Regulation of arterial blood pressure:
effects of arterial stiffness and respiration

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Declaration of originality

I hereby declare that the work presented in this thesis has not been submitted for a higher degree to any other university or institution. To the best of my knowledge this submission contains no material previously published or written by another person, except where due reference is stated otherwise. Any contribution made to the research by others is explicitly acknowledged.

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Conflict of interest

The research was conducted in the absence of any commercial or financial relationships that could be constructed as potential conflict of interest.

Abstract

Background. Baroreceptors (arterial stretch receptors) regulate blood pressure (BP). Arterial stiffness increases with age, leading to decreased baroreflex sensitivity (BRS) and altered autonomic nervous system (ANS) feedback. Slow breathing, through stretch receptor activation and BRS and ANS feedback, is said to lower BP. This study aimed (i) to confirm relationships between BRS, ANS, and arterial stiffness, and (ii) to quantify haemodynamic changes with slow breathing.

Methods. In 30 healthy subjects (20-66 years, 15 female), arterial stiffness was determined by carotid-femoral pulse wave velocity (cfPWV) and aortic augmentation index (aAIx), measured non-invasively. R-R interval and finger BP was continuously measured during 10 minutes each free breathing, and 0.1Hz breathing rate with 50% and 70% inspiration. BRS was estimated from spontaneous changes in systolic BP and corresponding interbeat interval (sequence technique). ANS (sympathetic and parasympathetic) input was measured through low and high frequency components of heart rate variability normalized to total power.

Results. BRS was not significantly correlated with cfPWV (R^2=0.06, p=0.223) but was with aAIx (R^2=0.15, p=0.036) and sympathetic activity (R^2=0.05, p=0.042). BP did not change with slow breathing (brachial systolic BP, free breathing: 117±13 mmHg, 0.1Hz/50% inspiration: 117±13 mmHg, 0.1Hz/70% inspiration: 118±13 mmHg, p=0.817), nor did BRS (p=0.103) but sympathetic activity increased (0.27±0.11, 0.41±0.19, 0.39±0.18 respectively, p<0.001).

Conclusion. BRS was correlated with aAIx but not with aortic stiffness as measured by cfPWV. Slow breathing increased sympathetic activity but did not alter BP. It is proposed that compensatory effects (sympathetic activity) overrides the BP reduction effects of slow breathing.

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List of Abbreviations

ABBR	abbreviation in full
AGE	Advanced glycation end product
AIx	Augmentation index
ANS	Autonomic nervous system
BP	Blood pressure
BRS	Baroreceptor sensitivity
aBRS	Aortic baroreceptor sensitivity
bBRS	Brachial baroreceptor sensitivity
cBRS	Central baroreceptor sensitivity
pBRS	Peripheral baroreceptor sensitivity
CAN	Cardiac autonomic neuropathy
cfPWV	Carotid femoral pulse wave velocity
DBP	Diastolic blood pressure
ECG	Electrocardiogram
ESH	European society of hypertension
FFT	Fast fourier transform algorithm
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
LF	Low frequency
MAP	Mean arterial pressure
Mϕ	Macrophage
MMP	Matrix metalloproteinase
PP	Pulse pressure
PWV	Pulse wave velocity
VLF	Very low frequency
VSMC	Vascular smooth muscle cells

1   Introduction

According to the World Health Organization, around 22% of adults globally had raised blood pressure in 2014 (www.who.int). Hypertension is defined as the systolic blood pressure above 140 mmHg and diastolic blood pressure above 90 mmHg (Mancia et al., 2013). The Framingham Heart Study which involved; the contribution of more than half a century of work, through which the investigators provided a significant insight into the epidemiology and the cardiovascular disease outcomes associated with hypertension, also showed the relation of systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial pressure (MAP) with cardiovascular disease outcomes (Franklin and Wong, 2013). Several alterations occur in the morphology and functions of the cardiovascular system with ageing, the arteries becoming stiffer and the aortic mechanical properties are altered. It is hypothesised that arterial hypertension with ageing is predominantly a result of loss of arterial compliance (Benetos et al., 1993; Kawasaki et al., 1987; O'Rourke, 1995).

1.1   Artery stiffness

Arteries are composed of three concentric layers from the lumen to the outside consisting of the tunica intima, tunica media, and tunica adventitia (Figure 1.). The innermost intimal layer is further made up of two layers: a basal layer and a musculoelastic layer. The basal lamina consists of endothelial cells lying on the basement membrane made up of a proteoglycan rich matrix and collagen fibers. The second layer of the intima, the musculoelastic layer, contains elastic fibers, collagen fibers and smooth muscle cells. The tunica media is composed of lamellar units of collagen fibers, elastic fibers, smooth muscle cells, mucopolysacharide, viscoelastic gel and ground substance. This layer forms the major bulk of the artery and contributes most to the elastic property of the artery. The outermost layer, the tunica adventitia, is made up of circumferentially arranged wavy collagen fibers with elastic fibers, loose connective tissue and fibroblasts.
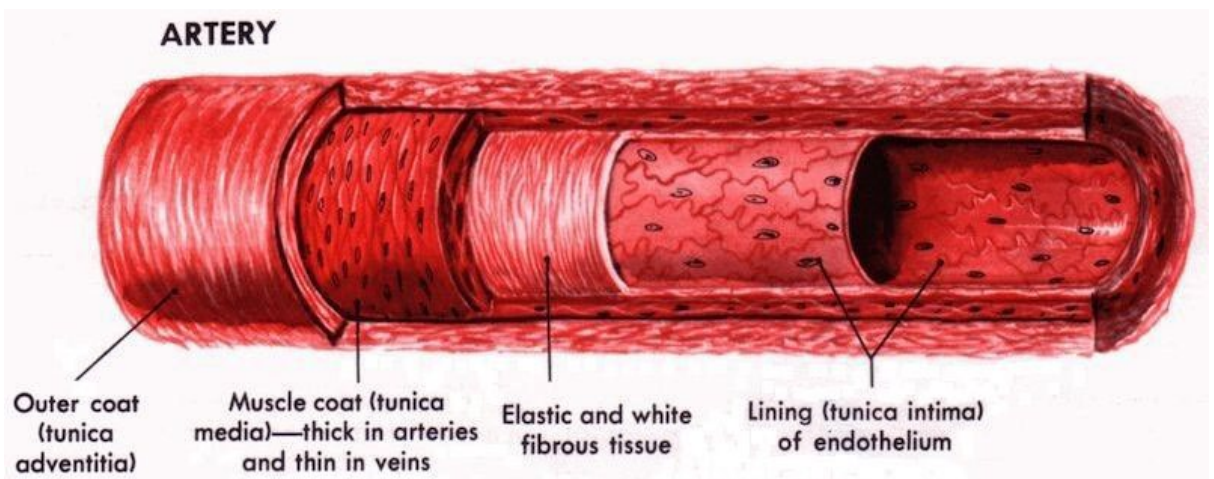
Figure 1: Structure of Artery (www.blendspace.com). The walls of arteries are made of three concentric layers, from lumen to outside, tunica intima composed of single layer of endothelial cells and small amount of subendothelial connective tissue, intima separated from media by dense elastic membrane. Tunica media is thickest layer and composed of smooth muscle cells, elastic fibres and connective tissue. Media separated from adventitia by dense elastic membrane external elastic lamina. Tunica adventitia composed of connective tissue, nutrient vessels and autonomic nerves.

Ageing, genetic and environmental factors are responsible for the structural and functional changes in the arterial wall (Figure 2) (Avolio, 1995). In the tunica media the elastic fibers undergo fatigue, fracture and degradation which then increase the load on the stiffer collagen fibers (Avolio et al., 2015). Calcification of the media (London, 2003), hypertrophy and accumulation of extracellular matrix can also take place. The endothelial cells in the tunica intima may decrease the release of vasodilator substance and increase the synthesis of vasoconstrictor substance (Sandoo et al., 2010).

One of the hallmarks of arterial ageing is stiffness, with ageing arteries thickening, dilating and becoming stiffer (Greenwald, 2007; Lakatta and Levy, 2003). As the arteries stiffen they lose their capacity to buffer the pulsatile volume output of the left ventricle. Increased level of stress on the arterial wall due to increase the aortic pressure will in turn increase the ventricular load with decrease in the myocardial perfusion and thus increasing the risk of cardiovascular events.
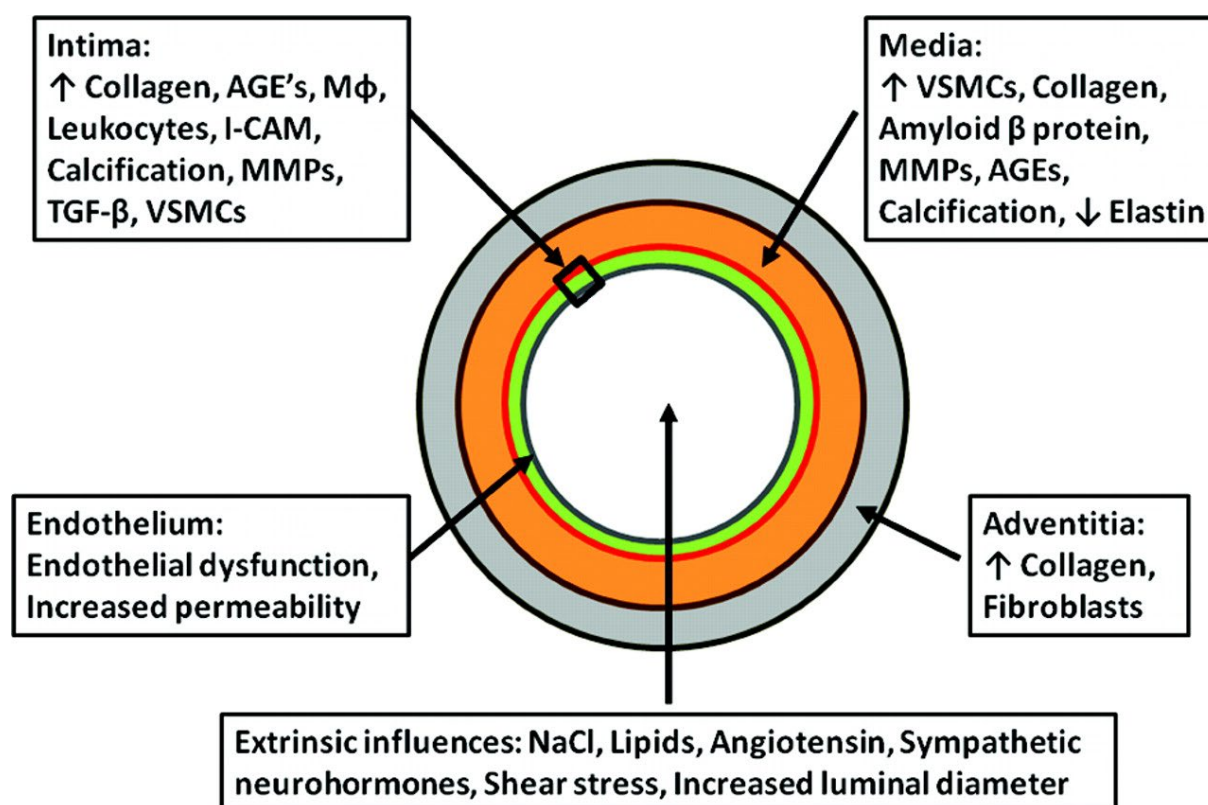
Figure 2: Causes of arterial stiffness (Zeiman et. al.) Summary of multiple known causes of increased arterial stiffness. AGE indicates advanced glycation end product. Mφ, macrophage: TGF-β, transforming growth factor-β and VSMC's, vascular smooth muscle cells, MMP matrix metalloproteinases, I-CAM intercellular cell adhesion molecule (Zieman, 2005).

The biomechanical properties of the arteries are measured using a variety of parameters and techniques, which has become important intervention to the epidemiological studies and cardiovascular research. There are many ways in which arterial stiffness can be measured non-invasively, including: measuring the pulse wave velocity (PWV) along an arterial length; measuring distensibility by; relating the change in diameter of the artery to distending pressure; and assessing arterial wave form features related to wave reflection. A commonly used non-invasive measurement of arterial stiffness is PWV. The pressure pulse generated during ventricular systole is propagated along the arterial tree; its speed is proportional to the elastic and geometric properties of the arterial wall, with PWV magnitude increasing with vessel stiffness (Farrar et al., 1991).

1.2 Baroreflex sensitivity

 An important nervous regulatory mechanism of blood pressure homeostasis is the baroreflex control system. The aortic arch and the carotid sinuses contain stretch receptors called the baroreceptors which detect changes in blood pressure (figure 3). These receptors then signal to the brain and through a negative feedback mechanism communicated through the sympathetic and parasympathetic system, regulate blood pressure and help maintain a continuous flow of blood to the brain and other vital organs. Arterial stiffening in the baroreceptor regions decreases the baroreceptor sensitivity by limiting the stretch and relaxation of the baroreceptors in response to the change in blood pressure (Kaushal and Taylor, 2002). Baroreflex sensitivity (BRS) is defined as the proportional reduction in the heart rate (HR) due to increased blood pressure. There is an increase in the sympathetic activity caused by impairment of arterial baroreflex (Guzzetti et al., 1988; Lucini et al., 1994; Pfeifer et al., 1983; Shimada et al., 1985).

Arterial hypertension is characterised by altered autonomic regulation, as indicated by reduced heart rate variability (HRV) (Lage et al., 1993), sympathetic hyperactivity (Curtis and O'Keefe, 2002; Gorman and Sloan, 2000) and blunted BRS (Pomeranz et al., 1985; Shannon et al., 1987). The impairment of baroreceptor function plays an adverse role in several cardiovascular diseases, hence the assessment of the baroreceptor sensitivity has gained importance in laboratory and clinical settings (Parati et al., 2000). Different methods are available for assessing the arterial baroreflex function, such as carotid sinus massage, electrical stimulation of carotid sinus nerves, occlusion of the common carotid artery, lower body negative pressure application, intravenous stepwise infusion of vasoactive agents, neck chamber technique and Valsalva manoeuvre. Most of them require the application of external stimulus to the subject under evaluation and have other limitations such as poor reproducibility, heavy interference with the neural mechanisms under evaluation, considerable invasiveness and non-negligible risk for the subject to be evaluated. These techniques are now replaced by newer non-invasive methods (Parati et al., 2000).

BRS can now be assessed without the use of any external intervention on the subject under evaluation both in laboratory conditions as well as to investigate dynamic features of baroreflex modulation of heart rate in daily life (Parati et al., 1996). These methods includes the sequence technique, R-R interval and SBP cross- correlation, modulus of R-R interval and SBP transfer function at 0.1Hz, squared ratio of R-R interval/ SBP spectral powers at 0.1 and 0.3Hz (α coefficient), closed loop R-R interval-SBP transfer function and statistical dependence of R-R interval on SBP fluctuations. All these methods are based on combined computer analysis of spontaneous blood pressure and heart rate fluctuations which justifies the term spontaneous baroreflex.
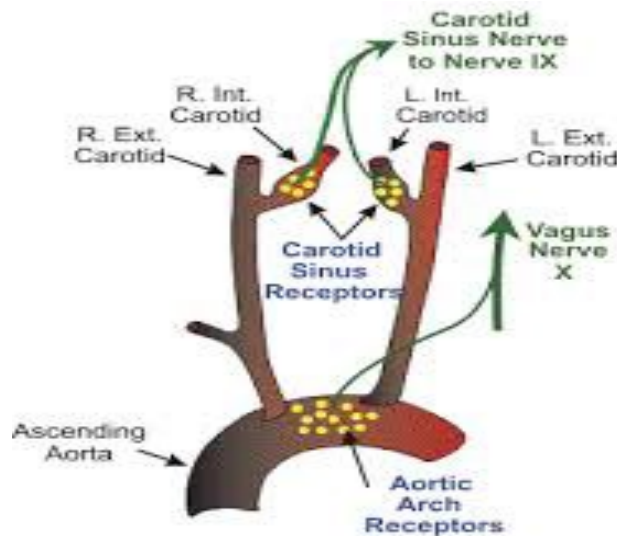
Figure 3: Location and innervation of Baroreceptors (www.cvphysiology.com). Most important Arterial baroreceptors are located in the carotid sinus and aortic arch, carotid sinus baroreceptors are innervated by the sinus nerve of Herring which is branch of glossopharyngeal nerve (1X cranial nerve). The aortic arch baroreceptors are innervated by the aortic nerve which combines with vagus nerve (X cranial nerve).

1.3 Slow breathing as a means of modifying blood pressure

Baroreflex sensitivity can be enhanced significantly by slow breathing both in the healthy and diseased individuals. The mechanism involved is through a relative increase in vagal activity, reduction in the sympathetic activity, small reduction in the heart rate and reduction in both systolic and diastolic blood pressures. Slow breathing also improves the resting oxygen saturation (Bernardi et al., 1998).

 The reduced breathing rate is compensated by increase in the tidal volume to maintain the per minute ventilation (Bernardi et al., 1998; Goso et al., 2001). Sympathetic activity increases with faster breathing rates and decreases with higher tidal volume (Goso et al., 2001; Naughton et al., 1998a). The lung inflation which increases with the decreasing breathing rates, stimulates the pulmonary stretch receptors and this neural activity serves as an input to the medulla and is integrated with the information about the level of arterial blood pressure generated by the arterial baroreceptors (Schein et al., 2001) and as a response to the elevated blood pressure levels or lung inflation, vasodilation occurs in a number of territories such as the limbs, skin, kidney and splanchnic vascular bed (Bernardi, 2002). A generalized decrease in the excitatory pathways regulating respiratory and cardiovascular system is induced by slow breathing. Both the respiratory system as well as the cardiovascular system shares similar control mechanisms, thus alterations in one of the systems will modify the functioning of the other. Therefore we can expect that the modification in the respiratory control would in turn affect the control of the cardiovascular system and as breathing is under voluntary control, it is theoretically possible to induce such changes by voluntary modifications of breathing.

The increase in the baroreflex sensitivity does not depend on the regularization obtained by controlling the breathing but it depends on slow breathing. Slow breathing done at the rate of 6

cycles/min for at least 10mins daily for weeks has been shown to decrease the blood pressure significantly (Harneet and Saravanan, 2014).

Slow breathing is said to improve BRS, through the increase in the vagal activity and decrease in the sympathetic activity.

1.4 Aims

The aims of this study are:

(1) To characterise the age related changes in arterial stiffness

(2) To study and confirm the previously found relationship between baroreceptor function, and arterial stiffness.

(3) To study and confirm the previously found relationship between baroreceptor function and sympathetic and parasympathetic activity measured using heart rate variability.

(4) To study and confirm the previously found relationship between baroreceptor function and sympathetic and parasympathetic activity measured using heart rate variability and arterial stiffness.

(5) To study the cardiovascular response to slow breathing.

2 Material and Methods

2.1 Subjects

Recruited participants were healthy without any history of respiratory or cardiovascular disease and were not taking any medicines that affected autonomic nervous regulation. Subjects were also excluded if they participated in sports at a competitive level. Subjects were asked not to consume caffeinated food or drink for five hours preceding the study and not to participate in heavy exercise for at least 3 hours preceding the study. All subjects provided informed, written consent, and the experimental protocol was approved by the Macquarie University Human Research Ethics Committee (Reference number 5201500263).

2.2 Experimental protocol

Information was collected on self-reported medical conditions and/or medications, and cardiovascular risk factors (modified questionnaire from Myers et al., 2009). Height and weight were measured. Standing height (cm) was measured using the altimeter with the participant's shoes removed and recorded to the nearest centimetres (cm). Body weight was measured using a digital scale and recorded to the nearest (kg).

Participants were then seated and instructed on how to breathe with a breathing metronome (Application name: Paced breathing pro, developer: Trex LLC) practiced the different breathing patterns and corrected if they were not inspiring or expiring at the required rate/depth. After at least five minutes seated rest, the blood pressure and carotid femoral pulse wave velocity (cfPWV) measurements were taken in seated position (SphygmoCor XCEL, AtCor Medical, Sydney, Australia). Blood pressure readings were repeated until two consecutive readings were within 5mmHg for both systolic and diastolic pressure. The same was applied to cfPWV measurements, with a limit of ±0.5 m/s. Then the participants were told to lie supine on the bed

and asked to rest for a period of five minutes, during which, the following devices were then attached:

- NexFin finger cuff

- electrocardiogram (ECG) leads

- Pulse oximeter

- Brachial cuff for SphygmoCor XCEL

- Femoral cuff for SphygmoCor XCEL

Body surface measurements were then made for the cfPWV measurement. After this the room was darkened by switching off the lights. Continuous recoding of blood pressure (NexFin finger cuff) and ECG was done for a period of 10 minutes without interruption, in the random order for the following breathing patterns:

- Free breathing

- 6 breaths/minute, with 50% of each breathing cycle being inspiration

- 6 breaths/minute, with 70% of each breathing cycle being inspiration

At the end of the breathing pattern brachial/aortic blood pressure measurement was recorded using the SphygmoCor XCEL device. The pulse wave velocity measurement was also taken with the SphygmoCor XCEL device. The hands and fingers were regularly checked for any signs of tissue hypoxia.

2.3   Blood pressure measurements

2.3.1   Pulse wave analysis

Several devices are available that allow the non-invasive recording of the arterial waveform and the generation of proximal aortic pressure profile. These devices have been validated in catheterization laboratories using invasive pressure measurements (Pauca et al., 2001). When using non-invasive pressure measurements they have been chosen to track changes in central aortic pressure. Pulse waveforms are either obtained using the tonometer (handheld or stationary), which captures the radial waveform or by oscillometric methods which uses a cuff encircling the limbs. Both methods produce a waveform conventionally from either the brachial artery (oscillometric cuff based assessment) or radial artery (tonometric assessment), which is subjected to a general transfer algorithm to produce a central pressure profile (figure 5).

A SphygmoCor 'XCEL unit (AtCor Medical, West Ryde, Australia) was used to perform the pulse wave analysis (Figure 4). This device records the volume displacement waveform from the brachial cuff, with the cuff inflated to 10mmHg below diastolic blood pressure (Butlin et al., 2012). Briefly the cuff was placed on the upper right arm to measure the brachial blood pressure and the volumetric displacement waveform to calculate central aortic blood pressure, augmentation index and augmented pressure. After the participant had been quietly resting for

five minutes, at least three consecutive measurements were taken with measurements repeated until two consecutive readings were within 5mmHg for both systolic and diastolic pressure.

2.3.2 Augmentation index (AIx)

Arterial pressure wave is a composition of forward pressure wave arising from the left ventricular ejection and backward (reflected pressure wave). Backward wave occurs due to impedance mismatch at the arterioles and branching points of arteries. The interaction between forward and reflected pulse wave is assessed by PWA and expressed as AIx. Normally the reflected wave arises at the aortic root during diastole and augments the coronary circulation increased stiffness in the aorta increases aortic PWV and the reflected wave returns faster and earlier at the aortic root during late diastole when the ventricle is still ejecting blood adding the reflected wave to the forward wave augmenting the central systolic pressure.

AIx is calculated as (augmentation pressure) divided by the pulse pressure (PP) X 100 to give a percentage value.

Figure 4: SphygmoCor XCEL (www.atcormedical.com). SphygmoCor XCEL provides measurement of central blood pressure or PWV. The brachial cuff is placed on the arm, over the brachial artery. This device records the brachial systolic and diastolic blood pressure, and then captures the brachial waveform. The brachial waveform is then analysed by the sphygmoCor brachial general transfer function to provide central aortic waveform and central blood pressure measurements like central aortic systolic BP, central PP, AIx.
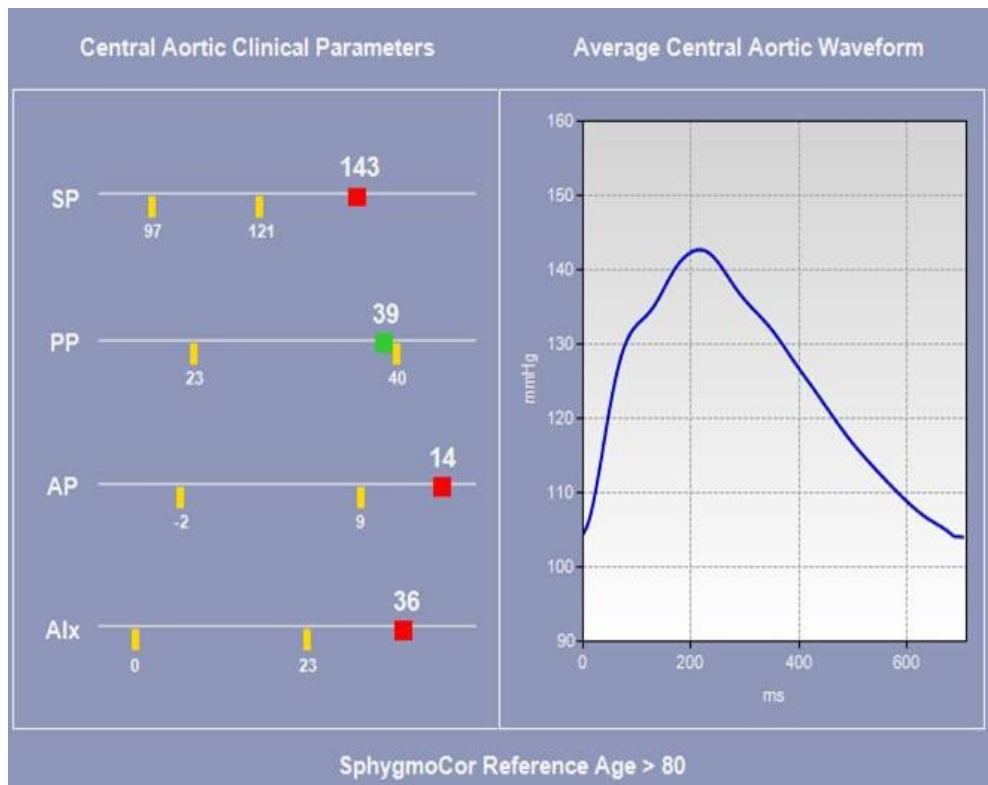
Figure 5: SphygmoCor XCEL report showing central clinical parameters and central aortic waveform (www.atcormedical.com)

2.3.2.1 Non-invasive continuous arterial pressure measurement

The NexFin device (Edwards Life Sciences, Irvine CA) measures the blood pressure continuously and non-invasively with an inflatable cuff placed around the middle finger at the middle phalanx (figure 6). This device is based on the volume clamp method of Penaz which works on the concept of pulsatile unloading of the finger arterial walls using an inflatable cuff with an inbuilt photoelectric plethysmograph (Penaz, 1985). Monitor calculates the cardiac output by the pulse contour method while the blood pressure is being continuously measured. The continuous finger blood pressure is transformed to a brachial artery waveform and the pulsatile systolic area is determined for each heartbeat. Using the arterial impedance, the device calculates cardiac output and stroke volume and determines the index values using patient's sex, height and weight (Akkermans et al., 2009; Eeftinck Schattenkerk et al., 2009). The device can be uncomfortable when used for long periods as the finger cuff pressure is greater than the venous pressure and occludes the venous return. In this study, only the finger systolic, mean, and diastolic values were used in analysis. Derived variables (brachial pressures, cardiac output, total peripheral resistance) reported by the NexFin devices were not included in the analysis.

Figure 6: NexFin (www.edwards.com). This device measures blood pressure continuously and noninvasively with an inflatable cuff around the middle phalynx. While continuously monitoring the blood pressure the monitor calculates the cardiac output by pulse-contour method. Using arterial impedance, the device calculates cardiac output and stroke volume and determines index values using the data of sex, height and weight.

2.4 Pulse wave velocity

PWV is the measurement of the speed of the pressure waves that travel along the arterial segments. In practice, pulse wave velocity is calculated as the distance / travelling time of the waves between the two measuring arterial sites of the pulse (figure 7). In a non-invasive study the two measurement sites should lie on the peripheral artery that can be palpated from the body surface (usually the carotid and the femoral arteries for aortic pulse wave velocity)(Hirata et al., 2006). SphygmoCor XCEL (AtCor Medical, West Ryde, Australia) measures the arterial stiffness and wave reflections characteristics were used to perform the Carotid femoral pulse wave velocity(cfPWV) .The XCEL device measures the augmentation index (AIx) using a partially inflated brachial cuff and the carotid-femoral pulse wave velocity (cfPWV) using partially inflated femoral cuff together with the carotid applanation tonometry (figure 8). The measurement of cfPWV and AIx with XCEL are valid, highly reliable and are not influenced by the side of the body on which the measurements are obtained (Hwang et al., 2014). This device is less time consuming unlike the other tonometry based devices because (1) the femoral and carotid signals are acquired simultaneously and (2) it does not require the acquisition of femoral artery applanation tonometry that oftentimes can be challenging due to the location of the measurement site. The cuff was placed over the femoral artery on the upper thigh region to capture the femoral waveform and the tonometer pressure sensor was placed over the skin of the neck to capture the carotid waveform. At least two consecutive measurements were taken for the seated and supine measurements.

The principal determinants of PWV can be described using the Moens-Korteweg equation that was derived in the 1920´s, and relates the velocity of the pulse wave travelling in a vessel to the material stiffness of that vessel.

$$PWV = \sqrt{\left(\frac{Eh}{2R\rho}\right)}$$

Where E is the Young´s modulus of the arterial wall in the circumferential direction, h is the vessel wall thickness, R is the vessel radius and ρ is the density of fluid (blood).

In a given blood vessel filled with blood having a fixed density, PWV is proportional to the square root of the Young´s modulus of elasticity of the velocity. In more simple terms, the stiffer the vessel the faster the PWV. The measurement of PWV is done by measuring the time taken for a pulse wave to travel a specified distance; the distance being divided by the time to give the velocity. Distance is usually measured making use of a tape, whilst timing is performed by measuring the interval between points on a pressure or flow waveform using the proximal and distal sensor. Usually the 'foot' of the waveform is used as the reference point since this is the part which is least affected by the wave reflections (Asmar et al., 1995). The time delay between the arrivals of the pulse wave at two sites is obtained by simultaneously measuring it or by recording the waveforms separately by comparing the time delay at both sites to a defined point on an ECG. Disadvantages with PWV measurement includes the inaccessibility of the central arteries hence making it necessary to compromise by using the nearest superficial arteries. There can also be some difficulty in estimating the actual arterial distance between recording sites using only surface measurements.

PWV will vary from one vessel to the other, with PWV greater in the peripheral arteries than the central arteries. For a healthy middle aged adult, the typical velocity in the ascending aorta would be of the order of 4m/s compared with the brachial artery where it is of the order 8m/s (Latham et al., 1985). This may be explained by the differences in the elastic properties of the various arteries in the body, the varying cross sectional area of the blood vessels and the modifying blood pressure profile along the arterial tree. With increasing distance from the heart along the arterial tree there is an increase in the SBP and hence the PP, the internal cross sectional area of the arteries decreases affecting vascular impedance and the ratio of collagen to elastin within the arterial wall increases.

2.4.1   Carotid femoral PWV

cfPWV has been recommended by the European society of Hypertension and European society of cardiology since 2007. This is the most simple, robust and reproducible non-invasive way to estimate aortic stiffness. The travel distance between the two arterial points should be accurate because even small differences will influence the absolute value of the PWV (Boutouyrie and Vermeersch, 2010; Laurent et al., 2006). However there is no general agreement as to how this distance is ideally measured in case of carotid femoral pulse wave velocity. There are different methods in which the distance can be measured which includes, direct distance between carotid and femoral measurement sites (Asmar et al., 1995b; Avolio et al., 2015b), subtract the distance from the sternal notch to the carotid measurement site from the total distance between the carotid artery to the femoral artery measurement site (Blacher et al., 1999b; Sugawara et al., 2008) or distance from the femoral site (van der Heijden-Spek et al., 2000; Mitchell et al., 2004; Weber et al., 2009). In this study, the subtraction method was used as this is the method recommended by the manufacturer (AtCor Medical) of the device used.

In the cfPWV measurements, the distance between the two arterial sites is measured, over the body surface, by making use of the tailor's tape (figure 9). In one of the studies (Huybrechts et al., 2011) the measurement of the distance between the different sites like the (common carotid – femoral artery, carotid artery and the suprasternal notch, carotid artery and the sternal notch, suprasternal notch and the femoral artery) was compared with real path length measured by MRI in which they showed that all the tape measured values for all the sites were significantly larger except for the common carotid and the femoral artery and the umbilicus femoral artery. Their study also showed that the measured tape values in females were significantly larger than the real MRI measurements.

Studies have also shown that the aorta becomes more tortuous with increasing age (Benetos et al., 2009; Salvi et al., 2004; Weber et al., 2009).

In persons with significant abdominal obesity or women with bigger bust size, performing correct straight tape measurements is more difficult and could result in an over estimation of the true arterial path. This overestimation will result in bias for the calculation of the absolute value for PWV.

In healthy subjects with less stiff arteries, the pressure wave reflected from the periphery reaches the aortic arch and auguments the pressure wave during the diastolic period. However, when the arteries are stiff, the velocity of the wave increases and the reflected wave reaches the heart earlier which is in systole, resulting in elevated SBP. As a result the ventricular load increases, leading to left ventricular hypertrophy and compromises coronary blood flow increasing the risk of myocardial ischemia (Sutton-Tyrrell et al., 2005). In man, it is independently associated with carotid and femoral plaques so also coronary calcification with and without coronary diseases (Oei et al., 2002). Elevated cfPWV is a risk factor for age related disorders like coronary disease, cognitive impairment and kidney diseases, suggesting close relationship between artery stiffness, reflected by cfPWV (Hofmann et al., 2014). Studies have confirmed that the cfPWV is a strong predictor of severity of coronary artery diseases and is associated with age, sex, and BP values (Hofmann et al., 2014).

Some of the limitations of cfPWV includes a high level of operator skill in the acquisition of tonometric waveforms, the need to expose the upper thigh (in the case of tonometric acquisition of the femoral pulse), difficulty in recording the femoral pressure waveforms in patients with metabolic syndrome, obesity and peripheral arterial diseases and inaccurate measurements in abdominal obesity and large breast size.
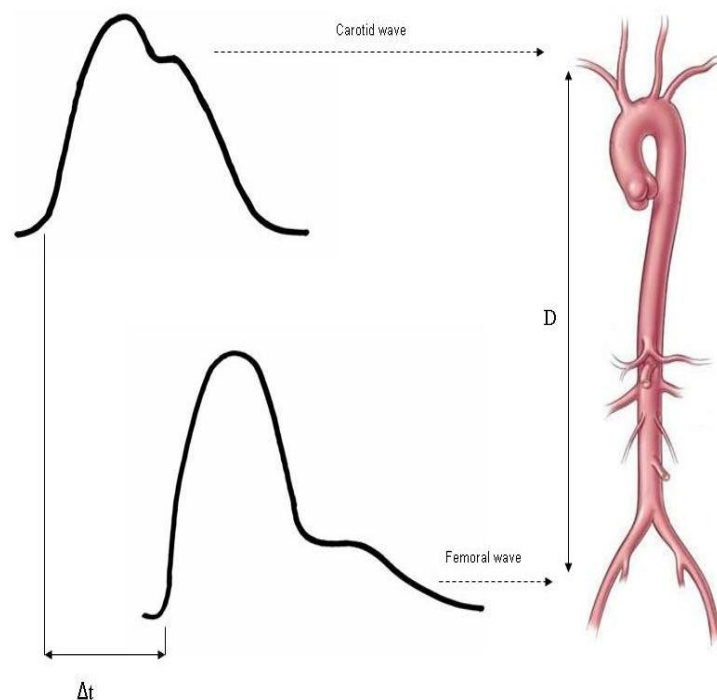
Figure 7: Pulse wave velocity determination, transit time is estimated by foot-to-foot method. The foot of the wave is defined at the end of diastole when the steep rise of the waveform begins. The transit time is the time of travel of the foot of the wave over a known distance. PWV=D (meters)/Δt (seconds).

Figure 8: cfPWV report showing the recorded carotid and femoral waveforms (top), the calculated cfPWV (13m/s, represented by blue dots), and where that cfPWV lies in comparison to a healthy population and European general population (www.atcormedical.com).

Figure 9: Measuring cfPWV (www.atcormedical.com). Cuff placed over the femoral artery to capture the femoral waveform and a tonometer placed over the skin of neck to capture the carotid waveform. Distance measurement made from carotid artery to suprasternal notch, suprasternal notch to the femoral cuff and from the femoral cuff to the femoral artery using tailors tape.

2.5   Electrocardiogram

A standard 3 lead electrocardiogram (ECG) was used to measure heart rate continuously for 10 minute periods during the three breathing patterns. Waveforms were recorded at 2 kHz. Recording and analysis, including identification of QRS peaks and heart rate variability analysis were done with LabChart Pro software and hardware (ADInstruments, Sydney, NSW, Australia)

2.6   Investigation of arterial baroreflex

The development of techniques that analyse the sensitivity of 'spontaneous' baroreflex control of heart rate has been an important step forward in the investigation of arterial baroreflex (Parati et al., 1996, 2000). The subject under evaluation does not require any external interventions in these techniques and can be used not only to assess BRS in standardized laboratory conditions but also to investigate the dynamic features of baroreflex modulation of heart rate in daily life. Some of the methods are (Parati et al., 2000):

- Sequence technique(sequences of beats where spontaneous systolic blood pressure changes are coupled with baroreflex mediated R-R interval changes).
- R-R interval – systolic blood pressure cross-correlation.
- Modulus of R-R interval- systolic transfer function at 0.1Hz.
- Squared ratio of R-R interval/ systolic blood pressure spectral powers at 0.1 and 0.3Hz (α coefficient).
- Closed-loop R-R interval- systolic blood pressure transfer function.
- Statistical dependence of R-R interval on systolic blood pressure fluctuations

2.6.1   The sequence technique

This technique is based on the computer identification in the time domain of spontaneously occurring sequences of four or more consecutive beats which is characterised by either a progressive increase in the systolic blood pressure and lengthening in the R-R interval or by progressive decrease in the systolic blood pressure and shortening in the R-R interval (Bertinieri et al., 1988; Parati et al., 1994, 1995, 1997). The slope of the regression line between the systolic blood pressure and the R-R interval changes is taken as an index of the sensitivity of arterial baroreflex modulation of heart rate (BRS). In this technique identification of sequences is done separately which is characterised by increases or reductions in the SBP, this allows the effects of spontaneously occurring baroreceptor activity to be separately assessed. The sequence method is based on strict threshold criteria because the sequences that are considered are only those which last for at least four beats and having consecutive systolic blood pressure and the R-R interval changes equal to or greater than 1mmHg and 5ms, respectively. The average BRS obtained by using the sequence method over periods of standardized behaviour and of sufficient duration (at least 10-15 min) is highly reproducible (Iellamo et al., 1994). The sequence method

largely reflects baroreflex control of cardiac vagal drive (Parlow et al., 1997). Studies which used this technique have documented high degree of variability which physiologically characterizes BRS in different behavioural conditions (Iellamo et al., 1994; Parati et al., 1994; Parlow et al., 1997). The sequence technique provides information on minute-to-minute variability of the effectiveness of baroreflex modulation of heart rate, or BRS values obtained reflects the baroreflex function even during a few seconds. The R-R interval-SBP sequences occurs unevenly over time, as they are related to daily life behaviours such an irregular occurrence provides a suitable estimate of the day and night BRS profile because the number of sequences observed over each hour of a 24hour blood pressure recording is high enough to obtain a large hourly database. This method also provides a separate assessment of the reflex R-R interval changes induced by baroreceptor stimulation (+RR/+SBP sequences) and baroreceptor deactivation (−RR/−SBP sequences).

In this experiment the BRS analysis, including calculation of the aortic pressure waveform from the peripheral pressure waveform, was conducted using BaroCor software (version 1.1, AtCor Medical, Sydney, Australia). Finger blood pressure waveforms (NexFin) and the ECG were recorded using ADInstruments LabChart equipment and software with custom scripts used to down sample the data to an appropriate format for analysis in the BaroCor software.

2.7 Heart rate variability

Heart rate variability (HRV) uses the fluctuations in the interval between normal heartbeats to assess autonomic control of the heart (Akselrod et al., 1981). These fluctuations are quantified by HRV analysis. HRV analysis is the ability to assess overall cardiac health and the state of the autonomic nervous system (ANS) responsible for regulating cardiac activity. HRV is a useful tool to investigate the sympathetic and parasympathetic function of the ANS. The presence of spontaneous, small, beat-to-beat variation in continuous recordings of arterial blood pressure and heart rate have represented recurrent findings in experimental and clinical medicine. Usually, increased HRV reflects increased autonomic modulation of the heart rate, the converse is also true. The low HRV values could reflect a lack of central modulation of heart rate or lack of response of the sinus node. HRV measurements are easy to perform, noninvasive, and have good reproducibility, if used under standardized conditions (Ge et al., 2002; Kleiger et al., 1991).

In most clinical applications, HRV is analysed by time, frequency domain, or nonlinear methods. Time and frequency domain indices of HRV are closely related (Kleiger et al., 1991) are based on normal-to- normal (N-N) interbeat intervals only.

Frequency domain method:

Various spectral methods have been used for the analysis of the tachogram since the early years. How the power (variance) distributes as a function of frequency is provided by the power spectral density (PSD) analysis. Method of calculation of PSD is nonparametric which is advantageous as it employs simple Fast Fourier transform algorithm (FFT) and has a high processing speed.

Spectral components

Spectral analysis of heart rate provides values of high and low frequency power (HF and LF) that are thought to be indicators of autonomic control of heart rate (Pagani et al., 1986; Pomeranz et al., 1985). Three main spectral components are distinguished in a spectrum calculated from short term recordings: very low frequency (VLF 0 - 0.04 Hz) associated with

slow regulatory mechanisms (thermoregulation and humoral control), low frequency (LF0.04 – 0.15 Hz) reflecting sympathetic and vagal activity and high frequency (HF 0.15 – 0.4 Hz) reflecting vagal activity. Another calculated parameter is the HF/LF ratio, which is considered to be the sympathovagal balance. The central frequency of LF and HF and the distribution of power are not fixed but varies in relation to changes in autonomic modulations of heart rate . HRV is non-invasive and technically appropriate, which makes this technique far more suitable for studies that involve large samples and/or repeated measurements.

In this experiment HRV was analysed as per the Task Force of the European Society of Cardiology guidelines (AHA and ESC, 1996). Detection of the RR interval from the ECG and subsequent analysis was performed using LabChart software (version 7.3.7, ADInstruments, Sydney, Australia). Standard HRV parameters were recorded including low frequency (0.04-0.15 Hz) and high frequency (0.15-0.4 Hz) power normalized to total power.

2.8 Breathing rate and load

In this experiment the subjects were made to breathe in three different patterns, and each pattern lasted for a period of 10 minutes which included

1) Free breathing.

2) 6 breaths/minute with half of each breathing cycle being inspiration(5.0sec inhale, 0.0sec hold, 5.0sec exhale)

3) 6 breaths/minute with 70% of each breathing cycle being inspiration(7.0sec inhale, 0.0sec hold, 3.0sec exhale)

The order of each of the breathing pattern was randomised (computer generated randomisation). An auditory cum visual signal metronome (App name: Paced breathing pro, developer: Trex LLC) was used to pace the breathing patterns. The breathing protocol included the following stages (fig.1):

1) Baseline (5min): subjects were made to lie supine at rest in a semi darkened room.

2) Paced breathing (10min): the paced breathing android application was shown to the subject on the mobile phone screen. Subject was instructed to inhale as the blue line moves up the pyramid and exhale as the blue line came down the pyramid. After 10 minutes of recording the metronome was continued to play with the breathing continued and simultaneously blood pressure measurements were taken. After the blood pressure was recorded, the metronome was stopped and the paced breathing too.

3) Recovery stage (5min): subject was told to rest and breath at their normal pace.
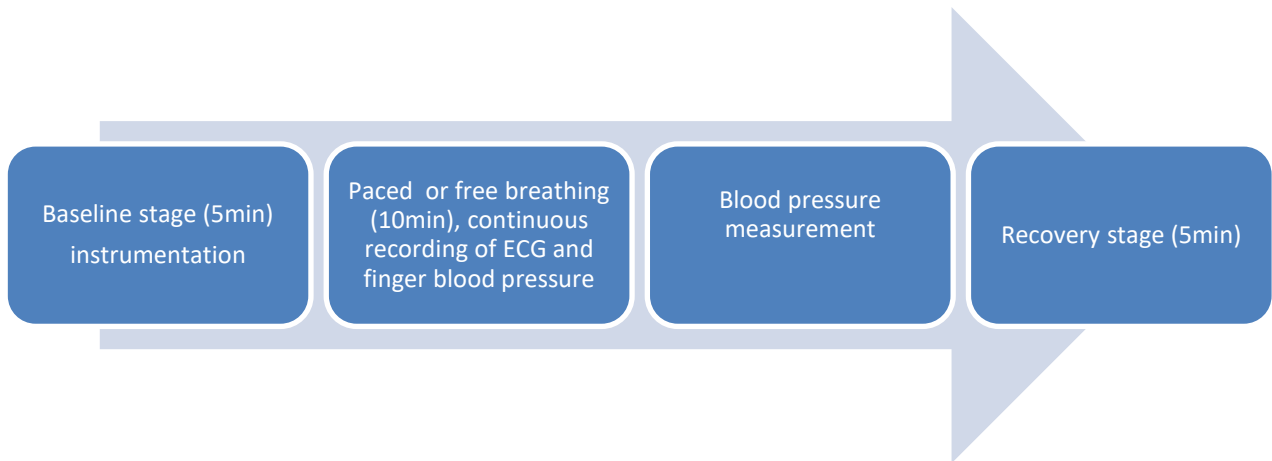
Figure 10: Paced or free breathing protocol. During baseline stage subjects lie supine at rest, paced/free breathing time the subject inhales and exhales according to the metronome and then after 10minutes the blood pressure recorded. During the recovery stage the subject is told to rest and breathe at their normal pace.

2.9 Data analysis

All measurements are reported as means ± standard deviation. Differences in blood pressure and cfPWV between the seated and supine position was analysed using paired t-tests. Differences in variables between different breathing rates was analysed using repeated measures analysis of variance. Age related changes in parameters was quantified using linear regression statistics. Data analysis was conducted in R, version 3.2.2.

3   Results

A total of 30 (19 females and 11 males) subjects were recruited varying in age from 20-66 years. Most of the subjects were nonsmokers (2 smokers) The demographics of the cohort are provided in Table 1.

Table 1: Demographics of subjects

Parameter	mean ± SD	minimum	maximum
height (cm)	169 ± 12	145	194
weight (kg)	70 ± 17	43	112

BMI (kg/m^2)	20 ± 4	18	33

3.1 Change in heart rate, systolic and diastolic blood pressure and cfPWV measurements with change in position from seated to supine.

Seated / supine measurements induced a change in DBP and a related change in cfPWV (Table 2). HR decreased significantly ($p<0.001$) from 79±12 to 67±11 bpm. All measures of brachial blood pressure recording, systolic, mean and diastolic (mmHg) decreased significantly. Brachial systolic blood pressure decreased from 126±11 to 117±14 mmHg ($p<0.001$) from seated to supine. Mean (96±8 to 86±8 mmHg, $p<0.001$) and the diastolic brachial blood pressure (81±7 to 72±6 mmHg, $p<0.001$) also showed a significant decrease. There was a decrease in aortic blood pressure systolic (113±10 to 106±11 mmHg, $p<0.001$), mean (96±8 to 86±8 mmHg, $p<0.001$), and diastolic (82±7 to 73±6 mmHg, $p<0.001$). The aortic augmentation index (AIx) increased (12±14 to 21±13%, $p<0.001$) with the change in position from seated to supine. cfPWV showed a significant decrease ($p<0.001$) with change in position (802±184 to 611±135, cm/s).

Table 2: Blood pressure and arterial stiffness parameters in seated and supine positions.

parameter	seated	supine	p
heart rate (bpm)	79 ± 12	67 ± 11	< 0.001
Brachial blood pressure (mmHg)			
Systolic	126 ± 11	117 ± 14	<0.001

Mean	96 ± 8	86 ± 8	<0.001
Diastolic	81 ± 7	72 ± 6	<0.001
Aortic blood pressure (mmHg)			
Systolic	113 ± 10	106 ± 11	<0.001
Mean	96 ± 8	86 ± 8	<0.001
Diastolic	82 ± 7	73 ± 6	<0.001
aortic AIx (%)	12 ± 14	21 ± 13	<0.001
cfPWV (cm/s)	802 ± 184	611 ± 135	<0.001

Abbreviations: bpm, beats per minute, AIx, augmentation index, cfPWV, carotid femoral pulse wave velocity.

Thus with the change is position from seated to supine all the measured cardiovascular parameters decreased except the AIx which showed an increase with the change in position.

3.2   Aim 1: Age related changes in arterial stiffness

Whilst the study was suitably powered to detect age-related changes in aortic and brachial systolic pressure and BRS (Table 3), the trend in age- related increase in arterial stiffness parameters cfPWV, augmentation index and pressure sensitivity of cfPWV had low p-values but were not significant for the sample size (Figure 11, Table 3 and Table 4)
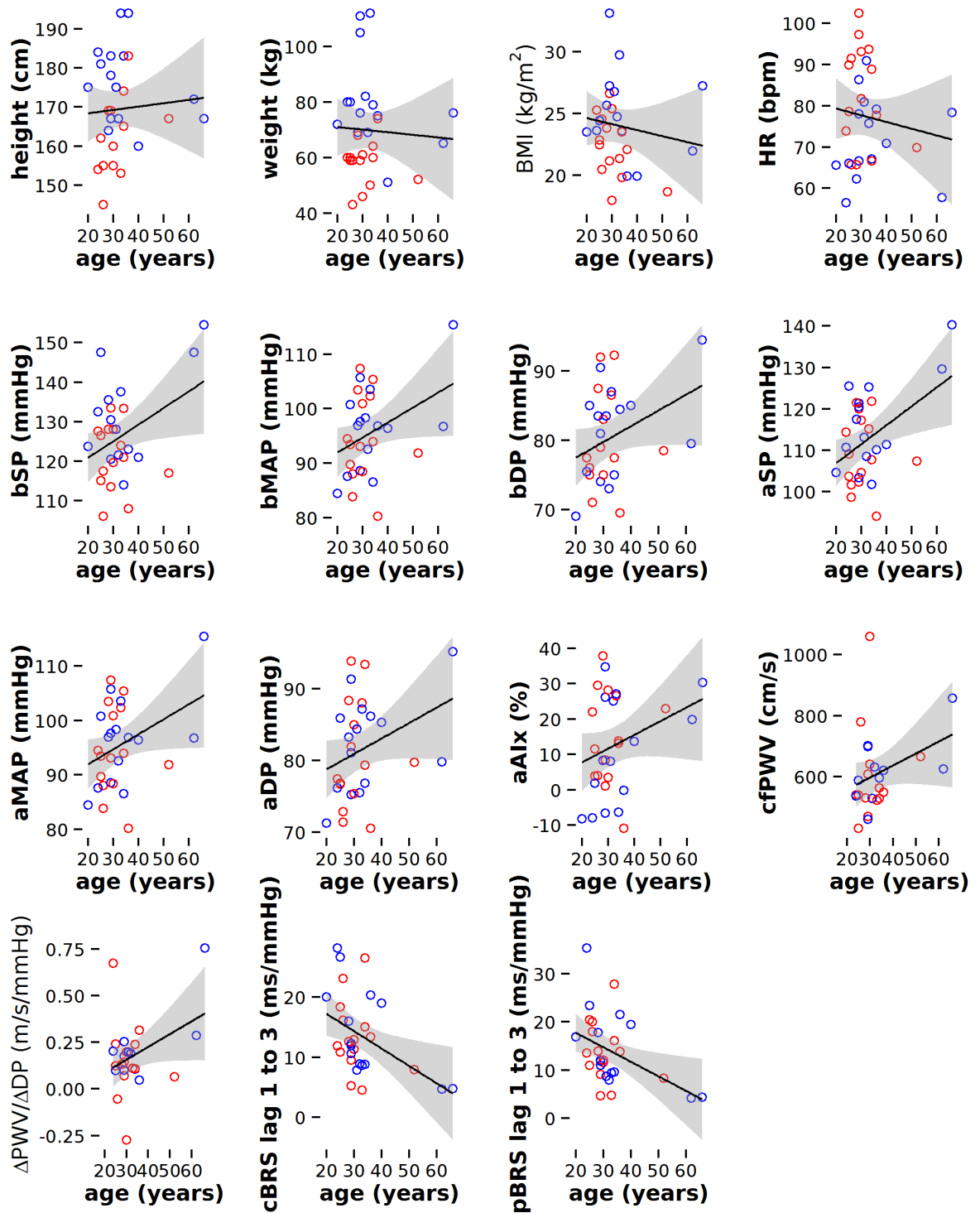
Figure 11. Exploratory plot of variables of height, weight, seated blood pressure, and BRS and HRV with free breathing with respect to age. Red points represent females and blue points males. The line indicates the least squared regression for both males and females with the 95% confidence interval shaded. Regression statistics provided in Table 4.

Abbreviations: BMI, basal metabolic index, HR, heart rate, aSP, aortic systolic blood pressure, aMAP, aortic mean arterial blood pressure, aDP, aortic diastolic blood pressure, bSP, brachial systolic blood pressure, bDP, brachial diastolic blood pressure, bMAP, brachial mean arterial blood pressure, aAIx, aortic augmentation index, cfPWV, carotid femoral pulse wave velocity, cBRS, central baroreceptor sensitivity, pBRS, peripheral baroreceptor sensitivity.

Table 3: Regression statistics with respect to age for plotted variables in figure 11

Variable	slope	intercept	R^2	p
height (cm)	0.086	167	0.01	0.695
weight (kg)	0.094	73	<0.01	0.764
body mass index (kg/m^2)	0.049	26	0.02	0.472
heart rate (bpm)	0.163	83	0.02	0.466
Brachial blood pressure (mmHg)				
Systolic	0.421	112	0.15	**0.032**
Mean	0.276	86	0.13	0.052
Diastolic	0.227	73	0.11	0.074
Aortic blood pressure (mmHg)				
Systolic	0.458	98	0.21	**0.01**
Mean	0.276	86	0.13	0.052
Diastolic	0.215	74	0.1	0.085
Aortic AIx (%)	0.39	0	0.08	0.125
cfPWV (cm/s)	3.92	481	0.1	0.123
ΔPWV/ΔDP (cm/s/mmHg)	0.007	0	0.14	0.065
BRS, average lag 1 to 3				
Aortic pressure (ms/mmHg)	0.287	23	0.2	**0.013**
Peripheral pressure (ms/mmHg)	0.3	24	0.19	**0.016**

Abbreviations: AIx, augmentation index, cfPWV, carotid femoral pulse wave velocity, PWV, pulse wave velocity, DP, diastolic blood pressure, BRS, baroreceptor sensitivity

Table 4: Regression statistics with respect to age for HRV variables plotted in figure 11

HRV variable	slope	intercept	R^2	p
NN (ms)	0.36	882	0	0.876
SD NN (ms)	-1.898	200	0.06	0.208
SD ΔNN (ms)	-2.888	252	0.06	0.208
RMSSD	-2.883	252	0.06	0.208
Total power (ms^2)	-709	5.03×10^4	0.06	0.188
Normalised HF power	0.034	29	0	0.869
Normalised LF power	-0.127	31	0.02	0.519
LF/HF ratio	-0.803	135	0.01	0.533

Abbreviations: NN, normal-to-normal interval, SDNN, standard deviation of NN interval, SDΔNN, standard deviation of the change in NN interval, RMSSD, squared root of the mean squared differences of the successive NN intervals, HF, high frequency, LF, low frequency.

3.3 Aim 2: Relationship between baroreceptor function and arterial stiffness

BRS, whether measured using aortic or peripheral systolic pressure (sequence technique) was not significantly correlated with cfPWV (aBRS p=0.864; bBRS p=0.625, Figure 12) or pressure sensitivity of cfPWV (aBRS p= 0.223; bBRS p=0.381 Figure 14). BRS was, however correlated with aortic AIx when calculated using aortic pressure (p=0.036) but not when using the brachial pressure (p=0.072, Figure 13, Table 5)

3.4 Aim 3: Relationship between sympathetic and parasympathetic activity and arterial stiffness

There was no relationship between normalized low frequency or high frequency power of HRV with cfPWV (Figure 15), aortic AIx (Figure 16), or pressure sensitivity of PWV (Figure 17). A summary of the regression statistics is included in Table 6.
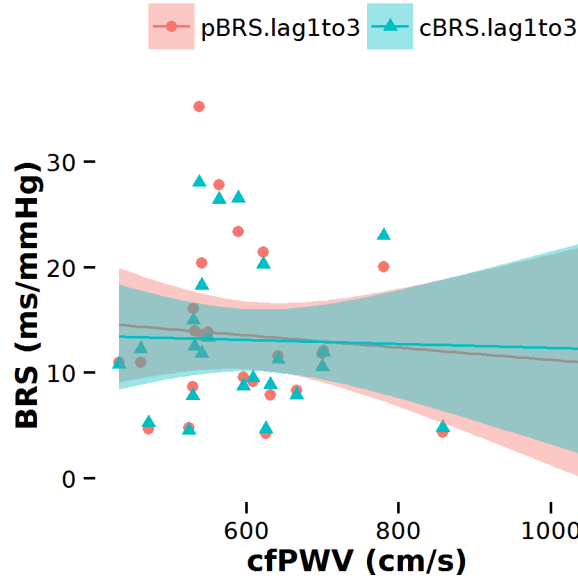
Figure 12: Relationship between cfPWV and baroreceptor sensitivity (BRS) as measured using peripheral systolic pressure (pBRS) and central systolic pressure (cBRS) using sequence technique. aBRS = -0.002 × cfPWV + 14 (R^2<0.01, p=0.864). bBRS =-0.006×cfPWV + 17 (R^2=0.01, p=0.625).
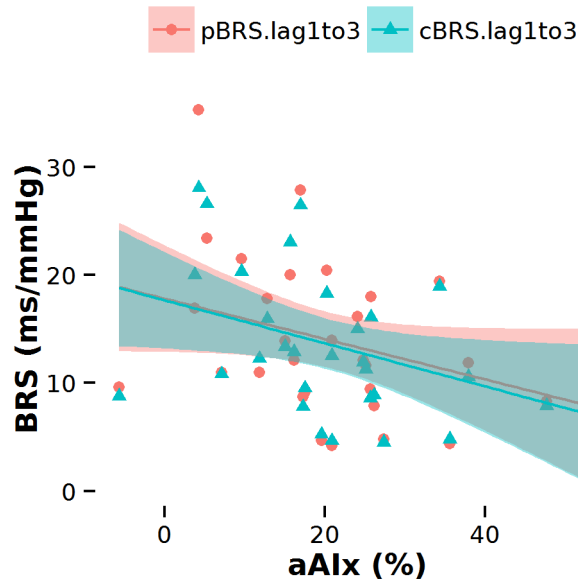
Figure 13: Relationship between supine augmentation index (AIx) and baroreceptor sensitivity (BRS) as measured using the peripheral systolic pressure (pBRS) and central aortic systolic pressure (cBRS) using sequence technique. aBRS = -0.199 × aAIx + 18 (R^2 =0.15, P=0.036). bBRS = -0.187 × aAIx +18 (R^2=0.11,p=0.072).
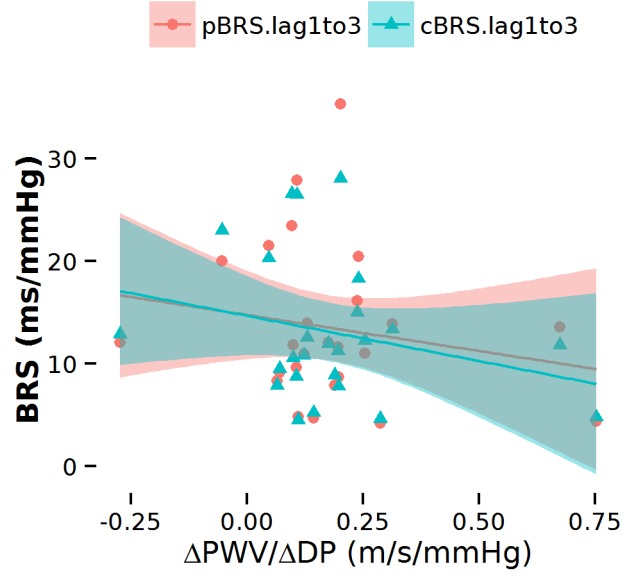
Figure 14: Relationship between pressure (diastolic) sensitivity of carotid femoral pulse wave velocity ($\Delta PWV/\Delta DP$) and baroreceptor sensitivity (BRS) as measured using the peripheral systolic pressure (pBRS) and central aortic systolic pressure (cBRS) using sequence technique. aBRS = -8.82 × $\Delta PWV/\Delta DP$ + 15 (R^2=0.06, p=0.223). bBRS = -7.016 × $\Delta PWV/\Delta DP$ + 15 (R^2 =0.03, p= 0.3 81).

Table 5: Linear regression statistics for the relationship between arterial stiffness parameters and baroreceptor sensitivity (BRS) calculated using the central aortic (c) and peripheral brachial (p) pressure values.

stiffness parameter	BRS (mean lag 1 to 3) (ms/mmHg)	slope	intercept	R^2	p
cfPWV (cm/s)	pBRS	-0.006	17	0.01	0.625
cfPWV (cm/s)	cBRS	-0.002	14	<0.01	0.864
aAIx (%)	pBRS	-0.187	18	0.11	0.072
aAIx (%)	cBRS	-0.199	18	0.15	**0.036**
$\Delta PWV/\Delta DP$ (m/s/mmHg)	pBRS	-7.02	15	0.03	0.381
$\Delta PWV/\Delta DP$ (m/s/mmHg)	cBRS	-8.82	15	0.06	0.223

Abbreviations: cfPWV, carotid femoral pulse wave velocity, aAIx, aortic augmentation index, ΔPWV, change in pulse wave velocity, ΔDP, change in diastolic blood pressure, pBRS, peripheral baroreceptor sensitivity, cBRS, central baroreceptor sensitivity.
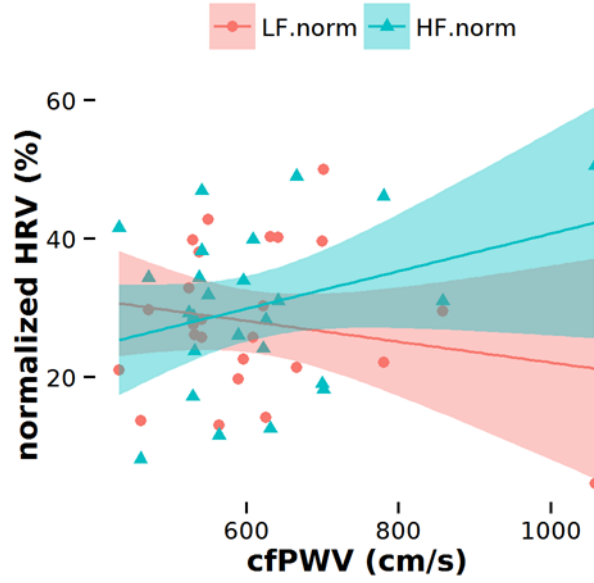
Figure 15: Relationship between carotid femoral pulse wave velocity (cfPWV) and normalized heart rate variability (HRV) in the low (LF) and high frequency (HF) ranges. . $HRV_{LF} = -0.015 \times cf PWV = 37$ ($R^2 = 0.03$, p = 0.371). $HRV_{HF} = 0.027 \times cfPWV + 14$ ($R^2 = 0.1$, p = 0.132).
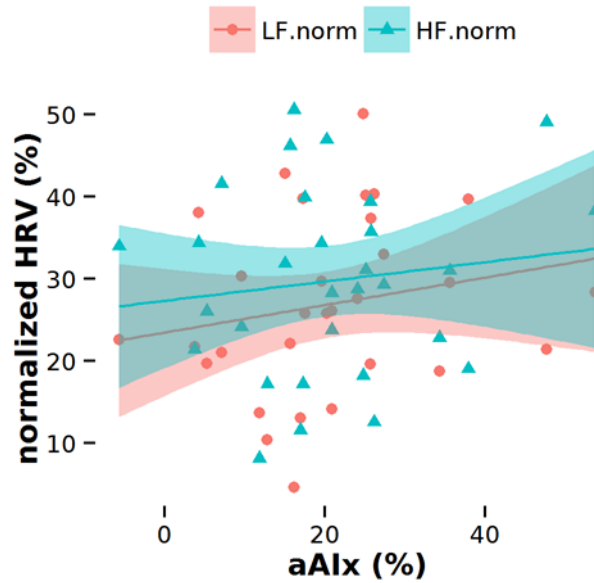
Figure 16: Relationship between aortic augmentation index (AIx) and normalized heart rate variability (HRV) in the low frequency (LF) and high frequency (HF) ranges. $HRV_{LF} = 0.167 \times AIx + 23$ ($R^2=0.04$, p = 0.293).$HRV_{HF} = 0.118 \times aAIx + 27$ ($R^2 = 0.02$, p = 0.485).
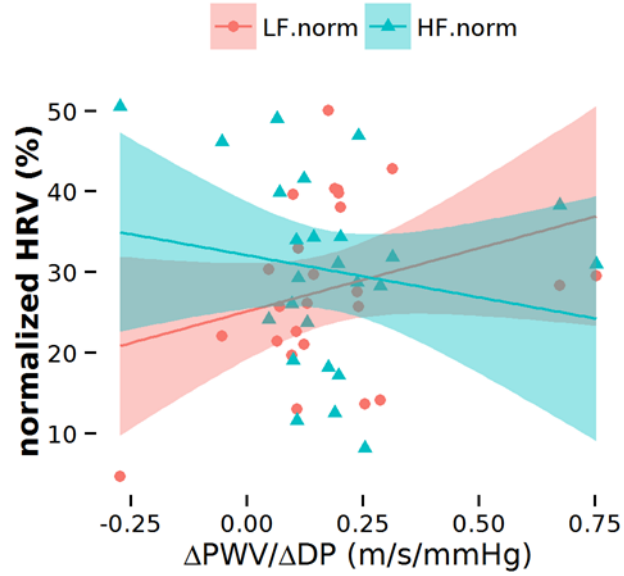
Figure 17: Relationship between pressure (diastolic) dependency of carotid femoral pulse wave velocity (ΔPWV/ΔDP) and normalized heart rate variability (HRV) in the low (LF) and high frequency (HF) ranges. HRV_{HF} = -10.443 × ΔPWV/ΔDP + 32 (R^2 = 0.03, p= 0.398).

Table 6: Linear regression statistics for the relationship between arterial stiffness parameters and heart rate variability (HRV) in low frequency and high frequency normalized power.

stiffness parameter	Normalized HRV	slope	intercept	R^2	p
cfPWV (cm/s)	LF	-0.015	37	0.03	0.371
cfPWV (cm/s)	HF	0.027	14	0.10	0.132
aAIx (%)	LF	0.167	23	0.04	0.293
aAIx (%)	HF	0.118	27	0.02	0.485
ΔPWV/ΔDP (m/s/mmHg)	LF	15.7	25	0.08	0.162
ΔPWV/ΔDP (m/s/mmHg)	HF	-10.4	32	0.03	0.398

Abbreviations: cfPWV, carotid femoral pulse wave velocity, AIx, augmentation index, ΔPWV, change in pulse wave velocity, ΔDP, change in diastolic blood pressure, HF, high frequency, LF, low frequency

3.5 Aim 4: Relationship between baroreceptor function and sympathetic and parasympathetic activity

The relationship between BRS and HRV is given in (Figure 18). There was no correlation between any of the parameters (Table 7), except that BRS calculated using the peripheral

waveform was significantly correlated with the low frequency (sympathetic) component of HRV, but with an R^2 value of 0.05.
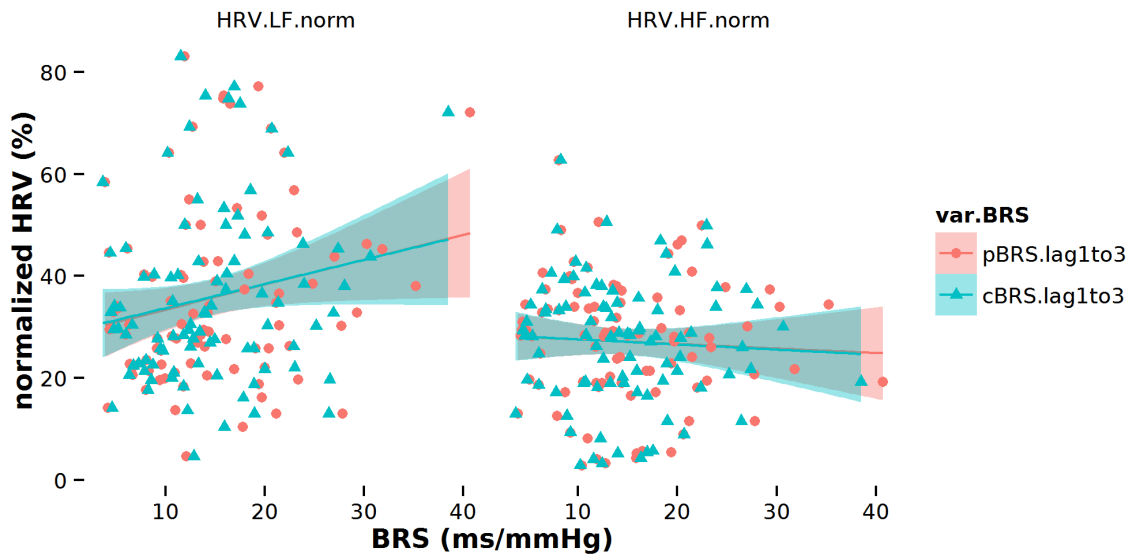
Figure 18: Relationship between baroreceptor sensitivity (BRS) measured using the sequence technique from the peripheral (p) and aortic (a) pressure waveform and heart rate variability (HRV) in the low frequency (LF) and high frequency (HF) ranges.

Table 7: Linear regression statistics for the relationship between baroreceptor sensitivity (BRS) calculated using the central (c) and peripheral (p) pressure values and heart rate variability (HRV) as plotted in Figure 18.

BRS (mean lag 1 to 3)	Normalised HRV	slope	intercept	R^2	p
cBRS	HF	-0.101	29	<0.01	0.597
pBRS	HF	-0.088	28	<0.01	0.61
cBRS	LF	0.473	29	0.04	0.074
pBRS	LF	0.488	29	0.05	**0.042**

Abbreviations: cBRS, central baroreceptor sensitivity, pBRS, peripheral baroreceptor sensitivity, HF, high frequency, LF low frequency.

3.6 Aim 5: Cardiovascular response to slow breathing

Breathing at 1Hz, whether at 50% or 70% inspiration, did not cause any change in aortic or brachial blood pressure relative to free breathing (Table 8), with the average change in systolic, mean, or diastolic pressure from free breathing being no more than 1 mmHg. Heart rate and aortic augmentation index also did not change with the 1Hz breathing patterns. The absolute

variability of heart rate (SD of NN) did increase with both the 1Hz breathing patterns relative to free breathing (Table 8). This increase in variability was reflected in an increase in the low frequency, but not the high frequency power of HRV (Table 8, Figure 19), indicating an increase in sympathetic nerve activity with the 1Hz breathing pattern.

Post-hoc tests revealed that there were no differences in any of the measured variables between the different breathing loads (50% and 70%) but significant differences were maintained between free breathing and 50%/1Hz and free breathing and 70%/1Hz.

BRS did not change with the breathing patterns, though there was a trend for BRS measured using both peripheral and central pressure waveforms at all lags to be higher with the 1Hz breathing pattern compared to free breathing. The number of BRS sequences increased significantly for all lags, both central and peripheral, other than lag 1 from the aortic pressure values (Table 9).
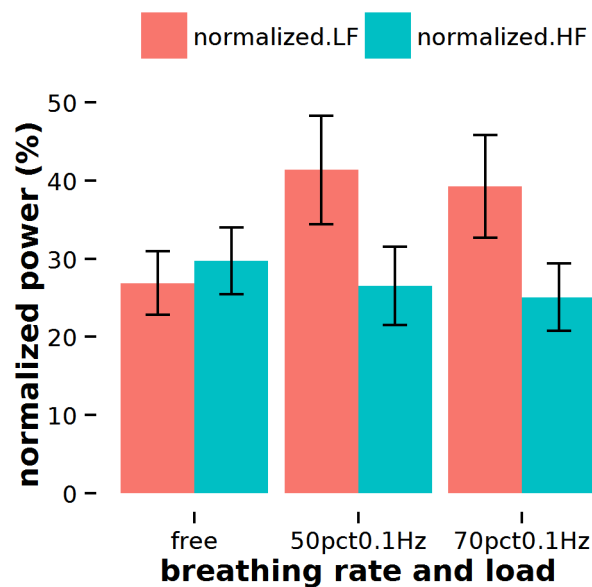
Figure 19: LF and HF (normalized to total power) for different breathing rates and loads.

Table 8: Blood pressure and heart rate variability with different breathing rates and loads.

Parameter	Free breathing	50% at 0.1 Hz	70% at 0.1 Hz	P value
Brachial blood pressure (mmHg)				
Systolic	117±13	117±13	118±13	0.817
Mean	85±7	84±8	85±7	0.467
diastolic	70±6	69±7	70±7	0.224
Aortic blood pressure (mmHg)				
systolic	105±10	104±11	106±11	0.526
Mean	85±7	84±8	85±7	0.467
Diastolic	71±6	70±7	71±7	0.234
Aortic AIx (%)	20±11	20±9	21±10	0.57
Heart rate (bpm)	68±9	71±11	72±10	0.085
Heart rate variability parameters				
NN (ms)	893±126	871±137	879±165	0.659
SD of NN (ms)	139±83	172±81	220±192	**0.038**
SD of ΔNN (ms)	158±127	198±121	202±123	0.122
RMSSD (ms)	158±127	198±121	202±123	0.122
Normalized LF power (%)	27±11	41±19	39±18	**<0.001**
Normalized HF power (%)	30±11	27±13	25±12	0.234
LF/HF power ratio (%)	108±70	357±581	328±517	**0.025**

Abbreviations: AIx, augmentation index, NN, normal-to-normal interval, SD of NN, standard deviation of normal-to-normal interval, RMSSD, squared root of the mean squared differences of the successive NN intervals, LF, low frequency power, HF, high frequency power.

Table 9: Baroreceptor sensitivity (BRS) parameters with different breathing rates and loads.

Parameter	Free breathing	50% at 0.1 Hz	70% at 0.1 Hz	P value
BRS, from finger pressure waveform (ms/mmHg)				
lag 1	14±7	15±8	15±7	0.103
lag 2	14±9	16±9	15±7	0.239
lag 3	14±7	16±9	15±7	0.121
mean lag 1 to 3	14±7	26±8	15±7	0.103
BRS, from derived aortic pressure waveform (ms/mmHg)				
lag 1	13±6	15±8	14±6	0.059
lag 2	14±7	16±8	15±6	0.129
lag 3	14±7	15±8	15±6	0.099
mean lag 1 to 3	14±7	15±8	15±6	0.068
Number of BRS sequences from finger pressure waveform				
lag 1	61±28	75±28	75±20	**0.032**
lag 2	50±33	71±29	70±23	**0.003**
lag 3	46±31	67±32	64±23	**0.002**
sum of lag 1 to 3	157±86	214±87	209±65	**0.004**
Number of BRS sequences from derived aortic pressure waveform				
Lag 1	63±29	76±19	76±19	0.062
Lag 2	51±32	72±29	72±21	**0.002**
Lag 3	47±31	69±31	65±22	**0.003**
Sum of lag 1 to 3	162±84	217±86	211±61	**0.006**

4   Discussion

4.1   Change in the blood pressure during seated and supine measurements and change in cfPWV

In the present study the change in blood pressure measurement was investigated in two positions, seated and supine. A significant decrease in the brachial and aortic blood pressure was observed from sitting to supine position. According to the American Heart Association recommendations for blood pressure measurement in humans and experimental animals, it has been stated that diastolic blood pressure measured while sitting is higher than when measured supine (by ~5mmHg) (Pickering et al., 2005), although there is less agreement about systolic pressure (JWM Lenders, 2003). Results from this study support the findings of prior studies which have shown similar results (Cavelaars, Tulen, van Bemmel, Mulder, & van den Meiracker, 2004; Gellman et al., 1990; Turjanmaa, Kalli, & Uusitalo, 1988). This decrease can be explained by the normal homeostatic mechanisms of BP. In response to the orthostatic challenge, both heart rate and total peripheral resistance increase to maintain central blood volume and adequate perfusion to the brain.

Our study showed an increase in cfPWV this may be due to the gravity effect on the blood column in the aortic trunk and the lower limbs and with the graded increase in the distending pressure which produces an increase in the regional stiffness resulting in increased pulse wave velocity with sitting (Avolio and Parati, 2011) . This result is similar to some previous Study by Xu et. al. who reported that carotid femoral pulse wave velocity showed an average increase by 21.5% from supine to upright position (Xu et al., 2015). Few studies to date have investigated the effects of measurement position on PWV. Hasagewa et.al. studied subjects aged 15-75 years in whom he measured the ankle and brachial cuff pressure both in supine and in standing position and compared it to the PWV. They found an increase in the PVW from 10.9-17.6 m/s when they changed the position from supine to standing (Hasegawa, 1979). A similar trend was seen in another study reported by Shimawaki et. al. were the baPWV increased from 11.3 to 15.8 m/s with change in position from supine to seated (Shimawaki, 2015). According to Hasagewa, standing causes the arteries of the lower extremities to expand because of the increase in the ankle blood pressure and compliance. This, in turn is associated with decrease in the elastic modulus of the arteries, which results in increased PWV in the arteries (Hasegawa, 1979).

 The study reported by Davis et.al shows that reflection parameters (reflection magnitude and augmentation index) decreases from supine to standing position whereas peripheral resistance increases in both young and aged adults (Davis et al., 2011), hence increase in the peripheral resistance was associated with increased wave reflection.

4.2   Age related changes in the arterial stiffness

There was an increase in the arterial stiffness with age show by increase in PWV. Arteries stiffen with both disease and age and this age-related increase in the arterial stiffness may then contribute to cardiovascular pathologies (Benetos et al., 2002). Another important modifiable factor that contributes to arterial stiffness is the mean arterial pressure (MAP) (McEniery et al., 2005; Protogerou et al., 2007; Segers et al., 2009). Possible explanation for the increase in the MAP with ageing is elastin fracture and degradation with a consequent increased loading on the stiffer collagen fibers. MAP has a greater impact on the small to medium sized muscular arteries leading to high peripheral vascular resistance (McEniery et al., 2007). Causes for the age-related stiffening of the arteries can be explained under two broad headings:

4.2.1 Changes in the elastin

The turnover rate of the elastic fibers in vivo is extremely low in vivo, and this longevity allows for the accumulation of age related changes caused by fragmentation, calcification, and matrix metalloproteinase (MMP)-degradation (Schlatmann and Becker, 1977). The elastin fibers lose functionality when they decay and shift the load onto the stiffer collagen fibrils which contributes to significant increases in arterial stiffness. Throughout the life time elastic fibers undergo fatigue and fragmentation due to the continuous pulsatile wall stress (Avolio et al., 2015; Greenwald, 2007). Calcification of the elastin fibers occurs due to direct binding of the calcium ions to the elastin fibers (Urry and Ohnishi, 1974; Urry, 1971). Studies conducted on aortic sections to study the effects of ageing have shown that the absolute elastin content remains relatively stable with age, the elastin concentration decreases and is accompanied by substantial increase in the collagen concentration (Fonck et al., 2009; Kanabrocki et al., 1960; Myers and Lang, 1946). Age is also associated with changes in the amino acid scale that contributes to decreased arterial compliance, caused by loss of elastin functionality. The compounds desmosine and isodesmosine are formed by four lysine amino acid molecules and are critical for crosslinking the elastin fibers to give them elastic properties (Davis and Anwar, 1970). The concentration of desmosine and isodesmosine and their crosslinks decrease with age (John and Thomas, 1972; Watanabe et al., 1996).

 In the healthy arteries the MMPs have a low basal activity so as to balance the absence of new elastin synthesis. The elastases MT1-MMP and MMP-2 activity are increased with age. MMP-2 has been found near the fragmented elastin fibers in the aorta (Bonnema et al., 2007; Wang et al., 2003; Yasmin et al., 2005).

4.2.2 Changes in collagen

Healthy arterial mechanics are governed by the elastin/collagen ratio. The collagen concentration in all the three layers of the arterial wall increases with age thus shifting the elastin/collagen balance. In the tunica media the vascular smooth muscle cells are replaced by the collagen fibers (Schlatmann and Becker, 1977). In individuals over the age of 50 years, collagen redistributes within the tunica media to bundle near the lamellae units (Greenberg, 1986; Schlatmann and Becker, 1977). The aged extracellular cells undergo morphological changes and resemble vascular smooth muscle cell phenotype which then express smooth muscle alpha actin and collagen 1, and this deposition of collagen contributes to intimal thickening (Fleenor et al., 2012). Collagen I and collagen III deposition in the tunica adventitia also increases with age and is accompanied by vessel stiffening (Fleenor et al., 2010). Increased collagen concentrations together with collagen crosslinking by non-enzymatic glycation also increases arterial stiffness. Glycation is a reaction occurring between reducing sugars and proteins, and directly stiffens tissues in addition to synthesising deleterious end products. Advanced glycation end products (AGEs) accumulates with aging and are harmful to the vascular health because they reduce nitric oxide availability. Nitric oxide is an important vasodilator used to maintain vascular tone and has anti-inflammatory effects on endothelium (Bucala et al., 1991; Decaterina et al., 1995; Xu et al., 2003, 2005).

There was an increase in the PWV seen with age this suggests that there are changes occurring to the collagen and elastic fibers in the arterial wall which is causing the increase in the speed of the pulse transmitted through the arterial segments.

4.2.3 Augmentation index (AIx)

Augmentation index was not significantly related to age in this study. Augmentation index is a derived parameter and it represents the summation of the outgoing and reflected pressure waves. Some investigators believe it to be a measure of arterial stiffness while, others say that it is an indirect measure of pulse wave velocity (Liang et al., 1998; O'Rourke et al., 2002; Yasmin and Brown, 1999). AIx is elevated in hypertension and influenced by age, body height, heart rate, gender, body fat distribution and insulin resistance (Giannattasio et al., 1997; Hayward and Kelly, 1997; Kelly et al., 1989; London et al., 1995, 2001; Wilkinson et al., 2000). There are numerous studies in which investigators have shown that an increase in the pulse wave velocity may not always be accompanied by increased AIx (Lacy et al., 2004). In a study of 60 type II diabetics, van Dijk et al demonstrated that AIx was not independently associated with brachial pressure from 24-hr ambulatory blood pressure monitoring. They concluded that brachial artery pulse pressure was determined by proximal aortic stiffness in a way which is not strongly influenced by peripheral pulse reflection (van Dijk et al., 2002). AIx was also found to be not correlated to age in patients with arthrosclerosis (Nurnberger et al., 2002).

However, AIx has been shown in large populations studies to increase with age, especially in the first few decades of adult life (McEniery et al., 2005). In the present study, the regression analysis showed an increase in the AIx of 3.9% per decade of life. However, the sample size did not yield a significant result.

4.3 Relationship between baroreceptor function and arterial stiffness parameters

Baroreceptors are the stretch sensitive receptors located in the walls of the carotid artery and the aortic arch. In our study baroreceptor function did not correlate with arterial stiffness. Change in the transmural stretch produced due to the change in the arterial blood pressure causes either activation or deactivation of these receptors within the walls of the carotid artery and aortic arch. Baroreceptors are not only sensitive to absolute changes in arterial pressure but also to the rate of pressure change (Chapleau and Abboud, 1987). Stiffness in the carotid artery and the aortic arch where the baroreceptors are located will affect the stretch and hence BRS (Monahan et al., 2001). This is because a higher pressure threshold and/or a more intense pressure change that is needed to distend the arterial wall (Bonyhay et al., 1996; Lage et al., 1993). In addition to structural vascular changes, functional mechanisms may contribute to alter baroreflex responses. Vascular compliance is an important determinant of the magnitude of deformation and hence, the same pulse pressure can result in increased baroreceptor firing (Kingwell et al., 1995). Baroreceptor activation is modulated by the activity of potassium channels and the sodium potassium pump, by paracrine factors like prostacyclin, platelet aggregation and by oxygen free radicals (Chapleau et al., 2001). Endothelial dysfunction (Bonetti et al., 2003), is strongly related to arterial stiffness, impairs prostacyclin (Chen et al., 1990), and enhances the formation of oxygen free radicals and platelet aggregation (Li et al., 1992) and in this way contribute to the impairment of BRS associated with vascular stiffness. Greater intima media thickness and vascular calcification in the baroreceptor regions of the carotid artery and the aortic arch is also said to reduce baroreceptor sensitivity (Chesterton et al., 2005; Gianaros et al., 2002). Exercise training improves endothelial function (Cameron and Dart, 1994; Silva et al., 1997) the major determinant of arterial compliance and reduces sympathetic activation which is another factor affecting the distensibility of the arteries (Coats et al., 1992; Grassi et al., 1994).

The current study did not reveal a relationship between cfPWV and BRS as was hypothesized, and given the p-values (0.63 and 0.86 for BRS measured using brachial and aortic pressure respectively), any moderate increase in sample size would be unlikely to reveal any

relationship. However, aortic AIx, a measure of wave reflection related to the stiffness of the systemic arteries was correlated with BRS measured using aortic pressure values and heart rate. BRS decreased with increasing AIx (indicating increasing stiffness of systemic arteries) at a rate of 0.199ms/mmHg per percent change in AIx.

4.4 Relationship between baroreceptor function and sympathetic and parasympathetic activity measured using heart rate variability

In our study there was no correlation between BRS and any of the parameters of HRV. The consensus is that the parasympathetic activity is largely reflected through high-frequency oscillations of power spectrum (HF), which are synchronised to the respiratory frequency (Pomeranz et al., 1985). The origin of low frequency (LF) spectral power component is less clear. Some investigators support that LF is a reflection of both parasympathetic and sympathetic modulation of heart rate (Akselrod et al., 1981), whilst others believe that LF is an indicator of sympathetic modulation (Malliani et al., 1991; Pagani et al., 1986). Nevertheless, the main physiological influence of the LF component is thought to be baroreceptor sensitivity (deBoer et al., 1987). Studies have shown that heart rate variability either by inappropriate activation of the sympathetic nervous system or lowered parasympathetic tone is related to increased risks of cardiovascular disease (Curtis and O'Keefe, 2002; Gorman and Sloan, 2000). HRV is influenced by various physiological factors including age (Shannon et al., 1987), postural changes (Pomeranz et al.) and time of the day (Malpas and Purdie, 1990). Pathophysiological conditions such as congestive heart failure (Saul et al., 1988), diabetic neuropathy (Weise and Heydenreich, 1990) and coronary heart disease (Hayano et al., 1990) is also associated with alterations in heart rate variability.

Reduced HRV is a reflection of increased sympathetic activity, which is a predisposing factor for myocardial ischemia and fatal arrhythmias, especially seen in post infarction patients (Kleiger et al., 1987; Malik and Camm, 1943).Whether reduced HRV plays a casual role or is merely a marker of risk, heart rate variability measures may identify individuals at increased risk for mortality (Tsuji et al., 1994).

Arterial hypertension is seen to be characterised by altered autonomic regulation, as indicated by altered autonomic regulation, as indicated by reduced HRV (Lage et al., 1993), sympathetic hyperactivity (Curtis and O'Keefe, 2002; Gorman and Sloan, 2000) and blunted BRS (Pomeranz et al.; Shannon et al., 1987). Reduction in the HRV and BRS has a notable clinical importance particularly in conditions like myocardial infarction or congestive heart failure (Curtis and O'Keefe, 2002; Gorman and Sloan, 2000). Shannon et.al. found that BRS was positively correlated with HRV (Shannon et al., 1987). Mario et.al. reported that the HRV and BRS are reduced in young undernourished subjects as compared with normal controls (Mario et al., 2003). Vitamin B12 deficiency associated with pernicious anaemia has been linked to reduction in HRV (both sympathetic and parasympathetic nerve components) (Aytemir et al., 2000; Sözen et al., 1998), which is reversed by vitamin B12 supplementation (Aytemir et al., 2000). A decrease in the BRS has been also found in hypothyroid rats(Foley et al., 2001). Millar et.al. showed that early autonomic repair of coarctation of the aorta does not prevent late childhood alterations in HRV and BRS (Millar et al., 2013), however the role of autonomic dysfunction in patients with repaired coarctation of aorta requires further investigation.

Some investigators have shown that carotid arterial elasticity lies in the causal pathway between risk factors and HF component. Thus in individuals who are prone to develop reduced carotid elasticity when exposed to risk factors (e.g. genetic propensity for atherosclerosis), there is a loss of elasticity which will in turn influence the baroreceptor function and hence HF component. On the other hand individuals, who have risk factors but are immune to their

harmful effects on elasticity will maintain a normal baroreceptor function, hence there is no association between risk factors and HF component (Koskinen et al., 2011).

Low vagal tone is associated with increased risk of coronary heart disease as well as with angiographic progression of coronary atherosclerosis and a predictor of cardiovascular prognosis in all(Curtis and O'Keefe, 2002; Dekker et al., 2000; Huikuri et al., 1999; Liao et al., 1997). The powerful mechanism for increased cardiovascular risk, related to low vagal activity, is altered regulation of peripheral inflammation via inflammatory reflex. Acetylcholine is released in abundant due to the activation of afferent vagal fibres which then interacts with the nicotinic receptors in the target organs. The activation of nicotinic receptors inhibits the release of cytokines and supress the peripheral inflammation (Andersson, 2005; Gidron et al., 2007). Increased vagal activity also has a protective action ischemia-related dysarrhytmias and an effect on reducing blood pressure (Legramante et al., 2007). There are many mechanisms whereby risk factors predispose to cardiovascular events, but the diminished vagal tone and activation of sympathetic nervous system is an important pathway through which cardiovascular risks are conferred (Curtis and O'Keefe, 2002).

4.5 Relationship between baroreceptor function, sympathetic and parasympathetic activity measured using heart rate variability and arterial stiffness

There was no relationship between normalized low frequency or high frequency power of heart rate variability with the arterial stiffness parameters. HRV is a sensitive indicator of baroreflex control, particularly of vagal control (Bernardi et al., 1994; Sleight et al., 1995). If HRV is a measure of baroreceptor function, then stiffness in the arteries would result in attenuated baroreceptor function and therefore reduced HRV. Investigators have shown that stiffness in the carotid artery wall correlates to all spectral indices of HRV both those that reflect vagal function as well as those that which reflect sympathetic activity, stiff arteries resulted in low HRV (Jensen-Urstad et al., 1997). Hayano et. al. have found that low HRV is associated with the extent or severity of coronary atherosclerosis (Hayano et al., 1990). La Rovere et.al. found a similar relationship between severity of coronary atherosclerosis and decreased baroreceptor function. HRV indices which mainly reflect autonomic cardiovascular imbalance favouring sympathetic activity over vagal tonus, are associated with increased aortic stiffness (La Rovere et al., 1998). HRV is a more precocious measure of presence of cardiac autonomic neuropathy (CAN) than clinical tests (Cardoso et al., 2014). Jaiswal et.al. reported in their study, strong association between cardiac autonomic dysfunction and both central and peripheral arterial stiffness (Jaiswal et al., 2013). Studies have found that increased arterial stiffness is linked to muscle sympathetic nerve activity (MSNA) independent of age, waist circumference, waist to hip ratio, heart rate, pulse pressure and blood pressure (Świerblewska et al., 2010). Sympathetic overactivity plays an important mechanistic role in the short term reduction of large arterial compliance and in the longer term contribute to association among arterial stiffening, left ventricular diastolic dysfunction and cardiovascular risk (Abhayaratna et al., 2006). On contrary Kosch et.al. demonstrated that increased sympathetic activity is related to decreased distensibility of muscular type brachial artery but not elastic type carotid artery (Kosch et al., 2002).

Arterial stiffness increases with age and HRV decreases with age. Stiffness in the carotid artery could be a factor responsible for reduced baroreceptor function (Kornet et al., 2002). Hajduczk and co-workers indicated that the decrease in the baroreceptor sensitivity with age does not result from the changed arterial wall properties because reflex inhibition of sympathetic nerve activity occurred despite a maintained increase in the baroreceptor activity (Hajduczok et al., 1991).

Studies have shown that after sympathectomy there is an upward shift of the distensibility pressure curve in the arteries and reduction in stiffness in the arteries, hence it can be inferred that the sympathetic nervous system exerts a marked tonic restraint on the arterial distensibility and this restraint involves both muscular and elastic arteries, thus provides the evidence of contribution of this factor in the modulation of mechanical properties of arteries (Mangoni et al., 1997).

Rodrigues et.al. Showed that reduced HRV predicts the progression of cardiac autonomic neuropathy and is associated with early atherosclerosis (Rodrigues et al., 2010). Cardiac autonomic neuropathy leading to inflammation represents the pathway through which the traditional risk factors promote or trigger the atherosclerosis process

4.6   Respiratory influences upon cardiovascular function

There is a close relationship between respiratory and cardiovascular processes mechanical and neurohormonal alterations in one system would affect those of the other in order to ensure homeostatic functioning. Mechanisms by which respiration modulate cardiovascular activity include(Grossman, 1983):

- Mechanical effects
- Lung inflation receptors
- Chemoreceptors
- Central mechanisms

Mechanical effects

The pumping action of the respiration pushes blood in and out of the heart. Inspiration serves to increase the venous return to the right heart and depending upon the degree of inspiration cardiac output will be altered by means of changes in heart rate and stroke volume(Grossman, 1983)..

Lung inflation receptors

Located in the bronchi and bronchioles, these are stretch receptors sensitive to changes in transpulmonary pressure and a linear relationship exists between tidal volume and receptor discharge (S.C. Gandevia, 1976).

Chemoreceptors

Located in the carotid and aortic bodies these receptors are sensitive to changes in blood concentration of oxygen, carbon dioxide and hydrogen which may be provoked by changes in respiratory variables (rate and depth of ventilation) (Grossman, 1983).

Central mechanisms

Central coupling mechanisms bind respiratory and cardiovascular processes together in such a manner that cardiovascular efferent discharge, both cardioinhibitory and vasomotor typically fluctuates in phase with central respiratory activity (Eckberg et al., 1980)

4.7   Respiratory parameters of significance

Respiratory modulation of cardiovascular activity is expressed through a number of parameters

- Breathing frequency: variation in breathing frequency is seen to produce inverse changes in heart rate variability independently of tidal volume (Hirsch and Bishop, 1981).
- Depth of ventilation: increased depth of ventilation causes augmentation of heart rate and heart rate variability, as well as generally increased blood flow to skeletal muscles but decreased flow to hands and feet(Hirsch and Bishop, 1981).
- Breath holding : breath-holding pauses between inspiration and expiration produces rapid and pronounced bradycardia (Daly et al., 1979).
- Muscular mode of ventilation: The relative contribution of thoracic (ribcage) and abdominal (diaphragmatic) muscle usage in the act of ventilation can vary widely between individuals and situations(De Troyer and Estenne, 1981).
- Alveolar carbon dioxide tension: considered to be a reflection of arterial CO_2 partial pressure, alveolar CO_2 pressure (end tidal) has been related to wide range of cardiovascular parameters.

4.8   Cardiovascular response to slow breathing

There was no significant change in blood pressure produced with slow breathing patters (free breathing, 50% inspiration at 0.1Hz and 70% inspiration at 0.1Hz). The reason for this might be that all subjects of our study were healthy normotensive subjects. Previous studies have shown a temporary fall in blood pressure following 10 minutes of slow breathing pattern but these studies were done on hypertensive individuals (Kaushik et al., 2006). Another study also performed on hypertensive individuals reported a fall in blood pressure following slow breathing exercises (Pinheiro et al., 2007). Other studies on healthy normotensive individuals reported a fall in blood pressure were conducted for a period of four weeks and above (Bhargava et al., 1988; Upadhyay Dhungel et al., 2008). Another study reported fall in blood pressure following five minutes of slow breathing 6breaths/minute, in which the subjects were seated erect on the floor with eyes closed and made to inhale through both the nostrils slowly upto the maximum for 4 seconds and then made to exhale slowly upto maximum through both the nostrils for 6 seconds, the inhalation exhalation time did not matter however the slow breathing procedure was different from the procedure we followed in our study (Pramanik et al., 2009). Sympathetic activity (low frequency power HRV) increased significantly with slow breathing.

4.8.1   Effects of consciously controlled slow breathing

A slow rate of breathing (in the range of 6 breaths/min, 0.1Hz) is said to have many favourable effects on the cardiorespiratory system (Bernardi, 2002) and is also proposed as the simplest physiological technique that can be used to augment baroreflex function (Bernardi et al., 2001; Goso et al., 2001; Joseph et al., 2005). It increases the resting oxygen saturation, improves ventilation/perfusion mismatching and increases exercise tolerance by reducing the sensation of dyspnea and fatigue (Bernardi et al., 1998). Other positive effect of slow breathing is that it significantly enhances the baroreceptor sensitivity, both in the healthy individuals as well as in the presence of chronic heart failure (Bernardi, 2002). It was inferred from the cross spectral studies that modulation exerted on the arterial baroreflex by slow breathing pattern would affect its cardiac, vagally mediated component, but study reported by Radaelli confirmed that slow controlled breathing was associated with enhanced reflex responses to carotid baroreceptor

stimulation and this enhancement was equally evident for bradycardic and depressor component of reflex as well as showed that the modulation extends to vascular, sympathetically mediated component of reflex (Radaelli et al., 2004).

During the process of slow breathing there is increase in the tidal volume which compensates for the reduced breathing rate in order to maintain the minute ventilation (Bernardi et al., 1998; Eckberg et al., 1985) and this is responsible for the autonomic changes occurring through the reduction in sympathetic activity (Goso et al., 2001). There is also a reduction in the blood pressure seen during slow breathing which is produced due to the reduced afterload which occurs secondary to the reduced sympathetic activity (Goso et al., 2001). Bilo G et. al. reported in their study that slow breathing at the rate of 6 breaths per minute was associated with improvement in ventilation efficiency shown by significant increase in blood oxygen saturation at high altitudes (Bilo et al., 2012). This favourable sympathovagal balance occurring during slow breathing is also linked to reduced chemoreflex activity (Ponikowski et al., 1997; Spicuzza et al., 2000).

The concept that slow breathing enhances BRS is mainly based on studies that have employed BRS quantification relying on spontaneous cardiovascular changes (e.g. alpha index) that quantifies the relationship between arterial blood pressure (BP) and R-R interval (cardiac period) changes occurring at respiratory frequency (Bernardi, 2002; Bernardi et al., 2001; Joseph et al., 2005), whereas there was no augmentation of BRS seen when it was assessed from drug induced blood pressure fluctuations evoked during modified oxford method (Tzeng et al., 2009). Horsman et. al. reported that apparent improvements in BRS associated with slow breathing reflects the frequency of blood pressure oscillations (i.e. the frequency-dependent nature of the baroreflex) and is not due to global effect of slow breathing (Horsman et al., 2015). Sheel et. al. reported that deep breathing produced inspiratory muscle fatigue which causes sympathetically mediated vasoconstriction and reduced flow in the resting limb (Sheel et al., 2001).

One of the studies has shown that exhalation feedback helps to concentrate on exhalation process and by this means slows down the respiration rate as a consequence also heart rate and thus can be useful tool for inducing relaxation and slowing heart rate (Zeier, 1984)

With all the above knowledge on the effectiveness of slow breathing, the purpose of the study was to test the hypothesis that slow breathing is responsible for lowering blood pressure and sympathetic activity but, in this study following the three different patterns of slow breathing there was no change in the blood pressure.

4.8.2 Free breathing versus slow breathing (50% inspiration at 0.1Hz and 70% inspiration at 0.1Hz)

During slow breathing pattern, there was sympathetic activation shown by an increase in the low frequency (LF) power. This may have been driven by arousal during prompted breathing compared to unprompted free breathing. In our study subjects were made to concentrate on the breathing metronome while performing the breathing patterns, this could have induced arousal and increased sympathetic nerve activity in our subjects. Arousal is known to depress the gain of baroreflex control of the sinus node (James Conway, M.D., Nicholas Boon, 2015).

Bernardi et. al. confirmed in their study that both respiration and sympathetic activity are capable of profoundly affecting the LF and (HF) components of RR variability. When sympathetic activity predominates as in conditions of stress, the LF of RR predominates regardless of the changes in respiration. In the absence of stress, respiration alone is capable of

completely altering the RR spectrum by increasing the LF component i.e. slow breathing. In conditions of stress a variable degree of sympathetic activation often interacts with concomitant effect of slowing of breathing, creating a predominance in the LF component which is partly explained by the increased sympathetic activity and partly by the slowed breathing. They confirmed that slow breathing along with mental stress generates an increase in the LF component in the RR spectrum regardless of the amount of stress involved in the task. In our study subjects were told to concentrate on the breathing metronome, which might have caused mild arousal and then they were asked to breathe in a particular pattern for a period of time which might have induced a little stress (Bernardi, M.D., 2011).

There is an increase in the tidal volume produced during controlled slow breathing which causes a mild hyperventilation (Sasaki and Maruyama, 2014). The other possible reason could be that the cortical input accompanied by the action of consciously controlled slow breathing induces alterations in circulatory regulation (Han et al., 1997). Controlled slow breathing requires mental concentration, which could tend to increase the LF component (Sloan et al., 1991). It has been seen in trials that mental stress produces an increase in the heart rate and blood pressure (Pagani et al., 1991; Yoshino and Matsuoka, 2005). There is a significant loss of vagal tone produced due to the discomfort experienced by the subjects in order to adjust the breathing rate (De Meersman et al., 1995). Breathing patterns are found to be highly sensitive to arousal (Blumenstein et al., 1995).

Regarding the inhalation-to-exhalation ratio (I:E ratio) in our study we had one pattern with equal time for inhalation and exhalation (5.0sec inhalation and 5.0sec exhalation) whereas the other pattern had a longer inspiration and quick expiration (7.0sec inhalation and 3.0sec exhalation) this type of pattern have been seen to be less effective in reducing physiological and psychological arousal than breathing in a pattern with short inspiration and longer and slower expiration (Cappo and Holmes, 1984).

HRV is said to be influenced by a number of non-pathological conditions like activity (Bernardi et al., 1996) and breathing (Bernardi et al., 1989; Sanderson and Yeung, 1996) increase in the LF-HF ratio indicates increased sympathetic activity and a decrease in this ratio indicates the increased vagal activity (Malliani, 2005; Taskforce, 1996)

Respiration should be analysed or measured in short sequences of data (4min-10 min) because the increase in the LF component of power spectrum may be produced due to change in breathing pattern or the normal sympathetic activation produce during mental tasks may be masked or increased by the addition of frequencies generated by unequal or slow breathing patterns. Therefore if respiration is not simultaneously analysed the changes in LF/HF ratio should not be taken as clear evidence of changes in autonomic tone (Bernardi, M.D., 2011).

5   Conclusion

This study examined a cohort of 30 subjects to quantify age-related changes in arterial stiffness, examine baroreceptor function relationships with other cardiovascular parameters, and to investigate the hypothesized blood pressure lowering effects of slow breathing.

Although a relatively small sample size for investigating age-related cardiovascular changes, increases in brachial and aortic systolic pressure and decreases in BRS with age were detected.

A recently derived novel index, the pressure sensitivity of cfPWV (ΔPWV/ΔDP) was calculated. There was a significant decrease in blood pressure with change in position from seated to supine, which is related to the normal hemostatic mechanism of blood pressure with

orthostatic challenge and the effect of gravity on the blood column between carotid and femoral sites, the height changing from seated to supine. This value, like other cardiovascular variables known to vary with age such as diastolic pressure and augmentation index, showed a trend toward increase with age, but did not reach statistical significance in the sample size studied.

Aortic stiffness, measured by cfPWV, did not indicate a role in determining BRS in this cohort of subjects. However, BRS was correlated with aortic AIx with BRS decreasing with increasing AIx. There was no relationship between normalized low frequency or high frequency power of HRV with any of the measured arterial stiffness parameters.

Slow breathing, especially 70% inspiration at 0.1Hz, is hypothesized to lower blood pressure by activating the pulmonary stretch receptors which inhibits the sympathetic activity leading to widespread vasodilation and decrease in peripheral resistance and thus lower the blood pressure. The data from the present study indicate that slow breathing at 0.1Hz, whether at 50% or 70% inspiration does not change blood pressure. Sympathetic activity was increased significantly with slow breathing. It is proposed that this increase in sympathetic activity is due to the mild arousal produced during concentration on the breathing metronome and mild stress produced to breathe in a particular pattern. It may be that unprompted slow breathing, more akin to meditative practice, may have a blood pressure lowering effect, though this may also be tied to altering of stress levels through meditation than modification of stretch receptors, and this remains to be studied. The results presented here indicate that prompted slow breathing does not have any benefit over supine rest with free breathing for blood pressure control.

Carotid intima- media thickness and/or prevalence of carotid atheromatous plaques, may modify baroreceptor sensitivity. Not directly assessing these by ultrasound is a limitation of the current work.

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