting ABP was obtained. Following that, baseline pulsatile ABP and HR were continuously recorded for 15 min by using photoplethysmography applied to a finger of the right hand, an ECG was recorded, and RR was measured using a respiratory belt. Thereafter, the participant was instructed to breathe slowly at 6 breaths/min with a sound aid for 5 min. After a rest period of

5 min and with the recordings on-going, the participant performed a squat-to-stand test, which involved standing by the couch for ~1.5 min then squatting for ~1 min before standing up and remaining upright for ~1.5 min. This manoeuvre was repeated three times (3 squats & 3 stands). Following this, the participant performed an acute mental stress test (Stroop Colour and Word Test) for ~3 min while recordings continued. Immediately following the mental stress test, the participant repeated the squat-to-stand test 3 times as indicated above. Finally, the participant reclined on the couch and rested for ~ 5 min before ending the recordings.

4.2.3 Measurements

As described in the general materials and methods chapter (Chapter 2, section 2.4.5), BRS was calculated by the sequence method from the relationship between SBP and R-R interval during spontaneous up and down sequences in the resting period (baseline), during slow breathing, and during mental stress. The sequence method was also used to calculate BRS during the squat to stand test (see Chapter 2, section 2.4.5). The pulse contour of the ABP recordings was used to compute SV, CO, and TPR offline at rest, during slow breathing and during mental stress as described in Chapter 2 (Section 2.4.1). Time-domain analysis of HRV was performed using the HRV analysis module in LabChart Software at rest, during slow breathing and mental stress (see Chapter 2, section 2.4.4).

4.2.4 Analysis of results

Data are expressed as Mean ± SEM. Microsoft Excel software (Version 2019) was used to extract the data from LabChart. It was also used to analyse and calculate BRS for each participant. Two-way ANOVA was used to compare the anthropometric measurements and cardiovascular baseline variables between the groups of participants. Two-way ANOVA was also done to test and compare the effect of sex and ethnicity on cardiovascular responses to

acute mental stress and slow breathing. Statistical significance was determined using Tukey post hoc test. A P value of less than 0.05 was assumed as statistical significance. Three Z score was conducted to detect outliers and values with a Z score ≤ -3 or ≥ 3 standard deviations from the mean were excluded. GraphPad Prism software (Version 9) was used to do all between groups comparisons.

4.3 Results

4.3.1 Anthropometric and baseline Characteristics

40 WE and SA men and women were included who met the predefined inclusion criteria. The participants were divided into four groups based on their ethnicity and sex: 9 WE males, 11 WE females, 10 SA males and 10 SA females. There were no significant differences in age between the groups (see Table 4.1). There were also no differences in BMI or WHR amongst the groups. In addition, IPAQ analysis showed that the weekly physical activity level was moderate and similar across groups. Furthermore, sphygmomanometer measurements of resting ABP and HR obtained prior to the start of the experiments showed no significant differences between the groups.

Table 4.1 Participants' anthropometric measurements at rest before the start of continuous recordings

Index	WEs		SAs	
	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
Age (years)	21.4±0.6	22.2±0.4	20.7±0.6	20.5±0.7
BMI (29kg/m^2)	23.6±0.9	22.7±0.9	23.1±1.0	25.1±1.6
WHR	0.8±0.0	0.8±0.0	0.8±0.0	0.8±0.0
Weekly Physical Activity Level	Moderate	Moderate	Moderate	Moderate
HR (beat/min)	68.3±4.3	73.3±2.8	68.5±3.4	84.0±5.8
SBP (mmHg)	119.4±1.7	115.5±2.9	116.5±2.5	115.7±4.2
DBP mmHg	71.3±1.7	72.0±2.4	68.3±2.2	77.7±2.7
mABP (mmHg)	87.4±1.5	86.5±2.3	84.4±1.8	90.4±3.0

Values are in Mean ± SEM. WEs: White Europeans; SAs: South Asians; WHR: Waist to Hip Ratio; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; mABP: mean arterial pressure; HR: heart rate.

4.3.2 Baseline measurements

As Table 4.2 shows, there were no differences in ABP, CO, or TPR across the groups at baseline while breathing spontaneously, and no differences in RR among the groups. Further, analysis of time-domain indices of HRV also showed that there were no differences between the groups and BRS was similar in all groups for up- and down-sequences. However, there were significant differences between SA men and women for HR and SV only: HR was higher in SA women than SA men (73.1±3.6 vs 59.9±2.8* beats/min; *: P= 0.02, SA men vs SA women; see Table 4.2), while SV was lower in SA women than SA men (72. 2±2.9 vs 90.9±3.6* ml; P=0.02). There were no significant differences in HR or SV between WE and SA men or between WE women and SA women.

Table 4.2 Sex differences comparisons of cardiovascular variables in WEs and SAs at rest (spontaneous breathing)

Index	Baseline			
	WEs		SAs	
	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
SBP (mmHg)	120.4±5.0	123.5±2.6	117.2±3.7	115.3±4.0
DBP (mmHg)	63.6±2.8	64.6±2.3	63.6±2.3	63.8 ±2.1
mABP (mmHg)	80.8±3.2	83.1±2.1	79.5 ±2.7	81.3±2.6
HR (beat/min)	59.4±3.4	69.0±2.8	59.9±2.8	73.1±3.6**
RRI (ms)	993.2±86.3	886.5±37.5	1020.9±51.3	838.5±37.2
RR (breath/min)	15.5±1.0	14.2±0.9	16.9±0.8	16.5±0.6
CO (L/min)	4.7±0.4	4.8±0.2	5.3 ±0.4	5.1±0.3
TPR (resistance unite)	1.13±0.09	1.15±0.08	0.98±0.07	1.07±0.05
SV (ml/beat)	81.7±5.4	72.2±5.3	90.9 ±3.6	72. 2 ±2.9**
UP-Sequence BRS (ms/mmHg)	1.1±0.1	1.2±0.1	1.0±0.13	1.1 ±0.1
Down-Sequence BRS (ms/mmHg)	1.2±0.1	1.2±0.0	1.2±0.1	1.2±0.1
SDRR (ms)	88.4±12.1	78.9±8.4	78.0±8.7	73.0±9.1
RMSSD (ms)	65.4±11.5	67.0±11.1	71.5±14.0	66.5±12.3
pNN50 (%)	30.0±7.4	33.6±5.9	39.7±8.6	38.5±7.4

Values are shown as Mean ± SEM. WEs: white Europeans; SAs: South Asians; SBP: systolic blood pressure; DBP: diastolic blood pressure; mABP: mean arterial pressure; HR: heart rate; RRI: R-R interval; RR: respiratory rate; BRS: baroreceptor reflex sensitivity; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume; SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. ******: significant difference between SA men and women ($p<0.05$).

4.3.3 Response to slow breathing

Table 4.3 and Figures 4.1, 4.2, and 4.3 summarise the mean values ± SEM at rest and during slow breathing, as well as the changes evoked in each variable by slow breathing. There were no differences during slow breathing in ABP, CO, TPR, and RR amongst the groups. However, ΔHR was different amongst the groups, there being an increase in HR in SA men but not in SA women (4.4 ± 0.9 vs -1.1 ± 1.6; P=0.03; Figure 4.1). There were no differences in ΔHR between WE and SA females, or between WE males and SA males during slow breathing. In addition, BRS and time-domain indices of HRV were not different during slow breathing amongst the groups nor were the changes evoked in these variables.

Table 4.3 Sex differences in WEs and SAs in cardiovascular variables at rest (spontaneous breathing) and in response to slow breathing exercise

	Baseline (Control) vs Slow Breathing			
	WEs		SAs	
Index	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
SBP (mmHg)				
Baseline	120.4±5.0	123.5±2.6	117.2±3.7	115.3±4.0
Slow Breathing	117.5±5.5	127.6±3.5	112.6±4.4	114.5±3.8
Δ	-2.9±2.5	4.1±1.9	-4.7±1.9	-0.8±2.6
DBP (mmHg)				
BL	63.6±2.8	64.6±2.3	63.6±2.3	63.8±2.1
SB	60.4±3.2	62.0±2.8	62.0±2.6	62.8±1.9
Δ	-3.2±1.3	-2.5±1.1	-3.1±1.6	-1.0± 1.4
mABP (mmHg)				
BL	80.8±3.2	83.1±2.1	79.5±2.7	81.3±2.6
SB	77.2±3.4	80.9±2.5	79.0±3.1	80.1±2.4
Δ	-3.6±1.5	-2.2±1.1	-3.8±1.5	-1.2±1.7
HR (beats/min)				
BL	59.4±3.4	69.0±2.8	59.9±2.8	73.1±3.6 **
SB	62.0±3.0	69.6±2.7	71.8±2.9	72.1±2.9
Δ	2.7±1.5	0.6±1.3	4.4±0.9	-1.1±1.6 **
RRI (ms)				

Table 4.3 Sex differences in WEs and SAs in cardiovascular variables at rest (spontaneous breathing) and in response to slow breathing exercise

Index	Baseline (Control) vs Slow Breathing			
	WEs		SAs	
	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
BL	993.2±86.3	886.5±37.5	1020.9±51.3	838.5±37.2
SB	945.4±79.3	883.9±36.9	857.2±34.1	855.9±34.3
Δ	-47.8±35.4	-2.6±14.4	-59.1±14.5	17.4±19.0
RR (breaths/min)				
BL	15.5±1.0	14.2±0.9	16.9±0.8	16.5±0.6
SB	6.2±0.1	6.7±0.2	6.9±0.3	6.7±0.3
Δ	-9.2±1.1	-7.5±0.9	-10.1±0.7	-9.8±0.7
CO (L/min)				
BL	4.7±0.4	4.8±0.2	5.3 ±0.4	5.1 ±0.3
SB	5.1±0.5	5.1±0.2	5.2±0.3	5.0±0.3
Δ	0.4±0.2	0.3±0.1	-0.1±0.1	-0.1±0.1
TPR (mmHg.s/mL)				
BL	1.13±0.09	1.15±0.08	0.98±0.07	1.07±0.05
SB	0.91±0.03	1.03±0.06	1.08±0.08	1.11±0.07
Δ	-0.05±0.27	-0.12±0.07	0.00±0.16	0.05±0.14
SV (ml/beat)				
BL	81.7±5.4	72.2±5.3	90.9 ±3.6	72. 2 ±2.9 **
SB	85.9±7.8	76.9±5.4	77.0±4.8	74.2±4.1
Δ	4.2±3.2	4.7±1.4	-1.4±1.8	2.0±2.2
Log up-sequence BRS (ms/mmHg)				
BL	1.1±0.1	1.2±0.1	1.0±0.13	1.1±0.1
SB	1.4±0.1	1.3±0.1	1.2±0.1	1.4±0.0
Δ	0.1±0.1	0.2±0.0	0.0±0.1	0.3±0.1
Log down-sequence BRS (ms/mmHg)				
BL	1.2±0.1	1.2±0.0	1.2±0.1	1.2±0.1
SB	1.1±0.1	1.2±0.0	1.2±0.0	1.3±0.0
Δ	0.0±0.0	0.0±0.1	-0.1 ± 0.0	0.0 ± 0.0
SDNN (ms)				
BL	88.4±12.1	78.9±8.4	78.0±8.7	73.0±9.1
SB	135.3±16.2	103.0±14.6	119.3±14.0	111.7±13.2
Δ	48.3±7.8	21.5±9.9	47.9 ± 8.1	38.6 ± 8.7
RMSSD (ms)				

Table 4.3 Sex differences in WEs and SAs in cardiovascular variables at rest (spontaneous breathing) and in response to slow breathing exercise

	Baseline (Control) vs Slow Breathing			
	WEs		SAs	
Index	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
BL	65.4±11.5	67.0±11.1	71.5±14.0	66.5±12.3
SB	101.1±16.2	82.2±14.8	88.9±12.9	80.6±11.5
Δ	41.5±10.3	10.5±7.0	23.7 ± 10.5	13.2 ± 9.5
pNN50 (%)				
BL	30.0±7.4	33.6±5.9	39.7±8.6	38.5 ±7.4
SB	42.1±5.2	33.7±4.6	38.6±4.6	36.5±4.6
Δ	12.4±5.0	2.7±4.5	3.7 ± 5.6	-2.3 ± 5.4

Values are shown as Mean ± SEM. WEs: white Europeans; SAs: South Asians; BL: baseline; SB: slow breathing; SBP: systolic blood pressure; DBP: diastolic blood pressure; mABP: mean arterial pressure; HR: heart rate; RRI: R-R interval; RR: respiratory rate; BRS: baroreceptor reflex sensitivity; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume; SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. Δ: the difference between values at rest and slow breathing. ******: significant difference between SA men and women ($p<0.05$).

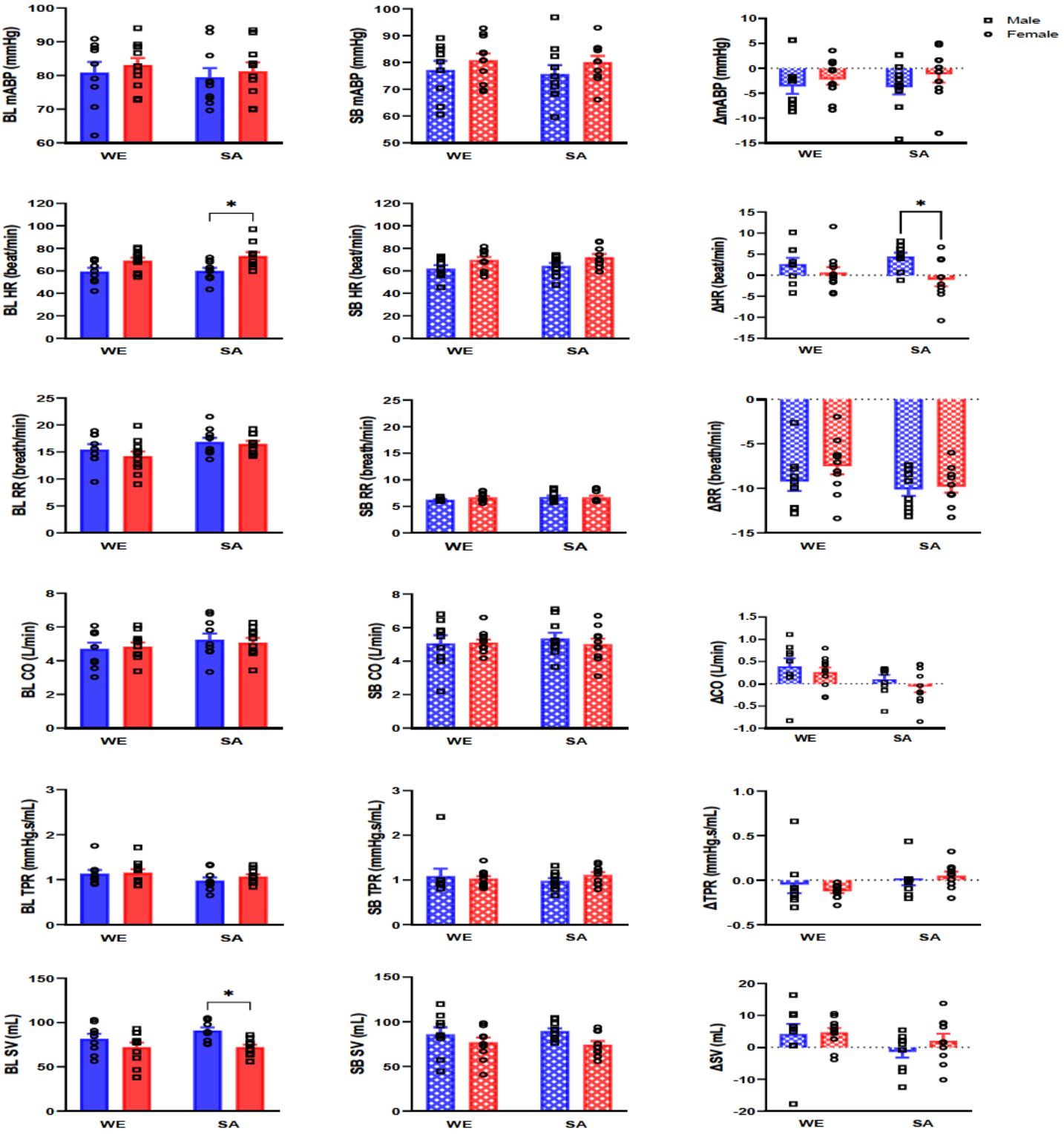


Figure 4.1 Hemodynamics sex differences in WEs and SAs at rest and during the slow breathing exercise.

WEs: white Europeans; SAs: South Asians; BL: baseline; SB: slow breathing; mABP: mean arterial pressure; HR: heart rate; RR: respiratory rate; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume. Δ: the difference between values at rest and slow breathing. *: significant difference ($p<0.05$).

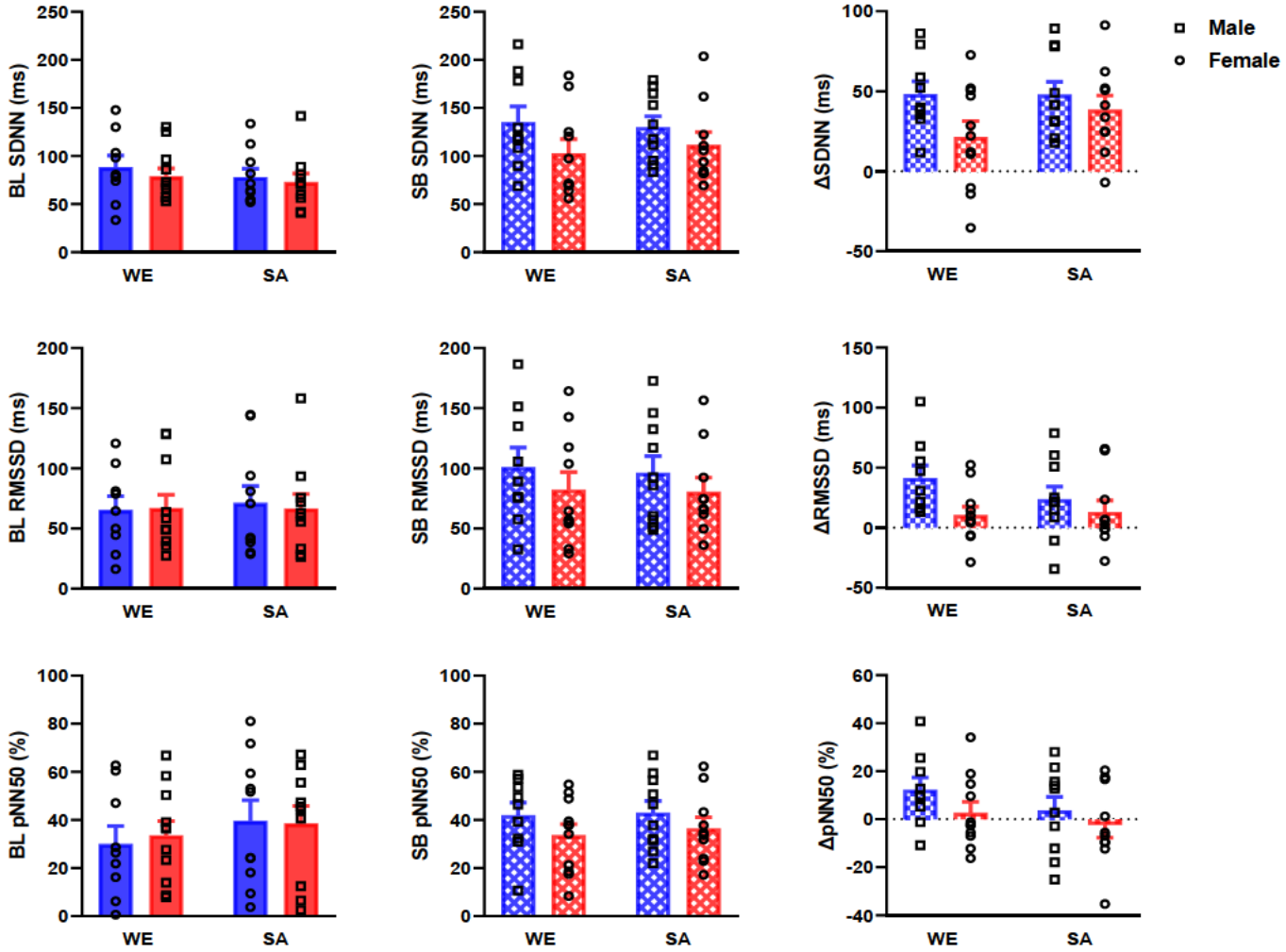


Figure 4.2 HRV comparisons and sex differences in WEs and SAs during baseline and slow breathing exercise.

WEs: white Europeans; SAs: South Asians; BL: baseline; SB: slow breathing; SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. Δ: the difference between values at rest and slow breathing.

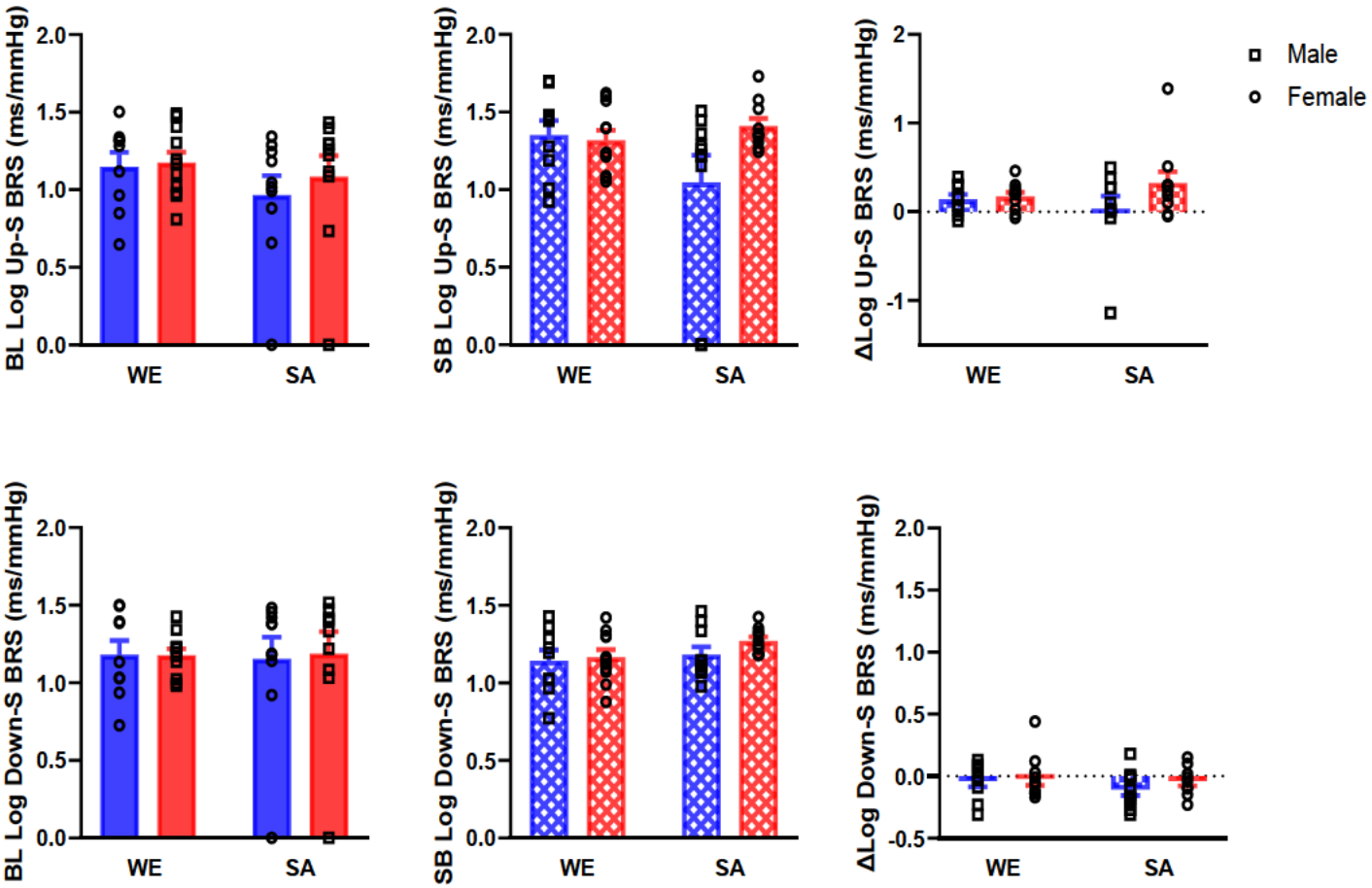


Figure 4.3 BRS Sex differences in WEs and SAs during baseline and slow breathing exercise.

WEs: white Europeans; SAs: South Asians; BL: baseline; SB: slow breathing; BRS: baroreceptor reflex sensitivity. Δ: the difference between values at rest and slow breathing.

4.3.4 Responses evoked by mental stress

A summary of the mean values at rest and during mental stress and the differences between those values are presented in Table 4.4 and in Figures 4.4, 4.5, and 4.6. There were no differences in the mean values of the hemodynamic variables or RR during mental stress among the groups and changes between baseline and mental stress values (Δ) evoked by mental stress were similar in all groups. Further, HRV and BRS values during spontaneous up- and down- SBP sequences did not differ during mental stress among the groups, neither were the changes evoked by mental stress different amongst the groups.

Table 4.4 Sex differences in WEs and SAs in cardiovascular response to mental stress test

	Baseline (Control) vs Mental Stress			
	WEs		SAs	
Index	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
SBP (mmHg)				
Baseline	120.4±5.0	123.5±2.6	117.2±3.7	115.3±4.0
Mental stress	156.2±5.5	155.3±6.6	135.7±5.1	144.8±7.1
Δ	35.9 ± 6.3	31.8 ± 5.6	18.5 ± 5.1	29.5 ± 6.6
DBP (mmHg)				
BL	63.6±2.8	64.6±2.3	63.6±2.3	63.8 ±2.1
MS	89.9±6.0	88.1±5.2	81.6±3.6	89.2±4.3
Δ	26.3 ± 5.8	23.5 ± 5.3	18.0 ± 4.5	25.4 ± 3.4
mABP (mmHg)				
BL	80.8±3.2	83.1±2.1	79.5 ±2.7	81.3±2.6
MS	108.9±5.9	108.6±5.6	97.7±3.8	108.3±4.9
Δ	28.0 ± 5.7	25.6 ± 5.6	18.3 ± 4.8	27.0 ± 3.9
HR (beat/min)				
BL	59.4±3.4	69.0±2.8	59.9±2.8	73.1 ±3.6 **
MS	71.9±3.8	82.6±3.8	71.4±3.3	86.8±5.2
Δ	12.6 ± 2.8	13.7 ± 3.4	11.5 ± 2.4	13.7 ± 2.7
RRI (ms)				

Table 4.4 Sex differences in WEs and SAs in cardiovascular response to mental stress test

Index	Baseline (Control) vs Mental Stress			
	WEs		SAs	
	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
BL	993.2±86.3	886.5±37.5	1020.9±51.3	838.5 ±37.2
MS	792.3±91.1	748.6±34.3	857.0±40.8	714.9±40.9
Δ	-200.8 ± 76.1	-137.9 ± 36.0	-163.9 ± 33.0	-123.7 ± 21.4
RR (breath/min)				
BL	15.5±1.0	14.2±0.9	16.9±0.8	16.5 ±0.6
MS	32.5±3.4	28.9±1.9	29.1±2.4	24.1±3.4
Δ	17.1 ± 3.6	14.7 ± 2.2	12.2 ± 2.2	7.6 ± 3.5
CO (L/min)				
BL	4.7±0.4	4.8±0.2	5.3 ±0.4	5.1 ±0.3
MS	6.2±0.7	5.6±0.4	5.5±0.5	5.2±0.5
Δ	1.1 ± 0.8	0.4 ± 0.4	0.3 ± 0.3	0.2 ± 0.3
TPR (mmHg.s/mL)				
BL	1.13±0.09	1.15±0.08	0.98±0.07	1.07±0.05
MS	1.32±0.27	1.34±0.14	1.21±0.20	1.38±0.14
Δ	0.60±1.32	0.36 ± 0.81	0.23 ± 0.49	0.31±0.33
SV (ml/beat)				
BL	81.7±5.4	72.2±5.3	90.9 ±3.6	72. 2 ±2.9 **
MS	80.1±11.4	62.3±5.1	77.1±6.5	60.6±3.9
Δ	-1.6 ± 9.6	-10.0 ± 6.6	-13.8 ± 6.1	-11.6 ± 3.5
Log up-sequence BRS (ms/mmHg)				
BL	1.1±0.1	1.2±0.1	1.0±0.13	1.1 ±0.1
MS	0.8±0.2	0.7±0.1	0.8±0.1	0.8±0.1
Δ	-0.3 ± 0.2	-0.4 ± 0.2	-0.3 ± 0.2	-0.3 ± 0.1
Log down-sequence BRS (ms/mmHg)				
BL	1.2±0.1	1.2±0.0	1.2±0.1	1.2±0.1
MS	0.6±0.2	0.6±0.1	1.0±0.1	0.9±0.1
Δ	-0.4 ± 0.1	-0.6 ± 0.1	-0.3 ± 0.1	-0.1 ± 0.2
SDNN (ms)				
BL	88.4±12.1	78.9±8.4	78.0±8.7	73.0±9.1
MS	63.8±8.7	57.6±6.2	71.9±9.5	55.2±5.1
Δ	-21.1 ± 6.7	-14.3 ± 6.3	-9.9 ± 8.6	-19.0 ± 7.8
RMSSD (ms)				
BL	65.4±11.5	67.0±11.1	71.5±14.0	66.5±12.3
MS	51.2±12.2	42.0±4.8	51.3±10.0	41.5±4.0

Table 4.4 Sex differences in WEs and SAs in cardiovascular response to mental stress test

Index	Baseline (Control) vs Mental Stress			
	WEs		SAs	
	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
Δ	-14.9 ± 10.2	-17.3 ± 10.0	-11.7 ± 10.4	-30.7 ± 9.9
pNN50 (%)				
BL	30.0±7.4	33.6±5.9	39.7±8.6	38.5 ±7.4
MS	22.6±6.8	26.4±6.7	28.8±6.5	16.7±3.9
Δ	-9.2 ± 5.3	-16.2 ± 5.0	-10.2 ± 4.7	-22.6 ± 4.6

Values are shown as Mean ± SEM. WEs: white Europeans; SAs: South Asians; BL: baseline; MS: mental stress; SBP: systolic blood pressure; DBP: diastolic blood pressure; mABP: mean arterial pressure; HR: heart rate; RRI: R-R interval; RR: respiratory rate; BRS: baroreceptor reflex sensitivity; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume; SDNN: mean of the standard deviations for all normal R-R intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. Δ: the difference between values at rest and mental stress. ******: significant difference between SA men and women ($p<0.05$). ##: significant difference between SA men and WE men ($p<0.05$). ++: significant difference between WE women and SA women ($p<0.05$).

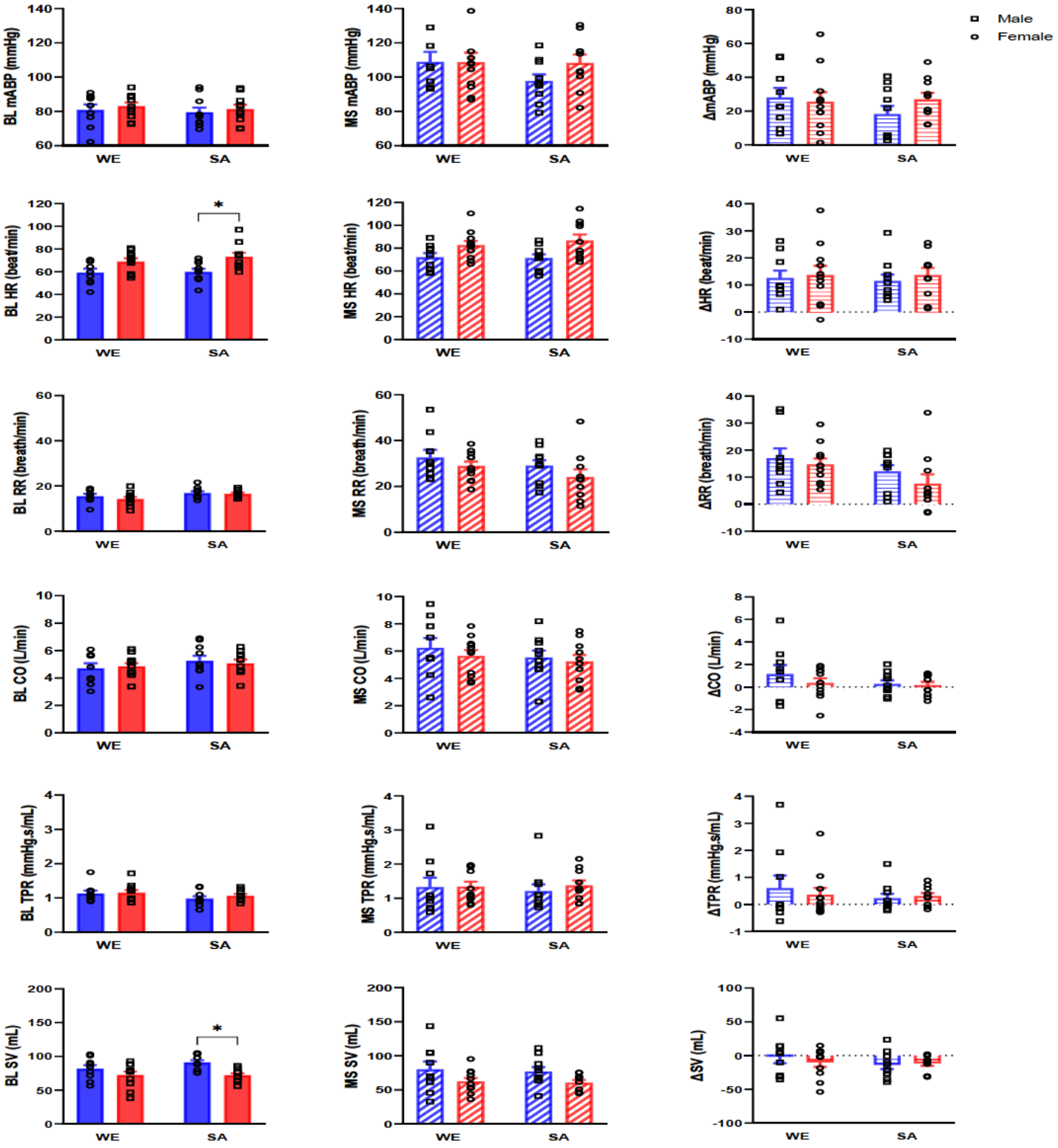


Figure 4.4 Hemodynamic changes evoked by mental stress in WE and SA men and women.

WEs: white Europeans; SAs: South Asians; BL: baseline; MS: mental stress; mABP: mean arterial pressure; HR: heart rate; RR: respiratory rate; CO: cardiac output; TPR: total peripheral resistance; SV: stroke volume. Δ: difference between values at rest and mental stress. *: significant difference ($p<0.05$).

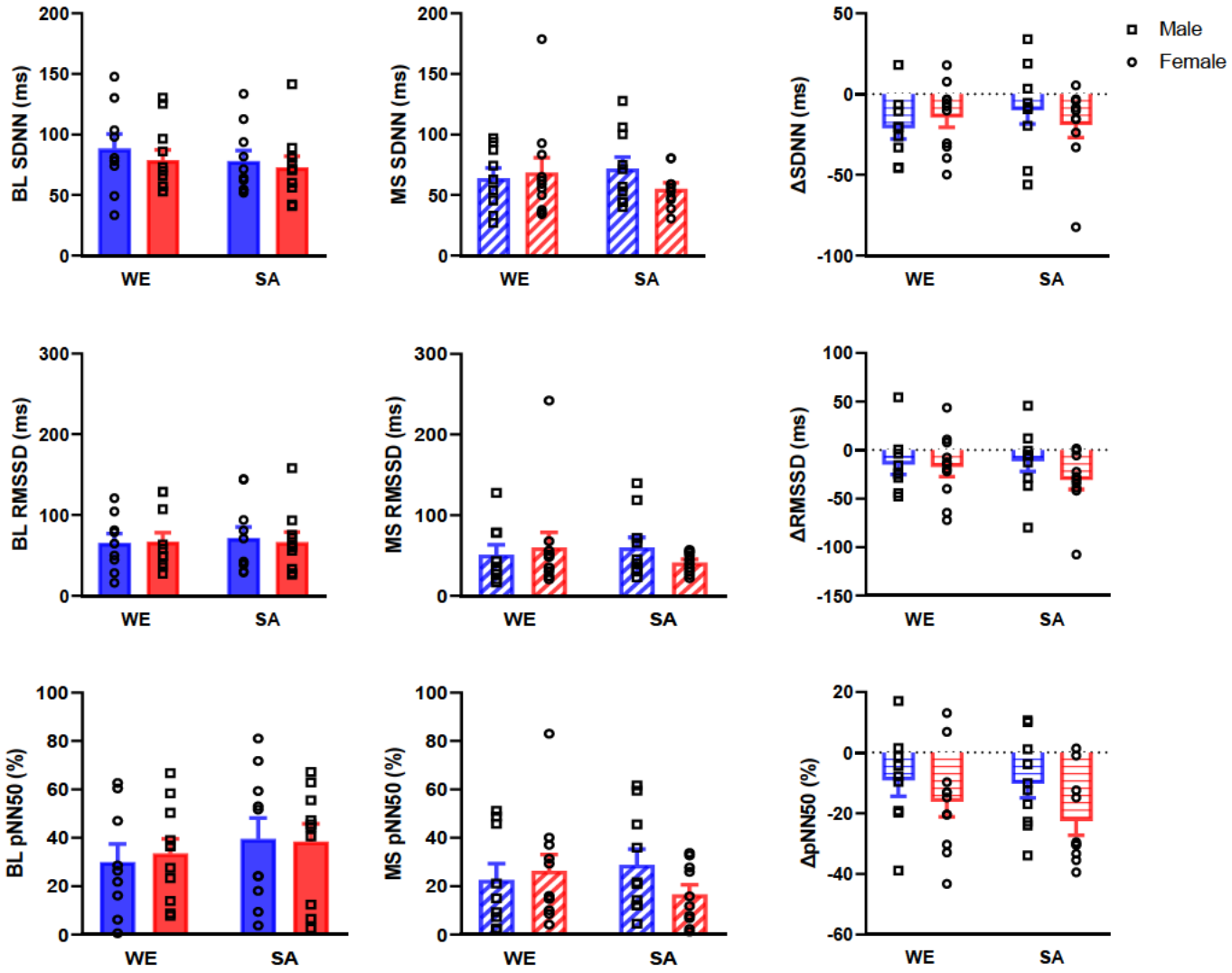


Figure 4.5 HRV changes and sex differences in WEs and SAs during baseline and mental stress.

WEs: white Europeans; SAs: South Asians; BL: baseline; SB: slow breathing; SDRR: mean of the standard deviations for all NN (R-R) intervals; RMSSD: Root mean square of successive RR interval differences; pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals. Δ: the difference between values at rest and mental stress.

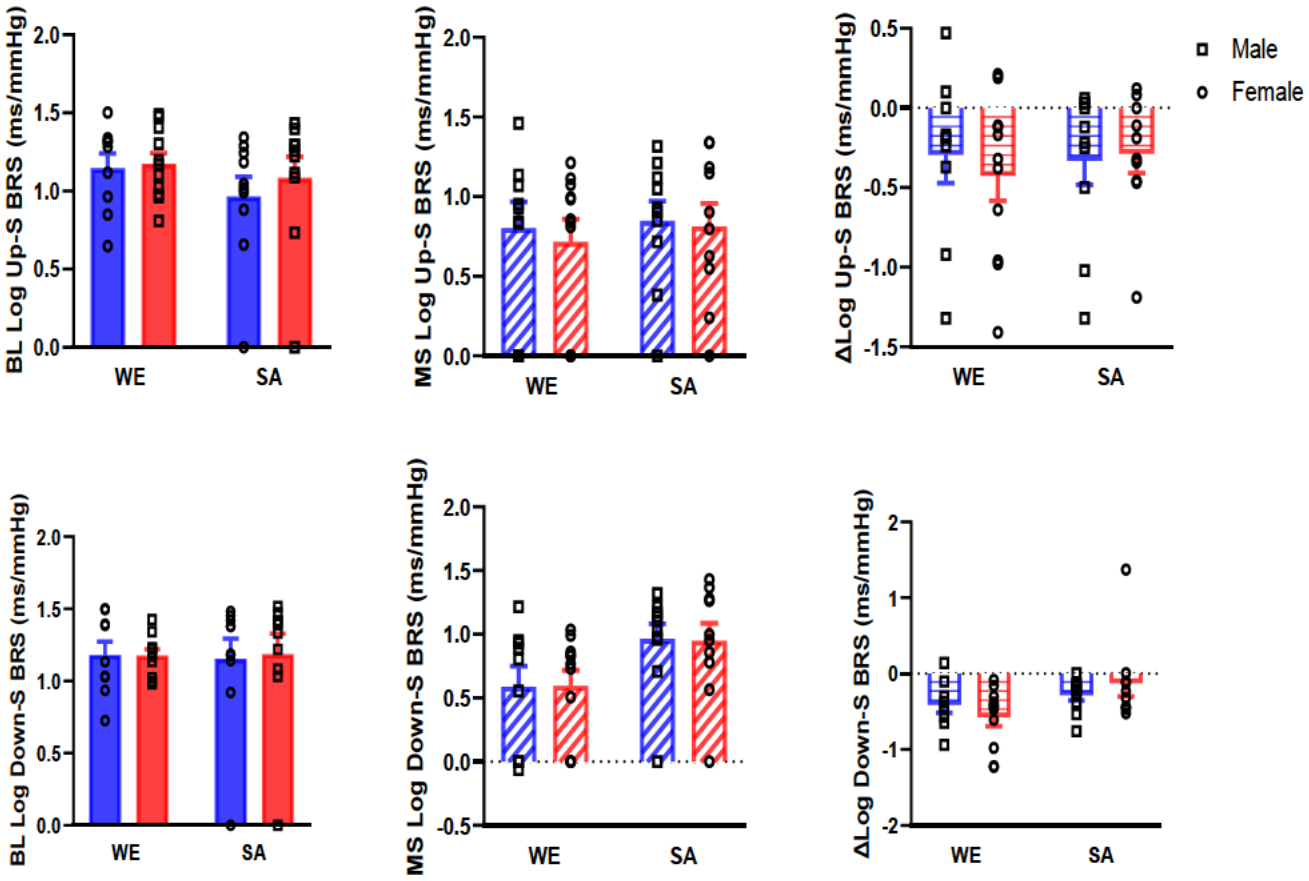


Figure 4.6 Changes in BRS during mental stress in WE and SA men and women.

WEs: white Europeans; SAs: South Asians; BL: baseline; SB: mental stress; BRS: baroreceptor reflex sensitivity. Δ: difference between values at rest and mental stress.

4.3.4.1 BRS during squat-to-stand test

Table 4.5 and figure 4.7 summarise the BRS results during the squat-to-stand test before and after the mental stress test. There were no differences among the groups in the tachycardic BRS recorded during squat to stand before mental stress. However, during the 1st squat to stand immediately after mental stress, BRS was higher in SA males than WE males (0.6 ± 0.1 vs 0.1±0.1* ms/mmHg; P=0.01, SA males vs WE males; Figure 4.7). Further, ΔBRS between BRS values during squat-to-stand before mental stress and 1st squat-to-stand after mental stress was suppressed in WE females relative to SA females (-0.3 ± 0.1 vs 0.1 ± 0.1* ms/mmHg; P=0.01). BRS values in the 2nd and 3rd squat-to-stand tests after mental stress did not differ amongst the groups. However, the mean ΔBRS for 1st – 3rd squat-to-stand was decreased in WE women but not in SA women (-0.2 ± 0.1 vs 0.0 ± 0.1* ms/mmHg; P=0.02; See Figure 4.7).

Table 4.5 Sex differences in WEs and SAs in BRS during squat-to-stand test between the mean of BRS values before and values after mental stress

	Baseline (Control) vs Mental Stress			
	WEs		SAs	
Index	Males (n=9)	Females (n=11)	Males (n=10)	Females (n=10)
BL (mBRS pre-MS)	0.4 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
1st stand (post-MS)	0.1 ± 0.1	0.2 ± 0.1	0.6 ± 0.1 ##	0.5 ± 0.1
Δ	-0.3 ± 0.1	-0.3 ± 0.1	0.0 ± 0.1	0.1 ± 0.1 ++
2nd stand (post-MS)	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Δ	-0.2 ± 0.1	-0.3 ± 0.1	-0.1 ± 0.1	-0.1 ± 0.1
3rd stand (post-MS)	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
Δ	-0.1 ± 0.1	-0.1 ± 0.1	-0.2 ± 0.1	0.1 ± 0.1
Mean (mBRS post-MS)	0.2 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
Δ	-0.2 ± 0.1	-0.2 ± 0.1	-0.1 ± 0.1	0.0 ± 0.1++

Values are shown as Mean ± SEM. WEs: white Europeans; SAs: South Asians; BL: baseline; MS: mental stress; BRS: baroreceptor reflex sensitivity; mBRS: mean baroreceptor reflex sensitivity. Δ: the difference between mean BRS before and BRS values after mental stress. ##: significant difference between SA men and WE men ($p<0.05$). ++: significant difference between WE women and SA women ($p<0.05$).

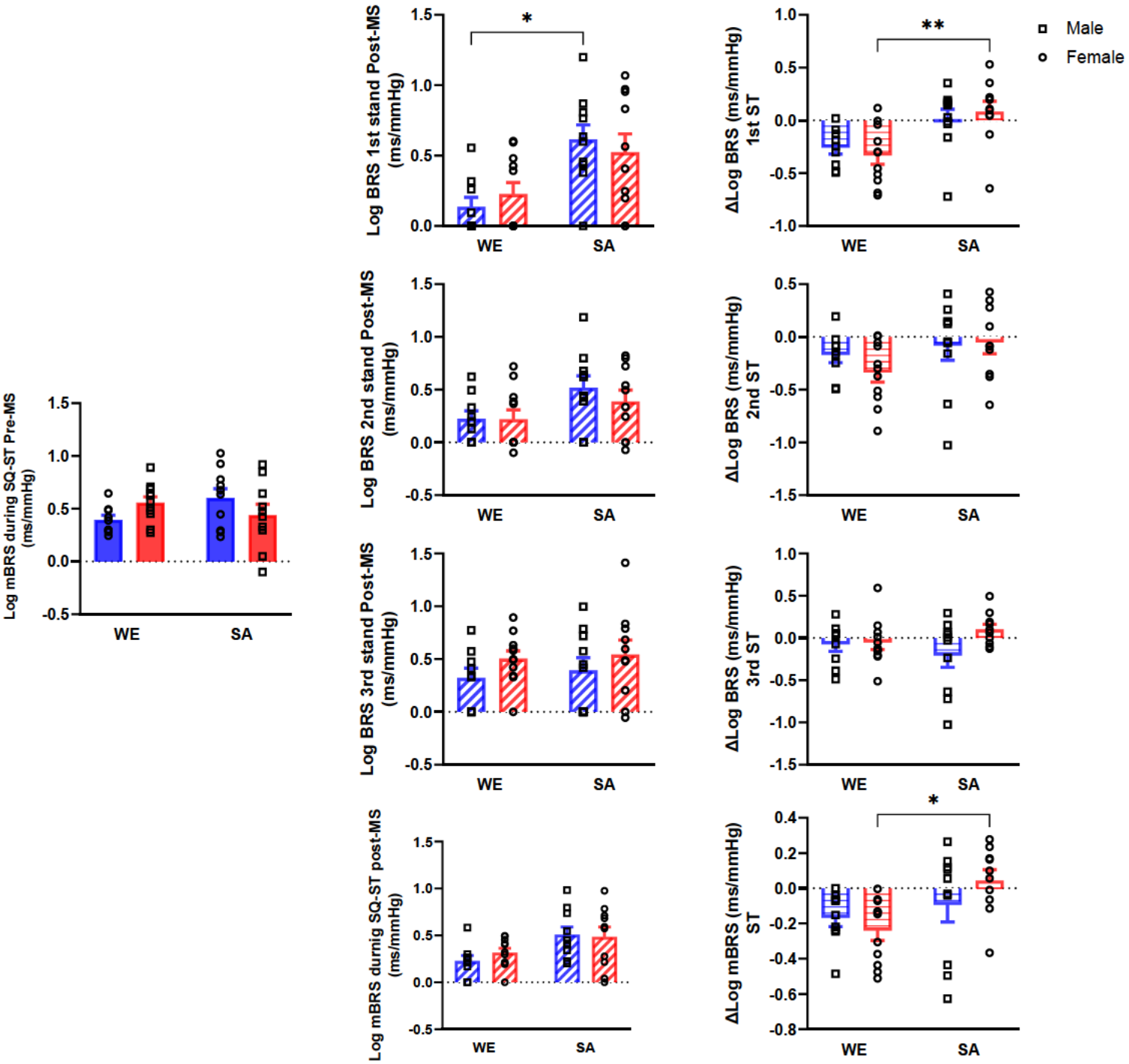


Figure 4.7 BRS comparisons during squat to stand test before and after mental stress test in WE and SA men and women.

WEs: white Europeans; SAs: South Asians; MS: mental stress; SQ-ST: squat to stand test; BRS: baroreceptor reflex sensitivity; mBRS: mean baroreceptor reflex sensitivity. Δ: the difference between values at rest and mental stress. *: $p<0.05$. **: $p<0.01$.

4.4 Discussion

The aim of the present study was to test for sex-dependent differences within and between young normotensive SAs and WEs adults in the cardiovascular responses evoked by acute mental stress and acute slow breathing. To the best of our knowledge, the present study is the first to investigate these issues through measuring systemic cardiovascular variables, RR, HRV, and BRS and RR at rest, during Stroop test and during slow breathing.

In summary, the results of the present study showed no sex-dependent differences in ABP, CO, TPR, RR, time-domain indices of HRV, or in the cardiac vagal component of BRS within or between WE and SA men and women at rest. HR and SV were also similar in SA and WE men and between WE men and women, but SA women had higher HR and lower SV than SA men. In addition, the present results showed that there were no sex-dependent differences in the haemodynamic variables, HRV or vagal BRS within or between WE and SA men and women during mental stress or during slow breathing. Nevertheless, the cardiac sympathetic component of BRS assessed by the squat-to-stand test was comparable between groups at rest but was higher in SA men than WE men immediately after mental stress. Moreover, ΔBRS was significantly smaller in SA women than WE women during the first squat-to-stand test immediately after mental stress.

4.4.1 Sex- and ethnic-dependent differences at rest (baseline values)

The outcomes of the present study showed no sex-dependent differences in ABP, CO, TPR, RR, HRV or in the vagal component of BRS between the groups. Multiple previous studies have tested sex differences between healthy adults, without taking ethnicity into account, but since the majority were performed in the UK, Europe or USA, it seems reasonable to assume they were mainly performed on WEs. Some of these studies reported similar findings to the present results in WEs, namely ABP [209-214], HR [214] ,CO [215], TPR [209, 215], RR [209],

HRV [216] and BRS did not differ significantly between young adult men and women [209, 212, 213]. By contrast, others reported that women had lower ABP [215, 217-222], higher HR [223, 224], lower SV and CO [209, 214, 222], and lower [225] or higher TPR [214]. It was also reported that women have lower HRV [212, 218, 224], and reduced vagal BRS as assessed using bolus injection of pharmaceutical agonists [226, 227]. Importantly, a systematic review and meta-analysis demonstrated that although HR is higher in women than men, women have lower HRV which reflects reduced vagal regulation of HR as judged by time- and frequency domain analyses [224]. It has also been noted that the higher HR and lower SV in women correlate with heart size generally being smaller in women than men [228]; this might explain the lower CO in women in some studies. On the other hand, the different outcomes for comparisons between TPR values in men and women may reflect the finding that TPR is closely related to MSNA and inversely related to CO in men, whereas in young women, the association between MSNA and TPR is counteracted by beta-adrenergic receptor stimulation, an effect that varies widely between women [214]. It seems likely that the variance of the present data from just 9 WE men and 10 WE women may have been too great to reveal disparities between the genders that have been apparent in other studies.

By contrast with the results from WE men and women, we did find that HR was significantly higher and SV was lower in SA women than SA men. Since CO values were similar in SA men and women at rest, it can be deduced that the lower SV in SA women compensated for the increase in HR, especially as mABP was not different between SA men and women. The results of Chapter 3 in which men and women were grouped together showed that RR was *higher* at rest in SAs than WEs (16.7±0.5 vs 14.8±0.7 breaths/min) and yet the vagal indices of HRV (SDNN and RMSSD) were *similar* in WEs and SAs. However, the results of the present sex-dependent comparisons indicate that RR at rest was *not* different between SA men and women, nor between WE men and women. Indeed, mean RR values appeared to

be higher in both SA men and SA women relative to WE men and women (Table 4.2) although any differences between ethnicities were not statistically significant. In other words, there is no reason to suggest that the higher resting RR in the *full* group of male and female SAs (Chapter 3) reflected higher RR in SA women than SA men. A similar argument can be made for vagal indices of HRV at rest, in that SDNN and RMSSD were not significantly different between SA men and women, nor between WE men and women and there were no sex-dependent differences between the ethnicities. Thus, from the data we have there is no reason to propose that the higher resting HR in SA women than SA men reflected greater respiratory-related inhibitory influences on cardiac vagal activity in SA women [224]. We cannot comment on whether the higher HR in SA women reflected higher cardiac sympathetic activity in SA women than men. Future studies should be performed on larger groups of young SA and WE men and women to give greater statistical power and to compare the vagal and sympathetic components of HRV in SA and WE men and women by using frequency- and time domain analyses, whilst assessing their relationship to resting RR.

Of the few studies which have compared young normotensive SA men and women, a study conducted in India by Satish *et al* [146] found there were no differences between the genders for ABP, time- and frequency-domains of HRV. However, in contrast to the present findings they found the HR to be similar in men and women. Another study conducted in India by Zachariah and Joseph [229] assessed time-domain indices of HRV in 30 young men and women (aged 17 – 20 years) and found no sex differences between men and women consistent with the present findings.

4.4.2 Effects of slow breathing

As far as we are aware no previous studies have compared the effects of slow breathing on haemodynamic variables, HRV, or BRS between genders within or between WE and SA

men and women. The results of Chapter 3 in which WE men and women and SA men and women were grouped in ethnicity-dependent groups, showed that acute slow breathing at 6 breaths/min was effective in reducing ABP in both ethnicities, but it was only in WEs that this was attributable to a decrease in TPR, presumably reflecting a decrease in sympathetic vasoconstrictor activity. In SAs, there were no significant changes in TPR or CO suggesting that the balance of changes in CO and TPR must have varied between individuals. The hypothesis tested in the present Chapter was that the effect of slow breathing on TPR was weaker in SA women than SA men and attenuated the change in TPR in the full SA group.

The results of the present study actually showed no sex-dependent differences within or between WEs or SA for the changes evoked by slow breathing in ABP, CO, SV or TPR. It was only the change in HR (a mean increase) which was smaller in SA women than SA men. Focussing on the variables of particular interest – ABP and TPR – in each ethnicity there seemed to be a trend for the fall in ABP induced by slow breathing to be smaller in females than males. It would be good to test this possibility in larger groups of subjects. Regarding, TPR, there were clearly differences between individuals. However, in WE men *and* women and in SA men, all except 1/9 WE men, 0/11 WE women and 1/10 SA men showed no change, or a fall in TPR, whereas in SA women, only 3/10 showed a fall in TPR. Thus, even though there were no statistically significant differences amongst the changes in TPR, the results are consistent with our hypothesis and the proposal that sympathetic vasoconstrictor activity in SA women is relatively insensitive to the inhibitory effects of slow breathing. It would be good to test the effects of slow breathing on ABP and TPR more rigorously in larger groups of participants and to test whether SA women may respond more strongly to longer periods of acute slow breathing, or to regular slow breathing training (see Chapter 5).

The finding that SA women showed smaller increases in HR during slow breathing than SA men is interesting. As discussed in Chapter 3, it is likely that slow breathing inhibits sympathetic vasoconstrictor activity as a reflex response to a larger tidal volume and stimulation of lung stretch receptors and/or to the inhibitory effects of reduced respiratory drive on sympathetic activity [186, 230]. Given lung stretch receptor stimulation also induces reflex tachycardia [230], raises the possibility that it may be this reflex that is blunted in SA women and that this may also explain their smaller reduction in TPR. This suggestion would be consistent with the evidence that lung volume and lung inflation is reduced in young SAs relative to young WEs, even in those with no respiratory disease (Chapter 1, section 1.3).

Turning finally to HRV and BRS, the results of Chapter 3 showed that the vagal indices of HRV were increased by slow breathing in the full groups of WEs and SAs, as was the vagal BRS to up-sequence changes in SBP. The present analyses give no reason to suggest that there were sex-dependent differences within, or between WEs and SA, in the beneficial effects of slow breathing on these vagal variables.

4.4.3 Response to mental stress

Studies exploring and comparing sex-dependent differences in hemodynamic, HRV and BRS changes evoked by mental stress within and between young normotensive SA and WE adults are also limited. In the present study there were no sex-dependent differences in the effects of mental stress on ABP, HR, SV, CO, or TPR, or in the effects on HRV within or between WEs and SAs. If it is assumed that the studies performed in the UK, Europe or US in which ethnicity was not reported were performed mainly on WE participants, then the present findings on WE men and women contrast with several reports that young normotensive men show a larger increase in ABP in response to mental stress and lower HRV during mental stress than normotensive women, whereas women show a larger increase in HR [134, 215, 220, 231].

However, while agreeing that women show larger increases in HR, others reported that the pressor response was not different between men and women [232]. In fact, in the latter study, women showed a larger increase in CO than men, reflecting the greater increase in HR but a fall in SV, together with a greater fall in TPR than men. It has also been reported that the pressor response to mental stress is greater in men, but that the change in MSNA does not differ between the genders [134]. It seems likely that the higher HR reported in women during mental stress is related to the higher HR and lower HRV reported at rest in most studies [31] [224] (see 4.4.2). Judging from the individual and mean data of the present study (Figure 4.4; 4.5), it may be that we did not reveal these effects in WE because the group sizes were too small, given the variance of the data.

However, the disparities between the outcomes reported for different studies for the change in ABP between genders may reflect differences between individuals in the balance of the changes in CO and TPR. This is particularly likely because the magnitude and direction of the change in TPR and ABP in response to mental stress is dependent on whether vasodilatation or vasoconstriction occurs in limb muscle and the direction of the limb vascular response is consistent within individuals [142, 233-235] (see section 3.4.3.1). Judging from the individual data for change in TPR evoked by mental stress in the present study, 3/9 WE men and 4/11 WE women showed increases in TPR, the remainder showing a decrease, indicating muscle vasodilatation (Figure 4.4). It seems rather unlikely that a larger study would reveal differences between the genders for direction or magnitude of change in TPR in WEs, but this should be tested.

Turning to the haemodynamic responses evoked by mental stress in SAs, to the best of our knowledge, no previous studies were conducted to test sex-dependent differences within SAs or between SAs and WE. The present data revealed no significant differences between

genders for the ABP, HR, SV, CO, or TPR values recorded during mental stress, nor for the changes evoked from baseline in these variables. Thus, the present data do not confirm our hypothesis that the pressor responses would be greater in SA women than SA men, or WE women. Nor were there greater increases in TPR in SA women than SA men or WE women. We recently reported that a higher proportion of SA men than WE men showed an increase in forearm vascular resistance (FVR) rather than a decrease in FVR in response to mental stress and this was correlated with a greater increase in ABP and with depressed endothelium-dependent dilatation in SA men [142]. Further, in another study, we showed that endothelium-dependent dilatation is blunted in young SA women relative to WE women, especially in SA women with hypertensive parents [144]. It is therefore interesting that in the present study, 60% of SA women (6/10) showed increases in TPR in response to mental stress, compared with only 36 % (4/11) of WE women (Figure 4.4) for this would be consistent with impaired muscle vasodilatation, or muscle vasoconstriction in response to mental stress in SA women. It will be important to test in larger group sizes whether *more* SA women do show larger increases in TPR and larger pressor responses in mental stress, consistent with their higher risk of CVD [5, 8]. The change in TPR is particularly relevant in this regard given the evidence that repeated vasoconstrictor responses to mental stress facilitate the development of hypertension [236, 237].

A characteristic feature of the alerting/defence response to mental stress is that the cardiac vagal component of the baroreceptor reflex is suppressed [132, 172]. The results described in Chapter 3 for whole groups of WEs and SAs showed that the BRS to up-sequences in SBP was inhibited during mental stress in both WEs and SAs, whereas the BRS to down-sequences was depressed in WEs only. The present analysis revealed no sex-dependent differences within or between WEs ad SAs for the effects of mental stress on BRS to up or down sequences, ie, BRS was apparently depressed in males and females in both ethnicities (Figure 4.6). Whether BRS to down-sequences (ie tachycardia induced by vagal inhibition) is less sensitive to defence

area inhibition in SA men and women than in WEs cannot be discerned from the present study and should be tested in larger groups.

A further aspect of the present results (see Chapter 3, section 3.4.3.3), is that we showed for the first time that the sympathetic component of cardiac BRS assessed by the squat-to-stand test was depressed immediately after mental stress in WEs but not in SAs. In the present study, after subgrouping according to sex and ethnicity, BRS during the first squat-to-stand immediately after mental stress was significantly greater in SA men than in WE men. Moreover, ΔBRS during the first squat-to-stand after mental stress was significantly smaller in SA women compared to WE women. Thus, taken together, these findings indicate that the sympathetic component of cardiac BRS is suppressed immediately after mental stress in both WE men and WE women but *not* in SA men or women. Given the sympathetic cardiac component of the baroreceptor reflex was inhibited immediately after mental stress in WE men and women, it seems reasonable to propose that this was a continuation of what was occurring *during* mental stress, consistent with evidence that the vagal component of BRS is depressed during and in recovery from mental stress [151] (see Chapter 3, section 3.4.3.3). Further, it seems reasonable to propose that the sympathetic *vascular* component of the baroreceptor reflex is also inhibited in both WE men and WE women consistent with previous evidence gained from recordings of renal sympathetic activity and MSNA [153, 238], and with the evidence that defence area activity inhibits the baroreceptor reflex at NTS – at an early point in the reflex pathway [132, 201].

On the other hand, the present study now provides evidence that the cardiac sympathetic component of the BRS as assessed by squat-to-stand test is preserved following mental stress both SA men and SA women at a time when the response is inhibited in WEs. Thus, there is no reason to suppose that the sympathetic BRS is inhibited during mental stress in SA men or

women. Clearly, it will be particularly important to establish whether the vascular component of the sympathetic arm of the baroreceptor reflex is preserved during and after mental stress in SA men and women.

Considering the functional relevance of these findings, it has been argued that inhibition of both the cardiac and vascular component of the baroreceptor reflex during the defence response to mental or environmental stressors ensures that the pressor component of the defence response is maintained and cardiac output is redistributed from renal and splanchnic beds where there is sympathetic constriction towards muscle in situations that are potentially life-threatening, so preparing the individual to fight or run away [132, 201]. Indeed, given the evidence that repeated pressor responses evoked by exposure to repeated environmental stressors or chronic daily stress increases the risk of hypertension [129], inhibition of the baroreceptor reflex as part of the response to mental stress would facilitate that process. Thus, the results of the present findings on young normotensive WE men and women, indicates that their pattern of haemodynamic response to mental stress and inhibition of the baroreceptor reflex puts them at future risk of hypertension if they experience repeated stressors.

By contrast, if it is assumed for the sake of argument that neither the sympathetic cardiac or vascular component of the baroreceptor reflex are inhibited in SA men and women during the alerting response, then their pressor response to mental stress would be effectively buffered by the baroreceptor reflex, limiting the increase in ABP and magnitude of the vasoconstrictor components. It would then follow that repeated exposure to environmental stressors and mental stress may not carry such a high risk of future hypertension for SA men and women as for WE men and women. This would contrast with one of the major hypotheses of the present PhD project. On the other hand, it can be argued that the present finding that the BRS to squat-stand and spontaneous down sequences in SBP is preserved in SA men and

women during and following mental stress would give the SAs better protection against orthostatic hypotension during and following mental stress than WEs [132, 239, 240]. In this context, it would be interesting to establish whether vasovagal syncope or emotional fainting is less common in SA men and women than in WE men and women.

4.4.4 Study limitations

The current study has a number of limitations. The participants included in the present study were healthy, normotensive, with similar heights, weights, BMIs, waist-to-hip ratios, and levels of physical activity, and they were students recruited from the College of Medical & Dental Sciences at University of Birmingham and from a narrow age range (18-25 years). However, only 40 healthy men and women were included with approximately 10 participants in each group. Thus, a positive aspect of the study is that the variance was limited by focussing on a select group. However, as noted in the discussion above, the variance within and between the groups limited the rigour of several of the comparisons which were important to the hypotheses tested. Thus, it will be important in future studies to increases the group sizes. It is also recognised that larger scale, multi-centre studies will be required to establish whether the findings of the present study hold for a wider sector of WE and SA men and women in the UK.

It was also a practical advantage that physical activity levels were self-reported rather than established in laboratory-based exercise studies, for this allowed the study to be non-invasive and helped facilitate participant enrolment. We also relied on self-reporting of menstruation to establish whether female participants were in the low oestrogen phase of the menstrual cycle. Previous reports have suggested that female hormones have an impact on cardiovascular responses including responses to mental stress [232, 241, 242]. Blood samples would have allowed oestrogen and progesterone to be measured and would also have allowed markers of oxidative stress, lipid profile, and insulin resistance to be tested in SAs and WEs. These tests might have shown if young SAs men and/or women show more CVD risk factors than WEs as others have reported in young SAs who seem perfectly healthy [205].

4.5 Conclusions

In the present study, there were no differences within WE men and women at rest for the recorded cardiovascular variables or RR, but young normotensive SA women showed higher HR than SA men. It is proposed this may reflect sympathovagal imbalance with increased vagal and sympathetic activity in SA women at rest. In response to acute slow breathing, there were no statistically significant sex-dependent differences within or between WEs and SAs in the haemodynamic changes, or in the effects on HRV or BRS. However, only a few individual SA women showed a fall in TPR during slow breathing, whereas most SA men and WE men and women showed a fall in TPR. This raises the possibility that SA women are more resistant to the inhibitory effects of acute slow breathing on sympathetic vasoconstriction and ABP. Similarly, there were no statistically significant sex-dependent differences within or between WEs and SAs for the haemodynamic responses to mental stress, although a larger number of SA women showed an increase rather than a fall in TPR during mental stress. This raises the possibility that the sympathetic vasoconstrictor effect of mental stress may be larger in SA women than in the other groups. Finally, although there were no sex-dependent differences within or between WEs and SAs in the inhibitory effects of mental stress on the vagal BRS, the cardiac sympathetic component of BRS was inhibited both in WE men and women but not in SA men and women. It is proposed that that the pressor response to mental stressors is buffered more effectively in SA men *and* women than in WE men and women. The possibilities are considered that repeated exposure to mental stress may not carry such a high risk of hypertension in SA men and women as in WE men and women and/or that SA men and women may be better protected than WE men and women against orthostatic hypotension and vasovagal syncope during mental stress.

Chapter 5: The effect of slow breathing on blood pressure regulation and autonomic function in healthy South Asian adults: A systematic review and meta-analysis

5.1 Introduction

As discussed in the general Introduction (section 1.4), autonomic influences play a crucial part in the homeostatic regulation of the cardiovascular system. One of the most frequently accepted and tested hypotheses in cardiovascular research is that essential hypertension is caused by an imbalance in sympathetic and parasympathetic regulation of the cardiovascular system [46-48]. This imbalance is characterised by increased sympathetic activity and possibly decreased parasympathetic activity [67]. Multiple lines of evidence demonstrate that young hypertensive people and those in the early stages of hypertension have increased sympathetic activity and a decreased cardiac vagal tone [49] and even in the offspring of hypertensives. Thus, it seems that this imbalance may be the forerunner of hypertension found in early and borderline hypertension as well as contributing to the maintenance of persistent hypertension [46].

As discussed in the General Introduction, hypertension is a major public health concern in South Asia, where it is the second leading cause of disability-adjusted life years lost, mainly as a result of the strong association between high blood pressure and the development of CVD [166]. Additionally, SAs are impacted by CVD, including hypertension, at a younger age than WEs [167-169]. In fact, over the last two decades, both the age- and sex-adjusted population mean BP and the prevalence of hypertension have risen across South Asia [243]. The prevalence of hypertension and other CVDs are not only high in native SAs, but also increased in migrant SAs living in western countries [20-22, 25, 164]. As discussed in the General Introduction (section 1.7), several non-pharmacologic approaches have been suggested to reduce the resting level of ABP, and slow breathing exercises are one of these approaches.

As discussed in the general introduction (section 1.6), the reduced vagal tone and increased sympathetic activity in hypertension is associated with reduced BRS and reduced

HRV [46-48, 67]. Reduced HRV is largely attributable to a reduction in respiratory inhibitory influences on cardiac vagal activity [244]. There is evidence that slow breathing exercise reduced ABP and increased BRS and HRV in healthy adults [158, 178, 209, 245, 246]. These effects of slow breathing training were also found in hypertensives. Several studies have demonstrated that slow breathing reduces ABP by stimulating several cardiovascular reflexes [123, 247], suggesting that slow breathing exercises reduce ABP and can be used to treat hypertension [125]. Further, it was reported that slow breathing at six breaths per minute (b/m) normalised the reduced HRV, BRS, and hypocapnia in hypertensive patients, as well as lowering ABP [67, 122]. Further, Joseph *et al* [67] reported that the reduction in ABP induced in hypertensives by a single session of breathing at 6 b/m was associated with an increase in vagal activity and a decrease in sympathetic activity, consistent with a change in autonomic balance. It also has been shown that slow breathing reduces mean ABP [181, 248, 249]. Additionally, some studies have shown that long-term regular practising of device-guided slow breathing has an antihypertensive effect, decreasing resting ABP in hypertensives [117, 123-125]. In this context, it is important to mention that there is limited evidence on the effects of slow breathing on BP regulation in SAs. However, behavioural approaches which involve slow breathing like meditation, yoga, and biofeedback such as are practiced in South Asia have shown some success in treating high ABP [123, 250-253]. Notably, Slow *pranayamic* breathing which is a type of yogic breathing that involves controlling breath movement has been shown to be effective in improving autonomic imbalance [184, 254].

Considering all the benefits of slow breathing mentioned above and knowing that SAs have a greater risk of developing hypertension and CVD, it is a reasonable hypothesis that slow breathing training could be a non-pharmaceutical therapeutic intervention for reducing the risk of hypertension and CVD in SAs, as well as the morbidity and mortality associated with these diseases. To date, no systematic review of published studies on this issue has been undertaken

to assess the effects of slow breathing on autonomic BP regulation in SAs. Therefore, the present review and meta-analysis was conducted to evaluate the overall effects of slow breathing on ABP, HRV, and BRS.

5.1.1 Objective

The aim of this systematic review and meta-analysis is to assess the impact of short and long-term practice of slow breathing on blood pressure regulation and autonomic function in healthy normotensive SA adults.

5.2 Methods

The protocol of this review was prospectively registered with the registration number CRD42022271708 in the international registry of systematic reviews (PROSPERO). The report of this systematic review was conducted in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [255], and the PRISMA checklist is available in Appendix 5.1.

5.2.1 Eligibility Criteria

5.2.1.1 Population

Studies on healthy normotensive SA men or/and women aged ≥ 18 years were considered for inclusion in this systematic review if slow breathing was used with at least one of the primary outcomes. South Asians are defined as those with ethnic roots originating from India, Pakistan, Bangladesh, Nepal, and Sri Lanka [4]. On the other hand, studies were excluded if conducted on subjects with a history of CVD, respiratory disease, kidney disease, or metabolic disease. Studies were also excluded if conducted on regular yoga practitioners as this may affect the results, or on athletes. In addition, smoking, pregnancy, and medications were considered as exclusion criteria. It is important to mention that hypertension was an inclusion criterion to compare the effects of slow breathing exercises on BP regulation between normotensives and hypertensives. However, it became an exclusion criterion due to the lack of studies that involved slow breathing training in hypertensive SAs.

5.2.1.2 Intervention

Studies were considered for inclusion if they performed any type of slow breathing training, including slow deep breathing exercises and slow yogic breathing exercises (e.g. *Pranayama*) at a frequency of ≤ 10 b/m for a minimum of 2 minutes per session. Studies

that performed either a single session or multiple sessions over a period of ≥ 4 weeks (long-term) of slow breathing were considered for inclusion.

The included studies were grouped into three groups based on the duration of slow breathing intervention, either short term (single session) or long-term (multiple sessions), and on the time at which the variables were measured and collected either during or immediately after the intervention. Group 1 comprised studies that involved a single session of slow breathing exercise and data were collected during baseline and during slow breathing training. Group 2 comprised studies that involved a single session of slow breathing exercise, but data were collected during baseline and immediately after slow breathing training. Group 3 comprised studies that involved long-term training of slow breathing.

Studies were excluded if the details of the slow breathing training were not provided. Studies were also excluded if the slow breathing exercise was performed with loaded resistance, or if the training's Respiratory Rate was < 4 b/m or > 10 b/m. Multiple interventions studies in which slow breathing overlapped with another intervention such as stretching were excluded.

5.2.1.3 The comparator

Spontaneous breathing (baseline) was the control status and the comparator, and slow breathing exercise as the intervention (delta change pre vs during or after).

5.2.1.4 Outcomes and data items

The primary outcomes were ABP, BRS, and time domain indices of HRV. Secondary outcomes were HR, frequency domain indices of HRV and RR. This review was conducted to assess and test the magnitude of change caused by slow breathing on these variables. Studies

that did not provide the values of at least one of the primary outcomes of interest before and during, or before and after slow breathing exercises were excluded.

5.2.2 Information Sources

A comprehensive search of the literature was conducted by searching the following electronic databases: Embase, MEDLINE, CINAHL, Cochrane Library, and Web of Science. The search queries were run without any restrictions on the date (from inception); the queries were run in December 2021.

5.2.3 Search strategy

The systematic review team carefully designed the search strategy and terms through the databases mentioned above and sources to obtain all available relevant studies. The following keywords and term combinations were used to search the literature: 1) Adult* OR Young adult* OR Human* 2) slow breath* OR breathing exercises OR slow breathing OR guided breathing OR device-guided OR qigong OR pranayama OR paced breathing OR loaded breathing OR controlled breathing OR resperate OR yogic breathing OR device-guided slow breath* OR ("yoga AND breath*") 3) blood pressure OR arterial pressure OR MAP OR ABP OR SBP OR heart rate OR HR OR heart function tests OR breathing rate OR respiratory rate OR respiratory function OR baroreflex sensitivity OR baroreflex OR BRS OR pressoreceptors OR HRV OR heart rate variability OR LF OR HF OR LF/HF OR SDRR OR RMSSD. These term combinations were combined with "AND". Full details of the search strategy and terms are provided in the study supplements (see Appendix 5.3).

Table 5.1 PICO format of the key concepts and their synonyms and alternatives

Key concept (PICO)	**Synonym**
P: Adults	Young adults, Humans
I: Slow breathing exercise	Breathing exercises, slow breathing, guided breathing, device-guided, Qigong, Pranayama, paced breathing, loaded breathing, controlled breathing, Resperate, yogic breathing, device-guided slow breathing, yoga breathing
C: Spontaneous breathing	Baseline breathing, uncontrolled breathing, normal breathing
O: Arterial Blood pressure, baroreceptor reflex sensitivity, heart rate variability, and respiratory rate	Arterial blood pressure, arterial pressure, ABP, SBP, MAP, DBP, HR, heart function tests, respiratory function, RR, baroreflex sensitivity, baroreflex, BRS, pressoreceptors, HRV, LF, HF, LF/HF, pNN50, SDRR, SDNN, RMSSD

PICO: Participant, Intervention, Comparator, and Outcome, ABP: arterial blood pressure, SBP: systolic blood pressure, MAP: mean arterial pressure, DBP: diastolic blood pressure, HR: heart rate, RR: respiratory rate, BRS: baroreceptor reflex sensitivity, HRV: heart rate variability, LF: low frequency, HF: high frequency, pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals, SDRR & SDNN: mean of the standard deviations for all R-R intervals, RMSSD: root-mean square differences of successive R-R intervals

5.2.4 Selection process

The obtained records from the above databases were imported into EndNote 9.1 to remove duplicates and then uploaded to Rayyan for the Systematic Reviews platform (http://rayyan.qcri.org/), which was used to facilitate the process of screening titles and abstracts amongst reviewers [256]. Abstracts were screened blindly by two independent reviewers for relevance using the predefined inclusion and exclusion criteria. Disagreements were resolved through discussion or by a third reviewer. After the reviewers had agreed on the relevance of studies, full texts were obtained and imported into EndNote and they were then screened for eligibility using the abstract screening method mentioned above. Relevant Randomised Control Trials (RCTs), Cohort, non-RCT, Longitudinal, and Case-series studies that met our predefined criteria and were available as full-text in English were included for full-text screening. Studies that did not provide the values of at least one of the outcomes of interest before and during or after slow breathing exercises were excluded. Case reports, reviews, paediatrics studies, and background studies were also excluded. Studies with incomplete data, irrelevant protocols and designs were also excluded.

5.2.5 Data collection process

Data extraction was carried out by one of the reviewers using the predefined inclusion and exclusion criteria, and the accuracy and consistency were checked by another reviewer. Extracted information from the included studies was arranged in a pre-defined table. This table contained: the author and year, the country where the study took place, sample size and participants' characteristics, inclusion and exclusion criteria, type and details of slow breathing exercise, and the results of the effects of slow breathing intervention on the cardiovascular variables mentioned above in comparison with control status.

5.2.6 Study Risk of bias assessment

To establish the validity of the selected articles, the risk of bias in the included studies was assessed using the appropriate tool as per the study design. The revised Cochrane Risk of Bias tool for RCTs (ROB2) was used for RCT studies, with the risk of bias for each study categorised as high, some concern, or low [257]. The Cochrane Risk Of Bias In Non-randomized Studies - of Interventions assessment tool (ROBINS-I tool) was used in the quality assessment of non-RCT studies, with the judgment of the risk of bias classified as low, no information, moderate, serious, and critical risk of bias [258]. The National Institute of Health (NIH) Quality Assessment Tool for Before-After (Pre-Post) Studies With No Control Group was used for pre-post studies [259]. The NIH quality assessment tools classify the quality of each study as good, fair, and poor. Methodological quality was assessed by two reviewers who had not been acquainted with each other's evaluation. After evaluating the selected studies, the results of the two reviewers were compared, and uncertainties or differences were discussed to reach a consensus. A third reviewer was consulted if a consensus could not be reached for a final decision.

5.2.7 Synthesis, effect measures, and certainty assessment

As indicated in 5.2.1.2, studies were grouped into three groups based on the duration of the intervention and on the time of assessment, and the analyses were carried out accordingly. The syntheses were carried out by assessing the Effect Size (ES) and 95% Confidence Interval (CI) within groups. Pooled ES was used to evaluate the overall effectiveness of the intervention. A P value of ≤ 0.05 was considered significant. Statistical heterogeneity between studies was assessed and reported using I^2.

In studies that reported only the median and interquartile range (IQR), the mean (M) and standard deviation (SD) were calculated using the tools reported by Wan, Wang [260]. Additionally, the Cambridge calculator (Effect Size Calculator (cem.org)) was used to calculate ES and standard error of ES (SEes). Microsoft Excel was used for data extraction and calculations before the analysis process. R Data Analysis Software was used for meta-analysis, and generating graphs. The Robvis (visualization tool) was used to plot the risk of bias assessment results (robvis (shinyapps.io)).

Data synthesis was conducted in accordance with the recommendations of the Cochrane collaboration. A narrative synthesis was carried out to assess and compare the effects of slow breathing exercises on the cardiovascular variables of interest in healthy normotensive SA adults. When homogeneous results of a minimum of two studies per group were available, a meta-analysis was conducted. Otherwise, the results of each study were reported individually.

5.2.8 Reporting bias assessment

Missing or unreported data were taken into consideration when the risk of bias assessment for each included study was carried out. As a part of reporting the results of the risk of bias, missing data were reported.

5.3 Results

5.3.1 Study selection

The initial database searches revealed a total of 4964 records, 1559 of which were removed as duplicates. The remaining 3405 records were screened by title and abstract, and 165 were considered for full-text screening. Of these, 10 records could not be retrieved, but 155 were obtained for screening. 147 studies were then excluded for the reasons listed in Figure 5.1. Ultimately, eight studies were included that met the inclusion criteria [120, 121, 182-184, 191, 261, 262]. The PRISMA flow diagram, shown in Figure 5.1, summarises the selection process.

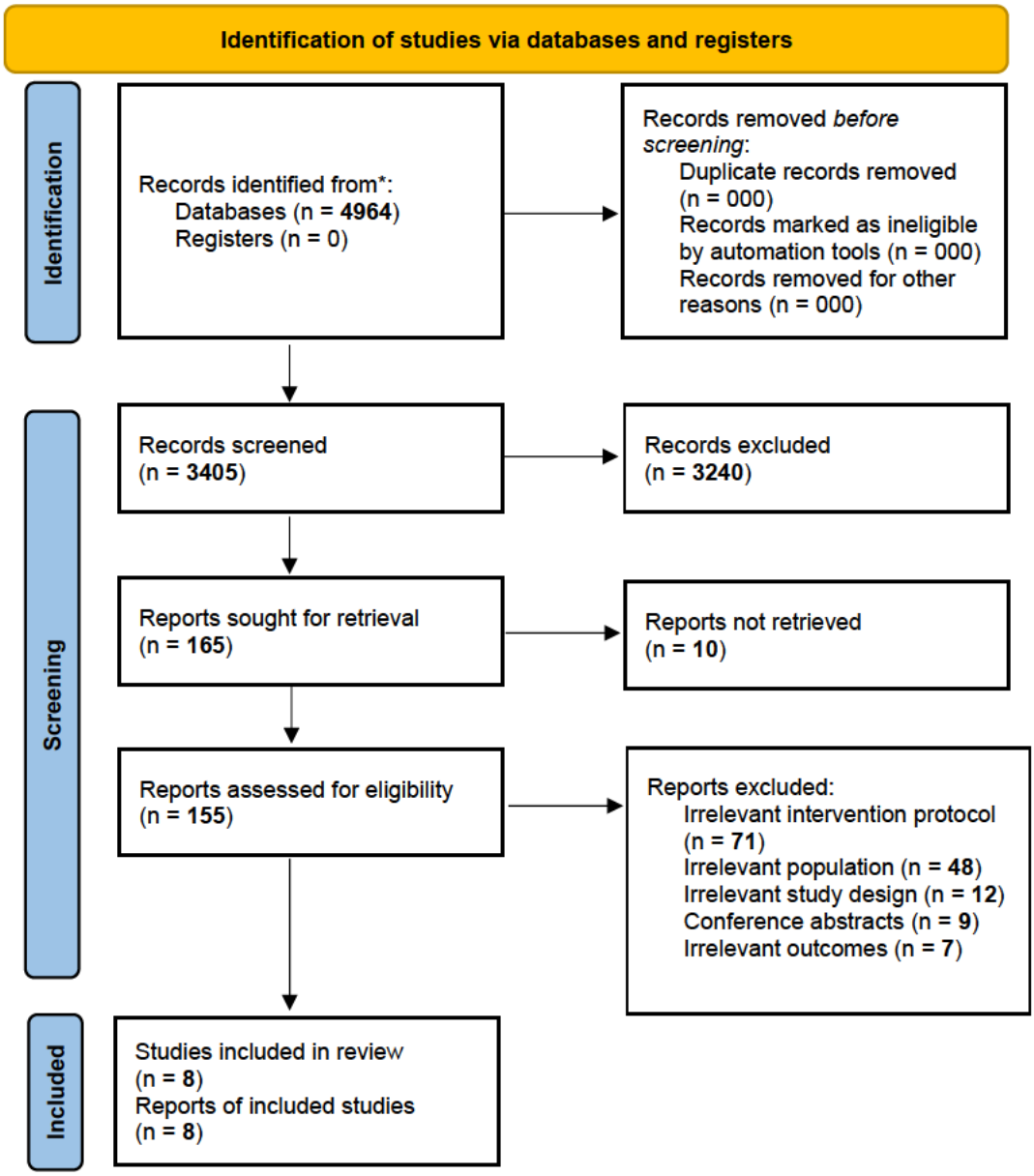


Figure 5.1 PRISMA flow diagram showing the selection process.

Notes: obtained from Page *et al* [255].

5.3.2 Study characteristics

A total of eight interventional studies were included in this review [120, 121, 182-184, 191, 261, 262]. Five of which were pre-post (with no control group) in design [182-184, 191, 262], two were non-RCTs [261, 263], and one was a RCT [121]. All included studies were conducted in India [121, 182, 183, 191, 261-263], except one which was conducted in Nepal [184]. From all included studies, the total number of participants included in this review was 187 young and middle-aged adults (140 men and 47 women). Each included study provided at least one variable of the primary outcomes of interest. ABP was reported in seven studies [121, 182-184, 261-263], HRV was reported in seven studies [121, 182, 183, 191, 261-263], and BRS was reported in two studies [182, 261]. Despite the variety of different types of breathing exercises amongst included studies, respiratory rate was 5-6 b/m in all the studies.

As described in 5.1.2.1, studies were divided into three groups on the basis of the time of assessment and duration of slow breathing training, Group 1 including studies which performed a single session of slow breathing, with data collected during slow breathing [182, 183, 191, 261], Group 2 comprising studies conducted in a single session of slow breathing, with data being collected immediately after slow breathing [182, 184, 262] and Group 3 being studies performed on long-term slow breathing training [121, 263]. The characteristics of each included study are shown in Table 5.2.

Table 5.2 Table of characteristics

Study	Country	N	Sex (M/F)	Age	BMI	Intervention	Assessment time	Outcomes
Anasuya *et al* [261] Non-RCT (Group 1)	India	40	27/13	31.1±7.3	23.5±1.7	5 min of SB (6 b/m)	During a single session of SB	ABP, HRV, BRS, HR
Helen Mary *et al* [183] (A) pre-post (Group 1)	India	30	18/12	26±4	NR	5 min of SDB (6 b/m)	During a single session of SB	ABP, HRV
Helen Mary *et al* [191] (B) pre-post (Group 1)	India	20	12/8	26±4	NR	5 min of SB (6 b/m)	During a single session of SB	HRV
Bhagat *et al* [182] pre-post (Group 1&2)	India	12	NR	30±3.8	NR	Yogic breathing: ANB (5 b/m)	During & immediately after a single session of SB	ABP, HRV, BRS, HR, RR
Chinagudi *et al* [262] pre-post (Group 2)	India	20	NR	36.55±2.9	NR	5 min of SB (6 b/m)	Immediately after a single session of SB	HRV, HR
Sharma *et al* [184] pre-post (Group 2)	Nepal	39	NR	25-35	NR	5 min of slow pace pranayama (6 b/m)	Immediately after a single session of SB	ABP, HR
Nagarajan [263] Non-RCT (Group 3)	India	8	5/3	26.3±3.9	24 ± 2.9	30 min daily of SB for 4 weeks (6 b/m)	After long term of SB training	ABP, HRV, HR, RR
Tharion *et al* [121] RCT (Group 3)	India	18	7/11	25.6±3.3	21.9±1.7	30 min daily of SDB for one month (6 b/m)	After long term of SB training	ABP, HRV, HR, RR

SB: slow breathing, SDB: slow deep breathing, b/m: breath/minute, ABP: arterial blood pressure, HRV: heart rate variability, BRS: baroreceptor reflex sensitivity, HR: heart rate, RR: respiratory rate, ANB: alternate nostril breathing

5.3.3 Risk of bias

For one study, the only RCT [121], the revised Cochrane risk of bias tool for RCTs, was used: there was an overall low risk of bias in this study. Two non-RCTs [121, 263] were assessed by the Cochrane risk of bias in non-randomized studies assessment tool: there was an overall moderate risk of bias in both studies (Figure 5.3). The remaining five interventional pre-post studies were assessed by the NIH tool for Before-After studies: there was an overall good quality in three studies [182, 183, 191] and poor quality in two studies [184, 262] (Figure 5.2).

Figure 5.2 Risk of bias results for pre-post studies: NIH assessment tool

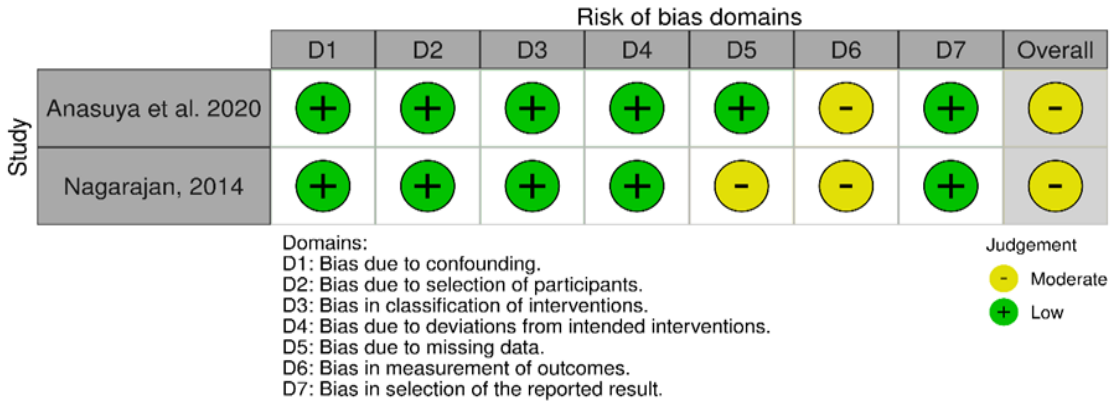


Figure 5.3 Risk of bias results of non-RCT included studies: ROBINS-I tool

5.3.4 Results of individual studies

The results of each included study are presented in Tables 5.3, 5.4, and 5.5.

5.3.4.1 During a single session of slow breathing (Group 1)

Four studies were included in this group [182, 183, 191, 261] with a total number of 102 participants. These studies included a single session of slow breathing, and data were collected *during* slow breathing.

Anasuya *et al* [261] recruited 80 participants, 40 of whom were yoga practitioners while 40 yoga-naïve individuals were controls. Only the yoga-naïve group was included in this review because regular practice of yoga was one of the exclusion criteria (section 5.3.1.2). The yoga-naïve group included 27 men and 13 women with a mean age of 31.1±7.3 years and a BMI of 23.5±1.7 kg/m2. In this study, they assessed the effects of 5 min of slow breathing at a frequency of 6 b/m on ABP (SBP, DBP, MAP), HRV (RRI, SDNN, RMSSD, pNN50, LF, HF, LF/HF), BRS (αLF BRS, αHF BRS, all sequence BRS), and HR. Helen Mary *et al* [183] (A) assessed SBP, RRI, and SDNN during 5 min of deep breathing at 6 b/m in 30 participants (18 men and 12 women) aged 26±4 years. Helen Mary *et al* [191] (B) also conducted another study to assess the impacts of slow breathing at 6 b/m on the frequency domain indices of HRV (LF and HF) in 20 participants (12 men, 8 women) with a mean age of 26±4 years. The last study in this group was conducted by Bhagat *et al* [182] to investigate the effects of a single session of slow yogic breathing at 6 b/m in 12 participants. They measured ABP (SBP, DBP, MAP), HRV (RRI, SDNN, RMSSD, pNN50, LF, HF, LF/HF ratio), BRS (αLF BRS, αHF BRS, Up-BRS, Down-BRS, All sequence BRS), and HR during slow yogic breathing.

5.3.4.2 Immediately after a single session of slow breathing (Group 2)

Three studies were included in this group [182, 184, 262] including a total of 71 participants. They were performed on a single session of slow breathing, and data were collected immediately after slow breathing.

Since Bhagat *et al* [182] provided a set of results collected immediately after the single session of slow breathing mentioned above (see section 5.4.2.1), this study was also included in this group. Chinagudi *et al* [262] recruited 20 participants with a mean age of 36.55 ± 2.9 years. They aimed to assess the effects of slow breathing on HRV: they measured SDNN, RMSSD, LF, HF, and LF/HF ratio immediately after 5 min of slow breathing at 6 b/m. Finally, Sharma *et al* [184] conducted their study on 39 participants aged 25-35 years. They investigated the effects of slow-paced *pranayama* (yogic nostril breathing) at a frequency of 6 b/m for 5 min on SBP, DBP, and HR.

5.3.4.3 After a long-term training of slow breathing (Group 3)

The two studies included in this group were ones that involved long-term training of slow breathing exercises [121, 263] with an overall sample size of 26 participants. Both studies recruited control groups without any intervention. However, only intervention groups that performed slow breathing exercises were included in this review as per the study design; one of the studies did not provide any data for the control group [120].

Both studies performed 30 min daily of slow breathing training for 4 weeks. Nagarajan [263] recruited 8 participants (5 men, 3 women) with a mean age of 26.3±3.9 years and BMI of 24 ± 2.9 kg/m2. They conducted a pre-post assessment of MAP, HR, RR, and SDNN. On the other hand, Tharion *et al* [121] included 18 participants, 7 of which were men and 11 were women. Their mean age and BMI were 25.6±3.3 kg/m2 and 21.9±1.7 years, respectively. They

assessed the effect of long-term training on HRV (RRI, SDNN, RMSSD, pNN50, LF, HF, LF/HF ratio), MAP, HR, and RR.

5.3.4.4 Effects of slow breathing on ABP, HR, and RR

Anasuya *et al* [261] provided the baseline and during slow breathing data for the yoga-naïve group. As shown in Table 5.3, during 5 min of slow breathing, there was an apparent reduction in mean ABP, and increases in BRS and time-domain indices of HRV, but the authors give no indication of whether these "changes" from baseline were tested for statistical significance. Hence, their data were used mainly for the meta-analysis

As shown in Table 5.3, during 5 min single session of slow breathing, Helen Mary *et al* [183] (A) found that SBP decreased significantly compared to baseline. By contrast, Bhagat *et al* [182] reported that 5 min of slow yogic breathing, induced no significant changes in SBP, DPB, or MAP compared to baseline. However, there was a significant increase in HR.

As shown in Table 5.4, Bhagat *et al* [182] also measured the same variables immediately after slow breathing but found no significant changes in SBP, DBP, MAP, HR, and RR. Similarly, Chinagudi *et al* [262] assessed HR before and immediately after 5 min of slow breathing, and found no change in HR. By contrast, Sharma *et al* [184] found that SPB and DBP decreased significantly immediately after 5 min of slow *pranayama* breathing, with no significant change in HR.

Only two studies that involved long-term slow breathing training were included in this review[121, 263]. They both reported a significant reduction in MAP and RR after 30 min of daily slow breathing training for one month. There was no significant change in HR in either study (see Table 5.5).

5.3.4.5 Effects of slow breathing on HRV

Three studies assessed HRV during a single session of slow breathing [182, 183, 191] (see Table 5.3). Helen Mary *et al* [183] (A) reported a significant increase in SDNN during slow breathing and in their other study, Helen Mary *et al* [191] (B) in which they tested the effects of slow breathing on frequency domain indices of HRV, their results showed a significant increase in the LF band and a significant decrease in the HF band during a single session of slow breathing. Bhagat *et al* [182] also assessed the time and frequency domains of HRV during slow breathing. They found a significant increase in SDNN, but a significant decrease in RRI and HF band with a significantly increased LF/HF ratio. There were no substantial changes in the other HRV variables (see Table 5.3).

Two studies assessed HRV immediately after a single session of slow breathing (see Table 5.4 [182, 262]). Bhagat *et al* [182] reported no significant changes in HRV after slow breathing. Chinagudi *et al* [262] found that the LF band and LF/HF ratio increased, while HF decreased significantly after slow breathing.

The two long-term studies also assessed HRV after one month of slow breathing training. One assessed SDNN only[263], but the other evaluated the time and frequency domain indices of HRV [121]. Nagarajan [263] showed that SDNN was significantly increased after one month of daily 30 min of slow breathing but, Tharion *et al* [121] showed no significant change in HRV.

5.3.4.6 Effects of slow breathing on BRS

Only two studies reported BRS data during [182, 261] or immediately after [182] a single session of slow breathing. Anasuya *et al* [261] reported BRS data before and during slow breathing but as mentioned above, they apparently did not make any within-group

comparisons, and/or did not report the results of such comparisons. Bhagat *et al* [182] assessed BRS before and during, and before and after slow breathing: their results showed no significant changes in BRS (see Tables 5.3 and 5.4).

Table 5.3 The results of group 1 studies (before and during a single session of SB)

Study	**N**	**Outcomes**	**Results**	
			Pre SB	**During SB**
Anasuya *et al* [261]	40	- **SBP** (mm Hg)	120.63 (111.78-129.72)	118.52 (113.07-128.37)
		- DBP (mm Hg)	63.15 (57.21-70.05)	61.13 (55.59-70.30)
		- MAP (mm Hg)	80.65 (74.07-87.26)	78.42 (71.87-85.31)
		- HR (bpm)	67.83 (64.39-75.71)	68.67 (64.60-75.03)
		HRV index		
		- RRI (ms)	902.73 (837.21-951.88)	882.41 (812.05-937.40)
		- SDNN (ms)	54.96 (42.13-74.24)	75.08 (62.52-106.45)
		- RMSSD (ms)	41.14 (33.61-57.54)	49.55 (39.80-71.93)
		- pNN50 (%)	9.74 (4.77-15.63)	12.31 (7.49-16.28)
		- LF (nu)	47.63 (41.38-61.14)	85.19 (78.99-91.04)
		- HF (nu)	45.54 (35.20-53.63)	12.90 (7.94-18.80)
		- LF/HF ratio	1.22 (0.90-2.0)	8.12 (4.88-13.66)
		BRS (ms/mm Hg)		
		- α-LF	3.98 (3-5.08)	5.34 (4.02-6.94)
		- α-HF	5.29 (3.87-7.16)	6.09 (4.57-8.42)
		- All sequence	16.42 (12.35-22.70)	18.57 (14.57-23.39)
Helen Mary *et al* [183]a	30	- **SBP** (mm Hg)	138 ± 20	126 ± 24 *
		HRV index		
		- RRI (ms)	902 ± 110	1009 ± 130
		- SDNN (ms)	36 ± 20	95 ± 28 *
Helen Mary *et al* [191]b	20	**HRV index**		
		- LF (nu)	30.2 ± 15.49	77.4 ± 12.8 *
		- HF (nu)	57 ± 23.3	14.1 ± 16.3 *
Bhagat *et al* [182]	12	- **SBP** (mm Hg)	108.73 ± 14.8	108.34 ± 15.6
		- DBP (mm Hg)	53.63 ± 12.8	54.60 ± 13.2
		- MAP (mm Hg)	71.45 ± 12.4	71.97 ± 12.6
		- HR (bpm)	76.4 ± 9.9	80.3 ± 7.9 *
		- RR (b/min)	19.5 ± 2.4	5.3 ± 0.2 *
		HRV index		
		- RRI (ms)	800.48 ± 105.1	762.67 ± 70.7*
		- SDNN (ms)	47.1 ± 18.5	82.2 ± 23.3*
		- RMSSD (ms)	40.8 ± 31.9	46.3 ± 20.3
		- pNN50 (%)	14 ± 15	15.9 ± 8.4
		- LF (Hz)	0.09 ± 0.02	0.08 ± 0.01
		- HF (Hz)	0.31 ± 0.05	0.17 ± 0.01*
		- LF/HF ratio	2 ± 1.5	12.1 ± 7 *
		BRS (ms/mm Hg)		
		- α-LF	2.63 ± 1.07	4.01 ± 1.81
		- α-HF	4.19 ± 1.65	2.99 ± 1.17
		- All BRS (sequence)	11.53 ± 6.5	12.3 ± 4.5
		- Up BRS (sequence)	11.41 ± 4.01	13.91 ± 4.33
		-Down BRS (sequence)	12.00 ± 5.00	11.12 ± 2.57

Values are in Median [IQR], or Mean ± SD. SBP: systolic blood pressure, MAP: mean arterial pressure, DBP: diastolic blood pressure, HR: heart rate, RR: respiratory rate, BRS:

baroreceptor reflex sensitivity, HRV: heart rate variability, RRI: R-R interval, SDNN: mean of the standard deviations for all NN (R-R) intervals, RMSSD: Root mean square of successive RR interval differences, pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals, LF: low frequency, HF: high frequency. * Statistically significant

Table 5.4 The results of group 2 studies (before and immediately after a single session of SB)

Study	**N**	**Outcomes**	**Results**	
			Pre	**Post**
Bhagat *et al* [182]	12	- **SBP** (mm Hg)	108.73 ± 14.8	106.33 ± 15.6
		- DBP (mm Hg)	53.63 ± 12.8	55.13 ± 11.6
		- MAP (mm Hg)	71.45 ± 12.4	68.78 ± 14.7
		- HR (bpm)	76.4 ± 9.9	78.3 ± 8
		- RR (b/min)	19.5 ± 2.4	17.4 ± 3.5
		HRV index		
		- RRI (ms)	800.48 ± 105.1	778.03 ± 77.2
		- SDNN (ms)	47.1 ± 18.5	57.71 ± 15.4
		- RMSSD (ms)	40.8 ± 31.9	31.7 ± 14.3
		- pNN50 (%)	14 ± 15	11 ± 11.9
		- LF (Hz)	0.09 ± 0.02	0.08 ± 0.02
		- HF (Hz)	0.31 ± 0.05	0.28 ± 0.06
		- LF/HF ratio	2 ± 1.5	3.3 ± 3.1
		BRS (ms/mm Hg)		
		- α-LF	2.63 ± 1.07	3.27 ± 1.67
		- α-HF	4.19 ± 1.65	3.95 ± 1.56
		- All BRS (sequence)	11.53 ± 6.5	12.1 ± 4.7
		- Up BRS (sequence)	11.41 ± 4.01	12.90 ± 5.20
		-Down BRS (sequence)	12.00 ± 5.00	12.14 ± 4.57
Chinagudi *et al* [262]	20	- HR (bpm)	80.02 ± 8.11	80.06 ± 6.19
		HRV index		
		- SDNN (ms)	44.89 ± 18.68	45.12 ± 26.33
		- RMSSD (ms)	29.96 ± 16.11	23.86 ± 12.92
		- LF (nu)	46.91 ± 14.51	60.79 ± 17.29 *
		- HF (nu)	43.56 ± 17.59	30.17 ± 13.52 *
		- LF/HF ratio	1.43 ± 0.84	3.06 ± 3.22 *
Sharma *et al* [184]	39	- **SBP** (mm Hg)	115.43 ± 10.79	107.84 ± 9.85*
		- DBP (mm Hg)	77.23 ± 7.50	73.17 ± 6.53*
		- HR (bpm)	74.23 ± 9.57	71.48 ± 8.03

Values are in Mean ±SD. SBP: systolic blood pressure, MAP: mean arterial pressure, DBP: diastolic blood pressure, HR: heart rate, RR: respiratory rate, BRS: baroreceptor reflex sensitivity, HRV: heart rate variability, RRI: R-R interval, SDNN: mean of the standard deviations for all NN (R-R) intervals, RMSSD: Root mean square of successive RR interval differences, pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals, LF: low frequency, HF: high frequency. * Statistically significant.

Table 5.5 The results of group 3 studies (before and after long-term training of SB)

Study	**N**	**Outcomes**	**Results**	
			Pre	**Post**
Nagarajan [263]	8	- MAP (mm Hg) - HR (bpm) -RR (b/min) **HRV index** - SDNN (ms)	82.33 ± 3.48 64.04 ± 3.07 16.63 ± 1.22 59 ± 6.02	79.17 ± 3.64* 63.52 ± 2.77 11.13 ± 1.88* 62.63 ± 4.95
Tharion *et al* [121]	18	- MAP (mm Hg) - HR (bpm) - RR (b/min) **HRV index** - RRI (s) - SDNN (s) - RMSSD (ms) - pNN50 (%) - LF (nu) - HF (nu) - LF/HF ratio	82.00 (80, 86.67) 68.79 (61.93, 72.78) 18.00 (17.00, 20.00) 0.88 (0.83,0.97) 0.06 (0.05, 0.07) 56.35 (36, 71.20) 30.55 (14.60, 45.20) 30.55 (14.60, 45.20) 54.35 (36.70, 71.70) 0.84 (0.40, 1.73)	–0.667 (–6.667, 1.333) * –0.07 (-5.73, 3.68) –2.50 (–4.00, –1.00) * +0.01 (–0.04, 0.07) +0.01 (–0.00, 0.02) +6.95 (–9.90, 17.10) +10.00 (–20.00, 52.00) +2.95 (–11.30, 10.70) –2.95 (–10.70, 11.30) +0.01 (–0.37, 0.32)

Values are in Median [IQR], or Mean ± SD. MAP: mean arterial pressure, HR: heart rate, RR: respiratory rate, HRV: heart rate variability, RRI: R-R interval, SDNN: mean of the standard deviations for all NN (R-R) intervals, RMSSD: Root mean square of successive RR interval differences, pNN50: proportion of NN50 divided by the total number of NN (R-R) intervals, LF: low frequency, HF: high frequency. * Statistically significant.

5.3.5 Results of meta-analyses

The data of each study were taken into the meta-analysis of at least one of the primary outcomes. The results are summarised in the Forest plots in Figures 5.4-5.8.

5.3.5.1 Slow breathing effects on ABP, HR, and RR

Three studies involving 82 participants reported at least one ABP outcome before and during slow breathing [182, 183, 261], two studies involving 70 participants reported HR results [182, 261]. Meta-analysis revealed no significant overall effect of slow breathing on ABP or HR, as shown in Figure 5.4 and appendix 5.2.

Two studies with 51 participants provided data of SBP, DBP, RR, and HR [182, 184], and one study with 20 participants reported HR before and after a single session of slow breathing [262]. As apparent in Figure 5.4, SBP decreased significantly after 5 min slow breathing (pooled ES= -0.54; 95% CI -1.08 to -0.01; P= 0.05; I^2 = 33%) compared to baseline values. However, there was no significant effect on DBP or HR (Appendix 5.2).

Only two studies, consisting of 26 participants, performed long-term slow breathing training [121, 263], both studies providing MAP, RR, and HR results before and after one month of training. The meta-analysis showed that long-term slow breathing significantly decreased MAP (pooled ES= -0.57; 95% CI -1.13 to -0.02; P= 0.04; I^2 = 0%; Figure 5.5) compared to baseline. The pooled analysis also showed a trend for a decrease in RR with pooled ES of -2.16 (CI -4.53 to 0.21; P= 0.07; I^2 = 88%; Figure 5.5). There was no significant overall effect of long-term slow breathing training on HR (Figure 5.5).

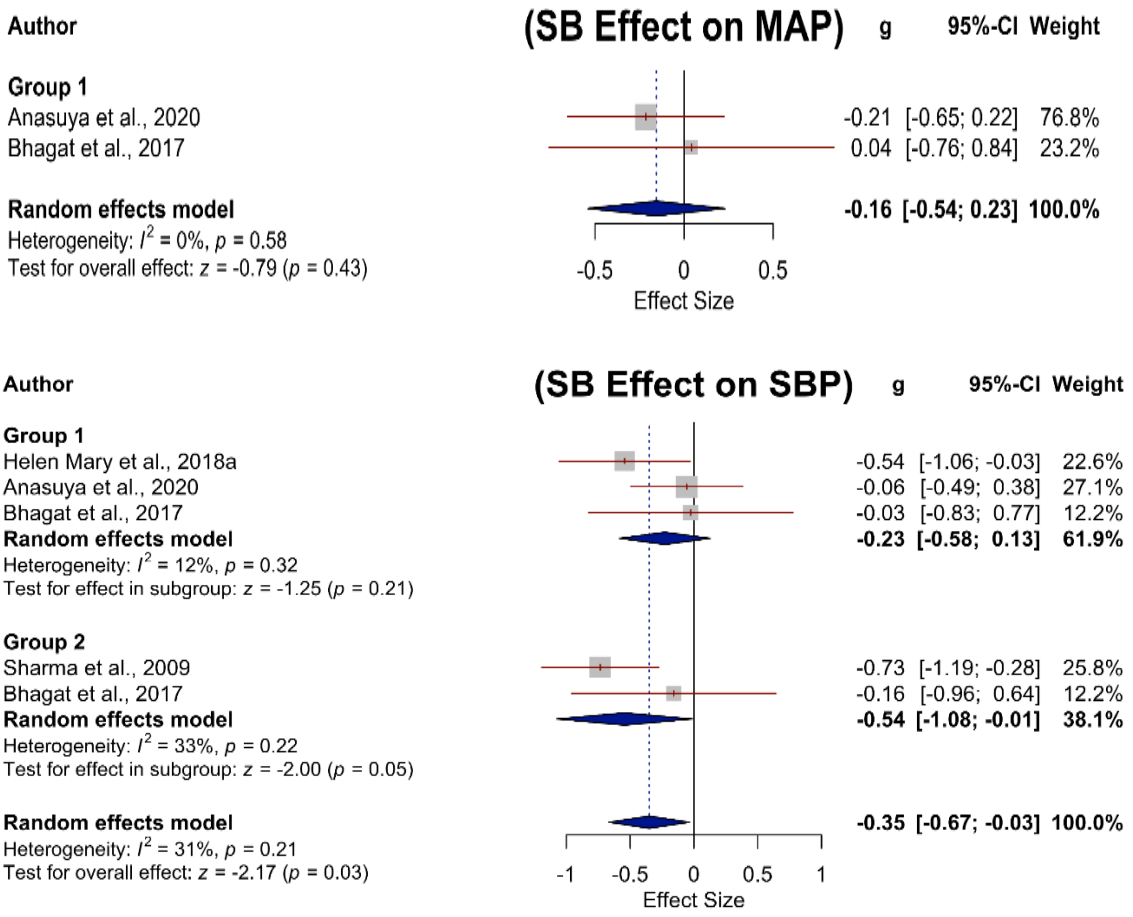


Figure 5.4 Forest plot of before vs during a single session, and before vs after a single session of SB effects on MAP and SBP in normotensive SAs.
Values are in ES and 95%-CI. SB: slow breathing; MAP: mean arterial blood pressure; SBP: systolic blood pressure; 95%-CI: 95% confidence interval; g: effect size (Hedges); I^2: statistical heterogeneity.

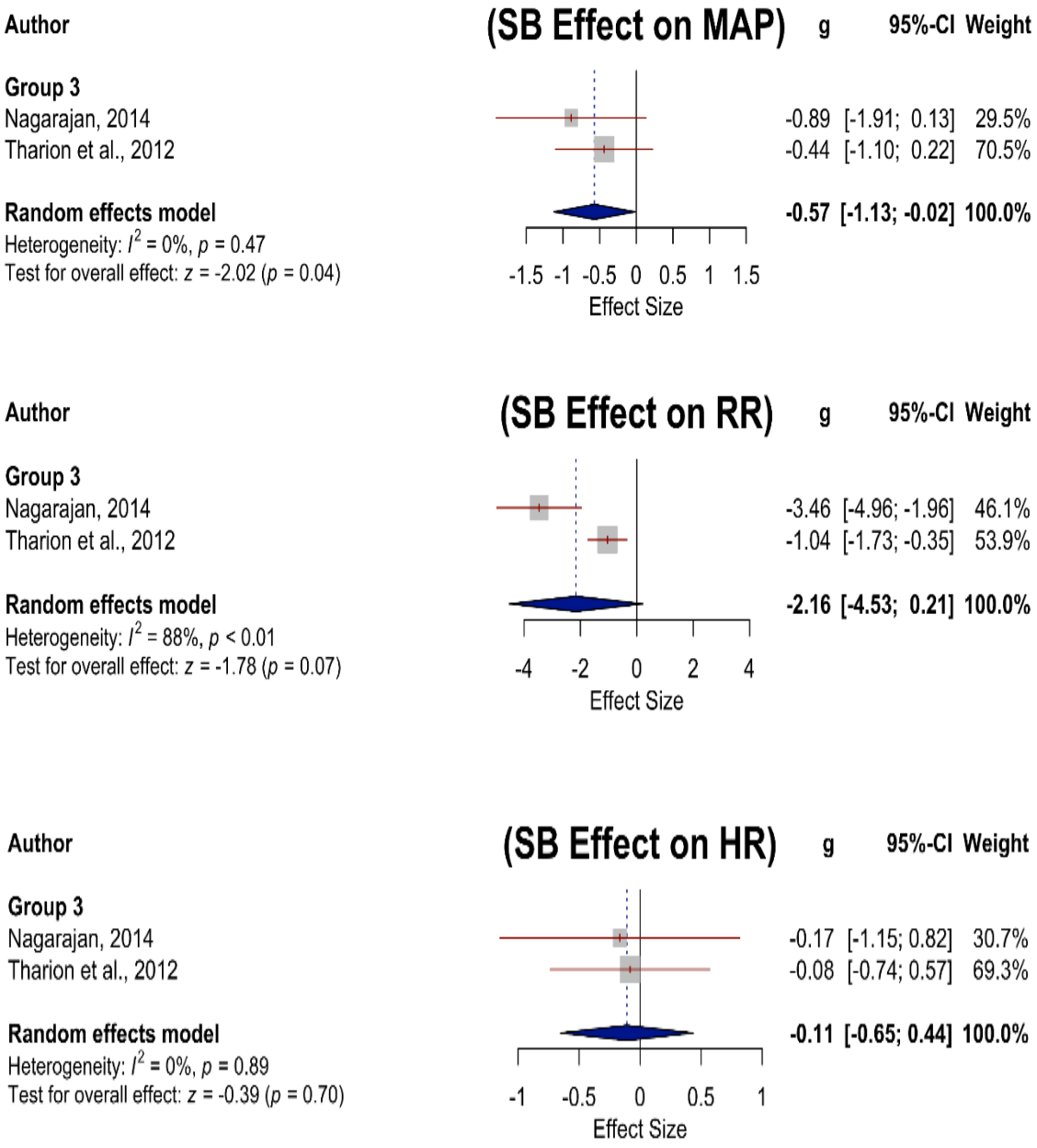


Figure 5.5 Forest plot of long-term (4 weeks) SB training effects on ABP, HR, and RR in normotensive SAs.

Values are in ES and 95%-CI. SB: slow breathing; MAP: mean arterial blood pressure; RR: respiratory rate; HR: heart rate; 95%-CI: 95% confidence interval; g: effect size (Hedges); I^2: statistical heterogeneity.

5.3.5.2 Slow breathing effects on HRV

Four studies in Group 1 [182, 183, 191, 261], consisting of 102 participants, assessed a minimum of two HRV variables during a single slow breathing session. As shown in Figure 5.6, slow breathing caused a significant overall increase in the time domain indices of SDNN (pooled ES= 1.61; 95% CI 0.65 to 2.85; P= 0.01; I^2 = 87%) and RMSSD (pooled ES= 0.39; 95% CI 0.00 to 0.78; P= 0.05; I^2 = 0%). There was no significant overall effect of slow breathing on pNN50 (Figure 5.6). However, meta-analysis also showed a significant increase in LF (pooled ES= 2.95; 95% CI 2.43 to 3.46; P= 0.01; I^2 = 0%) and LF/HF (pooled ES= 1.65; 95% CI 1.21 to 2.10; P= 0.01; I^2 = 0%), and a significant decrease in HF (pooled ES= -2.48; 95% CI -3.04 to -1.91; P= 0.01; I^2 = 26%) during slow breathing (Figure 5.7).

Two studies in Group 2, with a total of 32 participants, reported HRV results before and after a single session of slow breathing [182, 262]. Pooled analysis indicated no significant overall effect of slow breathing on both SDNN or RMSSD, but there was a significant increase in LF/HF ratio with an overall ES of 0.63 (95% CI 0.13 to 1.13; P= 0.01; I^2 = 0%; Figure 5.6).

The two long-term studies of Group 3 [121, 263] reported SDNN data before and after a month of slow breathing training. Overall, long-term slow breathing training resulted in a significant increase in SDNN with a pooled ES of 0.81 (95% CI 0.24 to 1.37; P= 0.01; I^2 = 0%; Figure 5.7).

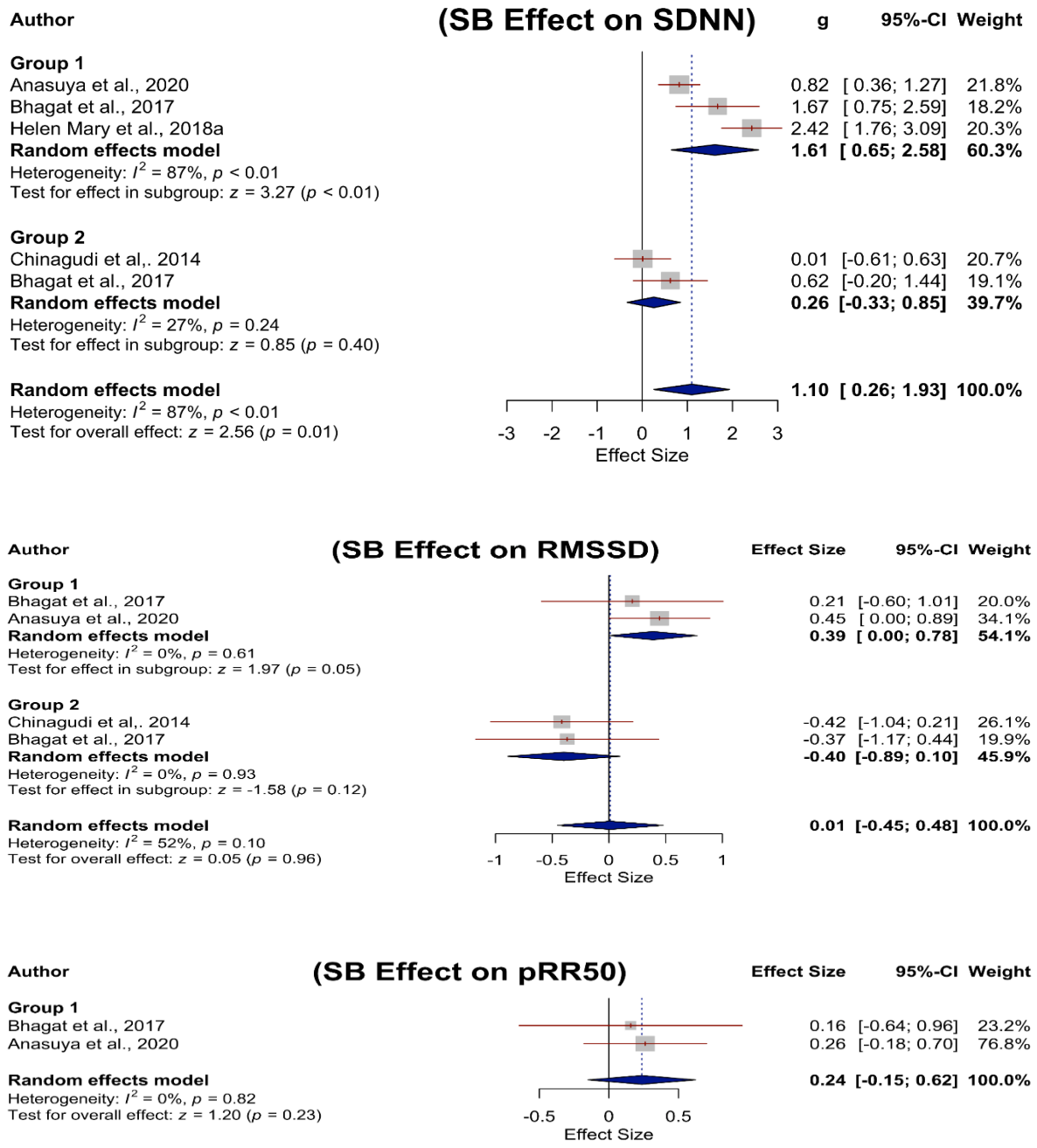


Figure 5.6 Forest plot of before vs, and before vs after during a single session of SB effects on time domain indices of HRV in normotensive SAs.

Values are in ES and 95%-CI. SB: slow breathing; HRV: heart rate variability; SDNN: mean of the standard deviations for all NN (R-R) intervals; RMSSD: Root mean square of successive RR interval differences; pRR50: proportion of NN50 divided by the total number of NN (R-R) intervals; 95%-CI: 95% confidence interval; g: effect size (Hedges); I^2: statistical heterogeneity.

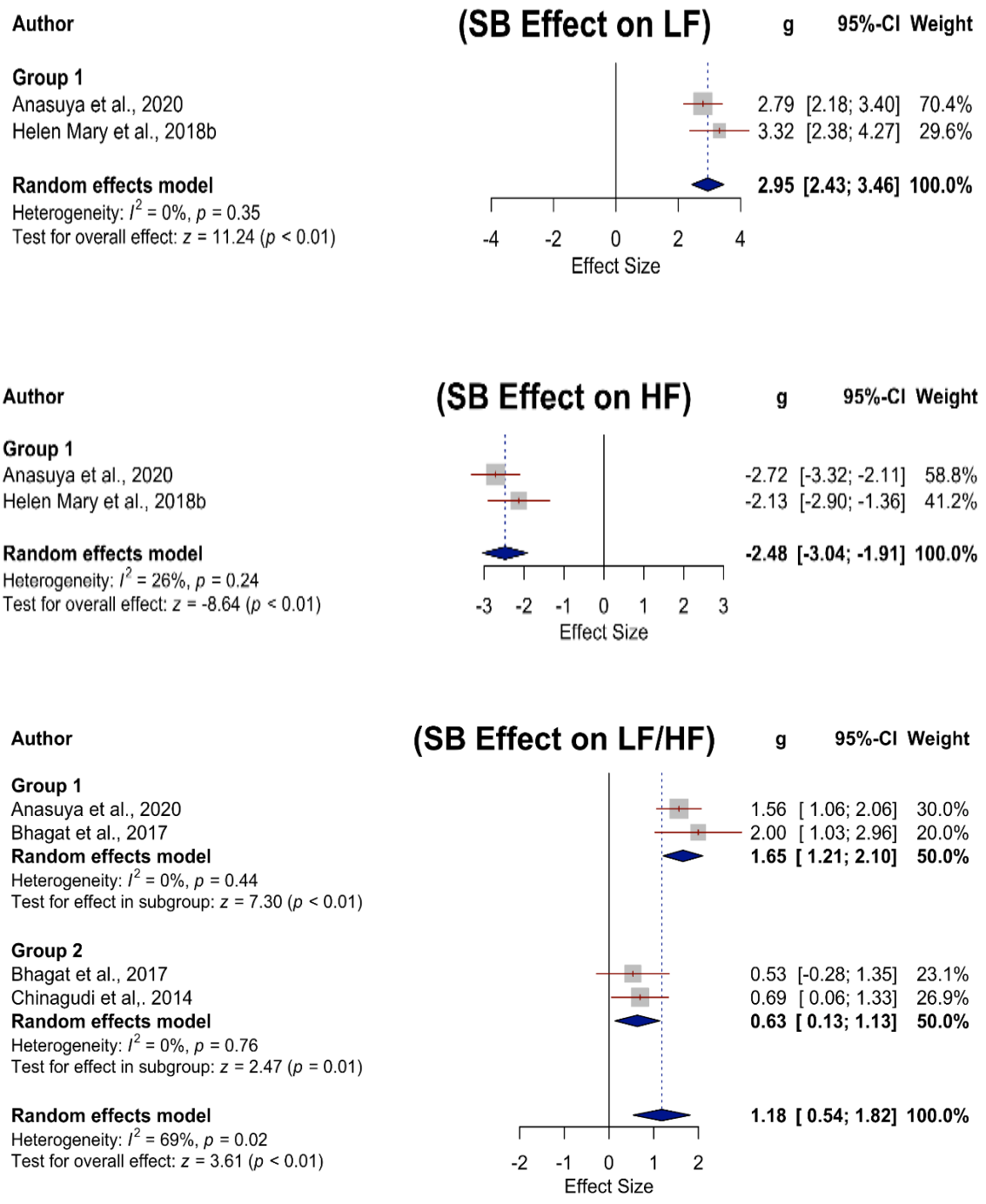


Figure 5.7 Forest plot of before vs, and before vs after during a single session of SB and its effects on frequency domain indices of HRV in normotensive SAs.

Values are in ES and 95%-CI. SB: slow breathing; HRV: heart rate variability; LF: low frequency; HF: high frequency; 95%-CI: 95% confidence interval; g: effect size (Hedges); I^2: statistical heterogeneity.

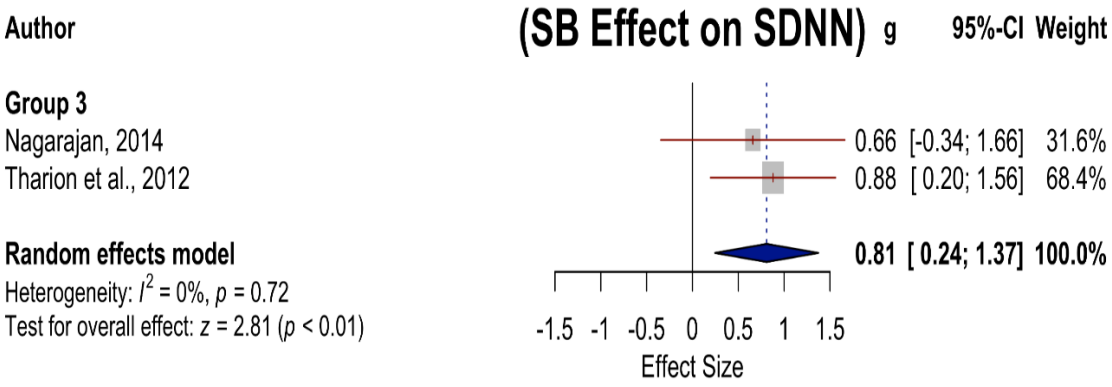


Figure 5.8 Forest plot of long-term training (4 weeks) of SB effects on HRV (SDNN) in normotensive SAs.

Values are in ES and 95%-CI. SB: slow breathing; HRV: heart rate variability; SDNN: mean of the standard deviations for all NN (R-R) intervals; 95%-CI: 95% confidence interval; g: effect size (Hedges); I^2: statistical heterogeneity.

5.3.5.3 Slow breathing effects on BRS

Only 2 studies with a total of 52 participants reported BRS results during slow breathing exercises (group 1) [182, 261]. Meta-analysis revealed a significant overall increase in α-LF BRS with a pooled ES of 0.77 (95% CI 0.37 to 1.17; P= 0.01; I^2 = 0%; Figure 5.9). However, we found no significant overall change in α-HF BRS or spontaneous BRS during slow breathing, (see Figure 5.9).

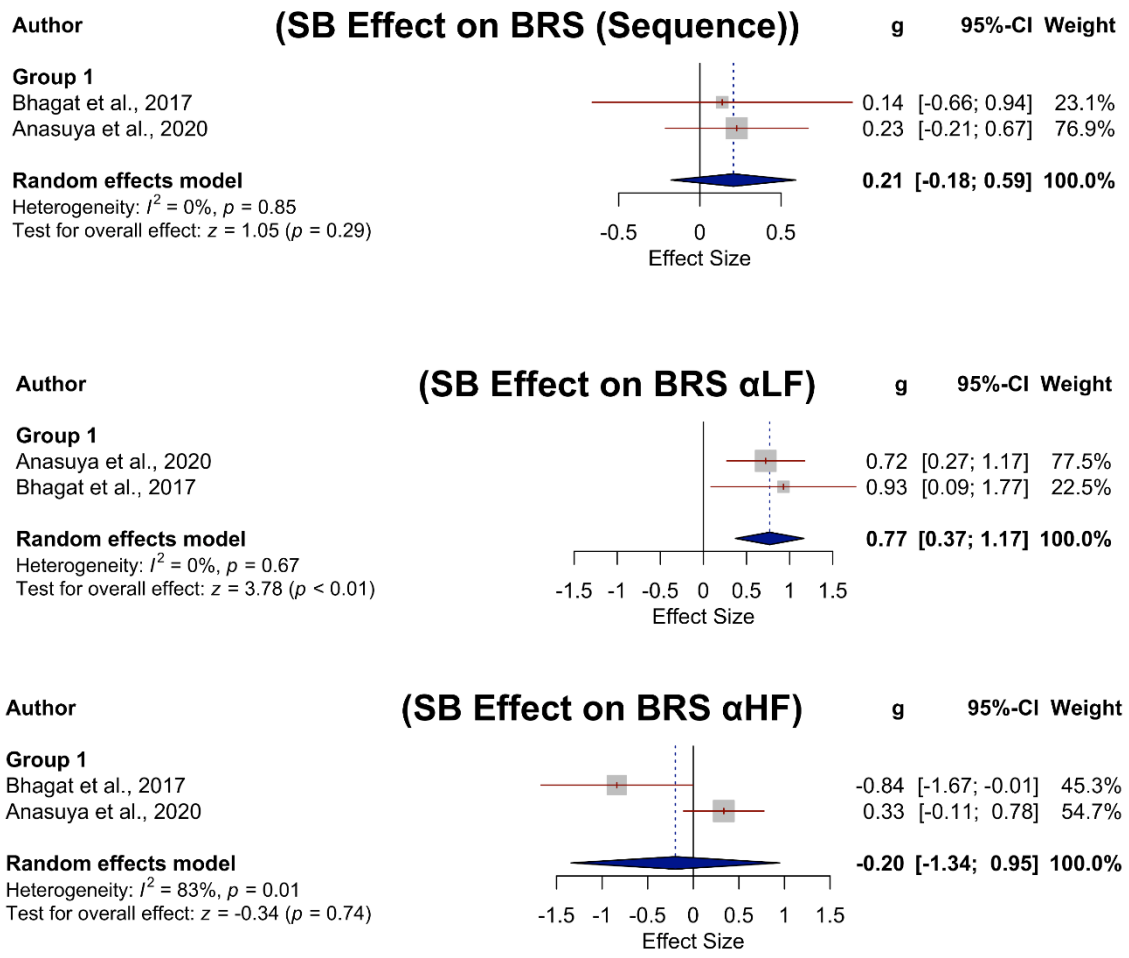


Figure 5.9 Forest plot of before vs during a single session of SB effects on BRS in normotensive SAs.
Values are in ES and 95%-CI. SB: slow breathing; BRS: baroreceptor reflex sensitivity; 95%-CI: 95% confidence interval; g: effect size (Hedges); I^2: statistical heterogeneity.

5.4 Discussion

It is well-established that SAs have a greater risk of developing CVD [22, 26, 166, 167, 264], including hypertension which is characterised by increased sympathetic activity and suppressed parasympathetic activity [46-48]. In hypertensive patients, several studies showed slow breathing at 6 b/min lowered ABP and normalised the reduced HRV, BRS and hypocapnia [67, 122]. The current review evaluates the possibility that slow breathing training might offer a non-pharmacological therapeutic intervention that could reduce the risk SAs have of developing hypertension and CVD and so reduce morbidity and mortality from these diseases. This systematic review and meta-analysis is the first to evaluate the effects of slow breathing exercises on ABP, HRV, and BRS in healthy normotensive SA adults.

Eight studies on SAs were included in this review [121, 182-184, 191, 261-263]. The included studies were heterogeneous in the time of assessment and duration of intervention. Therefore, when the meta-analysis was carried out, studies were classified into three groups: *during* a single session of slow breathing [182, 183, 191, 261], *immediately after* a single session of slow breathing [182, 184, 262], and *after* a long-term of slow breathing training [121, 263]. Studies were also heterogeneous in the types of slow breathing exercises, but all participants were required to perform breathing exercises at a respiratory rate of 5-6 b/m.

5.4.1 Main Outcomes

Overall, our meta-analysis on SAs revealed a significant overall increase in α-LF BRS during a single session of slow breathing, that was associated with a significant increase in RMSSD and SDNN during training. While increased in RMSSD indicates increased vagal activity [92, 100], increased SDNN is taken to be an estimate of overall HRV [100]. We found no significant effect on ABP *during* slow breathing. However, there was a significant decrease in

SBP *after* a single session of slow breathing, suggesting an acute beneficial effect in reducing SBP. Most importantly, the meta-analysis results showed that long-term (1 month) slow breathing training decreased MAP and increased HRV. There was also a trend for a decrease in spontaneous respiratory rate after long-term slow breathing training. This is important because the decreased spontaneous respiratory rate would need to be accompanied by an increased tidal volume in order to maintain normal minute ventilation. As a result, the Hering-Breur's inflation reflex would be enhanced leading to increased vagal discharge [263]. According to Narkiewicz, Van De Borne [265], decreased spontaneous respiratory rate in normotensive men was associated with decreased MSNA leading to decreased central sympathetic activity. Hence, a possible increase in vagal activity and decrease in sympathetic activity accompanying the decreased spontaneous respiratory rate might be the possible explanation of the reduced ABP following long-term slow breathing training.

5.4.2 Slow breathing effects on ABP

The pooled analysis of the results of three studies [182, 183, 261] indicated no overall effectiveness on ABP *during* 5 minutes of slow breathing. However, amongst these studies Bhagat *et al* [182] included 12 participants and reported no significant change in ABP during alternate nostril breathing (yogic breathing). This type of breathing involves using one nostril for inhalation for 4 seconds, followed by breath-hold for 2 seconds, then using the other nostril for exhalation for 4 seconds (5 b/m) and *vice versa,* rather than simply breathing in the usual manner at a reduced frequency. Further, a second study by Helen Mary et al [261] did not conduct or report results of within-group analysis. Thus, the results of the third study on 30 participants is of particular interest: they found that slow deep breathing (6 b/m) decreased SBP significantly [183]. It seems reasonable to propose that the inconsistency between the findings of the studies may be due to the variations in slow breathing exercise type and/or the number

of participants involved and to suggest that further studies similar to that conducted by Helen Mary *et al* [183] should be performed.

The present meta-analysis also demonstrated a significant decrease in SBP (P= 0.05; I^2 = 33%) *following* 5 min of slow breathing. However, this finding is inconclusive since only two studies were included in this analysis [182, 184], and one of them was of poor quality when assessed for risk of bias. The type of slow breathing intervention and the total number of participants in these studies was also heterogeneous: Bhagat *et al* [182] studied alternate nostril breathing in 12 participants, while Sharma *et al* [184] studied slow pace *pranayama* in 39 participants.

The most important outcome of the meta-analysis on SAs in relation to the working hypothesis is that long-term training of slow breathing for one month (30 min daily) significantly decreased MAP (P= 0.04; I^2 = 0%). The absence of statistical heterogeneity here, suggests consistency of the results and indicates the effectiveness of long-term slow breathing training in reducing BP. However, the fact that only two studies were included [121, 263], with a total of 26 participants, may limit the strength of our findings.

In a recent systematic review and meta-analysis conducted by Chaddha, Modaff [266], they included 17 studies with a total number of 1115 participants whose ethnicity was not indicated, to assess the effects of ≥ 4 weeks device- and non-device-guided slow breathing on ABP in hypertensive and prehypertensive patients. They demonstrated that slow breathing significantly reduced both SBP by -5.62 mmHg (95% CI -7.86 to -3.38; p < 0.001) and DBP by -2.97 mmHg (95% CI -4.28 to -1.66; p < 0.001). Interestingly, they found that the non-assisted slow breathing (*pranayama*) showed an even greater effect in lowering ABP than device-guided breathing. However, the authors reported high heterogeneity for all analyses they carried out. Further, another systematic review and meta-analysis by Mahtani, Nunan [267]

found a similar effect of ≥ 4 weeks of training of device-guided slow breathing on both SBP (-3.67 mmHg; 95% CI -5.99 to -1.39; P= 0.002) and DBP (-2.51 mmHg; 95% CI -4.15 to -0.87; P= 0.003) in 494 hypertensives from Israel, the United States, and the Netherlands. However, the authors reported some concerns about the included trials and concluded with a recommendation for further larger independent trials with a longer duration of intervention to validate the intervention. In addition, Zou, Zhao [268] conducted a meta-analysis on 6 RCTs consisting of 269 subjects and reported that patients with CVD showed a significant decrease in their resting SBP (-6.36 mmHg; 95% CI -10.32 to -2.39), DBP (-6.39 mmHg; 95% CI -7.30 to -5.49), and HR (-1.72 mmHg; 95% CI -2.70 to -0.75) compared to the controls when they practised voluntary slow breathing over a period of two weeks to six months.

Importantly, there is limited evidence on the effects of slow breathing on blood pressure regulation in SAs, especially hypertensive SAs. Only one study conducted by Adhana, Gupta [269] on hypertensive SAs was identified and was excluded due the fact that it was the only study that met our inclusion criteria before hypertension became an exclusion criterion due to the lack of studies (see section 5.2.1.1). Interestingly, they found that long-term (12 weeks) slow breathing training significantly decreased SBP (-12 mmHg; P< 0.001), DBP (-7 mmHg; P< 0.001), HR (-4.7 beats/min; P< 0.001), and RR (-3 breaths/min; P< 0.001) in SAs with uncontrolled essential hypertension [269]. Indeed, further studies investigating the effects of slow breathing on normotensive and hypertensive SAs and comparing the effectiveness of slow breathing between normotensive and hypertensive SAs will be needed.

5.4.3 Slow breathing effects on HRV

The present results showed that during slow breathing the time domain indices of HRV changed in a way that indicated that sympathetic activity decreased, and vagal activity was enhanced. Thus, there was a positive effect on HRV through increasing RMSSD (P= 0.05)

during a single session of slow breathing, indicative of an increased vagal activity, and the analysis showed no heterogeneity ($I^2 = 0\%$), suggesting the consistency of the results. Although SDNN, which is taken to be an estimate of overall HRV [100] also increased during slow breathing, a high degree of heterogeneity was noted, indicating that this results is inconsistent and imprecise.

By contrast, the pooled analysis of HRV data in the frequency domain showed an increase in LF (P= 0.01; I2 = 0%) and LF/HF (P= 0.01; I2 = 0%), but a decrease in HF (P= 0.01; I2 = 26%) during slow breathing. As HF reflects vagal activity [92, 100], this finding apparently contradicts the findings of the time domain indices during slow breathing. The possible explanation for this is that when the respiratory rate drops below 9 b/m (0.15 Hz), the power due to respiration which is usually in the HF range (0.15 – 0.4 Hz), shifts to the LF range (0.04 – 0.15 Hz) [92, 263, 270]. Therefore, the results of frequency domain analysis are not comparable during slow breathing [263].

The outcome of the meta-analyses of two long-term studies [121, 263] are likely to be of more physiological significance for they showed that long-term slow breathing training in SAs increased HRV, involving a significant increase in SDNN with no heterogeneity (P= 0.01; $I^2 = 0\%$). This is important because it was associated with a decrease in MAP (see Figure 5.5 & 5.8). Thus, it seems likely that an improved HRV induced by slow breathing training contributed to improvement of cardiac autonomic regulation and enhanced sympathovagal balance, and so to the decrease in ABP. However, as the overall sample size in this analysis was only 26 participants, further trials will be needed to confirm long-term slow breathing effects on HRV in SAs.

5.4.4 Slow breathing effects on BRS

During a single session of slow breathing in SAs, the meta-analysis revealed an overall increase in α-LF BRS with a pooled P = 0.01 with statistical heterogeneity of I^2 = 0%, that was associated with increased vagally-mediated HRV. This pattern might have been expected to lead to a decrease in ABP [270]. The fact that there was no overall decrease in ABP may have been influenced by the limited number of participants and studies included in the analysis, as only two studies (52 participants) reported data on BRS.

5.4.5 Study limitations

Some limitations should be considered when considering the findings of the present review. A primary limitation is the variation in the training duration, time of data collection and the type of outcomes recorded in the included studies, which led to classifying the studies into three separate groups. The fact that seven of the eight included studies were from a single SA country (India) is another limitation. In addition, the limited number of published studies and the small number of participants may have influenced and limited our findings. Also, all the participants of the included studies were healthy young or middle-aged SA adults. Therefore, we were unable to conduct subgroup comparisons between normotensive and hypertensive SAs for the effectiveness of slow breathing due to a lack of published studies on hypertensive SAs.

5.5 Conclusion

Despite the limited number of studies, our findings in healthy normotensive adult SAs suggest that slow breathing increases HRV and BRS during a single training session and decreases SBP following slow breathing. Slow breathing also increased HRV and reduced MAP after 4 weeks of training. Thus, after conducting the first review on the effectiveness of slow breathing on automatic regulation of ABP in SAs, it is reasonable to propose that slow breathing training may offer a costless non-pharmacological intervention that helps to decrease risk of developing hypertension and other CVDs amongst SAs. Further studies involving more participants on young, middle-aged, and old SAs are needed to elaborate these findings. In addition, further studies on healthy and CVD SAs patients with different age ranges are also needed. The outcomes of this systematic review are consistent with the hypothesis that a single period of slow breathing or long-term slow breathing training would reduce mean ABP in SAs.

Chapter 6: General discussion

6.1 Summary of project aims

The overall aim of the present PhD project was to investigate whether young normotensive SA adults show early markers of automatic dysfunction that may lead to future hypertension and cardiovascular disease and to assess the beneficial effects of acute and long-term slow breathing on autonomic function and regulation of ABP, especially in SA adults. Thus, a set of three studies were designed to achieve this objective. The first study was designed to evaluate the autonomic regulation of ABP and the baroreceptor reflex regulation of ABP and HRV of young, healthy non-smoking SAs at rest, during acute slow breathing, as well as during and after acute mental stress, in comparison to a matched group of WEs. Then, a second study was carried out with the aim of investigating whether there are sex- and ethnic-dependent differences among and between young normotensive SAs and WEs adults in the cardiovascular responses to acute slow breathing and acute mental stress.

Finally, a systematic review was designed to assess the effects of acute slow breathing and long-term slow breathing training on the autonomic regulation of ABP in healthy normotensive and hypertensive SA adults, which might offer a non-pharmacological therapeutic intervention for reducing ABP and reducing the risk that hypertension carries of further CVD and the morbidity and mortality of such diseases. Unfortunately, published studies assessing the effects of slow breathing in hypertensive SAs are limited and we were only able to identify one study that met our inclusion criteria. This meant it was not possible to conduct a meta-analysis on studies involving hypertensive SAs. Therefore, the systematic review and meta-analysis were carried out on studies involving healthy SA adults which met our inclusion criteria.

6.2 Main outcomes

The results of Chapter 3 revealed several novel findings. As discussed in section 3.4.1, under resting conditions, there were no ethnic differences in systemic cardiovascular variables between SAs and WEs. Furthermore, BRS assessed during up- and down-sequences in SP which reflect changes in cardiac vagal activity [49] and time-domain indices of HRV were not different between the two ethnicities. These findings were contrary to our initial hypothesis and showed that a mixed-sex group of young normotensive SAs do not show elevated ABP and TPR or reduced HRV and BRS at rest in comparison to matched WEs. However, SAs did show higher RR than WEs under resting conditions. According to Joseph *et al* [67], RR is increased in hypertensive individuals, with no change in $ETCO_2$, and this was attributed to raised peripheral chemoreceptor activity as shown by Trzebski, Tafil [271]. Further, both resting hyperventilation and the increased sympathetic drive in essential hypertension have been attributed at least in part to peripheral chemoreceptor stimulation [113, 180]. Thus, given the increased risk SAs have for hypertension, it will be important in future studies to measure tidal volume and $ETCO_2$ in young SAs at rest and to investigate whether young SAs also have raised peripheral chemoreceptor activity.

In addition, as discussed in section 3.4.2.1, acute slow breathing decreased ABP in WEs and SAs. However, TPR was decreased in WEs during slow breathing, but not in SAs. The reduction in TPR in WEs may be attributed to a reduction in sympathetic nerve activity to the vasculature, as it has been reported that slow breathing decreased MSNA in normotensive and early hypertensive subjects [186-188]. Further, as discussed in section 3.4.2.2, the time-domain indices of HRV increased *during* slow breathing, and BRS increased during up-sequences but not down-sequences in both WEs and SAs. Thus taken together, these findings indicate that the ability of slow breathing to inhibit vagal activity and enhance respiratory-dependent

changes in vagal activity is comparable in WEs and SAs, but the ability of reduced respiratory drive to decrease sympathetic vasoconstriction is less effective in SAs than in WEs.

On the other hand, mental stress, raised ABP, HR, and RR in both WEs and SAs (see section 3.4.3). This is consistent with the typical pattern of alerting or defence response [131-133]. However, there was an increase in TPR in SAs but not WEs *during* mental stress. suggesting that SAs showed greater sympathetically mediated vasoconstriction which contributed to the elevation of ABP. It has been reported that the direction of the change in TPR during mental stress depends on the magnitude of the change in limb vascular resistance [131, 197]. Thus, the increase in TPR in SAs indicates that the muscle vasodilatation of the defence response *during* mental stress was blunted or reversed to vasoconstriction. These findings raise the possibility that exaggerated sympathetic vasoconstrictor responses to stressors may facilitate future hypertension in SAs, for several previous reports have shown that exaggerated pressor responses to laboratory stressors are associated with the future development of essential hypertension [129, 194-196], and the association between exaggerated response to stressors is particularly strong in young individuals who are known to be at high risk of developing essential hypertension [170, 173, 175, 176].

A further novel finding was that during mental stress, time-domain indices of HRV were reduced, while the vagal BRS decreased during up-sequences in both WEs and SAs. However, BRS during down-sequences was reduced in WEs only *during* mental stress, and cardiac sympathetic component of BRS was reduced in the squat-to-stand test in WEs, but not in SAs immediately *following* mental stress. As discussed in Chapter 3, section 3.4.3.3, these findings indicate that even though inhibition of the baroreceptor reflex is considered to be a characteristic feature of the defence response, this process seems to be defective in young SAs particularly during falls in ABP given the cardiac vagal and sympathetic components of the

baroreceptor reflex were not inhibited in SAs *during* and immediately *after* mental stress. These findings suggest that young SAs may be better than WEs at buffering changes in ABP in response to stressors, but importantly, they suggest that SAs are better at producing further baroreceptor-mediated reductions in vagal activity and increases in sympathetic activity at a time when these effects are already occurring as part of the defence response. As discussed in section 3.5, persistence of these aspects of the baroreceptor reflex may give SAs better protection against vasovagal syncope or emotional fainting following a defence response [(132)], but would also be intrinsically pro-hypertensive in stressful situations. This issue would be interesting to explore in future studies.

An important part of the context in which the present study was performed was that SA women have a higher prevalence of CAD than SA men or WE women. Thus, the results of Chapter 4, in which the WE and SA groups were considered on the basis of sex were of particular interest. These analyses revealed no sex-dependent differences within or between WE and SA males and females in ABP, TPR, RR, time-domain indices of HRV, or the cardiac vagal component of BRS under resting conditions. However, whilst WE and SA men had similar HR and SV, and there were similar values between WE men and women at rest, SA women had greater HR and lower SV than SA men which may represent a sympathovagal imbalance in SA women at rest. Future studies on larger samples of young SA and WE men and women with greater statistical power will be necessary to fully evaluate the sympathetic and vagal components of HRV using frequency- and time-domain analysis and to investigate whether sympathovagal imbalance is already present in young SA women at rest.

During slow breathing (see section 4.4.2), there were also no sex-dependent differences in the haemodynamic variables, HRV, or vagal BRS among or between WE and SA men and women. Nevertheless, although the majority of SA men and WE men and women showed a

reduction in TPR *during* slow breathing, only a few SA women showed a reduction in TPR in response to slow breathing, suggesting that SA women may be more resistant to the inhibitory effects of slow breathing on sympathetic vasoconstriction and ABP. Future studies assessing the effects of slow breathing on ABP and TPR in a larger sample size are required to properly test this possibility. It would also be important to establish whether SA women react more strongly to longer duration, acute slow breathing or to regular slow breathing training.

In addition, as discussed in section 4.4.3, there were no statistically significant sex-dependent differences among or between WEs and SAs for haemodynamic responses to mental stress. However, a greater proportion of SA women showed an increase in TPR than a decrease during mental stress, raising the possibility that the sympathetic vasoconstrictor impact of mental stress may be greater in SA women than in SA men and WE women. This issue is especially important in view of evidence that frequent vasoconstrictor responses to mental stress increase the risk of future hypertension and CVD [236, 237]. Clearly, studies with larger sample size will be important to test whether young SA women generally show larger pressor responses and a greater rise in TPR in response to mental stress consistent with their higher risk of CVD and CAD [5, 8].

Given the baroreceptor reflex is generally expected to be inhibited during and after the defence response to mental stress [132], an important novel finding was that although the cardiac sympathetic component of BRS as measured by the squat-to-stand test was similar across groups at rest, it was greater in SA men than WE men immediately after mental stress, and the ΔBRS was significantly smaller in SA women than in WE women. Thus, not only do we have evidence that inhibition of the cardiac and vagal components of the BRS by mental stress is defective in SAs relative to WEs as discussed above and in section 4.4.3, but the sex -dependent comparisons indicate that this process is particularly defective in SA women. There is certainly

a need for future studies with larger groups of SA and WE men and women to test these possibilities and their functional significance.

Turning to the results of the systematic review (Chapter 5), the meta-analysis on healthy SA adults showed a significant increase in α-LF BRS, in association with increased RMSSD and SDNN during a single session of slow breathing indicating increased vagal activity [92, 100], and improved overall HRV [100]. The pooled analysis also showed a significant fall in SBP immediately following acute slow breathing. Thus, acute slow breathing is immediately effective in decreasing ABP. Importantly, the meta-analysis also revealed that four weeks of slow breathing training reduced MAP and increased HRV significantly in healthy SA adults. indicating that the decrease in ABP may be attributed at least in part to improved sympathovagal balance. Thus, the findings of the systematic review and meta-analysis agreed with our initial hypothesis that acute slow breathing and long-term slow breathing does decrease ABP in SA adults.

In view of these outcomes, it will be important for future studies to assess whether acute slow breathing has similar beneficial effects in hypertensive SAs and whether slow breathing training may offer a non-pharmacological intervention to help reduce the morbidity and mortality of hypertension and CVDs in SAs.

6.3 General limitations

The limitations of each study are reported separately in the relevant chapters (see sections 3.4.4, 4.4.4, and 5.4.5). However, an important overall limitation of the current PhD project should be highlighted. The initial aims of the current PhD project were to examine and compare autonomic modulation of ABP in SAs and WEs, as well as the effects mental stress and slow breathing on a variety of systemic hemodynamic variables and BRS. We were also

planning to conduct experiments to study the mechanisms underlying any differences that might be found between WEs and SAs. Finally, we were planning to examine the impact of inspiratory muscle training on respiratory and cardiovascular function in SAs and WEs. However, because to the Covid-19 epidemic, laboratory-based experimental work was halted after the first study and could not be restarted throughout the PhD funding period. As a result, a systematic review and meta-analysis was designed to investigate the effects of acute slow breathing and long-term slow breathing training on ABP regulation and autonomic function in healthy normotensive SA adults.

6.4 General conclusion

Young SAs men and women showed an exaggerated pressor response to mental stress relative to young WE men and women as reflected by increased TPR; the rise in TPR seemed to be more pronounced in SA women. These findings are consistent with mental stress facilitating the development of future CVD in SAs relative to WEs and in SA women in particular. Interestingly, in SAs, mental stress was associated with less suppression of the sympathetic component of BRS during squat-to-stand test than in WEs, indicating they are better at buffering falls in ABP and may be less vulnerable to vasovagal syncope, but raising the possibility that they are also at risk from the pro-hypertensive effect of further tachycardia. Further, the fact that acute slow breathing decreased ABP and increased vagal activity in both young SAs and WEs but decreased TPR in WEs only, suggests that slow breathing may be less effective as a potential therapy for reducing ABP in SAs than WEs. Nevertheless, the fact that the systematic review and meta-analysis showed that acute slow breathing does decrease ABP and increase vagal activity, and that four weeks of slow breathing training is able to decrease ABP and improved HRV in healthy SA adults, indicates that slow breathing training is

worthwhile exploring as a potential preventative therapy in young SAs and as a curative therapy in hypertensive SAs.

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Appendices

Appendix 2.1 Participant information sheet

Institute of Clinical Science College of Medical and Dental Sciences University of Birmingham

Is evidence of cardiovascular and respiratory disease already present in young healthy South Asians and White Europeans: can this be modified by respiratory training?

Investigators: *Mobarak Alqahtani Supervisors: Prof. Janice M Marshall & Dr Andrew Coney*

Participant Information Sheet 1

Thank you for your interest in this study. This information sheet is designed to give you an overview of what the study is about and your potential role in it. If you have any further questions, please do not hesitate to contact the investigator.

Background – What is already known?

The risk of cardiovascular disease (CVD) is much greater in South Asians, with ethnic roots in India, Pakistan, Bangladesh, Sri Lanka, or Nepal, than in Caucasians (White Europeans) and the occurrence of high blood pressure and diabetes is particularly high. These are all diseases that are associated with poor functioning of the endothelial cells that line the blood vessels. From our recent studies, we have evidence that the ability of the endothelial cells to release substances that dilate blood vessels is impaired in young South Asian men relative to young White European men, even though they show no obvious signs of high blood pressure or diabetes. However, we also have recent evidence that young South Asian men show blunted dilator responses, but exaggerated constriction of blood vessels and increases in blood pressure in response to environmental stimuli such as repeated sounds, or immersing one leg in cool water. These responses not only involve vasodilator substances released from the endothelial cell lining, but also the influences of the nerve fibres that supply the blood vessels and heart. These are the sympathetic nerve fibres, which constrict blood vessels and increase heart rate, and parasympathetic nerve fibres (called the vagus), which slow heart rate. Our evidence to date raises the possibility that the influences of the sympathetic fibres may be relatively more important in South Asians than in White Europeans.

It is also recognized that respiratory diseases, such as chronic bronchitis and asthma are commonly accompanied by CVD, and respiratory disturbances that occur during sleep are a common feature of CVD. In fact, respiratory disease is accompanied by disturbances in the way the sympathetic and parasympathetic nerve fibres regulate the cardiovascular system. On the other hand, in CVD, the normal influences of breathing on the sympathetic nerve fibres that regulate the cardiovascular system are exaggerated while those exerted on the parasympathetic (vagus) are depressed. Given that both respiratory disease and CVD develop progressively over many years, an obvious question is whether some individuals are predisposed to develop respiratory and/or cardiovascular disease and whether this can be recognized even in young adults. This is a particularly important question in relation to the occurrence of different diseases in different ethnicities because South Asians not only have a greater risk of CVD than White Europeans, but they have smaller lungs and greater risk of developing more serious respiratory disease.

With this background, the overall aims of the project are to establish whether even in young adulthood, healthy South Asians (SAs) show impaired sympathetic and parasympathetic regulation relative to young adult White Europeans (WEs), including impaired ability to control blood pressure by what is called the baroreceptor reflex, and disturbed ability to regulate blood pressure, heart rate and blood flow in response to common everyday stimuli. In particular, we want to establish whether any impairments can be attributed in part, to disturbed respiratory influences on the cardiovascular system. Further aims will be to establish whether a regular slow breathing, or inspiratory muscle
training over 4-5 weeks can produce benefits particularly in South Asians. As a first step, we plan to test the baroreceptor reflex at rest, during standing up and during mental stress.

Who can take part?

We are seeking young healthy (male or Female), non-smoker, non-obese volunteers for the study, aged between (18-25) yrs. You need to be either of South Asian ethnicity (originating from India, Pakistan, Sri Lanka, Nepal or Bangladesh), or of White European (Caucasian) ethnicity. You also need to be free of cardiovascular, respiratory conditions, or metabolic disease (diabetes, etc) and not to be taking medications. We require both your parents to be of the same ethnicity i.e. both South Asian, or both White European. We also need to gather information about your parent's cardiovascular history. We intend to recruit 10-12 subjects in each group.

Benefits – What do we want to know?

Even though there may be no long lasting benefits for you, the results of the study will give us new understanding of differences in cardiovascular regulation that exist between apparently healthy South Asians and White Europeans and will tell us the extent to which they reflect differences in the ways that breathing pattern influences the cardiovascular system. This will help us design studies to test whether training programmes involving slow breathing or strengthening respiratory muscles can help normalise the relationships between respiratory and cardiovascular systems and so avoid the development of CVD.

What will the study involve?

You will be asked to come for a first visit to the Medical School, in order to complete a routine health questionnaire. You will also have the opportunity to ask any questions that you might have regarding the study. If for any reason you are not suitable for the study, you will be told. However if you are accepted and are willing to participate, you will be given a dietary questionnaire to complete in your own time. We will also use this first visit to familiarize you with the laboratory equipment, study protocol and Participant Information Sheet. You will also be asked to do standard lung function tests, which means breathing into different devices.

You will be given the Participant Information sheet to read in your own time. If you feel comfortable with the experiment and understand the procedure, you will then be asked to sign the consent form.

Following this first visit, you will be requested to visit the laboratory on another occasion for an experimental session lasting roughly 1 and half hours. We will endeavour to make the time of this session as convenient for you as possible. If you incur travel expenses as a direct consequence of attending the laboratory for this project, then your travel expenses will be reimbursed on submission of an appropriate receipt.

What will you have to do *before* the visit for the experiment?

You will be asked not to consume any non-steroidal anti-inflammatory drugs (such as aspirin, ibufrofen, nurofen, paracetamol, panadol, etc) for 1 week before your visit, or consume any alcohol or exercise vigorously for at least 24 hours prior to your visit. You will also be asked not to consume any caffeinated drinks/caffeinated medication (tea, coffee, Red Bull, flu medicine, etc), or heavy meals for at least 12 hours prior to each visit.

What will you have to do *during* the visit for the experiment?
 You will be asked to recline on a couch and we will record your resting blood pressure from your non-dominant arm, by using a standard blood pressure monitor with a device called sphygmomanometer (non-invasive procedure).
 We will continuously record your blood pressure via a cuff placed on a finger. Small probes will also be placed on your chest to record your heart rate. An elasticised belt will be placed

around your chest to record your respiratory rate. Also, we may use a non-invasive device called capnography that measures your carbon dioxide breath by breath. (These are all non-invasive techniques).
 Your blood pressure and other recordings will be re-assessed while you are breathing slowly at 6 breaths/min – you will be given a sound signal to help you.
 You will be requested to stand up, crouch (squat) to the floor for about 15 seconds or so and then stand upright again.
 You will also be asked to undertake a mental stress test which will involve mental arithmetic, for example taking a two digit prime number like 13 from a 3 or 4 digit number and then from the remainder and so on.

Experimental protocol:
 We will then ask you to recline on a couch and will record your resting blood pressure with a standard blood pressure monitor three times or until it settles.
 From then on, blood pressure, heart rate and breathing will be recorded continuously as described above while you rest on the couch for 10-15 minutes. This will allow us to calculate your resting baroreceptor reflex.
 We will then ask you to breathe at 6 breaths/min with the aid of a sound signal, while you rest on the couch for about 5 minutes so that we can again calculate the baroreceptor reflex.
 Approximately 5 minutes later, we will ask you to stand by the couch. When the recordings have settled, we will ask you to squat to the floor as described above and then stand up. When you stand, we will ask you to remain still, using the couch for support if you wish. Meanwhile, we will make continuous recordings
 After this, you will be asked to do the Mental arithmetic stress test for 2-3 minutes, During this we will again make continuous recordings so that we can assess the baroreceptor reflex.
 You will then be asked to stand upright, squat and stand as described above.

Can you change your mind about participation?
It is entirely up to you to decide whether or not you take part in the study. If you decide to take part, but later change your mind you can withdraw at any time up to 2 weeks after the experimental protocol. Any usable data will be included in the study unless you ask otherwise: if you request that it is destroyed we will do this. All of the data collected and retained with your permission may be held by the University for research purposes on a secure database for up to 10 years. This is in accordance with University's research guidelines.
Are there any risks involved?
Any risks attached to this study are minimal.
You might feel faint, or experience dizziness when you stand up from the crouch. However, this is unlikely and is very uncommon. But, if you do experience any side effects, we will take care of you.

If we notice that your blood pressure or heart rhythm seem to be outside the normal range, we will suggest that you visit your GP. Similarly, if your blood glucose or insulin levels are outside the normal range, we will tell you and suggest you visit your GP.

What will happen to the results of the research study?

The results of the study will be used to contribute to publication of a PhD thesis and in papers published in medical science journals. All the data collected will be stored on a password protected database. All data will be strictly confidential and will be made anonymous so that you cannot be recognized from it.

Are you interested?

Having read though this information, if you are interested in participating in this study, please contact;

Mobarak Alqahtani
College of Medical and Dental Sciences University of Birmingham Email:
Supervisor: Professor Janice Marshall
Institute of Clinical Sciences College of Medical and Dental Sciences University of Birmingham Email:
Supervisor: Dr Andrew Coney
Institute of Clinical Sciences College of Medical and Dental Sciences University of Birmingham Email:

Appendix 2.2 Healthy Volunteer Screening Questionnaire and sports & physical activity status

Is evidence of cardiovascular and respiratory disease already present in young healthy South Asians and White Europeans: can this be modified by respiratory training?

Investigators: *Mobarak Alqahtani, Dr.Andrew Coney and Prof. Janice M Marshall*

Healthy Volunteer Screening Questionnaire

Please remember, all the information will be treated in the strictest of confidence.

VOLUNTEER INFORMATION

Subject No (for researcher only):_____
Name:_____
Date of Birth:_____ BMI:_____ Kg/m^2
Address:_____ Resting Heart Rate:_____ B/min
_____ Resting Blood Pressure:_____ mm Hg
_____ Dominant Arm: _____ (R or L)
_____ Arm Circumference (Dominant):_____ cm
Phone No:_____ Arm Circumference (Non-dominant):_____ cm
Email Address:_____ Max. Contraction (Dominant) _____ Kg
Height:_____ cm Max. Contraction (Non-dominant)_____ Kg
Weight:_____ Kg Waist circumference _____ cm

MEDICAL HISTORY

Do you currently smoke? **Yes / No**
Have you ever smoked? **Yes / No**
If yes, please provide details of when you quit?
_____
Do you, or have you ever used ANY drugs for recreational purposes? **Yes / No**
Are you taking ANY medication? **Yes / No**
If yes, please give details:
_____
Are you allergic to non steroidal anti-inflammatory drugs (NSAIDs) e.g. Aspirin, Nurofen, Ibuprofen, Naproxen, etc?
Yes / No

If yes, please give details:

_____

Do you, or have you ever suffered from any of the following?

- Cardiovascular Disease **Yes / No**
- Hemodynamic / Clotting Disorder **Yes / No**
- Metabolic Disease (e.g. Diabetes) **Yes / No**
- Respiratory Disease **Yes / No**
- Liver Disease **Yes / No**
- Kidney Disease **Yes / No**
- Ulcers (e.g. Stomach) **Yes / No**
- Any Other Chronic Medical Condition **Yes / No**

If yes to any of the above, please give details:

_____

_____

Does your family have a history of hypertension (high blood pressure)?
Yes / No
If yes, please give details_____

_____

_____

Does your family have a history of any other of the disease listed above?
Yes / No
If yes, please give details:

_____

_____

Have you ever fainted (*e.g. on standing, following fasting, during exercise)*? **Yes / No**
If yes, please give details: _____

Do you consume alcohol? **Yes / No**
If yes, how many units of alcohol do you consume per week?_____
Units
Note: *Half a pint of ordinary strength larger/beer/cider, or one small glass of wine, or one pub measure of spirits (e.g. a shot of tequila), or half a bottle of alcopop (e.g. Smirnoff Ice) is equal to **1 Unit.***
Roughly, how many cups of coffee, tea, cola or any other caffeinated/energy drink (e.g. Red Bull) do you drink in a day?

_____ Cups

Do you take dietary supplementation (Vitamins, Botanicals, Amino acids, etc)? **Yes / No**
If yes, what do you take and how often?

_____

How often do you eat citrus fruits?

_____

How many portions of fruit and vegetable do you have daily?

_____

How often do you eat fish rich in oil (Omega 3), eg mackerel, tuna, herring, salmon, sardines, or consume Fish oil supplements?

Do you use salt when cooking and/or do you add salt when eating food? Please give details

Do you use ready meals or buy pre-packed sandwiches? Please give details

How often do you eat snacks such as crisps, peanuts? Please give details

Have you taken part, or do you intend to take part, in any other trial within the three months prior to and following this particular study (e.g. other studies, blood donor)?
If yes, please give details? **Yes / No**

ETHNICITY

WHAT IS YOUR ETHNIC GROUP? Choose one section from (a) to (e) and tick the appropriate box to indicate your cultural background	
(a) WHITE ☐ British ☐ Irish ☐ Other White background ……	(b) BLACK or BLACK BRITISH ☐ Caribbean ☐ African ☐ Other Black background …….
(c) ASIAN or ASIAN BRITISH ☐ Indian ☐ Pakistani ☐ Bangladeshi ☐ Other Asian background ……..	(d) MIXED ☐ White and Black Caribbean ☐ White and Black African ☐ White and Asian ☐ Oher mixed background ……
(e) CHINESE or OTHER ETHNIC GROUP ☐ Chinese ☐ Any other mixed background	**Please indicate here**

Country of origin of your Parents:

Grandparents:

Have you lived in the UK since birth?
If not, please, indicate the time period residing in UK: (yrs.)

SPORTS & PHYSICAL ACTIVITY STATUS

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

1A. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?
Think about only those physical activities that you did for at least 10 minutes at a time.
_____ days per week ›

1B. How much time in total did you usually spend on one of those days doing vigorous physical activities?
_____ hours _____ minutes

or

- none

2A. Again, think only about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.
_____ days per week ›

2B. How much time in total did you usually spend on one of those days doing moderate physical activities?
_____ hours _____ minutes

or

- none

3A. During the last 7 days, on how many days did you walk for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.
_____ days per week ›

3b. How much time in total did you usually spend walking on one of those days?
_____ hours _____ minutes

or

- none

The last question is about the time you spent sitting on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

4. During the last 7 days, how much time in total did you usually spend sitting on a week day?
____ hours _____ minutes

DECLARATION:

I declare that all of the above information is correct to the best of my knowledge. I understand that this information will be treated in the strictest of confidence.
Signature:_____ Date:_____

Thank you for showing interest in this study.

Appendix 2.3 Healthy Volunteer's Consent Form

Is evidence of cardiovascular and respiratory disease already present in young healthy South Asians and White Europeans: can this be modified by respiratory training?

Investigators: *Mobarak Alqahtani (PhD Cardiovascular Science Student), Dr. Andrew Coney and Prof. Janice M Marshall.*

Healthy Volunteer's Consent Form

Please read this form carefully and sign it once one of the above named, has explained fully the aims and procedures of the study to you.

…… I have voluntarily agreed to take part in this study.

…… I confirm that I have been given a full explanation by at least one of the above named and that I have read and understood the information sheet given to me.

…… I have been given the opportunity to ask questions and discuss the study with one of the above investigators on all aspects of the study and have understood the advice and information given as a result.

…… I agree to comply with the reasonable instructions of the investigators and will notify them immediately of any unexpected unusual symptoms.

…… I understand that any data and/or samples collected will be strictly used for scientific research/study.

…… I authorize the investigators to disclose the results of my participation in the study but not any details which could lead to my identification (e.g. name, contact details).

…… I understand that information about me recorded during the study will be kept secure. If data is transferred to others it will be made anonymous.

…… I understand that the data collected and/or results of the study may be published in a scientific journal and that if published, my identity will be protected.

…… I authorize the investigators to disclose to me any abnormal test results.

…… I understand that I can ask for further instructions and/or explanations at any time.

…… I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing.

…… I confirm that I have disclosed all the relevant medical information before the study.

Please note that a copy of this form will be provided for you to keep. If you have any questions, require further instructions and/or explanations, please do not hesitate to contact Mobarak Alqahtani at [redacted]

Volunteer's Name:_____

Signature:_____

Date:_____

Investigator's Name:_____

Signature:_____

Date:_____

Subject Number:_____

PLEASE, DO NOT FORGET TO BRING THE CONSENT FORM WITH YOU ON THE PREARRANGED DATE OF EXPERIMENT.

Appendix 3.1 Abstract presented at the British Irish Hypertension Scientific meeting (BIHS 2019) and published in the Journal of Human Hypertension.

P-15 Baroreflex regulation of arterial blood pressure is disturbed in young South Asians relative to White Europeans, particularly during mental stress

Mobarak Alqahtani[*1], Andrew Coney[1], Janice Marshall[1]

[1]University of Birmingham, Birmingham, United Kingdom

Introduction: South Asians (SAs) have greater risk of cardiovascular disease than White Europeans (WEs); this is generally associated with endothelial dysfunction [1]. In view of our recent evidence that young SAs show greater sympathetic activation than WEs during mental stress [2], we investigated autonomic regulation of arterial blood pressure (ABP) in both ethnicities.

Methods: In 8 WEs and 8 SAs (4/4 men/women in each group; 19–24 years), ABP, heart rate (HR), ECG and respiration were recorded at rest, mental stress (Stroop test); baroreflex sensitivity (BRS) by sequence method and squat-stand test, heart rate variability (HRV) by spectral analysis were calculated.

Results: At rest, WEs and SAs had similar mean ABP (mABP, 82.2 ± 2.1 vs. 82.0 ± 4.2 mmHg), HR (70.1 ± 3.4 vs. 64.5 ± 6.5 beats/min) and BRS (up-sequence: 1.1 ± 0.09, down-sequence: 1.2 ± 0.05 vs. 1.2 ± 0.04 and 1.3 ± 0.06 ms/mmHg). However, low/high frequency power ratio (LF/HF) was 1.4 ± 0.05 in WEs (34.0 ± 6.2%/28.4 ± 5.1%), but only 0.7 ± 0.1 in SAs (26.5 ± 1.7 %/ 41.7 ± 4.3 %), the HF component tending to be greater in SAs ($P = 0.07$: SAs vs. WEs). Mental stress evoked similar increases in mABP and HR, but BRS was less depressed in SAs than WE especially during down-sequences (to 0.69 ± 0.11 vs. 1.03 ± 0.05* ms/mmHg, *: $P < 0.05$, WE vs. SA), while LF was increased in both SAs and WEs (to 42.9 ± 5.9* and 42.8 ± 4.8 %), but HF was decreased in SAs only (to 29.0 ± 3.4* %). Further, immediately following mental stress, BRS during squat-stand was depressed in WEs, but not SAs (0.6 ± 0.04 to 0.3 ± 0.09* vs. 0.6 ± 0.09 to 0.5 ± 0.11 ms/mmHg.

Conclusions: Even in young adult SAs, baroreflex regulation of ABP is disturbed particularly during mental stress with SAs showing exaggerated vagally- and sympathetically-mediated tachycardia relative to WEs. This may contribute to their increased risk of hypertension and associated cardiovascular disease

Appendix 3.2 The abstract presented at the Future Physiology 2019 Symposium

PC78

Comparisons between young South Asians and White Europeans in the effects of acute mental stress and slow breathing on baroreflex sensitivity and heart rate variability

M. Alqahtani, J. MARSHALL and A. Coney

University of Birmingham, Birmingham, UK

South Asians (SAs) have a greater risk of cardiovascular disease including hypertension, (CVD) than White Europeans (WEs). Whether this reflects disturbances in autonomic control of the cardiovascular system and whether this might be present an early adulthood has not been tested. Thus, we performed studies on young WEs and SAs (10, 8 respectively, aged 19-24; equal numbers of men and women in each group) in which arterial blood pressure (ABP), heart rate (HR), ECG and respiration were recorded at rest, during mental stress (3 min Colour Stroop test) and during 5 min slow breathing (6 breaths/min). Baroreflex sensitivity (BRS) was calculated by the sequence method as change in R-R interval evoked by spontaneous up and down sequences in systolic pressure (SP) at rest and during mental stress and as the relationship between R-R interval and the fall in SP evoked by standing from a squat position before and following mental stress. Heart rate variability (HRV) was computed in the time- and frequency-domains during mental stress and slow breathing. Comparisons within and between WEs and SAs were done by paired and un-paired t-tests respectively.

At rest, WEs and SAs had similar mean ABP (mABP, 82.2±2.1 vs 82.0±2.8 mmHg), HR (70.1±3.4 vs 64.5±4.5beats/min) and BRS (up-sequence: 1.1±0.09, down-sequence: 1.2±0.05 vs 1.2±0.04 and 1.3±0.06ms/mmHg). Mental stress evoked similar increases in mABP and HR in WEs and SAs, but was accompanied by less depression of BRS in SAs than WE especially during down-sequences (to 0.69±0.11 vs 1.1±0.07*ms/mmHg, *: P<0.05, WE vs SA). Further, in the time domain, RMSSD and pRR50, indices of vagal activity, were depressed during mental stress in SAs only, whereas following mental stress BRS during squat to stand, an index of cardiac sympathetic activity was depressed in WEs only (0.6±0.04 to 0.3±0.09* vs 0.6±0.09 to 0.5±0.11ms/mmHg). During slow breathing, ABP tended to decrease in both WEs and SAs (P,0.05; P=0.09 respectively), but SDRR increased in SAs only.

These results suggest that baroreflex regulation of ABP is disturbed in young adult SAs relative to WEs, with SAs showing more persistent vagally-mediated

tachycardia in response to falls in ABP during mental stress than WEs. Moreover, whereas, WEs showed depressed baroreflex sympathetically-mediated tachycardia for several minutes following mental stress, this was not the case in SAs. Such disturbances in SAs may be early markers of future CVD and hypertension in SAs. However, the finding that increased vagal activity in young SAs but not WEs raises the possibility that practice of regular slow breathing may help restore respiratory-cardiovascular interactions in SAs and so help limit CVD.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

Appendix 3.3 The abstract presented orally at the Experimental Biology international conference 2021 and published in FASEB Journal.

THE FASEB JOURNAL

Physiology | Free Access

Evidence that Young Normotensive South Asians Show Early Signs of Disturbed Arterial Blood Pressure (ABP) Regulation of in Acute Mental Stress Relative to White Europeans

Mobarak Alqahtani, Andrew Coney, Janice Marshall

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TOOLS SHARE

Abstract

The risk of cardiovascular disease (CVD) including hypertension is higher in South Asians (SAs) than White Europeans (WEs). But, whether disturbances in autonomic control of the cardiovascular system are present in early adulthood in SAs has not been tested. Thus, we performed studies on young 20 WEs and 21 SAs aged (21.9±0.36, 20.7±0.41 respectively, equal numbers of men and women in each group) in whom arterial blood pressure (ABP), electrocardiogram (ECG), heart rate (HR) and respiration were recorded at rest, during mental stress (3 min Colour Stroop test) and during 5 min slow breathing (6 breaths/min). Stroke volume (SV), cardiac output (CO), and total peripheral resistance (TPR) were computed from the pulse contour. Baroreflex sensitivity (BRS) was calculated by the sequence method as change in R-R interval evoked by spontaneous up and down sequences in systolic pressure (SP) at rest and during mental stress and as the relationship between R-R interval and the fall in SP evoked by standing from a squat before and at 0, 3, 6 min following the stress test. HRV was computed in time-domain during mental stress and slow breathing. Comparisons within and between WEs and SAs were done by paired and un-paired t-tests respectively. At rest, WEs and SAs had similar mean ABP (mABP, 86.9±1.42 vs 87.1±1.77mmHg), HR (71.1±2.45 vs 76.2±3.56 beats/min), CO (4.8±0.21 vs 5.2±0.21L/min), SV (76.5±3.86 vs 81.5±2.97ml), TPR (1.14±0.06 vs 1.01±0.04 resistance units), and BRS (up-sequence: 1.2±0.05, down-sequence: 1.2±0.05 vs 1.0±0.09 and 1.2±0.09ms/mmHg). Further, mental stress evoked similar increases in mABP and HR in WEs and SAs but was accompanied by an increase CO in WEs and by an increase in TPR in SAs. Concomitantly, vagal indices of HRV were depressed during mental stress in SAs only (RMSSD: 66.2±7.8 to56.2±11.4 and 67.6±8.7 to 50.0±6.5*ms, *: P<0.05 within WE or SAs). Further during up-sequences in SP, BRS was depressed in both SAs and WEs during mental stress, but during down-sequences, BRS was depressed in WE only (to 0.59±0.10 vs 0.96±0.08*ms/mmHg). Also, following mental stress, BRS during squat to stand, an index of vagosympathetic activity, was depressed in WEs only (0.5±0.04 at rest to 0.3±0.04* vs 0.5±0.06 to 0.5±0.06ms/mmHg). On the other hand, slow breathing, tended to decrease ABP in both WEs and SAs (82.1±1.8 to 79.2±2.1mmHg: P<0.05; 79.4±2.0 to 77.4±2.0mmHg: P~0.09 respectively) and increased vagal indices of HRV in both WEs and SAs (66.3±8.6 to 86.0±13.2*ms; 67.6±8.7 to 86.48.8*ms). These results suggest that during and after mental stress, young normotensive SAs show greater sympathetic vasoconstriction than WEs, and attenuated respiratory modulation of HR, while their ability to evoke baroreflex tachycardia by vagal inhibition and sympathetic activation is preserved relative to young WEs. Such disturbances in would be expected to increase the risk of future CVD and hypertension in SAs, particularly in those with high levels of daily stress. The finding that slow breathing increased vagal activity in young SAs raises the possibility that regular slow breathing may help to normalize autonomic regulation of ABP in SAs and limit future CVD.

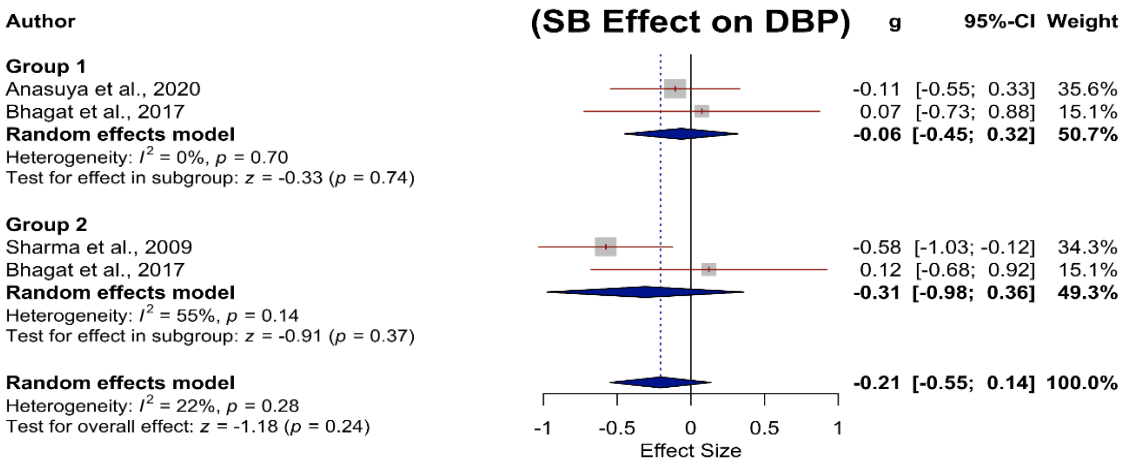
Appendix 5.1 Prisma checklist

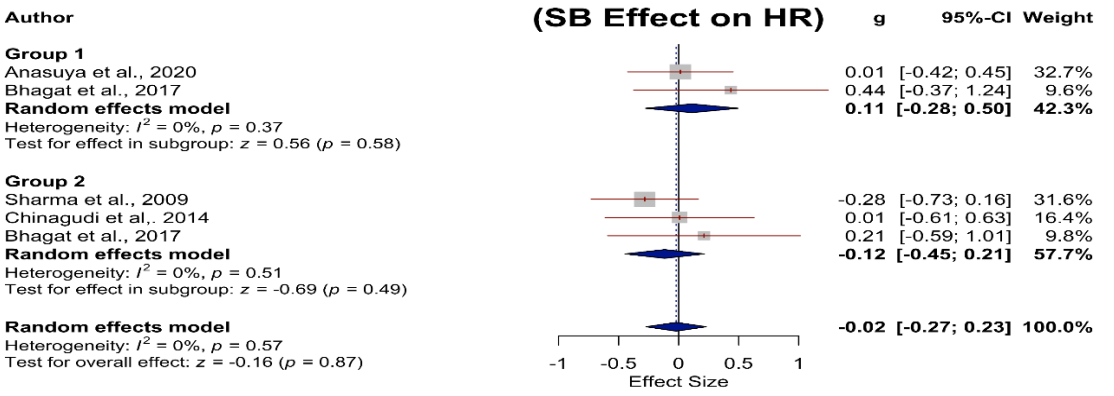
Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2&3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	4-6
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	6
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	7&8
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	9
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	9
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	11
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	11&12
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	8
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	8
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	12
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	12&13
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	12&13
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	12&13
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	12&13
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	12&13

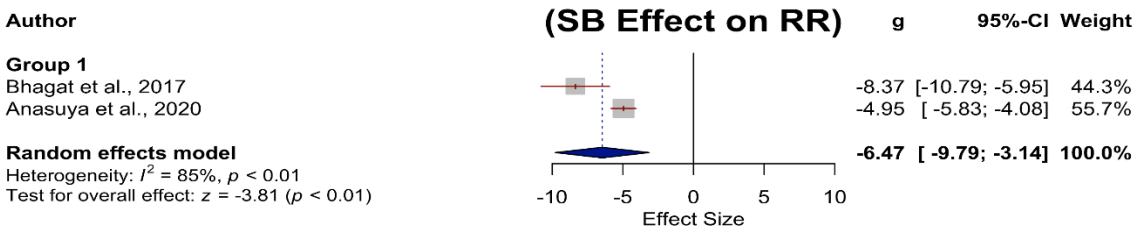
Section and Topic	Item #	Checklist item	Location where item is reported
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	12&13
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	13
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	14&15
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	NA
Study characteristics	17	Cite each included study and present its characteristics.	16&17
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	18&19
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	20-28
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	29-37
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	29-37
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	29-37
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	29-37
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	38-43
	23b	Discuss any limitations of the evidence included in the review.	38-43
	23c	Discuss any limitations of the review processes used.	38-43
	23d	Discuss implications of the results for practice, policy, and future research.	38-43
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	7
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	7
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	

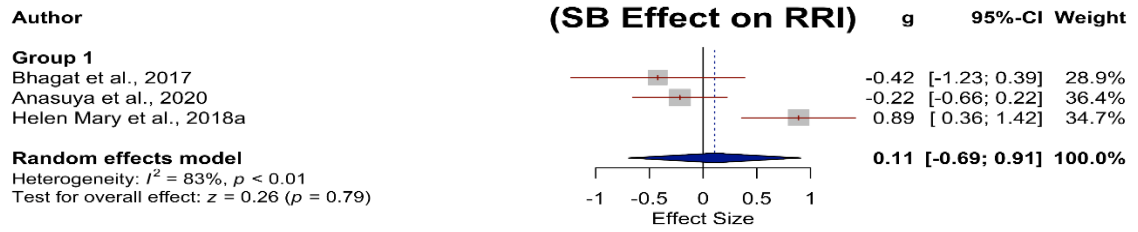
Section and Topic	Item #	Checklist item	Location where item is reported
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Appendix 5.2 DBP, HR, RR, and RRI during and immediately after a single session of SB









Appendix 5.3 Search strategy and terms

Medline:

1 exp Young Adult/ or exp Adult/ or Adult*.ti,ab. or Young Adult*.ti,ab.
2 exp Humans/ or Human*.ti,ab.
3 (Slow Breath* or deep Breath*).mp.
4 exp Breathing Exercises/
5 (Breathing Exercises or "slow breathing" or "guided breathing" or device-guided or Qigong or pranayama or "paced breathing" or "loaded breathing" or "controlled breathing" or Resperate or "yogic breathing").ti,ab.
6 RESPERATE*.mp.
7 (DEVICE-GUIDED SLOW BREATH* or DEVICE GUIDED SLOW BREATH*).mp.
8 exp Respiratory Rate/
9 exp Arterial Pressure/ or exp Blood Pressure/ or ABP.ti,ab. or SBP.ti,ab. or MAP.ti,ab. or DBP.ti,ab.
10 exp Heart Rate/ or HR.ti,ab.
11 exp Arterial Pressure/ or exp Blood Pressure/ or ABP.ti,ab. or SBP.ti,ab. or MAP.ti,ab. or DBP.ti,ab.
12 exp Heart Rate/ or HR.ti,ab.
13 exp Heart Function Tests/
14 RESPIRATORY FUNCTION*.ti,ab.
15 exp Pressoreceptors/ or exp Baroreflex/
16 (BAROREFLEX SENSITIV* or BRS).mp.
17 (HRV or HEART RATE VARIABILITY or RMSSD or SDRR or SDNN or SDSD or pNN50 or high frequency or HF or low frequency or LF or LF*HF ratio).ti,ab.
18 exp Yoga/
19 breath*.ti,ab
20 18 and 19
21 1 and 2
22 3 or 4 or 5 or 6 or 7 or 20
23 8 or 9 or 10
24 11 or 12 or 13 or 14 or 15 or 16 or 17
25 21 and 22 and 23 and 24

Embase:

1 exp Young Adult/ or exp Adult/ or Adult*.ti,ab. or Young Adult*.ti,ab.
2 exp Humans/ or Human*.ti,ab.
3 (Slow Breath* or Deep Breath*).mp.
4 exp Breathing Exercises/
5 (Breathing Exercises or "slow breathing" or "guided breathing" or device-guided or Qigong or pranayama or "paced breathing" or "loaded breathing" or "controlled breathing" or Resperate or "yogic breathing").ti,ab.
6 RESPERATE*.mp.
7 (DEVICE-GUIDED SLOW BREATH* or DEVICE GUIDED SLOW BREATH*).mp.
8 exp breathing rate/
9 exp Arterial Pressure/ or exp Blood Pressure/ or ABP.ti,ab. or SBP.ti,ab. or MAP.ti,ab. or DBP.ti,ab.
10 exp Heart Rate/ or HR.ti,ab.
11 exp Arterial Pressure/ or exp Blood Pressure/ or ABP.ti,ab. or SBP.ti,ab. or MAP.ti,ab. or DBP.ti,ab.

12 exp Heart Rate/ or HR.ti,ab.
13 exp heart function test/
14 exp respiratory function/
15 exp pressoreceptor reflex/ or Baroreflex.mp.
16 (BAROREFLEX SENSITIV* or BRS).mp.
17 (HRV or HEART RATE VARIABILITY or RMSSD or SDRR or SDNN or SDSD or pNN50 or high frequency or HF or low frequency or LF or LF*HF ratio).ti,ab.
18 exp Yoga/
19 breath*.ti,ab
20 18 and 19
21 1 and 2
22 3 or 4 or 5 or 6 or 7 or 20
23 8 or 9 or 10
24 11 or 12 or 13 or 14 or 15 or 16 or 17
25 21 and 22 and 23 and 24

CINAHL

S1 (MH "Adult+") OR AB adult* OR TI adult* OR (MH "Young Adult") OR TI Young Adult*
S2 (MH "Human") OR AB Human* OR TI Human*
S3 TX SLOW BREATH* OR deep breath* OR deep breath exercis*
S4 (MH "Breathing Exercises+") OR AB Breath* Exercis* OR TI Breath* Exercis*
S5 TI (Breathing Exercises OR "slow breathing" OR "guided breathing" OR device-guided OR Qigong OR pranayama OR "paced breathing" OR "loaded breathing" OR "controlled breathing" OR Resperate OR "yogic breathing") OR AB (Breathing Exercises OR "slow breathing" OR "guided breathing" OR device-guided OR Qigong OR pranayama OR "paced breathing" OR "loaded breathing" OR "controlled breathing" OR Resperate OR "yogic breathing")
S6 TX RESPERATE*
S7 TX DEVICE-GUIDED SLOW BREATH* OR TX DEVICE GUIDED SLOW BREATH*
S8 (MH "Respiratory Rate") OR TI breath* rate OR AB breath* rate
S9 ((MH "Arterial Pressure+") OR (MH "Blood Pressure+") OR (MH "Systolic Pressure") OR (MH "Diastolic Pressure")) OR TI (Arterial Pressure or Blood Pressure or ABP or SBP or MAP or DBP) OR AB (Arterial Pressure or Blood Pressure or ABP or SBP or MAP or DBP)
S10 TI (Heart Rate or HR) OR AB (Heart Rate or HR) OR (MH "Heart Rate+")
S11 ((MH "Arterial Pressure+") OR (MH "Blood Pressure+") OR (MH "Systolic Pressure") OR (MH "Diastolic Pressure")) OR TI (Arterial Pressure or Blood Pressure or ABP or SBP or MAP or DBP) OR AB (Arterial Pressure or Blood Pressure or ABP or SBP or MAP or DBP)
S12 TI (Heart Rate or HR) OR AB (Heart Rate or HR) OR (MH "Heart Rate+")
S13 (MH "Heart Function Tests+") OR (MH "Respiratory Function Tests+")
S14 (MH "Pressoreceptors") OR TI Barore* OR AB Barore* OR TI pressore* OR AB pressore*
S15 AB BRS OR TI BRS OR TI BAROREFLEX SENSITIV* OR AB BAROREFLEX SENSITIV*
S16 (MH "Heart Rate Variability") OR TI ("Heart Rate Variability" OR "HRV" OR "RMSSD" OR "SDRR" OR "SDNN" OR "SDSD" OR "pNN50" OR "high frequency"

OR "HF" OR "low frequency" OR "LF" OR "LF*HF ratio") OR AB ("Heart Rate Variability" OR "HRV" OR "RMSSD" OR "SDRR" OR "SDNN" OR "SDSD" OR "pNN50" OR "high frequency" OR "HF" OR "low frequency" OR "LF" OR "LF*HF ratio")

S17 (MH "Yoga+")
S18 TI breath* OR AB breath*.
S19 S17 AND S18
S20 S1 AND S2
S21 S3 OR S4 OR S5 OR S6 OR S7 OR S19
S22 S8 OR S9 OR S10
S23 S11 OR S12 OR S13 OR S14 OR S15 OR S16
S24 S20 AND S21 AND S22 AND S23

Web of Science

1 TOPIC: (adult*) OR TOPIC: (young NEAR adult*)
2 TOPIC: (Human*)
3 TOPIC: (SLOW NEAR/3 BREATH*) OR TOPIC: (deep NEAR/3 breath*) OR TOPIC: (deep* NEAR/3 breath* NEAR/3 exercis*)
4 TS=(Breath* Exercis*)
5 TOPIC: (RESPERATE*)
6 TOPIC: (DEVIC* NEAR/3 GUID* NEAR/3 SLOW NEAR/3 BREATH*)
7 TS=("breathing exercises" OR "breathing exercise" OR "slow breathing" OR "guided breathing" OR device-guided OR Qigong OR pranayama OR "paced breathing" OR "loaded breathing" OR "controlled breathing" OR Resperate OR "yogic breathing")
8 TS=(breath* NEAR/3 rate) OR TS=(Respira* NEAR/3 rate)
9 TS=(Arteri* NEAR/3 Press*) OR TS=(Blood NEAR/3 Press) OR TS=(Systo NEAR/3 Pressure) OR TS=(Diasto NEAR/3 Pressure) OR TS=(ABP) OR TS=(SBP) OR TS=(MAP) OR TS=(DBP)
10 TOPIC: (Heart NEAR/3 Rat*) OR TOPIC: (HR)
11 TS=(Arteri* NEAR/3 Press*) OR TS=(Blood NEAR/3 Press) OR TS=(Systo NEAR/3 Pressure) OR TS=(Diasto NEAR/3 Pressure) OR TS=(ABP) OR TS=(SBP) OR TS=(MAP) OR TS=(DBP)
12 TOPIC: (Heart NEAR/3 Rat*) OR TOPIC: (HR)
13 TOPIC: (Heart NEAR/4 Funct* NEAR/4 Test*)
14 TOPIC: (Respira* NEAR/4 Funct* NEAR/4 Test*)
15 TOPIC: (Baroreflex*) OR TOPIC: (Pressoreceptor*) OR TOPIC: (Baro* NEAR reflex) OR TOPIC: (Baro*) OR TOPIC: (Presso*) OR TOPIC: (Presso* NEAR reflex)
16 TOPIC: (BARO* NEAR SENSITIV*) OR TOPIC: (BRS)
17 TS=(Heart Rat* Variab*) OR TS=(Heart NEAR rat* NEAR Variab*) OR TS=(HRV) OR TS=(RMSSD) OR TS=(SDRR) OR TS=(SDNN) OR TS=(SDSD) OR TS=(pNN50) OR TS=(high NEAR/4 freque*) OR TS=(HF) OR TS=(low NEAR/4 freque*) OR TS=(LF) OR TS=(LF*HF NEAR/4 rat*)
18 #2 AND #1
19 #7 OR #6 OR #5 OR #4 OR #3
20 #10 OR #9 OR #8
21 #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11
22 #21 AND #20 AND #19 AND #18

Cochrane:

1 MeSH descriptor: [Young Adult] explode all trees
2 MeSH descriptor: [Adult] explode all trees
3 MeSH descriptor: [Humans] explode all trees
4 Slow breathing
5 Slow breath*
6 MeSH descriptor: [Breathing Exercises] explode all trees
7 MeSH descriptor: [Yoga] explode all trees
8 Resperate*
9 Device-guided slow breath*
10 Device guided slow breath*
11 Tidal volume* OR VT OR TV
12 MeSH descriptor: [Tidal Volume] explode all trees
13 MeSH descriptor: [Respiratory Rate] explode all trees
14 MeSH descriptor: [Arterial Pressure] explode all trees
15 Arterial Pressure OR Systolic OR Diastolic OR Mean Arterial Pressure OR ABP OR SBP OR DBP OR MAP
16 MeSH descriptor: [Blood Pressure] explode all trees
17 MeSH descriptor: [Heart Rate] explode all trees
18 MeSH descriptor: [Heart Function Tests] explode all trees
19 MeSH descriptor: [Respiratory Function Tests] explode all trees
20 MeSH descriptor: [Pressoreceptors] explode all trees
21 MeSH descriptor: [Baroreflex] explode all trees
22 BAROREFLEX SENSITIV* OR BRS
23 HRV or HEART RATE VARIABILITY or RMSSD or SDRR or SDNN or SDSD or pNN50 or high frequency or HF or low frequency or LF or LF*HF ratio
24 #1 OR #2 OR #3
25 #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10
26 #11 OR #12 OR #13 OR #14 OR #15 OR #16 #17
27 #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23
28 #24 AND #25 AND #26 AND #27