The Influence of Sex on the Relationship Between Arterial Mechanical Properties and Cardiovagal Baroreflex Sensitivity

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ABSTRACT

Cardiovagal baroreflex sensitivity (cvBRS) demonstrates a strong relationship with arterial mechanical properties. Both cvBRS and arterial mechanics differ by sex such that males demonstrate greater cvBRS, yet lower large artery elasticity than females. Whether the relationship between cvBRS and arterial mechanics is similar in males and females remains unexamined. As a result, it is unclear whether arterial mechanics contribute to sex differences in cvBRS. This study investigated the cross-sectional relationship between cvBRS and arterial mechanical properties of the common carotid, carotid sinus and aortic arch (AA) in 36 (18 females) young, healthy normotensives. The cvBRS-arterial mechanics relationship did not reach statistical significance and did not differ by sex. Both cvBRS and AA distensibility were greater in females than males. Sex differences in cvBRS were eliminated after controlling for AA distensibility. These findings suggest that in this sample, AA elasticity may contribute to the greater cvBRS in females than males.

KEY WORDS: cardiovagal baroreflex sensitivity, arterial mechanical properties, sex differences, blood pressure regulation, cardiovascular system

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ABBREVIATIONS

AA	*aortic arch*
ACh	*acetylcholine*
ANS	*autonomic nervous system*
AV	*atrio-ventricular*
BMI	*body mass index*
BP	*blood pressure*
BRS	*baroreflex sensitivity*
CCA	*common carotid artery*
cfPWV	*carotid-femoral pulse wave velocity*
CI	*confidence interval*
CO	*cardiac output*
CS	*carotid sinus*
CV	*cardiovascular*
cvBRS	*cardiovagal baroreflex sensitivity*
CVD	*cardiovascular disease*
CVLM	*caudal ventrolateral medulla*
DBP	*diastolic blood pressure*
ECG	*electrocardiogram*
FFT	*fast Fourier transform*
HF	*high-frequency*
HR	*heart rate*
HRT	*hormone replacement therapy*
HT	*hypertension*
LF	*low-frequency*
LVH	*left ventricular hypertrophy*
MAP	*mean arterial pressure*
MI	*myocardial infarction*
NTS	*nucleus of the solitary tract (nucleus tractus solitarius)*
OC	*oral contraceptive*
PP	*pulse pressure*
PWV	*pulse wave velocity*
RRI	*R-R interval*
RVLM	*rostral ventrolateral medulla*
SA	*sino-atrial*
SBP	*systolic blood pressure*
TPR	*total peripheral resistance*
T1D	*type 1 diabetes*
T2D	*type 2 diabetes*
WHR	*waist-to-hip ratio*

CHAPTER I: INTRODUCTION

The arterial baroreflex is a critical neuro-cardiovascular (CV) reflex responsible for regulating arterial blood pressure (BP) homeostasis. Regulation of short-term BP homeostasis is required for maintaining organ perfusion (most importantly brain perfusion), in response to BP challenges such as orthostasis, exercise or psychological stress. Through negative feedback the arterial baroreflex responds to acute fluctuations in arterial BP by modulating factors that determine BP (e.g. cardiac output and total peripheral resistance; CO and TRP, respectively). The cardiovagal BR branch of the arterial baroreflex is responsible for modulating beat-by-beat heart rate (HR), contributing to changes in CO.[1, 2]

In healthy individuals, BP alterations are indirectly detected by baroreceptors, afferent mechano-sensitive nerve fibres that terminate at the carotid sinus (CS) and the aortic arch (AA).[3, 4] Rises and falls in BP induce mechanical deformation of the artery that evokes neural depolarization and alters the firing rate of the baroreceptors.[5] Afferent nerve fibres carry impulses to the brainstem where the signal is processed and an efferent signal is elicited. Efferent nerves travel from the brainstem to the sino-atrial (SA) and atrio-ventricular (AV) nodes of the heart in order to modulate HR.[1] The anatomy of the cardiovagal baroreflex can be divided into two components. The initial stage of the baroreflex involving the transduction of BP into mechanical deformation is referred to as the arterial component, while the transduction of vessel stretch into changes in HR, including afferent, central and efferent activity, is referred to as the neural component.[6]

Cardiovagal baroreflex sensitivity (cvBRS) measures the efficiency of HR regulation via the cardiovagal baroreflex by measuring the time interval between R peaks

(R-R interval; RRI) in response to a change in systolic blood pressure (SBP).[7] Investigators use a variety of techniques to assess cvBRS. The current study employs a spontaneous method known as cross-spectral analysis, which utilizes a transfer function to estimate the relationship (or gain) between SBP and RRI changes.[8] Regardless of the method, a greater cvBRS implies increased efficiency of the cardiovagal baroreflex to alter HR in response to changes in SBP. cvBRS is regarded as a comprehensive index of autonomic function. In fact, evidence suggests that cvBRS may predict CV morbidity and mortality in patients with cardiovascular disease (CVD)[9] and relatively healthy individuals.[10] Thus, beyond short-term BP regulation, the cardiovagal baroreflex may be a critical mechanism involved in maintaining total CV health.

Given that arterial deformation is required to elicit the cardiovagal baroreflex cascade,[5] investigators have examined the relationship between arterial mechanical properties and cvBRS. Due to its superficial location and simple anatomy, many studies have focused on the association between common carotid artery (CCA) mechanics and cvBRS. CCA distensibility, a measure of arterial elastic properties,[11] demonstrates a strong relationship with cvBRS.[12, 13] However, baroreceptors are located in the CS and the AA.[3, 4] Findings[14, 15] (including unpublished studies from our laboratory) suggest that these locations may provide greater insight to the relationship between arterial mechanical properties and cvBRS. To date, few studies have investigated the relationship between cvBRS and CS mechanics,[14] while only one has assessed AA mechanics[15] using ultrasonography.

Although not universal,[16-18] findings suggest that sex differences exist in cvBRS such that males often demonstrate greater cvBRS than females.[19-21] Investigators have

attributed disparities in cvBRS to differences in either the arterial or neural components of the cardiovagal baroreflex. Intriguingly, studies report that females may have greater AA, CS and CCA distensibility than males.[22-24] However, the finding that females have greater arterial distensibility, yet demonstrate lesser cvBRS compared to males, contradicts the evidence suggesting that arterial distensibility is positively associated with cvBRS.[12, 13] Thus, further research examining sex specific BP regulation strategies is warranted. To date, no study has examined whether the relationship between cvBRS and arterial mechanical properties is similar in males and females. As a result, whether arterial mechanical properties contribute to sex differences in cvBRS is not known.

Therefore, the objective of this study was to examine the influence of sex on the relationship between arterial mechanical properties and cvBRS in young healthy individuals. We had two hypotheses. First, cvBRS will demonstrate a positive linear correlation with CCA, CS and AA distensibility while exhibiting a negative linear correlation with cfPWV. Second, compared to females, males will demonstrate a stronger relationship between arterial mechanical properties and cvBRS.

CHAPTER II: LITERATURE REVIEW

2.1 The Arterial Baroreflex

The arterial baroreflex is a critical neuro-CV reflex responsible for maintaining arterial BP homeostasis. Through negative feedback the arterial baroreflex responds to acute fluctuations in arterial BP by modulating factors that determine BP (i.e. CO and TPR, respectively).[2] This occurs through two independently regulated pathways, the cardiovagal (cardiac) baroreflex and the sympathetic (vascular) baroreflex (illustrated in Figure 2-1).[2] Both pathways respond to the same stimulus, changes in arterial BP. BP alterations are indirectly detected by baroreceptors, afferent mechano-sensitive nerve fibres which terminate in the adventitia of the CS and the AA.[1, 4, 25] Rises and falls in BP induce mechanical deformation of the artery (i.e. arterial diameter changes) that evokes neural depolarization and alters the firing rate of the baroreceptors.[5, 26, 27] The glossopharyngeal (cranial nerve IX) and vagus nerve (cranial nerve X), which innervate the CS and AA, respectively, allow afferent impulses to travel to the brainstem.[28, 29] These nerves form excitatory synapses with neurons located in the nucleus of the solitary tract (NTS) where glutamate is the primary neurotransmitter.[28, 30]

In the brainstem the arterial baroreflex differentiates into the cardiovagal and sympathetic baroreflexes, which provide differential regulation of arterial BP. The sympathetic baroreflex involves a neural projection from the NTS to neurons located in the caudal ventrolateral medulla (CVLM), which sends inhibitory signals to neurons located in the rostral ventrolateral medulla (RVLM).[31] RVLM neurons send signals to sympathetic preganglionic neurons of the intermediolateral cell column, which in turn stimulate sympathetic ganglia via acetylcholine (ACh). Sympathetic ganglia are

responsible for stimulating effector organs including vascular smooth muscle of arteries, primarily through norepinephrine binding of alpha-adrenergic receptors.[1, 30, 32] This is the dominant pathway involved in the regulation of arterial BP;[33] however, it will not be the focus of this thesis.

The cardiovagal baroreflex pathway originates as the NTS neurons synapse with vagal preganglionic neurons located in the ventrolateral portion of the nucleus ambiguus.[1] The cholinergic neurons extend to the cardiac neurons via the right and left efferent vagus nerve branches which terminate (releasing ACh) at the SA and AV nodes to exert a negative chronotropic effect (decelerate HR) within a fraction of a second.[7, 34] Rapid vagal control of HR is accomplished due to the micro-structural organization of the SA and AV nodes. These ganglia have high cholinesterase concentrations, which enable ACh to undergo rapid hydrolysis.[29] Additionally, potassium channel opening occurs rapidly via ACh muscarinic receptor binding, independent of second messengers, which delay transduction.[29]

The anatomical and neurochemical organization of the central nervous pathways allows negative feedback that simultaneously inhibits sympathetic and stimulates vagal activity, such that elevated BP elicits a decrease in TPR, venous return, HR and CO, ultimately causing a decrease in BP.[35] Alternatively, a decrease in arterial BP results in sympatho-excitation and vagal inhibition, restoring BP via the opposite response.[35] These mechanisms ensure short-term BP homeostasis via the arterial baroreflex. Thus, the arterial baroreflex is a key mechanism in maintaining organ perfusion, especially brain perfusion, during BP challenges such as orthostasis, exercise or psychological stress. The ability of the arterial baroreflex to modulate HR will be the focus of this thesis.

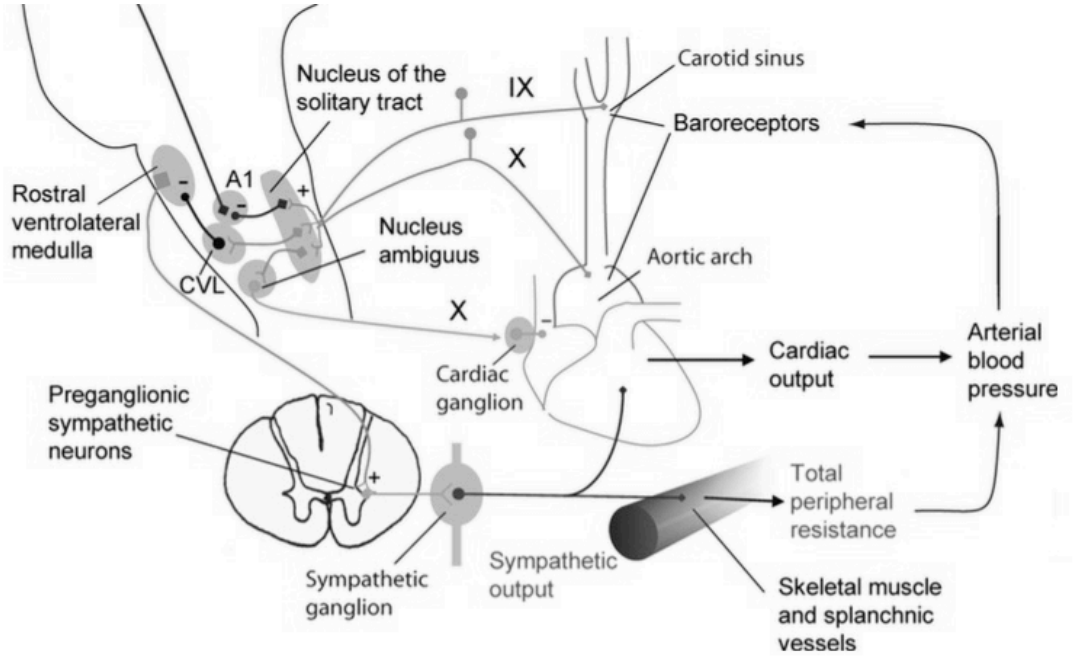

Figure 2-1. Organization of the cardiovagal and sympathetic baroreflex pathways, end organ responses and hemodynamic changes. Note: X, cranial nerve X; IX, cranial nerve IX; A1, noradrenergic neurons; CVL, caudal ventrolateral medulla; +, stimulatory; - inhibitory. Taken from Benarroch et al.[1]

2.2 Measuring the Cardiovagal Baroreflex

Baroreflex sensitivity (BRS, milliseconds/ millimeters of mercury; ms/mmHg) measures the efficiency of HR regulation via the arterial baroreflex by measuring the time interval between R peaks in response to a change in SBP.[7] Although the arterial baroreflex is a closed-loop system (i.e. HR changes feed-forward causing BP changes), cardiovagal BRS is defined as a change in RRI in response to a change in SBP (stimulus variable). Over a wide range of BP's, the relationship between RRI and BP demonstrates a sigmoid pattern.[36] The slope of the linear portion of the sigmoid relationship quantifies BRS.[37]

Although there are both vagal and sympathetic influences on the heart, experimental evidence suggests that BRS measures only vagally mediated changes in HR evoked by the cardiovagal baroreflex. Two lines of evidence confirm this notion. First, only vagal innervation of the heart can occur as rapidly as changes are observed[38] and

second, atropine abolishes the prompt bradycardic response to an acute BP increase.[39] Thus, the term *cardiovagal BRS (cvBRS)* will be used throughout this document to refer to the efficiency of the cardiovagal baroreflex to modulate HR.

2.2.1 Components of the Cardiovagal Baroreflex Pathway

Traditional methods examine the cardiovagal baroreflex as a single entity; however, the cardiovagal baroreflex pathway is composed of two components, arterial and neural. Consequently, observed changes in cvBRS can originate in either component. The initial stage of the baroreflex is the transduction of BP into arterial stretch, referred to as the arterial component (referred to as the mechanical component in other texts). The transduction of vessel stretch into changes in HR, including afferent, central and efferent activity, refers to the neural component.[6] Simultaneous beat-by-beat carotid ultrasonography allows researchers to quantify the relative contribution of these components to cvBRS.[40] This methodological alteration provides a mechanistic understanding of cvBRS. Although the current study does not employ this method, proper knowledge is essential, as significant evidence regarding cvBRS has resulted from its use. Additionally, understanding that the arterial system (specifically the CS and AA) is a component of the cardiovagal baroreflex is critical, as this study investigates the relationship between arterial mechanical properties and cvBRS in young healthy individuals. Current research employs numerous techniques to estimate cvBRS.

2.2.2 Pharmacological Methods

The Oxford and Modified Oxford techniques are pharmacological methods used to measure cvBRS. These methods involve experimentally induced changes in arterial BP through vasoactive agents. Smyth and colleagues observed an acute increase in arterial BP followed by a rapid decrease in HR following the injection of angiotensin.[41] This is known as the Oxford technique. At the time, the Oxford technique involved intravenous doses of 0.25 μg of angiotensin, an α_2-adrenergic agonist, until BP increased by 25-35 mmHg.[41] The investigators plotted the linear relationship between SBP in millimeters of mercury (input; x-axis) and a one beat-delayed RRI in milliseconds (output; y-axis). The slope of the regression equation, was used as an estimate of cvBRS. A steeper slope indicates a greater cvBRS, such that an increase in cvBRS reflects a greater increase in RRI for a one mmHg increase in SBP.[7] However, the use of angiotensin as a pressor agent provided confounding results as it was found to have direct cardiac effects.[34, 42] Thus, angiotensin was replaced by phenylephrine, a selective α_1-agonist. In a similar fashion, 1-2 μg/kg (typically 50-200 μg) bolus injections of phenylephrine were given to healthy participants until SBP increased between 20-30 mmHg.[42]

The Oxford technique is limited by its ability to only assess changes in HR in response to rising arterial pressures. This drawback motivated investigators to devise an approach to assess responses in HR to falling SBP's. Initially implemented by Pickering and colleagues in 1972 and further modified by Ebert and Cowley in 1992, the Modified Oxford technique explores cardiovagal baroreflex control of both rising and falling arterial BP.[6, 43] Vasodilator agents evoke arterial BP reductions via vascular smooth muscle relaxation. Commonly, the procedure involves sequential bolus injections of 100

μg of sodium nitroprusside to decrease SBP followed by 150 μg of phenylephrine hydrochloride to increase SBP 60 seconds later.[6] Baroreflex slopes calculated during falling BP are often less steep than those derived from increasing arterial BP, suggesting differential regulation of HR during positive and negative BP changes.[44]

Pharmacological methods of measuring cvBRS have several strengths.[34] Briefly, the arterial BP change evoked by a vasoactive agent provides a natural physiological stimuli which does not require cooperation from the participant or utilize unusual equipment (e.g. neck chamber). Additionally, pharmacological methods provide a large pressure stimuli over a short period of time which serves to open the closed-loop system between BP and HR.[34] However, these methods are not without their drawbacks. The main objection against the use of pharmacological BP manipulation is its capacity to alter the reflex itself. For example, evidence suggests that phenylephrine may induce carotid vasoconstriction,[45] in addition to having central nervous[46] and cardiovagal inhibitory effects.[47] Moreover, although BP changes induced by pharmacological stimuli are a natural stimuli, they are much greater than those occurring during spontaneous fluctuations and may not reflect physiological BP.[7] Lastly, injections of vasoactive substances may lack physiological selectivity. That is, a pressure stimulus evoked by pressor or depressor drugs may stimulate other pressure reflexes such as the cardiopulmonary receptors, which may provide misleading results.[7] Thus, other methods of assessing cvBRS have been designed to overcome the limitations of pharmacological methods.

2.2.3 The Neck Chamber Method

As an alternative to pharmacological methods, cvBRS can be assessed using a variable pressure neck chamber. The neck chamber was first used by Ernsting and Parry in 1957 in a series of experiments with the aim of understanding the function of the cardiovagal baroreflex.[2, 34, 48] Although many chamber designs exist, all allow mechanical manipulation of the carotid baroreceptors through quantifiable changes in carotid transmural pressure. Carotid baroreceptors detect increases in chamber pressure (neck pressure) as decreased arterial BP, which elicits a reflex increase in HR. Alternatively, decreases in chamber pressure (neck suction) are detected as hypertensive stimuli by the baroreceptors and results in a bradycardic response.[7, 49] Typically, 2-4 trials of neck pressure/suction are conducted which include random ordered 5-second pulses of pressure ranging from +40 to -80 mmHg. The cardiac response is typically observed 2-3 seconds after application of pressure.[2] Similar to pharmacological methods, the neck chamber technique calculates cvBRS by measuring the slope of the regression of RRI on neck pressure values. In contrast, the neck chamber technique is able to quickly induce rapid neck suction or pressure which allows the investigator to estimate sinusoidal stimulus-response curves.[2, 48]

Stimulus-response curves allow the calculation of several derived variables that provide the investigator with more insight into the function of the cardiovagal baroreflex.[2] For example, the response range is a measure of the minimum and maximum elicited RRI changes, whereas operating range is the minimum and maximum changes of SBP. The centring point is the point where the RRI change is equal for increases or decreases in SBP and is also known as the maximal gain. The operating point is the RRI

or SBP before stimulus. Threshold and saturation refer to the points at which no further changes in RRI occur in response to changes in SBP.[2]

The neck chamber method is advantageous in CV research as it allows the investigator to have intricate control over pressure stimuli applied to the carotid baroreceptors. This includes the ability to regulate the type and amount of pressure stimulus applied in addition to both the timing and duration of the pressure exposure.[50] Furthermore, as this method is non-invasive and does not rely on the administration of vasoactive drugs, the direct cardiac and smooth muscle effects of these drugs do not confound the response. Conversely, it is argued that pressures within the carotid artery and sinus may not be equal to those recorded in the neck chamber. It has been observed that only 64-83% and 86-89% of neck suction and pressure, respectively, are transmitted to the carotid.[2, 34, 48] However, complete transmission of pressure, may depend on variations in both chamber design and inter-subject carotid variations. Last and most importantly, the neck chamber approach of cvBRS measurement only provides the investigator with the capacity to measure CS baroreceptor mediated HR responses to pressure and does not attempt to quantify the contribution of AA baroreceptors.[2] Thus, when employing the neck chamber approach, a combination of methods must be used to measure integrated cvBRS. The following summarizes less invasive techniques to measure cvBRS.

2.2.4 Spontaneous Methods

In contrast to previously mentioned techniques that require extensive laboratory protocol and equipment, researchers often utilize computer based-techniques which analyze

spontaneously occurring fluctuations in arterial BP and RRI to calculate cvBRS.[7] Due to this, both the sequence method and spectral analysis methods are termed spontaneous techniques of cvBRS measurement. These methods are relatively simple, cheap and non-invasive. Both rely on spontaneous or naturally occurring parallel changes in RRI in response to changes in BP, rather than external modulation of BP to induce an RRI response. As a result, these methods do not suffer from the limitations that accompany the use of vasoactive drugs or neck chamber devices. Unlike previous techniques, spontaneous methods examine the baroreflex as a closed-loop system.[49] Thus, it cannot be disregarded that changes in HR may feed-forward, contributing to changes in BP.

2.2.4.1 The Sequence Method

The sequence (time domain) method, described by Parati et al.,[51] utilizes computer identification of three or more consecutive beats in which increases or decreases in SBP are accompanied by lengthening or shortening of RRI. To be considered a valid sequence for the calculation of cvBRS, the change in SBP and RRI must be at least one mmHg and six ms, respectively.[51] Typically, the accepted correlation between changes in SBP and RRI is 0.85, yielding a high specificity. Once again, the slope of the regression line relating changes in SBP to RRI serves as an estimate of cvBRS. As these sequences occur many times over the course of a trial, the average of the slopes are calculated to obtain the estimate of cvBRS.[43] cvBRS estimates can be calculated for all SBP/RRI changes or separately for bradycardic responses to rises in SBP and tachycardic responses to falls in SBP, providing an advantage over spectral methods. It has been demonstrated that changes in RRI are the result of baroreflex-mediated response to spontaneous changes in

SBP, as SBP-RRI sequences are abolished in sino-aortic denervated cats.[52] Analysis of spontaneous fluctuations in SBP and RRI can also yield an index of baroreflex effectiveness.[43] This is accomplished by calculating the ratio between the number of valid SBP-RRI sequences and the total number of SBP sequence pressure changes.[43] This index allows the researcher to quantify how many spontaneous series of SBP changes are actually compensated for by baroreflex-mediated RRI modulations. Hence, this index serves to provide complementary information of spontaneous baroreflex function.

2.2.4.2 The Spectral Method

Spectral methods used to analyze cvBRS are founded on the observations of rhythmic BP and HR oscillations related to respiratory and vasomotor activity by Hale, von Haller, Ludwig and Mayer.[53] The spectral (frequency domain) method is based on the assumption that parallel oscillations in SBP and RRI are mediated by the cardiovagal baroreflex.[54] These oscillations occur at two main frequency domains; low-frequency (LF) and high-frequency (HF). The LF exists between 0.04-0.15 Hz, while the HF exists between 0.15 - 0.40 Hz. However, early hemodynamic investigations often included three domains, low (0.02 to 0.06 Hz), mid (0.07-0.14 Hz), and high (0.15-0.40 Hz). Autonomic drug blockade yielded insight to the origins of each domain for RRI and SBP.[55, 56] Briefly, changes in the LF domain are mediated by both sympathetic and parasympathetic activity and are commonly induced by changes in body temperature or mental tasks. The mid-frequency domain is related to BP control mechanisms which are vagally mediated. The HF domain is also vagally regulated and is related to respiration, provided the respiratory frequency does not exceed mean RRI or HR.[55, 56]

Generally, upon collection of approximately 5 minutes (128-1024 beats) of beat-by-beat RRI and SBP, the data are converted from the time to the frequency domain via Fast Fourier Transform (Figure 2-2) or autoregressive modeling and then analyzed using either the alpha-coefficient technique or transfer function spectral analysis. The former, initially used by Pagani et al. provides spectral indices of LF and HF cvBRS by calculating the square root of the ratio between RRI and SBP at each spectral power.[57] Alternatively, the transfer function spectral analysis technique of cvBRS measurement is used, the following outlines details pertinent to this method.

The transfer function spectral analysis method of computing cvBRS was devised by Robbe et al. in the mid 1980's.[8] This method computes cvBRS as the average transfer function gain between the spectral powers (area under the curve) of SBP and RRI in the frequency range of 0.07-0.14 Hz (Figure 2-2), though the LF range varies by investigation. For example, O'Leary et al. have previously utilized an LF range of 0.03 - 0.15 Hz,[58] while others have used 0.04 – 0.15 Hz.[59, 60, 61] To calculate the gain between RRI and SBP the transfer function method divides the cross spectrum of RRI and SBP by the input autospectrum, SBP.[62] To ensure reliability, the linear relationship between SBP and RRI oscillations must be greater than a coherence threshold of 0.5 (Figure 2-2). Similar to the Oxford method, transfer function analysis reflects vagal control of HR and compares well with cvBRS estimates from a number of other methods.[8, 63]

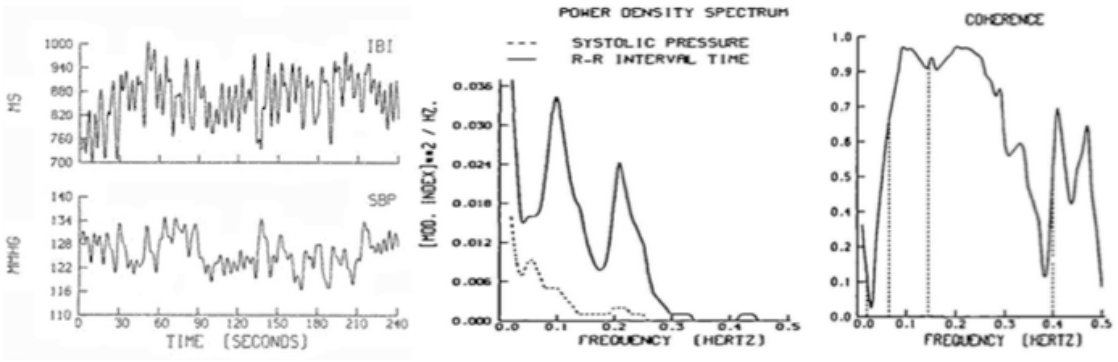

Figure 2-2. RRI (referred to as IBI; inter-beat interval) and SBP in the time domain (left), Power Spectral Density of RRI and SBP in the frequency domain (centre), coherence of SBP and RRI in LF domain (right). Adapted from Robbe et al. [39].

For example, Robbe et al. found a strong correlation (r = 0.94) between cvBRS obtained via transfer function spectral analysis and the phenylephrine method in eight healthy, young men.[8] In support of this, Lord et al. found that cvBRS values obtained through transfer function analyses were similar to the phenylephrine method, albeit this transfer function tended to overestimate cvBRS by approximately 17%.[64] In a study comparing the sequence technique to the spectral method (alpha-coefficient), Hughson et al. identified that both methods yielded similar cvBRS and were highly correlated (r = 0.85).[65] In addition, estimates of cvBRS via transfer function analysis demonstrate moderately low test-retest coefficients of variation. For example, Lord et al. found that the intra-subject coefficient of variation between two trials performed in the same day were 13.5% and 17.8 % in the LF and HF regions, respectively.[64] However, this group identified that the intra-subject coefficient of variation was greater (approximately 25% for LF and HF) over the course of a week for both LF and HF.[64] Similar investigations in both healthy and clinical populations have yielded comparable estimates of intra-subject coefficient of variation's ranging from 19% to 34%.[66-68] Based on these findings, investigators should employ caution upon detection of small cvBRS differences as they

may be a result of the inherent variability of the measure itself rather than a physiological difference between groups. Although this may be a limitation, spectral analysis and other spontaneous methods have several advantages.

Spontaneous methods are simple, non-invasive and relatively inexpensive.[63] In contrast to rigorous laboratory methods that elicit large changes in SBP, spontaneous methods provide reliable cvBRS estimates that are indicative of physiological cardiovagal baroreflex-mediated HR changes.[54] Moreover, these methods do not suffer from disadvantages specific to pharmacological methods such as central nervous or cardiac effects. Thus, estimates are not confounded by a drug-altered baroreflex.[63] Furthermore, spontaneous methods analyze only the baroreflex control of HR, while vasoactive methods may investigate the response of other receptor populations such as the cardiopulmonary receptors.[43] That being said, these techniques are criticized on their ability to only assess HR changes in response to BP fluctuations, while it is known that TPR plays a major role in BP regulation.[34] Lastly, in comparison to the sequence technique, spectral methods are limited by their reliance on longer recording periods (approximately 5 minutes) and their inability to examine baroreflex hysteresis (i.e. HR responses to both BP increases and decreases).[8, 69]

2.2.4.3 Autoregressive Moving Average Models

Autoregressive moving average models serve as a more complex alternative to the spectral analyses mentioned above. These mathematical models allow estimation of cvBRS using biological signals such as BP, RRI and respiration frequency among others. Autoregressive models adjust for the complex interactions which occur between BP and

HR in a closed loop system such as measuring baroreflex-mediated changes in RRI while controlling for feed-forward fluctuations in BP resulting from RRI changes. Beyond this, autoregressive models are able to account for the physiological impact of respiratory frequency on HR and BP.[43, 70]

In summary, a variety of techniques are employed to measure cvBRS, each with inherent strengths and limitations. Although some researchers hold invasive measures in higher regard than spontaneous techniques, it is evident that none are interchangeable. Ideally, cvBRS should be measured using a battery of techniques in order to provide the greatest knowledge regarding the cvBRS of a given sample. An in depth review by La Rovere et al. suggests that the average value of cvBRS (phenylephrine) is 15 ms/mmHg in healthy individuals.[7] However, extensive research suggests that various CV pathologies and physiological factors affect cvBRS. The subsequent section elaborates on these relationships.

2.3 Prognostic Significance of cvBRS

A deteriorated autonomic nervous system (ANS), characterized by increased sympathetic activity and low vagal activity, is a hallmark of many diseases.[71] Measurement of cvBRS, as a marker of ANS activity, has clinical relevance as it provides prognostic information regarding CV disease's (CVD) including myocardial infarction (MI) and heart failure.[50] Seminal studies by Schwartz et al. probed the prognostic implications of cvBRS by exploring sudden cardiac death in canines. Their findings suggest that the cardiovagal baroreflex is diminished in most canines 30 days after MI and dysfunctional baroreflex activity may lead to ventricular fibrillation.[72, 73] These studies encouraged human

investigations by La Rovere and colleagues[74] as well as others[75-79] in populations with previous MI's, namely the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) study.[9] This study demonstrated that diminished cvBRS (i.e. less than 3.0 ms/mmHg) was independently associated with increased total CV mortality (Relative Risk, 95% Confidence Interval (CI); 2.8, 1.24-6.16).[9]

Likewise, cvBRS demonstrates significant prognostic capacity in chronic heart failure populations.[80] Mortara et al. identified that cvBRS was the independent predictor of cardiac death or transplantation (Hazard Ratio, 95% CI; 2, 1.06-3.48) in 228 patients with chronic heart failure.[81] While La Rovere et al. and Mortara et al. used invasive vasoactive methods to assess cvBRS in patients with CVD, more recently Pinna and colleagues measured cvBRS non-invasively via the transfer function gain of the LF domain. The researchers demonstrated that a cvBRS less than 3.1 ms/mmHg was associated with a three-fold increased risk (Hazard Ratio, 95% CI; 3.2, 1.7-6.0) of suffering a cardiac event in 228 stable heart failure patients.[82] Compared to the phenylephrine method, cvBRS estimation by transfer function requires less technical expertise and due to its less taxing approach can be used prospectively in high-risk populations. Kiviniemi et al. expanded the predictive capacity of cvBRS (Valsalva) by examining both CV and non-CV related deaths in middle-aged individuals at risk of CVD without having suffered an MI or stroke.[10] Their findings suggest that a cvBRS less than 3 ms/mmHg significantly predicted both CV and all-cause mortality (Hazard Ratio, 95%CI; 9.1, 3.8-21.7 and 2.4, 1.1-5.4, respectively).[10] Taken together, these results suggest that regardless of the method used, a cvBRS less than ~3 ms/mmHg is indicative of CV event risk in individuals with or without chronic CVD, compared to those with cvBRS >3 ms/mmHg.

Thus, cardiovagal baroreflex control of autonomic activity may be important in determining overall health. This underscores the significance of cvBRS measurement both in the laboratory and clinical practice.

2.4 Cardiovascular Risk Factors and cvBRS

As mentioned, end-organ damage resulting from chronic CVD is associated with reduced cvBRS. It has been established that several risk factors are associated with the development of CVD such as hypertension (HT), obesity, diabetes mellitus and dyslipidemia. In order to further understand the relationship between baroreflex-mediated regulation of HR and CVD, researchers have examined the interplay between individual CVD risk factors and cvBRS. The following section reviews the association between cvBRS and BP, body composition, diabetes as well as lipids. It should be recognized that the current study investigates young, healthy individuals free of CVD and CVD risk factors. However, CVD risk factors are negatively associated with cvBRS even below clinically significant values. Therefore, it is necessary to understand the association between CVD risk factors and cvBRS when considering exclusion criteria and covariates for a study.

2.4.1 The Association Between Arterial BP and cvBRS

There is a well-known association between arterial BP and cvBRS. The inverse cvBRS-BP relationship exists in normotensives, such that as an individual's BP increases, their cvBRS decreases. This was demonstrated by Hesse et al. who found a moderate, negative association ($r = -0.49$) between ambulatory BP and cvBRS in 50 young, healthy,

normotensive individuals.[83] Furthermore, research consistently demonstrates that individuals with HT (i.e. SBP ≥ 140 and/or diastolic BP (DBP) ≥ 90) have diminished cvBRS compared to normotensives.[42, 84] For example, Parati et al. determined that compared to normotensives, hypertensive individuals have dramatically reduced (-38%) cvBRS (sequence technique) as determined by 24 hour BP and HR recordings.[51] Similarly, Takeshita and colleagues compared cvBRS (phenylephrine) between three groups of participants, young (< 20 years) individuals with normal BP, young individuals with borderline HT and older individuals (> 48 years) with HT.[85] Interestingly, they observed a graded decrease in cvBRS by BP group. Normotensives had the greatest cvBRS followed by borderline hypertensives and clinical hypertensives (16, 9, 5 ms/mmHg, respectively).[85] This confirms that cvBRS is attenuated in individuals with high BP prior to established HT and emphasizes the age-independent association between elevated BP and diminished cvBRS. Collectively, these findings provide support for the relationship between arterial BP and cvBRS in both normotensives and hypertensives.

Due to the cross-sectional design of the aforementioned studies the temporality of the arterial BP-cvBRS relationship remains unclear. Denervation studies provide insight to this relationship, although findings are not consistent. While Cowley et al. identified that sino-aortic denervation altered only short-term control of BP in canines,[86] Smit and colleagues found that some patients experienced chronic elevated BP after complete carotid baroreceptor denervation.[87] Observations by Smit et al. suggest that baroreflex dysfunction may precede HT, indicating that the arterial baroreflex may be in part responsible for the pathogenesis of HT.

2.4.2 The Association Between Body Composition and cvBRS

Obesity is an independent risk factor for CV morbidity and mortality.[88] Although relatively few investigations explore the association between body composition and cvBRS, it is evident that baroreflex-mediated HR modulation is reduced in both obese individuals with several comorbidities as well as obese normotensives with no comorbidities.[89, 90] Beske et al. identified that cvBRS was lower in overweight and obese males with high body fat (> 20 kg) compared to age-matched controls with lower body fat (< 20 kg). Furthermore, they found that compared to those with lower abdominal visceral fat, age and body weight matched males with higher abdominal visceral fat (visceral to subcutaneous fat ratio >0.4) demonstrated reduced cvBRS.[90] Moreover, an investigation by Alvarez et al. found that weight loss accompanying a 3-month caloric restriction diet intervention in overweight and obese males was associated with improvements in cvBRS.[91] These findings identify an inverse association between body mass and cvBRS. Additionally, they suggest that abdominal visceral fat may be involved in the mechanism contributing to diminished cvBRS.

2.4.3 The Association Between Diabetes and cvBRS

Similar to overweight and obesity, individuals with both type one and type two diabetes (T1D and T2D, respectively) are at a higher risk of CVD and mortality.[88] Moreover, these individuals demonstrate a reduced ability to regulate HR in response to BP changes. In their investigation comparing middle-aged, healthy normotensives to age-matched diabetics (both T1D and T2D) with and without autonomic neuropathy, Frattola et al. identified that diabetics have reduced cvBRS (alpha-coefficient) compared to healthy

individuals.[92] This has been confirmed by others, such as Bernardi et al.[93] and Rosengard-Barlund et al.[94] who examined individuals with T1D. However, these studies did not control for SBP, which was greater in diabetic than healthy participants. Additionally, these studies did not consider factors accompanying diabetes such as insulin treatment, insulinemia or hypo/hyper glycaemia, which may be involved in cvBRS reductions.[93] Nonetheless, these findings offer insight to the relationship between diabetes mellitus and the impaired baroreflex-mediated regulation of HR.

2.4.4 The Association Between Serum Lipids and cvBRS

Dyslipidemia, a lipid metabolism disorder, is a well-known metabolic risk factor of CVD[88] and may be associated with a reduced cvBRS. The literature regarding the effect of elevated serum lipids on cvBRS is inconsistent, as studies exploring acute and chronic dyslipidemia on cvBRS provide conflicting results. Gadegbeku et al. examined the effect of acute (one hour) increases in plasma free fatty acids and triglycerides (via lipid and heparin infusion) on cvBRS in obese individuals with HT and lean normotensives matched for age, sex and ethnicity.[95] Compared to baseline, cvBRS was reduced post-lipid infusion in both groups and this reduction was correlated with plasma increases in free fatty acids but not triglycerides.[95] These findings suggest that plasma lipid concentrations may be partly responsible for cvBRS reductions in individuals with dysregulated lipid metabolism. Conversely, a study by Monahan et al.[96] found no effect of acute hyperlipidemia on cvBRS. As Monahan et al. employed a control group and examined baroreflex-mediated HR responses to both rises and falls in BP (phenylephrine and nitroprusside), it is suggested that plasma lipid concentrations may not have an acute

effect on cvBRS. Both Piccirillo et al. and Koskinen et al. examined the effect of chronic dyslipidemia on cvBRS, yet found contradictory results. While Piccirillo et al. found that individuals with higher levels (>5 millimoles) of low-density lipoprotein had lower cvBRS compared to those with lower levels (≤ 5 millimoles) of low-density lipoprotein,[97] Koskinen et al. did not find a difference in cvBRS between individuals with hypercholesterolemia and controls.[98] Therefore, adverse serum profiles may be associated with reduced cvBRS; however, conflicting data by several investigations limit a definitive conclusion.

In summary, it is evident that risk factors contributing to CVD are associated with cvBRS. Currently, the mechanism by which cvBRS is reduced by CV risk factors is inadequately understood. As the distension of baroreceptor harbouring vessels (i.e. CS and AA) is required to initiate the baroreflex signaling cascade,[26] it is hypothesized that arterial mechanical properties are implicated in impaired cvBRS. However, as both arterial and neural components are involved in baroreflex regulation of HR, it is possible that CV risk factors affect both components.

2.5 The Influence of Aging on cvBRS

A multitude of complex CV alterations accompany aging, including functional and structural changes to the heart, vasculature and the ANS.[99] Due to this, age is commonly controlled for by age-matching or in statistical models. It has been well documented by numerous studies that cvBRS decreases with age.[6, 99-101] Bristow et al.[84] and later Gribbin et al.[42] examined the relationship between age and cvBRS in both normotensives and hypertensives. Gribbin et al. noted that the slope of the line relating RRI to SBP (i.e.

gain) decreased with age in both sexes (19-66 years).[42] Similarly, Rudas et al. confirmed that age is inversely correlated with baroreflex slopes in response to rising and falling pressures (r = -0.73, -0.77, respectively).[44] These findings indicate that aging diminishes an individual's ability to regulate HR in response to changes in BP.

Many investigations of varying sample sizes have replicated these findings using both invasive (i.e. Oxford and Modified Oxford) and non-invasive methods (i.e. Neck Chamber, Valsalva, Spontaneous) of determining cvBRS.[17, 21, 102-114] Of note, Parati et al. investigated cvBRS in 8 young (24 years) and 8 old (64 years) individuals over a 24-hour period using sequence and spectral techniques. Both techniques yeilded consistently greater cvBRS in young compared to older individuals.[115] These findings have been confirmed by several studies utilizing large population representative samples.[17, 21, 102, 112, 113] For example, Kardos et al. identified that age explained 21% of the variation of cvBRS obtained by the sequence method in 1134 males and females.[17] The abundance of evidence regarding this topic indicates that human aging has a degenerative effect on cvBRS, though the mechanism responsible for this decline is not adequately understood.

While some hypothesize that age-associated reductions in neural signaling are responsible for diminished cvBRS, others argue that the decline in cvBRS is due to reduced arterial elasticity with age. To elucidate the mechanism, both Hunt et al. and Monahan et al. measured cvBRS while performing carotid ultrasound to measure arterial distension. This allowed them to control for the transduction of BP changes into arterial stretch and measure both arterial (Δ diameter/ Δ pressure) and neural (Δ RRI/ Δ diameter) components of cvBRS in addition to conventional cvBRS.[40, 107, 116] While Hunt et al. found that the reduction of cvBRS in old compared to young, sedentary males was

attributed to reductions in both arterial and neural components, Monahan et al. identified that an attenuated arterial component explained age-related cvBRS reductions.[85, 86] Therefore, it is probable that age effects both components of the baroreflex pathway. This claim is supported by several lines of experimental evidence which demonstrate age-related reductions in cardiac responsiveness due to diminished muscarinic receptor concentrations in addition to decreased distensibility caused by arterial thickening.[99, 101]

While it has been consistently demonstrated that cvBRS is diminished with aging, there is inadequate information regarding whether the effect of aging on cvBRS is similar in both sexes. Of the studies that include both males and females, many are not designed to test potential sex differences in aging. In their study of individuals aged 23-77 years, Laitinen et al. reported that cvBRS was lower in females than males, though it appears that the sex difference was driven by younger participants, as no sex difference was observed in the oldest age group (60-77 years).[21] It is hypothesized that sex hormones are responsible for the difference in cvBRS between males and females. Thus, menopause may be responsible for the lack of observed difference in the 60-77 age group.[117] These observations are supported by experimental evidence by Credeur et al. who examined cvBRS in young and old females.[114] Herein it was identified that older females have a reduced ability to make HR adjustments and rely on changes in TPR in response to BP changes, whereas younger females rely on the HR changes to accommodate BP fluctuations.[114] Previously, it was found that men rely more on TPR than HR to regulate BP[118] and assuming that this is not altered throughout a male lifespan, it can be hypothesized that sex differences in BP regulation become less apparent with age.

However, to date, no study has examined BP regulatory mechanism changes in males with age.

In summary, abundant evidence suggests that aging compromises the efficiency of the baroreflex to modulate HR, which likely occurs due to reductions in the structure and function of both the arterial and neural baroreflex components. Research indicates that aging may affect cvBRS sensitivity in females more so than males due to the systemic physiological changes accompanying menopause. However, more female specific research is required, as compared to males, the knowledge regarding CV regulation in females is lacking. Therefore, the following section details our current knowledge regarding the influence of sex on cvBRS.

2.6 The Influence of Sex on cvBRS

Sex is a major determinant of CV function in both health and disease. It is well established that the prevalence of CVD and HT is greater in males than females.[119] These findings are suggestive of a long-term female-specific protective mechanism, most often attributed to ovarian hormones, estrogen and/or progesterone. This theory is strengthened by the diminished protective effect in post-menopausal females, who demonstrate increased prevalence of CVD.[119] Alternatively, females more so than males demonstrate orthostatic intolerance, underscored by a greater tendency for syncope.[120, 121] Syncope may be caused by inefficient BP regulation via the arterial baroreflex in response to falling BP induced by orthostasis.[122] Although paradoxical, these findings are suggestive of sex differences in both acute and chronic CV regulation.

Experimental evidence provides insight to sex differences in HR and BP regulation. Based on the findings that males rely more on TPR to regulate BP and females demonstrate an attenuated TPR response,[33, 123] Kim et al. investigated the relative contribution of the cardiovagal and sympathetic baroreflexes to BP regulation in males and females.[118] Using the neck chamber method they discovered that compared to males, females demonstrate a greater reduction in BP in response to simulated HT due to a greater bradycardic response, while males relied more on TPR alterations.[118] This suggests that HR regulation is more important in females than males for short-term BP homeostasis.

Several human studies have evaluated sex differences in cvBRS. A review of the present literature exploring sex differences suggests that cvBRS (ms/mmHg) may range from 13[19] to 24[20] in females and 16[18] to 46[20] in males. Many of these inquiries conclude that females demonstrate decreased cvBRS compared to males,[17, 19-21, 124] while others have found no statistical difference.[16, 18, 59, 67, 112, 125, 126] Moreover, no study has found greater cvBRS in females than males. This disparity may exist due to varying methodologies, as numerous techniques have been employed to investigate this topic (i.e. pharmacological[19-21], neck chamber[124], Valsalva[124], lower body negative pressure[124] and spontaneous methods[16, 17, 59, 67]). Additionally, inconsistent findings may result from a lack of continuity in covariate selection between studies. While most groups control for age, SBP, DBP, HR, and body mass index (BMI), many fail to control for menstrual phase of female participants[16, 17, 20, 21, 59, 67, 112, 125, 126], oral contraceptive (OC) use [16, 20, 21, 59, 67, 112, 125, 126], aerobic fitness[16, 18, 20, 59, 67, 112, 125, 126] or relative fat mass.[16-18, 20, 21, 59, 67, 112, 125, 126] The most thorough investigation of sex differences in cvBRS was conducted by

Beske and co-workers.[19] Analysis of 25 (11 males, 14 females; ~ 26 years old) participants using the modified Oxford technique yielded significantly lower cvBRS values in females compared to males (13 and 20 ms/mmHg, respectively). Unlike other studies, eumenorrheic, premenopausal female participants were tested only if they were non-OC users and exclusively during early follicular phase of the cycle (days 3-5). These criteria reduce inter-individual variability, as cvBRS may fluctuate across the female menstrual cycle. Although it is disputed, based on these findings it appears that females demonstrate lower cvBRS than men. This difference may exist due to sex hormones, as testosterone is the dominant sex hormone in males, while estrogen and progesterone are dominant in females. While complex laboratory methods can be used to modulate male and female sex hormones, the simplest way to examine the effect of female sex hormones is to investigate cvBRS at different menstrual stages in addition to pre- and post-menopause. As males do not experience equivalent hormone-related fluctuations across their lifespan, the following section will only highlight the effect of female hormone fluctuations on cvBRS.

2.6.1 The Influence of Female Sex Hormones on cvBRS

The influence of menstrual cycle phase on cvBRS remains unclear, as several human studies examining this topic yield conflicting results. Some report that baroreflex-mediated changes in HR vary across the female reproductive cycle,[127-129] while the majority have found no difference.[127, 130-134] The incongruence of these findings may stem from the method of cvBRS measurement (i.e. pharmacological vs. non-pharmacological) or time at which cvBRS measurements occurred.[135] For example, Cooke et al.[130] found

that cvBRS remained constant across four phases (follicular, pre-ovulatory, early and late luteal) of the female cycle using the neck chamber method, while Tanaka and co-workers[127] observed cvBRS (via Modified Oxford) to fluctuate across three phases (early follicular, pre-ovulatory, mid-luteal) of the cycle. As sex hormone concentrations (i.e. estrogens and progesterones) influence the phases of the reproductive cycle, it is speculated that they may directly modulate the cardiovagal baroreflex.

Recently, through elaborate techniques involving gonadotropin releasing hormone suppression and administration of estradiol, Wenner et al.[136] found an improvement in cvBRS only in those women who were prone to orthostatic intolerance. Conversely, using similar methods, Brunt et al.[137] found that estradiol and progesterone injections (individually and in combination) had no effect on cvBRS in healthy women. These findings demonstrate that female sex hormones may have an effect on cvBRS, though further research is required to improve our understanding on the topic. In order to reduce potential variability arising from ovarian hormone concentrations, it is common practice to test females in the same menstrual phase. Beyond this, investigators may also control for the effect of OC's on cvBRS.

OC's are sources of exogenous estrogens and progesterones which are widely used by women.[138] Limited research has explored the effect of exogenous ovarian hormones on cvBRS. This could be due to the fact that OC's present a variety of challenges in CV research. Exogenous estrogens and progesterones delivered from OC's differ from endogenous circulating hormones. For example, some differences may include the ratio of estrogen to progesterone, the physiological potency and the static concentration of exogenous hormones.[129, 135] Additionally, findings may lack

generalizability as hormone profiles vary between OC's. Thus, ensuring all participants receive the same type of OC, although challenging, may improve internal validity.[135] Research has identified that during the hormonal low phase (i.e. follicular) associated with placebo (i.e. sugar pill) ingestion, OC users had greater cvBRS than during the hormonal high phase (i.e. mid-luteal).[129] However, this investigation does not allow one to assess the true effect of OC's on cvBRS because it does not include non-OC using control group. A recent investigation by Wilczak et al. comparing cvBRS (sequence method) between OC using and non-OC using young females provides inconclusive results. This study identified statistically different cvBRS between groups during controlled breathing, yet failed to do so during spontaneous breathing.[139] Thus, although OC's appear to be a useful tool in cvBRS research due to their effect on hormonal concentrations, currently there are no definitive conclusions regarding their effect on cvBRS.

Similar to OC use, limited research explores the effect of menopause on cvBRS. Although they did not directly examine the effect of menopause, Laitinen et al. identified that sex differences in cvBRS that were present in young and middle-aged individuals were not apparent in 60-77 year olds.[21] Assuming that women in this age group would have undergone menopause, this finding suggests that diminished sex hormone concentrations may be responsible for the similar cvBRS values in this age group. However, Laitinen et al. included older females taking hormone replacement therapy (HRT), which makes it difficult to assess the effects of estrogen or progesterone in this study. Likewise, Gerritsen et al. also failed to identify a sex difference in cvBRS in a sample with an average age of 63 years.[67] Other researchers examining the effect of

menopause on CV regulation also support these findings. Both Credeur et al. and Barnes et al. investigated baroreflex regulation of BP in young and post-menopausal older women. Both groups identified that post-menopausal females regulate BP differently than younger female controls. That being said Credeur et al. found that young females relied on HR to alter BP and post-menopausal females relied on TPR, whereas Barnes et al. found that post-menopausal females relied more on HR responses to restore BP.[114, 126] Therefore, it can be cautiously concluded that sex hormones may be implicated in differences in cvBRS and BP regulation between males and females. Due to the lack of research devoted to examining human sex differences across the life span, in addition to the heterogeneity of current studies, an analysis of animal model research may assist in further elucidating the effect of sex hormones on CV regulation.

Laboratory investigations employing rat models confirm that compared to males, females demonstrate a reduced cvBRS. Beyond this, El-Mas et al. found that although females have lower cvBRS than males, ovariectomized female rats demonstrated a further reduction in cvBRS. Reductions in cvBRS in ovariectomized females were attributed to the absence of estradiol, as treatment with 17 β-estradiol attenuated the cvBRS reduction in this group.[140] Moreover, Mohamed et al. identified that the positive effect of 17 β-estradiol on cvBRS was centrally mediated and involved the aortic nerve.[141] Indeed, estrogen has a variety of central effects, as estrogen receptors are expressed on many central integration pathways of mice including the NTS, RVLM and the paraventricular nucleus of the hypothalamus.[142] In contrast, the effect of progesterone on the cardiovagal baroreflex has received less attention. Findings from one study suggest that the progesterone metabolite 3 α-hydroxy-dihydroprogesterone, which is elevated in

pregnant rats, may be responsible for reduced cvBRS.[143] Considering these points, it can postulated that estrogen and progesterone may have opposing effects on cvBRS, which may be responsible for observed cvBRS differences in males and females. However, further research is required to understand the effect of female hormones on cvBRS.

Unfortunately, limited research in animal models explores the effect of testosterone on cardiovagal baroreflex control of HR. El-Mas et al. found that testosterone reductions caused by castration were associated with decreased cvBRS in male rats compared to controls. Depleted testosterone was suggested to be responsible for the reduction in cvBRS, as testosterone supplementation was associated with restored cvBRS similar to control rats.[144] Similar to estrogen, testosterone is hypothesized to exert its effects centrally, as androgen receptors are expressed on various segments of the brainstem responsible for cardiovagal baroreflex integration.[144] Therefore, testosterone may be partially responsible for cvBRS differences in males and females, but further research is required.

In summary, laboratory data provide evidence to suggest that males have greater cvBRS than females. However, this finding is disputed. It is hypothesized that sex hormones may be responsible for these differences. The influence of female sex hormones have been examined utilizing both physiological female hormonal fluctuations (i.e. menstrual cycle and menopause) and fluctuations induced by pharmaceuticals (i.e. OC's). Results from clinical studies provide heterogeneous results that hinder a definitive conclusion regarding the role that sex hormones play in baroreflex regulation of HR, while research in animals provides greater insight on the topic. Indeed many physiological factors influence cvBRS and many of these differ by sex. One example is

the mechanical properties of the arterial system, which will be detailed in the following section.

2.7 The Arterial System

The vasculature is paramount to the CV system. It is comprised of arteries and veins of varying sizes in addition to a multitude of capillary networks.[29] In regards to the focus of this thesis, this section will elaborate on the structure, function and mechanical properties of arteries. An abundance of research examines arterial vessels, as the health of our arteries often serves as a marker of overall CV health.[145] Arterial structure becomes altered in aging and disease, rendering them less elastic or distensible. Reduced elasticity is accompanied by impaired arterial function, which may lead to health consequences such as CVD.[146, 147] More importantly (in this thesis), the CS and AA arteries deform in response to BP changes and initiate the neural cascade responsible for baroreflex-mediated adjustments in HR.[99, 101] Thus, the elasticity of the arterial system is associated with effective HR regulation by the cardiovagal baroreflex.

2.7.1 Arterial Structure

All arteries are comprised of three concentrically layered histological components that surround the lumen. From innermost to the outermost, there is the tunica intima, tunica media and tunica adventitia.[148] The intima consists of a one cell thick endothelial layer comprised of endothelial cells that are fixed to the basal lamina. The vascular endothelium is in direct contact with the blood and has the ability to release soluble mediators which participate in modulating vascular tone, coagulation and thrombosis.[149]

Beyond the intima lies the tunica media, which is composed of smooth muscle cells and is surrounded by a lattice network of elastin and collagen. Smooth muscle cells allow this layer to contract causing vasoconstriction, thereby modulating blood flow.[150] The outermost layer is the tunica adventitia and is dominantly composed of fibro-elastic connective tissue with lymphatic and small blood vessels (i.e. vaso vasorum) in larger arteries.[151] More in depth discussions of arterial anatomy are available elsewhere.[148, 149, 151, 152]

2.7.2 Arterial Function

The composition of an artery, that is, the ratio of smooth muscle to elastin and collagen, influences the function of an artery. The composition and function, varies by arterial region. The two main functions of the arterial system are to deliver blood from the heart to the periphery (i.e. conduit function) and to dampen the oscillations from intermittent ejections from the left ventricle to ensure constant flow and pressure (i.e. cushioning function).[153] Large elastic arteries, most notably the aorta and its branches, have a high proportion of elastin, allowing them to accommodate high pressures from the left ventricle and maintain continuous blood flow through diastole. Large muscular arteries have a high proportion of smooth muscle and are responsible for delivering blood to all organs. Smaller muscular arteries known as arterioles also have a large amount of smooth muscle enabling them to control blood flow by altering vascular resistance.[151] The efficiency of the arterial system depends on the elasticity of the vasculature. The composition of the arterial walls changes due to a variety of factors and as the elastic properties of the vasculature diminishes, arteries are said to stiffen.

2.7.3 Alterations in Arterial Mechanical Properties

Diffuse structural and functional changes in the vessel wall are responsible for reduced arterial elasticity and vascular stiffness. Degenerative arterial changes (i.e. thickening and stiffening) are referred to as arteriosclerosis, whereas atherosclerosis is a specific type of arteriosclerosis resulting from endovascular inflammatory disease, lipid oxidation and plaque formation.[154] For example, age-related arteriosclerosis primarily affects the aorta and central elastic arteries due to the chronic pulsatile stress evoked by blood ejected from the heart.[154, 155] Reductions in central artery elasticity are attributed to the deterioration of the media layer, which involves diminished elastin organization and thickness as well as increases in collagen and calcification.[156] Declines in elasticity are accompanied by increased pulse wave reflection which contributes to SBP amplification, increased arterial stress and further arteriosclerotic alterations.[11] Chronic adaptations to reductions in arterial elasticity include increased lumen diameter, increased wall thickness and endothelial dysfunction.[151] As a result, a cycle of vascular adaptations and resultant rises in SBP and pulse pressure (PP) arise which may lead to HT and CV complications such as left ventricular hypertrophy (LVH), aneurysms, atherosclerosis and MI's.[151, 154] A variety of techniques are used to measure arterial stiffness and elasticity.

2.8 Measures of Arterial Mechanical Properties

Numerous methodologies can be employed to assess systemic, regional and local arterial elasticity as well as stiffness. It should be noted that elasticity and stiffness cannot be used interchangeably as they refer to two unique vascular indices. Stiffness quantifies the resistance of a vessel to pressure deformation, whereas elasticity is the inverse of stiffness

and refers to the change in arterial area due to a change in pressure.[153] Due to the non-linear pressure-diameter relationship of an artery, arterial stiffness is pressure-dependent, meaning that stiffness is increased due to higher distending pressures.[150] Therefore, investigators should report the BP at which the stiffness measures were estimated and make comparisons cautiously between groups with differing BP's.[157]

There are a variety of measurements to estimate both arterial stiffness and elasticity at the systemic, regional and local level. Estimations of vascular mechanical properties often rely on models of circulation that encompass several assumptions. In brief, two principle models of the arterial system exist, the Windkessel and Propagative.[155] The Windkessel model views the arterial system such that the large elastic arteries maintain steady state flow via cushioning properties and the peripheral arteries provide peripheral resistance.[155] However, this model has been criticized due to the fact that it makes two unrealistic assumptions.[11] First, it assumes that the arterial tree has separate cushioning and conduit functions, which is not the case as elastic properties are dispersed throughout the aorta and its main branches. Second, it assumes that pulse wave travels at infinite velocities with similar amplitudes throughout the arterial tree. This is not correct as physical properties of the arteries change towards the periphery in healthy individuals, which leads to amplification of the pressure wave.[11] In contrast, a more realistic model of the arterial system is the Propagative model, which perceives the arterial system as a tube in which one end receives blood from the heart and the other exerts resistance.[155] In this model the pulse wave generated from the heart is propagated towards the periphery, becoming amplified as arteries stiffen and as the number of bifurcations increase. Reflections travel centrally and meet the incident pressure waves,

thus resultant pulse waves are a summation of incident and reflected waves.[155] The amplification phenomenon describes the occurrence of larger pulse waves in peripheral than central arteries due to the greater number of reflection sites (i.e. bifurcations) and larger resultant waves.[11] Pulse waves are used to assess systemic and regional stiffness and will be further discussed below.[11, 155]

2.8.1 Local Arterial Measures

Assessment of local vascular properties often focuses on either the carotid artery since it is a location of frequent atherosclerosis and the aorta, as it is responsible for vascular afterload resisting left ventricular ejection and hence, an important health predictor. A major advantage of employing local estimates of arterial elasticity or stiffness is that diameters and pressures are measured directly by ultrasonography and applanation tonometry, respectively. The force of blood on the vessel wall is known as circumferential wall stress ($\sigma=F/A$; F, force; A, area), and is directly proportional to the vessel pressure and radius while inversely proportional to the thickness. The resulting deformation of the vessel wall due to the stress of BP is known as strain ($\varepsilon = L – L_0/L_0$; L, final length; L_0, initial length).[154] The ratio of stress to strain is quantified by the Young's elastic modulus, which estimates the elastic properties of the vessel wall by considering wall thickness, although assuming homogeneity of the vessel wall.[154] Compliance refers to the absolute change in vessel area due to a change in pressure, while distensibility considers relative changes in area for a given pressure.[11] Inverse to distensibility is Peterson's elastic modulus, which indicates the pressure change responsible for the relative increase in lumen area.[155] Lastly, the β-stiffness index estimates arterial

compliance independent of distending pressure.[155] These parameters rely on the assumption that the cross section of the artery is circular.[158] The aforementioned indices can be calculated using the formulas provided in table 2-1.

2.8.2 Regional Arterial Measures

Researchers often employ pulse wave velocity (PWV) as a non-invasive measure of regional stiffness (i.e. mechanical properties of an arterial segment) due to its inherent simplicity and reproducibility.[11] PWV refers to the speed at which an arterial pulse wave travels through the vasculature.[155] The force of the blood ejected from the left ventricle is transmitted into a pulse wave, which travels to distal sites of the body through the arterial wall. The speed at which a pulse wave travels provides an index of arterial stiffness, as stiffer arteries transmit pulse waves faster than arteries that are more elastic.[153] Therefore, PWV's are greater in stiffer arteries.

PWV is calculated by measuring the time taken for pulse waves to travel a measured distance (formula Table 2-1), most commonly between the carotid and the femoral arteries (carotid-femoral PWV; cfPWV), which provides an index of stiffness for the entire aorta.[11] Pulse waves are measured transcutaneously at two different arterial sites either simultaneously or subsequently depending on equipment availability. If collected simultaneously, transit times (seconds) are measured between the feet of the two wave forms (i.e. the point at the end of diastole when the steep rise of the wave form begins), whereas when collected separately, the electrocardiogram (ECG) R-wave is used to measure the transit times of the separate pulse waves which are then subtracted from one another.[159] Pulse waves are commonly obtained via applanation tonometery, doppler

ultrasound, photoplethysmographic sensors and magnetic resonance imaging.[151] A measuring tape is used to measure the distances between anatomical landmarks and pulse wave recording sites. Commonly, the distance from the sternal notch to the carotid site is subtracted from the distance from the sternal notch to the femoral artery, although variations do exist.[151]

Table 2-1. *Equations used to calculate arterial mechanical properties*

Index	Formula
Young's modulus / Incremental elastic modulus (mmHg/cm^2)	$E_{inc=}\frac{3\left(\frac{1+\pi\left(\frac{dmin}{2}\right)^2}{\pi\left(\frac{de}{2}\right)^2-\pi\left(\frac{di}{2}\right)^2}\right)}{\frac{CSA}{\pi\left(\frac{dmin}{2}\right)^2\cdot PP}}$
Compliance (cm^2·mmHg^{-1})	$Compliance=\frac{\pi\left(\frac{dmax}{2}\right)^2-\pi\left(\frac{dmin}{2}\right)^2}{PP}$
Distensibility (mmHg^{-1})	$Distensibility=\frac{\pi\left(\frac{dmax}{2}\right)^2-\pi\left(\frac{dmin}{2}\right)^2}{\pi\left(\frac{dmin}{2}\right)^2\cdot PP}$
Peterson's elastic modulus (mmHg)	$Peterson=\frac{\pi\left(\frac{dmin}{2}\right)^2\cdot PP}{\pi\left(\frac{dmax}{2}\right)^2-\pi\left(\frac{dmin}{2}\right)^2}$
B stiffness (non-dimensional)	$ß=\frac{\ln\left(\frac{SBP}{DBP}\right)}{\left(\frac{dmax-dmin}{dmin}\right)}$
PWV (m/s)	$PWV=\frac{D}{\Delta t}$

Note: mmHg, millimetres Mercury; cm, centimetres; Einc, Young's modulus; dmin, minimum diastolic diameter; de, external diameter; di, internal diameter; CSA, cross-sectional area; PP, pulse pressure; dmax, maximum systolic diameter; SBP, systolic blood pressure; DBP, diastolic blood pressure; D, distance; t, time. View in text citations above.

2.9 Prognostic Significance of Aortic Mechanical Properties

Estimations of aortic stiffness are clinically significant, as the buffering capacity of the aorta is responsible for many pathophysiological adaptations that occur in CVD. In a healthy individual, the blood ejected from the left ventricle applies an outward force causing the aorta to distend. During diastole the elastic component of the aorta causes it to return to its original area, propelling the blood forward and assisting the heart in the delivery of blood systemically. However, with progressive stiffening, the ability of the aorta to accommodate blood and propel blood forward is reduced, thereby increasing myocardial workload.[153] The relationship between central arterial stiffness and cardiac function is known as ventricular-arterial coupling and is a major determinant of CV health.

Epidemiological research suggests that aortic stiffness (via cfPWV) has predictive utility in assessing CV events (e.g. MI) and mortality. It is non-invasive, simple and demonstrates high reproducibility. For these reasons, cfPWV is considered the 'Gold-Standard' in measuring arterial stiffness.[11] A recent meta-analysis of 17 longitudinal studies (average follow up 7.7 years) examining aortic PWV (11 of which utilized cfPWV) in 15,887 individuals found that the pooled relative risk of fatal and non-fatal CV events linearly increases from the first to third tertile of aortic PWV. CV events included MI, stroke, revascularization and aortic conditions. Moreover, the relative risk of total CV events, CV mortality and all-cause mortality were 2.26 (95% CI: 1.89-2.70), 2.02 (95% CI: 1.68-2.42) and 1.90 (95% CI: 1.61-2.24), respectively for subjects with high compared to low PWV's. Lastly, the investigators identified that a 1m/s increase in

aortic PWV predicted a 15% greater risk of CV events, mortality and all-cause mortality, after controlling for CV risk factors.[160]

Similar investigations have confirmed the CV predictive ability of cfPWV in a variety of populations such as those with T2D, essential hypertensives, end-stage renal disease, elderly individuals and the general population.[11] Likewise, AA stiffness assessed by ultrasonography is associated with CVD outcomes such as LVH and HT.[161, 162] These results demonstrate the utility of both local and regional measures of aortic properties in predicting both fatal and non-fatal CV events in addition to all-cause mortality and serve to highlight the role of aortic stiffness in CVD sequalae. Hence, in their Expert Consensus document, Laurent and colleagues postulated that aortic stiffness is an intermediate endpoint for CV events.[11]

2.10 Cardiovascular Risk Factors and Arterial Mechanical Properties

In addition to the numerous arterial alterations that occur in CVD, arterial mechanical properties are also modified by CV risk factors such as HT, obesity, diabetes mellitus and dyslipidemia. As previously mentioned these risk factors are associated with modifications in the efficiency to elicit baroreflex-mediated HR changes. As the arterial component of the cardiovagal baroreflex contributes to cvBRS, it is likely that alterations to arterial mechanical properties are responsible for cvBRS variations. The following section provides insight to the relationship between CV risk factors and arterial mechanical properties. As mentioned, the current study focuses on young, healthy individuals; however, understanding the potential effect of CV risk factors on arterial

mechanics is important when considering exclusion criteria and including covariates in multivariate models.

2.10.1 The Association Between Arterial BP and Arterial Mechanical Properties

Arterial BP is highly associated with arterial functional properties[163] and investigations demonstrate that indices of arterial mechanical properties are altered in HT.[164] For instance, Liu et al. identified that aortic compliance was reduced in individuals with HT compared to normotensives across a wide range of BP.[165] A longitudinal study by Benetos et al. extends this finding as they identified that compared to normotensive individuals, age-related increases in aortic stiffness (cfPWV), were greater in treated hypertensives after adjusting for sex, age and baseline PWV.[166] Further research has identified that arterial stiffening may be implicated in the pathogenesis of high BP and HT.[167] For example, in a longitudinal study, Dernellis and Panaretou identified that increased arterial stiffness proceeds the development of HT.[168] Specifically, this group found that mechanical properties of the ascending aorta such as strain, distensibility and β-stiffness index predicted the development of HT within 4 years after controlling for traditional CVD risk factors.[168] Thus, based on these observations it can be concluded that a relationship exists between arterial mechanical properties and high BP; however, due to the conflicting findings, it appears that bidirectional causality may exist and further research is required to elucidate the relationship.

2.10.2 The Association Between Body Composition and Arterial Mechanical Properties

Total body fat and to a greater extent central fat mass are major risk factors of CVD.[88] It is hypothesized that central fat mass may cause CVD by its negative effect on arterial health, motivating researchers to investigate this relationship. One such study was conducted by Ferreira et al. in which they explored the effect of central and peripheral fat mass as well as lean mass on the mechanical properties of large elastic arteries in 336 adults (average age 36.5 years).[169] Using dual-energy x-ray absorptiometry to assess body composition it was found that central fat mass had an adverse effect on large artery mechanical properties.[169] This was based on the inverse association between central fat and both CCA as well as femoral artery distensibility, femoral artery compliance and a tendency toward a positive association with aortic stiffness (cfPWV) after controlling for sex, height, and BP.[169] In contrast, they found that peripheral fat and lean mass had protective effects on arterial properties, as they were associated with favourable estimates of distensibility, compliance and the stiffness of some arteries.[169] Furthermore, a recent inquiry by Ferreira et al. confirms and extends the knowledge regarding the effect of central fat mass on arterial properties by demonstrating that subcutaneous trunk fat was negatively associated with large artery elasticity (compliance and distensibility) while being positively related to arterial stiffness (Young's elastic modulus) independent of aerobic capacity and metabolic syndrome.[170] Taken together, these data offer evidence of the deleterious effect that central fat mass has on large artery mechanical properties in adults and factors that promote central body fat accumulation should be considered as arterial stiffness risk factors.

2.10.3 Diabetes and Arterial Mechanical Properties

A variety of evidence supports the theory that both T1D and T2D are associated with impaired arterial function including increased stiffness and reduced elasticity.[171] To further the knowledge on this topic, Giannattasio et al. explored the effect of T1D on arterial properties in their longitudinal study of 80 males and females (average age 35 years), of whom 60 individuals had T1D unaccompanied by any micro- or macro-vascular disease and were otherwise healthy.[172] The group found that at baseline, compared to healthy controls, participants with T1D demonstrated lower distensibility of the abdominal aorta and radial artery as well as greater radial and CCA wall thickness.[172] Furthermore, after 23 months, T1D participants demonstrated significant reductions in both radial and aortic distensibilities while the controls showed no changes.[172] These findings suggest that individuals with T1D have altered arterial mechanical properties, which become progressively worse in a short duration. In a similar manner, individuals with T2D demonstrated poorer arterial mechanical properties compared to healthy controls.[173] This was observed in a study examining aortic stiffness and CCA intima-media thickness in 271 participants with T2D and 285 age-matched controls.[173] Findings from this study illustrate that T2D patients demonstrate both greater aortic stiffness and CCA intima-media thickness than healthy controls; thus, suggesting that individuals with T2D may have altered vascular properties.[173] Collectively, these findings demonstrate that arterial mechanical properties are reduced in individuals with diabetes mellitus, compared to healthy individuals.

2.10.4 The Association Between Serum Lipids and Arterial Mechanical Properties

In addition to the previously mentioned CVD risk factors, dyslipidemia may also be associated with deleterious arterial changes. This was explored in a study by Toikka et al. which examined the serum lipid profile in relation to the elastic properties of large arteries (e.g. thoracic aorta and CCA) in young men.[174] Individuals with a low high-density lipoprotein to total cholesterol ratio demonstrated lower CCA compliance compared to age and body mass matched participants with a high high-density lipoprotein to total cholesterol ratio.[174] Moreover, multivariate regression analyses revealed that serum oxidized low-density lipoprotein level independently predicted CCA compliance, while univariate analysis showed an inverse correlation between oxidized low-density lipoprotein and ascending aortic compliance.[174] These results suggest that unhealthy serum lipid levels may be detrimental for the elasticity of large arteries. Moreover, a study by Ferrier et al. exploring the effects of a three-month cholesterol lowering therapy (Atorvastatin) on mechanical properties of the large arteries found that systemic arterial compliance was greater after the protocol compared to the placebo.[175] However, the increase in arterial compliance was accompanied by improvements in both serum lipid profiles and BP, which may have accounted for the observed change. Together, these findings suggest that serum lipid profiles may influence arterial properties; thus, lipid-altering pharmaceuticals may serve as a treatment for arterial stiffening.

2.11 The Influence of Aging on Arterial Mechanical Properties

As mentioned, age-associated arteriosclerosis is an unavoidable process responsible for arterial stiffening across a lifespan.[154, 163] The effects of aging are more prominently observed in large elastic arteries, in which buffering capacity is an important determinant of total CV health.[163] Investigations have examined the effect of age-related changes in arterial structure on measures of arterial mechanical properties. For example, Benetos et al. examined the effect of aging on the elastic properties of the CCA and the femoral artery (i.e. distensibility, compliance, diameter, distension) using ultrasonography in males and females aged 23-71 years.[176] Based on the strong inverse correlation between age and CCA distensibility ($r = -0.70$, $p < 0.0001$) and the absence of this association for the femoral artery, it was concluded that CCA but not femoral elasticity decreases with age.[176] A more recent study by Mitchell et al. extends these findings by examining the effect of aging on aortic stiffness (cfPWV).[177] Their findings suggest that much like the CCA, age-related arterial stiffening occurs in the aorta.[177] Based on these observations, it can be concluded that aging is accompanied by central arterial alterations that reduce arterial distensibility and increase stiffness.

2.12 The Influence of Sex on Arterial Mechanical Properties

Research has identified sex differences in the arterial mechanical properties of several large arteries. Importantly, investigators have found the mechanics of baroreceptor harbouring vessels (i.e. CS and AA) to differ between males and females. Using magnetic resonance imaging, Nethononda et al. examined sex differences in proximal and abdominal aortic elasticity of young (20-29 years), healthy adults. The results indicated

that the distensibilities of the ascending, proximal descending and abdominal aorta, were greater in females than males, implying that females have greater aortic elasticity than males.[22] Similarly, Rose et al. found descending aortic distensibility to be greater in young females than males, whereas no sex difference was found in the elasticity of the ascending aorta in healthy individuals.[178] These results are also supported by Waddell and colleagues who found females to have a greater proximal aortic distensibility index than lipid, HR and BP matched males.[179] Lastly, Sonesson et al. found the abdominal aorta to be stiffer in males than females aged similarly to individuals in our sample.[180] Collectively, these findings indicate that females may have greater AA distensibility than males.

Sex differences in aortic stiffness have also been examined using cfPWV, though cfPWV does not appear to differ by sex. For example, Smulyan et al. found that cfPWV was the same in males and females. Although this suggests that regional aortic stiffness is not different in males and females (11.6 and 11.2, respectively), Smulyan et al. recruited middle-aged normotensives and hypertensives.[181] Consistent with this are findings from a population-based study by Filipovsky and colleagues. This study found that young males and females (≤ 35 years) had similar cfPWV, yet males demonstrated a non-significant tendency towards greater cfPWV.[182] These data suggest that younger males may have greater (non-statistical) aortic stiffness than females, which supports previous observations that females have greater AA elasticity. Thus, these studies suggest that males and females have similar regional aortic stiffness, although some evidence implies that males may tend towards having greater regional aortic stiffness.

Researchers have also investigated the effect of sex on the arterial mechanical

properties of the CCA and the CS. In a large sample of 2,195 individuals (35-55 years), Vermeersch et al. compared the distensibility of the CCA between males and females. In their youngest group of individuals (~38 years), CCA distensibility was similar by sex, indicating that males and females do not differ in CCA elasticity.[183] Moreover, findings by Giltay et al. extend these observations, as it was found that CCA distensibility was similar in young males and females.[184] However, some inconsistencies exist in the literature regarding sex differences in CCA distensibility. While Koskinen et al.[24] found greater CCA distensibility in females than males in population sample of young individuals, Van Merode et al.[185] found greater CCA distensibility in young (20-29 years) males than females.[185] Therefore, although inconsistencies exist in the literature, several studies suggest that males and females have similar elastic properties at the CCA.

Considerably less research has investigated the effect of sex on CS elastic properties. To the best of our knowledge, only one study[23] has compared CS mechanics in males and females, though this study did not examine local mechanical properties. To examine sex differences in CS properties, Schulz et al.[23] analyzed angiograms from 5,395 individuals ranging from healthy to 50% carotid stenosis. It was observed that significant differences in plaque distribution existed between males and females such that females were more likely to have maximum stenosis in the external carotid while males had maximum stenosis in the internal carotid artery. Based on this, it may be concluded that the internal carotid artery is more elastic in females than males. Moreover, Schulz et al. identified that CCA bifurcation anatomy was different between males and females, which may be implicated in the different patterns of maximal stenosis. Specifically, it was found that while males most often had smaller internal and external carotid arteries compared to

their CCA (i.e. smaller outflow area), female's internal and external carotid arteries were often similar in area to their CCA's (i.e. larger outflow area).[23] Although this study did not measure arterial distensibility, it provides insight to arterial mechanics at the CS and the CCA bifurcation. These findings also support previously mentioned studies which found females to have greater arterial elasticity than males. The scarcity of research in this area underscores the need for further studies investigating CS mechanics in young, healthy males and females.

2.12.1 The Influence of Sex Hormones on Arterial Mechanical Properties

Sex hormones may be implicated in differences in large artery mechanical properties between males and females. This has been explored in both clinical research studies employing non-invasive measures of arterial elasticity and animal model research examining the direct vascular effects of sex hormones. In 10 healthy, young women, Hayashi et al.[132, 186] found that carotid arterial compliance was associated with hormonal concentration such that compliance increased from the menstrual phase to the ovulatory phase, and was attenuated in the luteal phase.[186] This suggests that changes in estrogen and progesterone concentrations may alter the elastic properties of large elastic arteries such as the CCA. This finding has been validated in animal studies which have identified the presence of estrogen and progesterone receptors on the CCA as well as the proximal and distal aorta.[187] Surprisingly however, a recent study by Vlachopoulos et al. demonstrated that testosterone may have a favorable effect on arterial mechanical properties as they found a moderate inverse relationship between testosterone and aortic stiffness (cfPWV).[188] Similar to estrogen, testosterone receptors are expressed in the

endothelium and vascular smooth muscles of large arteries; however, some researchers hypothesize that the effect of testosterone is via 17 β-estradiol which testosterone is converted to by the enzyme aromatase.[187] Although both male and female sex hormones appear to promote arterial elasticity, they may be implicated in the sex differences observed in large artery elasticity.

2.13 The Relationship between Arterial Mechanical Properties and cvBRS

Researchers have attributed alterations in cvBRS to changes in either the neural or arterial component of the cardiovagal baroreflex. As the cardiovagal baroreflex cascade is initiated by arterial deformation (i.e. increases or decreases in diameter),[5, 26] it is hypothesized that the mechanical properties of barosensitive vessels (e.g. CS and AA) are in part responsible for cvBRS. Therefore, differences in arterial mechanics between groups (e.g. males and females, hypertensives and normotensives) may be responsible for variances in cvBRS. For example, HT and aging are associated with reductions in both arterial elasticity and cvBRS.[116] Additionally, exercise is accompanied by concomitant improvements in both cvBRS and arterial elasticity.[116] Although, some researchers provide evidence that arterial elasticity plays a dominant role in determining cvBRS, others suggest that neural alterations are also responsible for influencing cvBRS.[107, 116]

Several investigations have explored the relationship between arterial mechanical properties and cvBRS. Studies by Angell-James[26] and others[5] provide some of the earliest evidence regarding the critical role of arterial distension in the cardiovagal baroreflex cascade. By manipulating extra and intra-aortic pressure of an excised aorta, Angell-James observed that afferent baroreceptor nerve activity elicited by increases in intra-

aortic pressure was abolished by simultaneously increasing extra-aortic pressure, which prevented the wall from distending.[26] Thus, this study identified vessel distension as the stimulus required to elicit the cardiovagal baroreflex cascade. As a result, it is hypothesized that the mechanical properties of baroreceptor harbouring vessels are associated with cvBRS.

Bonyhay and colleagues were among the first to investigate this relationship in humans. This study examined CCA distensibility and cvBRS in 19 healthy, young adults (mean age 22 years). CCA distensibility obtained by ultrasonography was an independent predictor of cvBRS (Oxford method) explaining 61% ($r = 0.78$) of cvBRS variability.[13] These findings are supported by Steinback et al. who assessed the relationship between early systolic maximal CCA distensibility and cvBRS.[12] This study found that decreases in maximal CCA distensibility elicited by head up tilt were associated with simultaneous reductions in cvBRS ($R^2 = 0.75$), independent of hemodynamic changes accompanying postural stress.[12] Findings by Steinback et al. suggest that reductions in arterial elasticity upon head up tilt may be implicated in attenuated cvBRS. Taken together, these findings support the concept that arterial mechanical properties are mechanistically linked to the efficiency of the cardiovagal baroreflex.

In addition to the above mentioned studies, several other investigations have examined the relationship between cvBRS and CCA mechanical properties. For instance, Monahan et al. performed a series of experiments examining changes in arterial elasticity and cvBRS with aging as well as endurance training. In one study, Monahan et al. found that in 47 healthy, sedentary males (19-76 years), age-related decreases in CCA compliance were positively and independently related to concomitant reductions in

cvBRS ($R^2 = 0.51$).[106] Moreover, middle-aged males participating in 13 weeks of regular aerobic exercise demonstrated increases in both cvBRS and CCA compliance ($r = 0.72$).[106] This study serves to suggest that changes in CCA compliance may be a core component of the mechanism underlying cvBRS alterations with aging and exercise. As their previous study did not examine neural changes, Monahan et al. conducted a similar study to investigate the extent to which arterial and neural alterations contribute to cvBRS changes with aging and exercise. In this cross-sectional study, they found that the cvBRS differences between old and young groups, as well as sedentary and endurance-trained groups, were attributed to changes in CCA compliance rather than neural.[116] While findings by Kaushal and colleagues support this conclusion, Hunt et al. have found that neural alterations may also determine cvBRS.[107, 189] Collectively, these data suggest that CCA elasticity and to a lesser extent neural modulation contribute to cvBRS alterations. The CCA is a superficial artery with simple anatomy allowing more manageable image acquisition by researchers. However, baroreceptors are located in the CS[3] and AA,[4] yet few studies have examined the relationship between cvBRS and the mechanical properties at these sites.

To date, the relationship between cvBRS and AA mechanics has only been assessed in one study. In a sub-sample of 17 young (20-23 years) individuals, Lenard et al. examined the association between AA distensibility, using a wave-tracking technique and cvBRS, via the sequence method.[15] It was found that AA distensibility was moderately correlated to cvBRS ($r = 0.73$), which confirms the cvBRS-arterial mechanics relationship and extends the understanding to AA mechanical properties.

Studies by Mattace-Raso et al. and Michas et al. have examined the relationship between cvBRS and aortic mechanical properties; however, both utilized cfPWV to estimate aortic stiffness and did not directly examine AA mechanics.[190, 191] In a large population study of 2,400 elderly adults (average age 71.7 years), Mattace-Raso and coworkers found that cfPWV independently and negatively predicted cvBRS after controlling for covariates such as age, sex, BP, HR, medications and CV risk factors.[190] Similarly, in a sample of 160 middle-aged normotensives and hypertensives, Michas et al. found an independent negative relationship between cfPWV and cvBRS.[191] These studies demonstrate that increases in aortic stiffness are associated with reductions in cvBRS. However, as cfPWV measures aortic regional stiffness, which is heterogeneous (i.e. stiffness increases towards the femoral artery),[11] then estimates of aortic stiffness using cfPWV may not be indicative of AA (i.e. the location of baroreceptors) mechanical properties. Also, because only older individuals with CV comorbidities were recruited for these studies, the relationship between cvBRS and cfPWV in young, healthy individuals remains unclear.

The relationship between cvBRS and the mechanical properties at the CS has only been investigated in two studies (in addition to unpublished studies from our laboratory). A study by Gianaros and coworkers examined the mechanical properties at the CCA, CS and internal carotid artery in males and females aged 40-70 years, free from CVD and HT. It was found that CS intima-media thickness (a marker of sub-clinical atherosclerosis) was associated with reduced cvBRS (after adjusting for BP and CV risk factors), whereas no association existed between cvBRS and CCA or internal carotid artery intima-media thickness.[14] These findings suggest that cvBRS is related to the

arterial mechanical properties at the CS but not at the CCA or internal carotid artery. This supports the cvBRS-arterial mechanical properties relationship, yet should be interpreted with caution as baroreceptors are located in the proximal internal carotid artery[3] and it would be expected that the mechanical properties at this location would also be related to cvBRS. Additionally, Lenard et al. have examined the relationship between CS mechanical properties and cvBRS. This study found that CS distensibility (measured at the proximal internal carotid artery) was moderately related to cvBRS ($r = 0.54$) in sub-sample of young individuals.[15] Although scarce, this evidence identifies a relationship between cvBRS and CS mechanical properties. Further research is required to substantiate these claims.

In summary, arterial deformation is required to initiate the baroreflex-signaling cascade responsible for HR alterations. Therefore, the mechanical properties of baroreceptor harboring arteries (i.e. AA and CS) are important in determining cvBRS. While the relationship between CCA mechanical properties and cvBRS has been consistently demonstrated, proportionately less research has explored cvBRS in relation to the mechanical properties of the CS and AA. Thus, extensive research is still required to add important knowledge to this area.

2.14 Study Rationale

The current study is warranted for two reasons. First, there are significant disparities between males and females such that males are studied disproportionately more than females. As a result, the Institute of Medicine urged clinicians and researchers to increase the knowledge regarding sex differences in human health and disease.[192] This

disparity also occurs in CV research, such that an incomplete understanding exists regarding sex-specific CV regulatory mechanisms. Findings suggest that sex differences exist in cvBRS such that males often demonstrate greater cvBRS than females.[19-21] Investigators hypothesize that disparities in cvBRS between the sexes may arise from differences in either the arterial or neural components of the cardiovagal baroreflex. However, females often demonstrate greater arterial elasticity.[22-24] This finding does not explain why females typically demonstrate lower cvBRS; given the strong relationship between arterial elasticity and cvBRS.[12, 13] However, to date no study has examined whether the relationship between arterial mechanical properties and cvBRS is similar in males and females. The majority of the investigations have examined only males,[15, 104, 106, 116] while those that include both sexes are limited to elderly females[190] or do not direct their research question to identifying sex differences.[193, 194] As a result, whether arterial mechanical properties contribute to sex differences in cvBRS is not known.

Second, few studies to date have examined cvBRS in relation to aortic or CS mechanics.[15, 190, 191, 14] Both Mattace-Raso et al. and Michas et al. identified that cfPWV was an independent predictor of cvBRS (spontaneous) after adjusting for a variety of CV risk factors in older adult populations. However, these studies failed to exclude participants with the following confounding attributes: post-menopausal females, females receiving HRT, smokers, BP medication use, hyperlipidemia, orthostatic hypotension or overweight/obesity. In addition, Lenard et al. found moderate correlations between cvBRS (sequence) and the distensibility of the AA and CS ($r = 0.73$, 0.54, respectively) in a sub-sample of young individuals. However, the conclusions made by this study are limited, as the technique used to calculate both AA as well as CS distensibility is

inadequate (i.e. using peripheral PP's) and it does not provide any participant information (e.g. sex, health status, BP). Moreover, Gianarros et al. found that CS intima-media thickness was associated with cvBRS in a sample of middle aged and elderly individuals (40-70 years). Taken together, these studies suggest that both AA and CS mechanical properties are associated with cvBRS. However, given the collective limitations of past studies, these findings are not generalizable to a young, healthy population. As such, a valid relationship between cvBRS and AA as well as CS mechanics remains to be examined. Lastly, a study performing both cvBRS measurement and a comprehensive assessment of arterial mechanical properties (e.g. AA, CS and CCA elasticity as well as regional aortic stiffness) may provide novel insight to a mechanism explaining sex differences in cvBRS.

2.15 Purpose

Previous studies have not examined if the relationship between arterial mechanical properties and cvBRS differs by sex. As a result, whether disparities in arterial mechanical properties contribute to sex differences in cvBRS is not known. Moreover, few investigations have tested the relationship between cvBRS and the arterial mechanics of the CS, AA and the entire aorta; however, the limitations suffered by these studies (e.g. older samples with comorbidities, inadequate calculation of arterial distensibility) motivates further research on this topic. In order to address these knowledge-gaps, the purpose of this study was to investigate the influence of sex on the relationship between cvBRS and the arterial mechanical properties of the CCA, CS, AA as well as the entire aorta in young, healthy individuals.

2.16 Hypotheses

1) It was hypothesized that cvBRS will demonstrate a positive linear correlation with CCA, CS and AA distensibility while exhibiting a negative linear correlation with cfPWV.
2) It was hypothesized that compared to females, males will demonstrate a stronger relationship between arterial mechanical properties and cvBRS.

CHAPTER III: METHODS

3.1 Study Participants

Thirty-six young, healthy, non-smoking, normotensive individuals (18 females; average 24 ±2, 19-28 years) gave informed written consent to participate in this study. Participants were excluded from analyses if they were taking BP or autonomic modulating medications, had an arrhythmia, BMI ≥ 30 kg/m^2 or had been diagnosed with CVD or its known risk factors (e.g. HT, diabetes, dyslipidemia) as these have been found to alter cvBRS.[42, 50, 90, 93]. Endurance athletes were excluded from this study. Testing occurred between 09:00-11:00 hours after a fasted rest. Participants refrained from physical activity, alcohol and caffeine consumption 12 hours prior to testing. Females were tested during the hormonal low phase of their menstrual cycle (i.e. days 1-7, menstruation). Both OC using and non-using females were included in this study.

3.2 Experimental Design

Participants reported to the Human Hemodynamics Laboratory at Brock University to participate in the experimental protocol. Prior to the experimental protocol, participants were invited to attend a familiarization session, which included a description of the experimental details, protocol and benefits as well as risks. At this time, participants filled out the medical history questionnaire, in addition to reviewing and signing the informed consent form approved by the Brock University Research Ethics Board. On the testing day, participants voided their bladder prior to data collection to prevent the effect of bladder distension on arterial BP.[195] Participants wore athletic attire for the experimental protocol. First, anthropometric measurements including height, body mass,

waist and hip circumferences as well as body composition assessment were conducted. Following anthropometric and body composition measures, participants assumed the supine position for 15 minutes in order to achieve a resting state. Participants were instrumented, followed by three manual BP measurements and a subsequent 10 minutes of beat-by-beat HR and BP collection. Following this, AA, CCA and CS ultrasonography as well as applanation tonometry were performed. Three manual BP's were taken post-data collection to ensure baseline BP values. This study received approval from the Brock University Research Ethics Board (14-017 O'LEARY, Appendix C).

3.3 Experimental Measures

3.3.1 Anthropometrics and Body Composition

A stadiometer (STAT 7X, Ellard Instrumentation Ltd., Monroe, WA, USA) was used to measure height (cm) and a digital scale (BWB-800S, Tanita Corp., Tokyo, Japan) was used to measure body mass (kg). BMI (kg/m^2) was calculated using height and mass. Waist and hip circumferences (cm) were measured using an inelastic tape at the iliac crest and widest part of buttocks, respectively. Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference. Body composition (i.e. proportion fat mass and absolute lean/fat mass) was assessed via air-displacement plethysmography (BODPOD, *Gold Standard,* Life Measurement Inc, Concord, CA, USA).[196, 197] Subjects were seated in the chamber and a minimum of two volume measurements were conducted. A third volume measurement was performed if the difference between the two previous measures was greater than 150 mL. The two closest measurements were averaged. The BODPOD was calibrated prior to volume

measurement. Participants were required to wear tight-fitting active wear to ensure valid measurement.

3.3.2 Cardiovascular Measurement

RRI, the time between successive heart beats, was obtained via one lead ECG. Beat-by-beat non-invasive BP was measured at the left middle finger via the Nexfin, placed at heart level (BMEYE, Amsterdam, the Netherlands). Continuous BP and RRI were recorded for ten minutes. Prior to and following data collection, three manual BP readings were taken at the right brachial artery using a stethoscope and sphygmomanometer. Beat-by-beat finger BP's were adjusted to the average brachial SBP and DBP taken prior to data collection.[198] BP and RRI were sampled using an online data analysis and acquisition system (Powerlab and Chart 7, ADInstruments) at 1000Hz, providing a resolution of 1ms.

3.3.3 Cardiovascular Analysis

Average brachial-adjusted finger (Nexfin) SBP and DBP were taken from the first minute of data collection. Mean arterial pressure (MAP; ⅓SBP + ⅔DBP) and brachial PP (SBP-DBP) were calculated. Continuous RRI and BP data were inspected for ectopic beats; however, no irregularities were identified for this sample of participants. Matlab (Mathworks, R2012b) was used to interpolate and resample the data using the mean cardiac frequency. Data were filtered using a low-pass Butterworth filter of 0.95 Hz and detrended to remove any linear trends. Fast Fourier Transform (FFT) was completed with the Welch method and a Hanning window sized ¼ of the signal length with ½ overlap.

LF (0.04 - 0.15 Hz) and HF (0.15 - 0.40 Hz) spectral areas were utilized for RRI and SBP. A coherence of ≥ 0.5 was employed for gain and phase relationships.[8] cvBRS was measured for the first five minutes of continuous data collection, using the mean transfer function gain of the LF domain.[58]

3.3.4 Arterial Measures

Following 10 minutes of continuous data collection, ultrasonography (Vivid I, General Electric Medical Systems, Netherlands) was used to measure arterial diameters of the right CCA (1-2 cm proximal to the bifurcation), the right CS (proximal internal carotid artery just distal to the carotid bifurcation) and the AA (between the brachiocephalic and left CCA branches) using a 4 MHz phased array transducer for the AA and a 12 MHz linear array transducer for CCA and CS.[12, 14, 199] The suprasternal view was used for AA imaging as it depicts the AA and the three supra-aortic vessels (i.e. brachiocephalic, left CCA, left subclavian).[200] A minimum of three five-beat image sequences were captured. Two high quality image sequences were analyzed. Diastolic and systolic arterial diameters were measured using EchoPAC software calipers (ECHOPAC7-002308, General Electric Medical Systems, New York) at the R-wave and at the end of the T wave, respectively.[201] Arterial diameter was measured at the adventitial-medial border of the near wall to the medial-adventitial border of the far wall. AA image sequences were collected in B-mode and analyzed in M-mode while CCA and CS images were collected and analyzed in B-mode.

Local PP's were derived from arterial waveforms of the left CCA and CS, obtained non-invasively by a hand held applanation tonometer (Millar Instruments,

Houston, TX). The tonometer was calibrated with an external device using a two-point calibration system. Central PP's were measured indirectly using a transfer function algorithm (central PP (mmHg) = 0.90 (brachial adjusted finger PP) – 12.5)[202] applied to brachial-adjusted finger PP's. Central PP's were obtained using this validated method[202] to estimate local AA pressures which otherwise requires invasive intra-arterial measurement, as the AA is a deep artery that cannot be applanated. Measures of central BP in this study were similar to age, sex and brachial BP reference values in a nationally representative meta-analysis.[203]

Arterial diameters and local PP were used to determine CCA, CS and AA distensibility ($mmHg^{-1}$) using the formula:[11, 198]

$$\text{Distensibility} = \frac{\pi\left(\frac{\text{dmax}}{2}\right)^2 - \pi\left(\frac{\text{dmin}}{2}\right)^2}{\pi\left(\frac{\text{dmin}}{2}\right)^2 \cdot \text{PP}},$$

where dmax indicates maximum diameter, dmin indicates minimum diameter and PP indicates pulse pressure. Calculation of arterial distensibility using local PP provides greater accuracy of arterial elasticity, which would be underestimated using PP obtained at peripheral sites (e.g. brachial artery) given that amplification of PP increases towards the periphery.[11] Distension (cm) and strain (%) of the AA, CS and CCA were calculated using the respective formulas:[15]

$$\text{Distension} = \text{dmax} - \text{dmin},$$

$$\text{Strain} = \left(\frac{\text{dmax} - \text{dmin}}{\text{dmin}}\right) \cdot 100\%,$$

where dmax indicates maximum diameter and dmin indicates minimum diameter.

Following ultrasonography, cfPWV was employed to estimate regional aortic stiffness. Using applanation tonometry (Millar Instruments, Houston, TX) proximal pulse waves were collected from the left CCA and distal pulse waves were collected at the left femoral artery, distal to the inguinal ligament. The R-wave of the ECG was used as the initiation point of the pulse wave as carotid and femoral tonometry were performed sequentially.[159] Pulse waves were band-pass filtered (5 - 30Hz) to identify the time at minimum DBP.[204] The time at the minimum DBP subtracted from the time at the R-wave of the QRS complex was used to calculate transit time (seconds) at each site.[11] Distances (m) were measured (using an inelastic tape) from the sternal notch to the site where CCA PP's were collected and from the sternal notch to the femoral PP location (using the umbilicus as an intermediate landmark).[151] cfPWV (m/s) was calculated by averaging a total of 15 beats using the following formula:[11]

$$PWV = \frac{(D_2 - D_1)}{(T_2 - T_1)},$$

where, D_2 is the femoral artery distance, D_1 is the CCA distance, T_2 is the CCA transit time and T_1 is the femoral artery transit time.

As a result of inadequate acquisition of PP, arterial diameters or pulse wave transit times, sample sizes varied by measurement site or arterial indices. An overview of sample sizes is provided in Figure 3-1. Attrition analysis suggested that no significant demographic, anthropometric, body composition or hemodynamic differences existed between individuals with and without AA distensibility data (data not shown).

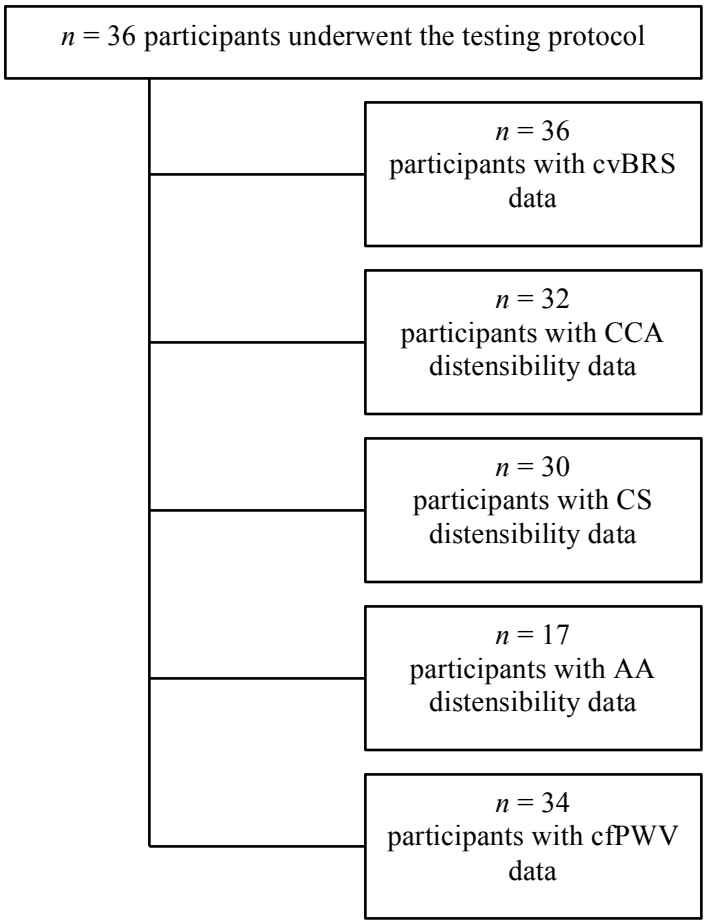

Figure 3-1. Overview of sample sizes by site and measure of arterial mechanical properties.

3.4 Statistical Methods

Descriptive statistics were computed for demographic, anthropometric and hemodynamic variables. Continuous variables were expressed as means (±SD). The categorical variable was expressed a frequencies (%). Shapiro-Wilk tests of normality were used to assess variable distribution. Independent sample *t* tests were performed to detect sex differences and Mann-Whitney tests of independent samples were used to detect sex differences in non-normally distributed variables. Pearson's tests of correlation were used to assess univariate correlates of cvBRS as well as the cvBRS-arterial mechanical properties relationship. Spearman tests of correlation were used to assess relationships in which one or both variables were non-normally distributed. Univariate correlations were stratified by sex. Simple linear regression was used to derive regression equations for cvBRS-arterial mechanical properties relationships. Sex by arterial mechanical properties interaction terms were made to evaluate whether the cvBRS-arterial mechanical properties relationship varied by sex. Multivariate linear regressions were performed to assess the effect of sex on cvBRS while controlling for physiologically relevant covariates found to differ by sex. Model one included sex and age. Models two, three and four included the previous model and further controlled for proportion of fat mass, WHR and SBP, respectively. Model five included sex and AA distensibility. Sample size calculations were performed using SAS (v9.4, SAS Institute, Cary, NC). All other statistical analyses were performed using SPSS (v20, SPSS Inc., Chicago, IL). Tests were two-tailed, $\alpha = 0.05$.

CHAPTER IV: RESULTS

4.1 Descriptive Statistics

Thirty-six young individuals (n = 18 females; average 24 ±2, 19-28 years) were recruited to participate in this study. Table 4-1 displays subject demographic, anthropometric and hemodynamic data for the sample as well as stratified by sex. Sex differences existed for all variables in Table 4-1 with the exception of RRI ($p = 0.29$). Compared to females, males were older, taller, had greater body mass, BMI, WHR and lean mass ($p < 0.05$). Females had greater fat mass and proportion of fat mass than males ($p < 0.05$). Compared to females, males had greater SBP (118 ±9 vs. 106 ±8 mmHg; $U_{Mann\text{-}Whitney}$= 49, $p < 0.01$), DBP (71 ±7 vs. 66 ±5 mmHg; $t_{(34)}$= -2.68, $p = 0.01$), MAP (87 ±7 vs. 79 ±5 mmHg; $t_{(34)}$= -3.89, $p < 0.01$) and brachial PP (47 ±9 vs. 41 ±6 mmHg; $t_{(34)}$= -2.47, $p = 0.02$). Of the females, 12 of 18 (66.7%) used OC's.

Table 4-1. *Sample demographics, anthropometrics and hemodynamics*

		Females	Males	p
n	36	18	18	
Age, *years*	24 (2)	23 (2)*	25 (2)	<0.01
Oral contraceptives	-	12 (66.7)	-	
Height, *cm*	172.6 (7.5)	167.2 (5.2)*	178.0 (5.0)	<0.01
Weight, *kg*	69.7 (11.4)	61.9 (6.5)*	77.5 (9.8)	<0.01
BMI, kg/m^2	23.3 (2.7)	22.2 (2.4)*	24.4 (2.5)	0.01
WHR	0.81 (0.06)	0.78 (0.06)*	0.84 (0.04)	<0.01
Lean Mass, *kg*	58.0 (11.6)	48.0 (3.8)*	68.0 (7.2)	<0.01
Fat Mass, *kg*	11.6 (4.9)	13.8 (4.3)*	9.4 (4.5)	<0.01
Fat Mass, *%*	16.9 (7.1)	22.0 (5.2)*	11.8 (4.7)	<0.01
SBP, *mmHg*	112 (10)	106 (8)*#	118 (9)	<0.01
DBP, *mmHg*	69 (7)	66 (5)*	71 (7)	0.01
MAP, *mmHg*	83 (7)	79 (5)*	87 (7)	<0.01
Brachial PP, *mmHg*	44 (8)	41 (6)*	47 (9)	0.02
RRI, *ms*	1058 (146)	1032 (118)	1084 (169)	0.29

Values are means (±SD). Independent sample *t* tests were employed unless otherwise stated. BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; RRI, R-R interval.
* indicates $p < 0.05$ vs. males.
# indicates Mann-Whitney U Test.

4.2 The Effect of Sex on cvBRS

Females had 32% greater cvBRS than males (25 ±11 vs. 19 ±7 ms/mmHg; $t_{(34)}$= 2.18, p = 0.04; Figure 4-1). The effect size of sex on cvBRS was 0.12 ($\eta^2_{partial}$) with a power of 0.56. cvBRS was 22 ±9 ms/mmHg for the sample.

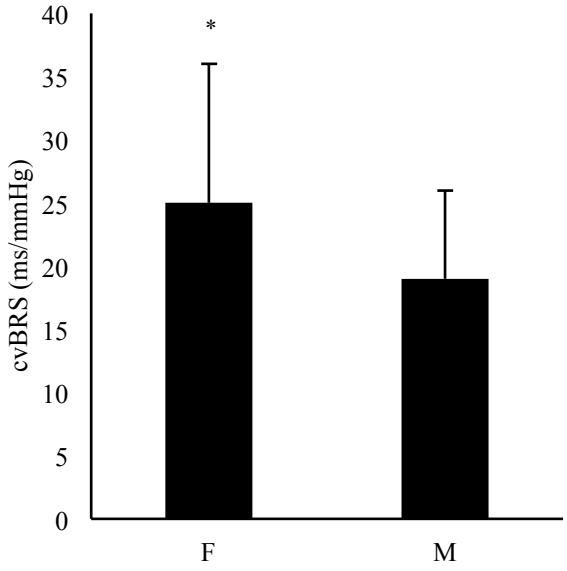

Figure 4-1. cvBRS by sex (F, female; M, male). Independent sample *t* tests were employed. Bars represent group means. Error bars represent SD. * indicates $p < 0.05$ vs. M.

4.3 Univariate Correlates of cvBRS

Table 4-2 displays the univariate correlates of cvBRS. cvBRS demonstrated a positive correlation with RRI ($r = 0.46$, $p < 0.01$) and inverse relationships with SBP and MAP ($r = -0.45, -0.35$, respectively, $p < 0.05$). Moreover, cvBRS exhibited a trend towards an inverse relationship with height ($r = -0.32$, $p = 0.06$). After stratifying by sex, only RRI remained significant in females and males ($r = 0.65, 0.58$, respectively, $p \leq 0.01$). There was a trend towards a positive relationship with WHR in females ($r = 0.45$, $p = 0.06$).

Table 4-2. *Univariate correlates of cvBRS*

				Females			Males		
	n	r	p	*n*	r	p	*n*	r	p
Age, *years*	36	0.12	0.49	18	0.33	0.18	18	0.32	0.19
Height, *cm*	36	-0.32	0.06	18	0.06	0.82	18	-0.37	0.13
Weight, *kg*	36	-0.19	0.28	18	0.34	0.17	18	-0.17	0.50
BMI, kg/m^2	36	0.00	1.00	18	0.27	0.28	18	0.04	0.87
WHR	36	-0.29	0.87	18	0.45	0.06	18	-0.36	0.14
Lean Mass, *kg*	36	-0.26	0.13	18	0.27	0.28	18	-0.01	0.97
Fat Mass, *kg*	36	0.19	0.27	18	0.30	0.22	18	-0.37	0.13
Fat Mass, *%*	36	0.28	0.10	18	0.24	0.35	18	-0.32	0.19
RRI, *ms*	36	0.46	<0.01*	18	0.65	<0.01*	18	0.58	0.01*
SBP, *mmHg*	36	-0.45	<0.01*	18	-0.28	0.26	18	-0.25#	0.32
DBP, *mmHg*	36	-0.22	0.21	18	-0.02	0.95	18	-0.17	0.51
MAP, *mmHg*	36	-0.35	0.03*	18	-0.15	0.55	18	-0.30	0.23

Pearson tests of correlation were employed unless otherwise stated. BMI, body mass index; WHR, waist-to-hip ratio; RRI, R-R interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
* indicates $p < 0.05$.
# indicates Spearman correlation coefficient.

4.4 The Effect of Sex on Arterial Mechanical Properties

Table 4-3 details the effect of sex on the local mechanical properties of the CCA, CS and AA. Males demonstrated both larger diastolic (0.66 ±0.05 vs. 0.63 ±0.03 cm; $t_{(34)}$= -2.12, p = 0.04) and systolic CCA diameters (0.72 ±0.05 vs. 0.69 ±0.04 cm; $t_{(34)}$= -2.11, p = 0.04). Females demonstrated a trend towards greater CCA PP than males (29 ±5 vs. 25 ±8 mmHg; $t_{(30)}$ = 1.84, p = 0.08). Males demonstrated a trend towards greater CCA distensibility than females (7.89 ±2.1 vs. 6.66 ±1.7 $mmHg^{-1}$ $x10^{-3}$; $t_{(30)}$= -1.85, p = 0.08). Males had larger systolic CS diameters (0.80 ±0.12 vs. 0.74 ±0.07; $U_{Mann\text{-}Whitney}$= 69, p = 0.04) and trended towards larger diastolic diameters (0.74 ±0.07 vs. 0.69 ±0.06 cm; $t_{(29)}$ = -1.76, p = 0.09) than females. No other sex differences existed for the CS.

In regards to AA mechanical properties, males had larger diastolic diameters (2.43 ±0.23 vs. 2.17 ±0.16 cm; $t_{(15)}$= -2.61, p = 0.02) and trended towards having larger systolic diameters (2.75 ±0.25 vs. 2.54 ±0.18 cm; $t_{(15)}$= -2.02, p = 0.06). AA PP was greater in males than females (30 ±8 vs. 24 ±5 mmHg; $t_{(34)}$= -2.47, p = 0.02). Both AA strain (16.8 ±3.6 vs. 13.2 ±2.9 %; $t_{(15)}$= 2.26, p = 0.04) and AA distensibility (16.5 ±6.0 vs. 10.5 ±3.8 $mmHg^{-1}$ $x10^{-3}$; $t_{(15)}$= 2.52, p = 0.02) were greater in females than males. Although only 17 individuals had AA distensibility data, the effect size of sex on AA distensibility was 0.30 ($\eta^2_{partial}$) with a power of 0.66. There were no sex differences in distension at any site.

Table 4-4 shows the effect of sex on regional aortic properties. There were no sex differences, with the exception of males having larger femoral distances than females (0.572 ±0.04 vs. 0.529 ±0.03 m; $U_{Mann\text{-}Whitney}$=53, p < 0.01) and trending towards greater carotid distance (0.081 ±0.02 vs. 0.072 ±0.01 m; $t_{(34)}$ = -1.82, p = 0.08).

Table 4-3. *Arterial mechanical properties by measurement site*

Common Carotid	*n*		*n*	Females	*n*	Males	p
Dd, *cm*	36	0.65 (0.04)	18	0.63 (0.03)*	18	0.66 (0.05)	0.04
Ds, *cm*	36	0.71 (0.05)	18	0.69 (0.04)*	18	0.72 (0.05)	0.04
Distension, *cm*	36	0.058 (0.012)	18	0.057 (0.009)#	18	0.059 (0.015)	0.82
Strain, *%*	36	8.9 (1.8)	18	9.0 (1.4)	18	8.9 (2.2)	0.94
PP, *mmHg*	32	27 (7)	16	29 (5)	16	25 (8)	0.08
Distensibility, $mmHg^{-1}x10^{-3}$	32	7.28 (2.0)	16	6.66 (1.7)	16	7.89 (2.1)	0.08
Carotid Sinus							
Dd, *cm*	31	0.71 (0.09)	15	0.69 (0.06)	16	0.74 (0.11)	0.09
Ds, *cm*	31	0.78 (0.10)	15	0.74 (0.07)*#	16	0.80 (0.12)	0.04
Distension, *cm*	31	0.062 (0.018)	15	0.060 (0.016)#	16	0.065 (0.020)	0.45
Strain, *%*	31	8.7 (2.2)	15	8.7 (2.2)	16	8.8 (2.3)	0.93
PP, *mmHg*	34	37 (7)	17	36 (10)	17	39 (4)	0.28
Distensibility, $mmHg^{-1}x10^{-3}$	30	4.98 (1.9)	15	5.32 (2.3)	15	4.63 (1.3)	0.32
Aortic Arch							
Dd, *cm*	17	2.31 (0.24)	8	2.17 (0.16)*	9	2.43 (0.23)	0.02
Ds, *cm*	17	2.65 (0.24)	8	2.54 (0.18)	9	2.75 (0.25)	0.06
Distension, *cm*	17	0.34 (0.072)	8	0.36 (0.076)	9	0.32 (0.066)	0.23
Strain, *%*	17	14.9 (3.6)	8	16.8 (3.6)*	9	13.2 (2.9)	0.04
PP, *mmHg*	36	27 (8)	18	24 (5)*	18	30 (8)	0.02
Distensibility, $mmHg^{-1}x10^{-3}$	17	13.3 (5.7)	8	16.5 (6.0)*	9	10.5 (3.8)	0.02

Values are means (±SD). Independent sample *t* tests were employed unless otherwise stated. Dd, diastolic diameter; Ds, systolic diameter; PP, pulse pressure.
* indicates $p < 0.05$ vs. males.
# indicates Mann-Whitney U test.

Table 4-4. *Transit times, distances and cfPWV*

	n		*n*	Females	*n*	Males	p
CTT, *s*	34	0.105 (0.01)	16	0.104 (0.01)	18	0.107 (0.01)	0.58
FTT, *s*	35	0.193 (0.02)	17	0.191 (0.02)	18	0.194 (0.02)	0.67
Carotid Distance, *m*	36	0.076 (0.01)	18	0.072 (0.01)	18	0.081 (0.02)	0.08
Femoral Distance, *m*	36	0.551 (0.04)	18	0.529 (0.03)*#	18	0.572 (0.04)	<0.01
cfPWV, *m/s*	34	5.51 (0.85)	16	5.27 (0.77)	18	5.72 (0.88)	0.13

Values are means (±SD). Independent sample *t* tests were employed unless otherwise stated. CTT, carotid transit time; FTT, femoral transit time. cfPWV, carotid-femoral pulse wave velocity.
* indicates $p < 0.05$ vs. males.
# indicates Mann-Whitney U Test.

4.4.1 Explaining Sex Differences in AA Distensibility

As mentioned, AA distensibility was greater in females than males ($p = 0.02$). In order to provide a mechanistic understanding of this observation, the sample size was restricted to individuals with AA distensibility ($n = 17$; Figure 4-2). This analysis revealed that AA strain was greater in females than males (16.8 ±3.5 vs. 13.2 ±2.9 %; $t_{(15)} = 2.26$, $p = 0.04$), while there was no sex difference in AA PP (males: 29 ±8 vs. females: 23 ±6 mmHg; $t_{(15)} = -1.65$, $p = 0.12$).

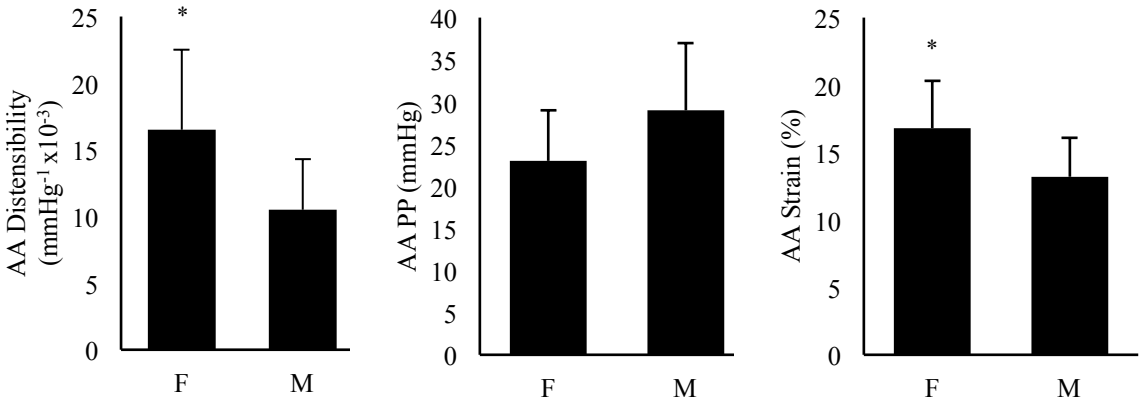

Figure 4-2. AA (aortic arch) distensibility (left), AA PP (pulse pressure; centre), and AA strain (right) by sex (F, female; M, male). Independent sample *t* tests were employed. $n = 8$ F, 9 M. Bars represent group means. Error bars represent SD.
* indicates $p < 0.05$ vs. M.

4.5 The Relationship Between Arterial Mechanical Properties and cvBRS

Table 4-5 presents the univariate correlations between arterial mechanics and cvBRS. When examining the entire sample, cvBRS correlated with PP at the CCA (r = 0.40, p = 0.03) and the AA (r = -0.37, p = 0.03). cvBRS and CCA strain demonstrated a trend towards a positive correlation (r = 0.31, p = 0.06). When stratified by sex, cvBRS was correlated with CCA distension ($r_{Spearman}$ = 0.48, p = 0.045) and strain (r = 0.51, p = 0.03) in males but not females. In males, both CS distension and PP demonstrated trends towards significant correlations with cvBRS (r = 0.47, p = 0.07; r = 0.48, p = 0.052, respectively). AA PP demonstrated a negative correlation with cvBRS (r = -0.37, p = 0.03).

Table 4-5. *Arterial mechanical property correlates of cvBRS*

				Females			Males		
	n	r	p	*n*	r	p	*n*	r	p
CCA distension, *cm*	36	0.27	0.11	18	0.19	0.44	18	0.48[#]	0.045[*]
CCA strain, *%*	36	0.31	0.06	18	0.23	0.36	18	0.51	0.03[*]
CCA PP, *mmHg*	32	0.40	0.03[*]	16	0.26	0.34	16	0.46	0.07
CCA distensibility, $mmHg^{-1}\ x10^{-3}$	32	-0.08	0.67	16	0.01	0.98	16	0.11	0.68
CS distension, *cm*	31	0.19	0.30	15	0.09	0.75	16	0.47[#]	0.07
CS strain, *%*	31	0.27	0.15	15	0.20	0.48	16	0.42	0.10
CS PP, *mmHg*	34	-0.18	0.31	17	-0.28	0.28	17	0.48	0.052
CS distensibility, $mmHg^{-1}\ x10^{-3}$	30	0.24	0.21	15	0.17	0.56	15	0.30	0.28
AA distension, *cm*	17	0.07	0.80	8	-0.27	0.52	9	0.14	0.72
AA strain, *%*	17	0.29	0.26	8	-0.18	0.67	9	0.45	0.23
AA PP, *mmHg*	36	-0.37	0.03[*]	18	-0.37	0.13	18	-0.24	0.35
AA distensibility, $mmHg^{-1}\ x10^{-3}$	17	0.42	0.09	8	0.20	0.63	9	0.23	0.55
cfPWV, *m/s*	34	-0.05	0.79	16	0.24	0.36	18	-0.16	0.54

Pearson tests of correlation were employed unless otherwise stated. PP, pulse pressure; CCA, common carotid artery; CS, carotid sinus; AA, aortic arch.
[*] indicates $p < 0.05$.
[#] indicates Spearman correlation coefficients.

Figure 4-3 depicts the relationships between cvBRS and distensibility of the CCA (*n* = 32), CS (*n* = 30) and AA (*n* = 17) in addition to the relationship between cvBRS and cfPWV (*n* = 34). These relationships did not reach significance, albeit the cvBRS-AA distensibility correlation was trending towards significance ($\beta_{AA\ distensibility}$ ±SE: 0.56 ±0.3, $R^2 = 0.18$, $p = 0.09$; $\eta^2_{partial} = 0.18$, power = 0.40). cvBRS did not correlate with CCA distensibility ($\beta_{CCA\ distensibility}$ ±SE: -0.37 ±0.9, $R^2 = 0.01$, $p = 0.67$), CS distensibility ($\beta_{CS\ distensibility}$ ±SE: 1.2 ±0.9, $R^2 = 0.06$, $p = 0.21$) or cfPWV (β_{cfPWV} ±SE: -0.52 ±1.9, $R^2 = 0.002$, $p = 0.79$). To address our second hypothesis, sex by arterial mechanics interaction terms were made. However, there were no significant findings ($p > 0.05$), suggesting the cvBRS-arterial mechanical properties relationship did not differ by sex.

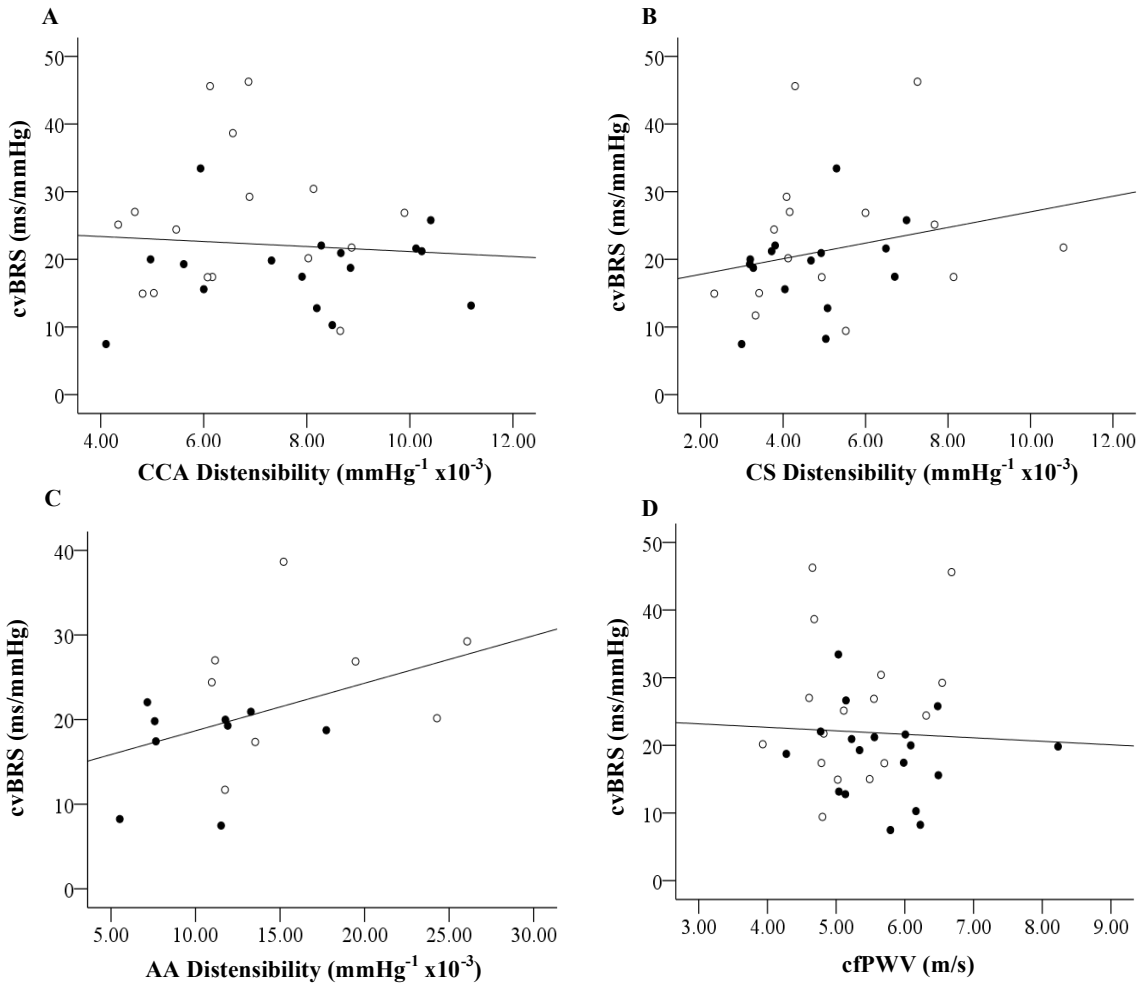

Figure 4-3. Correlation between cardiovagal baroreflex sensitivity (cvBRS) and CCA (common carotid artery) distensibility (A; *n* = 32), CS (carotid sinus) distensibility (B; *n* = 30), AA (aortic arch) distensibility (C; *n* = 17) and cfPWV (D; *n* = 34).
○ indicates females.
● indicates males.

4.6 Multivariate Correlates of cvBRS

In order to assess sex differences in cvBRS while controlling for covariates that were found to differ by sex, five multivariate regression models were created (Table 4-6). Model one included sex and age. Model two expanded on model one with the addition of proportion of fat mass. Model three included model two and added WHR to examine whether body fat distribution differences between males and females were responsible for cvBRS differences. The effect of sex remained significant in models one ($p < 0.01$), two ($p = 0.048$) and three ($p = 0.04$). Model four extended model three by including SBP. The effect of sex was not significant in model four ($p = 0.21$). Likewise, the effect of sex was no longer significant once AA distensibility was added to model five ($p = 0.19$). There were no significant sex interaction terms and therefore these were not included.

Table 4-6. *Multivariate correlates of cvBRS*

	Model 1	Model 2	Model 3	Model 4	Model 5
Sex, *female/ male*	-9.2 (3.1)*	-9.4 (4.6)*	-11.4 (5.0)*	-6.9 (5.3)	-5.5 (4.0)
Age, *years*	1.4 (0.7)	1.4 (0.7)	1.2 (0.7)	1.2 (0.7)	-
Fat Mass, *%*	-	-0.03 (0.3)	-0.04 (0.3)	-0.003 (0.3)	-
WHR	-	-	30.3 (30.7)	28.3 (29.4)	-
SBP, *mmHg*	-	-	-	-0.3 (0.2)	-
AA distensibility, $mmHg^{-1}\ x10^{-3}$	-	-	-	-	0.3 (0.4)
n	36	36	36	36	17
Adjusted R^2	0.17	0.14	0.14	0.21	0.18

Values are $\beta_{unstandardized}$ (±SE). Mutivariate linear regression was performed. WHR, waist-to-hip ratio; SBP, systolic blood pressure; AA, aortic arch.
* indicates $p < 0.05$.

CHAPTER V: DISCUSSION

5.1 Introduction

This study aimed to investigate the influence of sex on the relationship between arterial mechanical properties and cvBRS in young, healthy individuals. This objective was identified given the following information. First, previous findings suggest that sex differences exist in cvBRS such that males often demonstrate greater cvBRS than females,[19] yet other studies report that females have greater AA, CS and CCA elasticity than males.[22-24] However, this contradicts the finding that cvBRS is positively related to arterial elasticity.[12, 13] Second, although investigators have attributed sex disparities in cvBRS to differences in either the arterial or neural components of the cardiovagal baroreflex, to date no study has examined whether the relationship between cvBRS and arterial mechanical properties is similar in males and females. As a result, whether arterial mechanical properties contribute to sex differences in cvBRS is not known.

This study tested two hypotheses. First, cvBRS will demonstrate a positive, linear correlation with CCA, CS and AA distensibility while exhibiting a negative linear correlation with cfPWV and second, males will demonstrate a stronger relationship between arterial mechanical properties and cvBRS than females.

There were three novel findings in the current study. First, females demonstrated greater cvBRS than males. Second, females demonstrated greater AA distensibility than males, yet no sex differences were observed for the mechanical properties of other locations. Third, when controlling for AA distensibility, sex differences in cvBRS were abolished. This suggests that in our sample, increased AA distensibility may contribute to greater cvBRS in females than males.

5.2 Sex Differences in cvBRS

This is the first study to report greater cvBRS in females than males. It was found that cvBRS measured by cross-spectral analysis was 32% greater in females than males in our sample. This indicates that compared to males, females more efficiently elicit baroreflex-mediated changes in HR in response to naturally occurring SBP fluctuations in the supine position. As cvBRS is predictive of CV mortality and morbidity in individuals with no previous CV events,[10] our findings support epidemiological observations that females have a lower prevalence of CVD than males prior to menopause.[119] Alternatively, given that decreased cvBRS may be a mechanism involved in orthostatic intolerance,[122] our findings are inconsistent with the observation that females have higher occurrences of syncope than males.[121]

Although our data appears to be at odds with previous findings suggesting that cvBRS is greater in males than females,[19-21] these specific studies assessed cvBRS using Oxford or modified-Oxford techniques, which induce large changes in BP. Perhaps differences in methods employed to measure cvBRS are responsible for this discrepancy. To our knowledge, no studies employing spontaneous methods (i.e. spectral or sequence techniques) in young individuals (< 30 years) report sex differences in cvBRS measured in the supine position.[17, 18, 59] Thus, our finding does not contradict studies employing similar methodologies.

Regardless of the technique used, either pharmacological or spontaneous, cvBRS reported in previous studies are in line with those found in the current study. In males, cvBRS was 19 ±7 ms/mmHg, which is identical to values reported by both Beske et al.[19] and Tank and colleagues.[59] cvBRS in females (25 ±11 ms/mmHg) was similar to values

reported by Abdel-Rahman and colleagues[20] (24 ±15 ms/mmHg). Although different experimental approaches were used to obtain cvBRS in previous investigations, studies suggest that cvBRS assessed by spectral analysis is highly correlated with pharmacological and sequence methods.[8, 65]

Several lines of evidence lend support to the observation that cvBRS is greater in females than males. First, an investigation by Tanaka et al.[127] indicated that females in the low hormone phase of their menstrual cycle (i.e. menstruation) have greater cvBRS than males in response to both spontaneous and sodium nitroprusside induced decreases in SBP. This suggests that compared to males, females (in the low hormone phase) more efficiently buffer drops in BP with baroreflex-mediated increases in HR. This observation supports the current finding as females in our sample were also tested during the low hormone phase. The current findings are also in line with those by Kim et al.[118] who found that compared to males, females demonstrated larger decreases in BP (driven by reductions in HR) in response to carotid pressor stimuli (via neck chamber), suggesting more efficient short-term HR regulation than males. This may occur because females rely on contributions from the cardiovagal baroreflex more so than changes in vasomotor tone by the sympathetic baroreflex in response to BP alterations.[118] Collectively, these findings suggest that in some samples, females have greater baroreflex-mediated HR regulation than males.

Likewise, studies exploring HR variability in males and females provide support to the current findings. Some[205, 206] but not all[207] indicate that females have greater vagal activity than males, which may explain the greater HR control demonstrated in this study. Specifically, Ryan et al. as well as Antelmi et al. identified that young, healthy females

had greater HF power and HF/LF ratio than males during both spontaneous and metronome paced breathing.[205, 206] As HF spectral power reflects vagal control of HR and given that the cardiovagal baroreflex modulates beat-by-beat HR via changes in vagal activity,[208] these findings support the observation that females demonstrated greater efficiency regulating HR than males. However, direct comparisons cannot be made with previous studies, as the current investigation did not measure HR variability.

5.2.1 Sex Differences in cvBRS: Mechanisms

Sex hormones may be implicated in the cvBRS sex differences observed in the present investigation. Several human studies report the positive effects of female sex hormones, particularly estrogen, on cvBRS. Alternatively, studies have also found that testosterone enhances cvBRS, proving that explaining sex differences in cvBRS is a complex matter warranting further research.

In a sample of post-menopausal females (average age 54 years), Huikuri and colleagues demonstrated that HRT is associated with increases in cvBRS compared to controls, suggesting that female ovarian hormones may improve baroreflex-mediated regulation of HR.[117] However, caution must be employed when interpreting the conclusions from this study as details regarding the type, concentration, duration of therapy, CV risk factors and hemodynamic profiles between groups were not reported. Additionally, Wenner et al. demonstrated cvBRS improvements in females with low orthostatic tolerance after short-term administration of estradiol, compared to their cvBRS during endogenous ovarian hormone suppression (i.e. low estrogen and progesterone).[136] Also, Barnes et al. found that post-menopausal females demonstrated

reduced cvBRS compared to young females, implying that the reduction of female sex hormones occurring during menopause may be responsible for the attenuated reflex control of HR.[126] In contrast, the positive effect of female sex hormones is not universal, as other human trials have found no effect on cvBRS.[137, 209] Similar studies investigating male sex hormones and BP regulation indicate that testosterone may promote cvBRS in males. For example, in elderly males with chronic heart failure, Caminiti et al. observed that 12-weeks of long acting testosterone administration was associated with increases in cvBRS, while no change was observed in the placebo group over the course of the trial.[210] Therefore, these studies provide evidence to suggest that sex hormones may account for sex differences in cvBRS. However, mechanisms underlying cvBRS sex differences are complex, as both female and male sex hormones are found to enhance cvBRS.

Studies in non-human models support findings in humans and add strength to the theory that sex differences in cvBRS may be due to sex hormones. Much like human studies, it appears that both female and male sex hormones improve cardiovagal baroreflex functioning. A study by El-Mas et al. demonstrated that female ovariectomized rats had reduced cvBRS compared to female controls and that estradiol administration was accompanied by increased cvBRS similar to age-matched control females and males.[140] Another study by El-Mas et al. identified that castrated rats demonstrated reduced cvBRS in response to rises in BP whereas testosterone replacement was associated with increases in cvBRS to the level of controls.[144] These data further the concept that male and female sex hormones improve cvBRS. However, testosterone may exert its effect on central nervous pathways or the arterial system via its conversion to estradiol by aromatase.[187]

Research indicates that sex hormones may exert baroreflex-protective effects via both the central nervous system and baroreceptor harbouring arteries. The latter will be discussed in a section forthcoming. Experimental evidence suggests that estrogen and testosterone receptors are located in brain regions responsible for baroreflex regulation of HR such as the NTS and nucleus ambiguus.[211] Injections of estrogen into the NTS are associated with increases in vagal tone.[142] Thus, estrogen may improve the functional capacity of central regions responsible for baroreflex control of HR. Estrogen may facilitate both ACh production via increased choline acetyl-transferase activity and central glutamatergic signaling.[142] Likewise, testosterone may facilitate central glutamatergic neurotransmission.[144] ACh and glutamate are critical neurotransmitters involved in baroreflex signaling at the neuro-effector synapses of the heart and central regions (e.g. NTS-nucleus ambiguus junctions),[142] respectively.

We did not directly measure sex hormones in this study, though females were tested during self-reported menstruation, a phase of low ovarian hormone concentrations.[212] Therefore, the magnitude of estrogen's effect on cvBRS may be underestimated compared to females being tested during early (high estrogen) or mid-luteal phases (high estrogen and progesterone). This speculation is based on the finding that baroreflex control of HR is improved with estrogen administration and cvBRS has been shown to improve from early to mid-luteal phases compared with the early-follicular phase in females.[127, 128] Despite that menstruation is accompanied by low concentrations of estrogen and progesterone, follicle stimulating hormone gradually increases throughout this phase.[212] To our knowledge, the effect of follicle stimulating hormone on cvBRS has not been investigated and its effect may have contributed to sex

differences. Moreover, plasma volume changes across the female menstrual cycle[128] and plasma volume may be lower in females during menstruation.[212] Changes in plasma volume across the female cycle may be caused by aldosterone, vasopressin and renin concentrations.[128] Although they may have been responsible for cvBRS sex differences, evidence regarding the effect of neurohormones on cvBRS is mixed[21, 213-215] and findings suggest that changes in plasma volume do not alter cvBRS.[216]

5.3 Sex Differences in Arterial Mechanical Properties

Previous studies have attributed disparities in cvBRS to differences in either the arterial (e.g. baroreceptor harbouring vessels) or neural (e.g. NTS) components of the cardiovagal baroreflex. Although this study did not examine the neural component, this is the first study to examine the relationship between arterial mechanical properties and cvBRS in both sexes. Therefore, this study provided a mechanistic understanding of sex differences in cvBRS. The current study examined mechanical properties at the CCA, CS, AA and of the regional aorta. Sex differences in these measures and mechanisms to explain the current findings are discussed below.

5.3.1 Sex Differences in Arterial Mechanical Properties by Location

5.3.1.1 Sex Differences in Arterial Mechanical Properties: AA Distensibility

Our main finding was that AA distensibility is greater in young, healthy females than males, which may have been due to greater AA strain in females as no sex difference in PP was found in sub-sample analysis. Given the importance of large artery elasticity on total CV health and the predictive ability of aortic stiffness, the current finding suggests

that young females may be at lower risk of CVD and CV outcomes than males.[217] This is congruent with literature that suggests females have a lower prevalence of CVD than males prior to menopause.[119]

To the best of our knowledge, this is the first study to demonstrate that females have greater AA distensibility between the brachiocephalic and left CCA branches. This finding is supported by others examining local aortic properties but is not universal.[201] Previous research by Nethononda et al. found that young females, aged 20-29 years, exhibit greater distensibilities of the ascending, proximal descending and abdominal aorta than young males using magnetic resonance imaging. Individuals in this sample (n = 92 males, 117 females) were similar to ours in that they were free of overt CVD, were normotensive, did not take CV medications and were not diabetic.[22] Although a small proportion of this sample were smokers, they were equally distributed between the sexes (3% males, 4% females), which likely would not have influenced their results. Similarly, Waddell and colleagues found that young females had a greater proximal aortic distensibility index, suggesting they had greater proximal aortic elasticity than young males, matched for lipids, HR and BP.[179] Similar observations have been made by Rose et al., who examined the ascending and descending aorta in relatively young, healthy males and females (average ages 33 and 36 years, respectively). Their findings suggest that females have greater descending aortic distensibility, while no sex differences in distensibility were present at the ascending aorta.[178] Moreover, Sonesson et al. found the abdominal aorta to be stiffer in males than females aged similarly to individuals in our sample.[180] Therefore, findings from our study and others indicate that aortic and specifically AA distensibility may be greater in females than males.

AA distensibility of females and males (16.5 ±6.0 and 10.5 ±3.8, respectively; average: 13.3 ±5.7 $mmHg^{-1}$ $x10^{-3}$) in the current study differ from those previously reported. Lenard et al. reported AA distensibility to be 4.58 ±1.0 $mmHg^{-1}$ $x10^{-3}$.[15] However, this study failed to include important data regarding participant characteristics such as BP, HR, sex, medication use, anthropometrics and body composition, making comparisons very difficult. Nethononda and colleagues reported the distensibility of the ascending and proximal descending aorta to be 7.80 ±2.5 and 6.20 ±1.7 $mmHg^{-1}$ $x10^{-3}$, respectively in males and 8.90 ±2.5 and 7.10 ±1.9 $mmHg^{-1}$ $x10^{-3}$ $mmHg^{-1}$ $x10^{-3}$, respectively in females aged 20-29 (average ~26) years.[22] These values are closer to ours than those reported by Lenard et al. and as mentioned with the exception of including a small number of smokers, the study sample is comparable to ours.

The discrepancy in AA distensibilities between the current study and those reported by Lenard et al. and Nethononda et al.[15, 22] may be methodological. The current study calculated distensibility with local PP's using a transfer function algorithm of brachial BP.[202] Acquisition of local PP for calculation of distensibility is recommended,[11] given that PP increases towards the periphery, leading to an underestimation of distensibility at central sites. For example, when calculating AA distensibility using peripheral PP (43 ±8 mmHg; 7.78 ±2.8 $mmHg^{-1}$ $x10^{-3}$), rather than AA PP (26 ±8 mmHg; 13.3 ±5.7 $mmHg^{-1}$ $x10^{-3}$), distensibility was similar to values reported by Nethononda et al. Our study is limited as we did not directly measure AA PP which requires invasive arterial catheterization. However, we are confident that the AA BP's obtained in this study via the transfer function algorithm are accurate as they were similar to age and sex adjusted values reported in a population based meta-analysis.[203]

5.3.1.2 Sex Differences in Arterial Mechanical Properties: cfPWV

The current study found males and females to demonstrate similar cfPWV. As cfPWV provides an index of regional aortic stiffness,[155] this finding indicates that the average stiffness of the aorta is similar in males and females. This finding is consistent with other studies, although direct comparisons are difficult given varying study populations. Ahimastos and colleagues found cfPWV to be similar in young post-pubescent males and females (average age 16 years). Although the participants in this study were younger than those recruited in the present study, similarities exist for a variety of anthropometric and hemodynamic variables such as height, brachial MAP and cfPWV (approximately 5.0 m/s).[218] In a sample of middle-aged individuals, Smulyan et al. found that cfPWV was the same in males and females. Although this suggests that regional aortic stiffness is not different by sex, supporting the present findings, Smulyan et al. recruited participants with both normal BP and essential HT, which may explain why their average cfPWV (~ 11.3 m/s) is much greater than the current study. Additionally, a population-based study by Filipovsky et al. found that young males (≤ 35 years) demonstrate a non-significant tendency towards greater cfPWV than females.[182] Lastly, findings by Segers et al. support the current findings as no sex differences in cfPWV were detected in a sample of middle-aged males and females.[219] Thus, the finding that males and females have similar cfPWV is supported by several studies in the literature.

There are two reasons why sex differences were found for the mechanical properties of the AA but not the entire aorta. First, distensibility and cfPWV measure different arterial properties.[11] While the former measures elasticity by quantifying the relative arterial distension in response to changes in local pressure, the latter measures

stiffness by assessing the speed at which pulse waves travel throughout a known length of the vasculature. Second, local AA distensibility was measured between the brachiocephalic and left CCA branches using estimated AA PP, while regional cfPWV was measured between the CCA and femoral arteries. Importantly, regional aortic stiffness increases from proximal to distal sites.[11] cfPWV provides an average estimate of stiffness for a heterogeneous vessel, which may have made it difficult to detect local differences in AA stiffness.

5.3.1.3 Sex Differences in Arterial Mechanical Properties: CCA distensibility

There were no significant sex differences in CCA distensibility, though males demonstrated a trend towards greater CCA distensibility than females ($p = 0.08$, $\eta^2_{parial} = 0.10$, power = 0.43). Sample size calculation determined that a sample of 82 individuals (41 females) was required to obtain a statistical difference in CCA distensibility by sex. Data from a study by Vermeersch et al. support the current finding, as it was observed that middle-aged males and females exhibit no differences in CCA distensibility.[183] Comparisons should be made with caution as the sample recruited by Vermeersch et al. did not display optimal health. Their study included a large proportion of smokers (17-24%) and individuals with high BMI as well as elevated BP. Moreover, findings by Giltay et al. support the current observations as it was found that CCA distensibility was similar in young males and females (< 30 years).[184] Similar to Vermeersch et al. this sample contained a large proportion of smokers but failed to report participant BP. Lastly, our finding is supported by Marlatt et al. who investigated CCA mechanical properties in both normotensive adolescents (6-18 years) and adults (18-49 years). While it was found

that females had greater CCA compliance than males, no difference was found for CCA distensibility in 291 males and 313 female adults.[220]

In contrast, studies by Koskinen et al.[24] and Van Merode et al.[185] found that CCA distensibility varies by sex, although the results from these studies contradict one another. Koskinen and colleagues found that females had greater CCA distensibility than males in a population representative sample of young individuals, which included smokers as well as those with metabolic syndrome.[24] Van Merode et al. examined CCA distensibility in healthy individuals 20-69 years old, stratified by 10-year age groups. In the 20-29 age group (n = 7 females,16 males), males demonstrated greater CCA distensibility, a pattern that remained consistent for the older age groups.[185] Therefore, while some studies suggest that no sex difference exists in CCA distensibility, findings by Van Merode and colleagues support our observation of a trend towards greater CCA distensibility in males.[185]

5.3.1.4 Sex Differences in Arterial Mechanical Properties: CS Distensibility

To our knowledge, this is the first study to compare CS distensibility between the sexes. The present study found CS distensibility to be similar in males and females. Both the CCA and CS were examined in this study to provide a comprehensive evaluation of arterial mechanical properties. Given that baroreceptors are located in the CS at the proximal internal carotid artery[3] it was hypothesized that arterial properties at this site may explain sex differences in cvBRS. Previous findings suggest that CS properties may better predict cvBRS than CCA properties.[14] Also because arterial composition and geometry varies from the CCA to CS[221] it was hypothesized that mechanical properties

may vary from the CCA to the CS. Indeed this was confirmed in the present study as it was shown that the CS was less distensible than the CCA (Appendix A-4).

As mentioned, previous research has not examined the effect of sex on the arterial mechanical properties of the CS. However, a study by Schulz et al.[23] suggests that sex differences may exist in CCA bifurcation and CS anatomy beyond absolute diameter. By analyzing angiograms from 5,395 individuals ranging from healthy to 50% stenosis, Schulz et al. identified that CCA bifurcation anatomy was different between males and females. Specifically, it was found that while males most often had smaller internal and external carotid arteries compared to their CCA (i.e. smaller outflow area), female's internal and external carotid arteries were often similar in area to their CCA's (i.e. larger outflow area). These anatomical differences persisted after adjusting for anthropometric and hemodynamic variables as well as CV risk factors. To add, Schulz et al. found that there were significant differences in plaque distribution between males and females, which may have resulted from differences in regional blood flow velocity caused by disparities in bifurcation anatomy. Schulz et al. identified that females were more likely to have their point of maximum stenosis in the external carotid while males more often had their point of maximum stenosis in the internal carotid artery.[23] Based on these findings and given that subclinical changes in arterial mechanical properties (e.g. distensibility) may precede adverse clinical manifestations,[151] these findings suggest that females may have greater CS distensibility than males. The current findings do not support this, as no sex difference was detected in CS distensibility. It is likely that our sample did not have significant differences in bifurcation anatomy, were too young or did

not have sufficient risk factor accumulation to demonstrate subclinical changes in arterial properties.

5.3.2 Sex Differences in Arterial Mechanical Properties: Mechanisms

The current study found that AA distensibility was greater in females than males, while it also identified a trend towards greater CCA distensibility in males. Moreover, no sex differences were detected for CS distensibility or cfPWV. Researchers have hypothesized that sex disparities in arterial mechanics may be attributed to sex hormones. However, few studies have investigated the effect of sex hormones on arterial mechanical properties and the conclusions made by these studies are limited as they did not examine arterial sites such as the CS or AA. As a result, research is inconclusive and cannot explain the complex sex differences in arterial mechanics identified in the present study (e.g. females had greater AA elasticity, yet demonstrated a tendency towards lesser CCA elasticity compared to males).

Many investigations have examined the effect of sex hormones on arterial mechanical properties. Sex hormones may partly contribute to sex differences in arterial mechanical properties, though it is suspected that other factors also influence arterial mechanical properties as both female and male hormones demonstrate protective effects. In a study of 10 healthy, young females, Hayashi et al. examined changes in arterial mechanical properties throughout the menstrual cycle as well as assessed the relationship between arterial properties and ovarian hormone concentrations. Based on their observation that CCA compliance and distensibility were greatest at the ovulatory phase and correlated positively with the ratio of estrogen to progesterone concentrations, it was

concluded that estrogen likely increases CCA elasticity.[186] Other studies also support the hypothesis that estrogen and/or progesterone facilitate increases in arterial elasticity. For example, Staessen and colleagues found that post-menopausal females had elevated cfPWV compared to pre-menopausal individuals, which persisted after controlling for factors including age, body composition, BP and both CV and hormone medications.[222] Thus, it was concluded that reductions in hormonal concentrations accompanying menopause may be responsible for declines in aortic elasticity. In addition, a cross-sectional investigation by Moreau et al. identified that post-menopausal females taking HRT for an average of 10 years demonstrated greater CCA distensibility than post-menopausal controls not taking any hormone medications. This suggests that exogenous estrogens or progesterones may improve CCA mechanical properties.[223]

In contrast, the protective effect of female sex hormones on arterial elasticity has not been demonstrated in all studies. In 12 healthy, young females, Willekes et al. found that both CCA and femoral artery distensibility were similar across the follicular, ovulatory and luteal phases on the menstrual cycle.[224] Likewise, Teede et al. conducted a two year randomized control trial on the effects of combined HRT on arterial measures in healthy, post-menopausal females. Their findings indicate that systemic arterial compliance as well as aortic and systemic PWV were not altered by exogenous sex hormones.[225] Thus, these studies suggest that ovarian hormones may not modulate arterial mechanical properties. Further research is required to improve the current understanding of sex hormones on arterial mechanics.

Likewise, studies have investigated the influence of male sex hormones on arterial mechanical properties. In 206 males followed for an average of approximately 12 years,

Hougaku et al.[226] observed an inverse association between testosterone concentration and CCA stiffness after controlling for variables such as age, BP and body composition. Moreover, when testosterone concentrations were measured eight years prior to CCA stiffness assessment, it was found that low testosterone predicted CCA stiffness, suggesting an positive effect of testosterone on arterial properties.[226] Similarly, an analysis by Dockery and colleagues found moderate inverse relationships between testosterone and cfPWV as well as carotid-radial PWV in elderly males free of CVD.[227] Although these studies suggest a potential protective influence of testosterone on arterial properties, further research is required to confirm these findings.

Male and female sex hormones may modulate arterial mechanical properties via several pathways. Using human and non-human models, researchers have identified that sex hormone receptors are located at various sites including the smooth muscle and endothelium of the aorta, CCA, internal carotid artery and coronary arteries.[228] Estrogen and progesterone have been shown to influence vessel remodeling by increasing the elastin to collagen ratio and increase endothelium-dependent vasodilation by increasing nitric oxide production.[187, 228] Moreover, ovarian hormones are responsible for increasing prostacyclin release and endothelium-derived hyperpolarizing factor production as well as decreasing endothelin-1 production and calcium influx.[228] Research indicates that testosterone exerts many CV and arterial-protective effects including promoting vascular relaxation via endothelium-derived hyperpolarizing factor release and has been shown to act directly on vascular smooth muscle to elicit relaxation and inhibit cell proliferation.[228] In contrast, studies suggest that testosterone may reduce the elastin to collagen ratio thereby having a detrimental effect on arterial properties.[187]

The literature does not provide an adequate mechanism to explain the findings generated by the current study. Some studies have found that estrogen may increase arterial elasticity. Although, this explains the finding that females had greater AA distensibility than males, it does not justify the tendency towards greater CCA distensibility in males than females. Conversely, studies examining the effect of testosterone in males may explain the non-significant tendency toward greater CCA distensibility in males than females. Therefore, in order to elucidate the role of sex hormones on arterial properties in humans, future studies must be conducted to clarify the heterogeneous findings that exist in the literature, in addition to extending this research to understanding the effect of sex hormones on arterial locations such as the AA and CS.

5.4 The Relationship between Arterial Mechanical Properties and cvBRS

To reiterate, the current study proposed the hypothesis that arterial mechanical properties will be associated with cvBRS. This was based on the several findings. Specifically, Lenard et al.[15] investigated the relationship between spontaneous cvBRS and the distensibility of the CS and AA in a subsample of 17 young individuals. They found that the distensibility of both sites were moderately correlated with cvBRS, reaffirming the importance of mechanical properties in baroreflex regulation of HR. Similarly, both Mattace-Raso et al.[190] and Michas et al.[191] found negative relationships between cvBRS and cfPWV in large samples of older adults with a variety of CV risk factors. Many investigations have also identified a significant relationship between the mechanical properties of the CCA and cvBRS.[12, 13, 189] Given that baroreceptor harbouring vessels are an integral component of the cardiovagal baroreflex,[1, 116] responsible for detection of SBP

changes and initiation of the baroreflex cascade, the relationship between these variables is widely accepted.

In the current study, although the relationship between cvBRS and arterial distensibilities (CCA, CS, AA) and cfPWV did not reach statistical significance, the association between AA distensibility and cvBRS was trending towards significance and the directionality of these relationships (with the exception of CCA distensibility) were consistent with the literature.[13, 15, 190] Calculation of sample size demonstrated that 37 individuals were required for the positive correlation between cvBRS and AA distensibility ($\eta^2_{parial} = 0.18$, power = 0.40) to reach statistical significance. However, in order for the relationships between cvBRS and CS ($\eta^2_{parial} = 0.056$, power = 0.24) as well as CCA distensibility ($\eta^2_{parial} = 0.006$, power = 0.070), 137 and 1287 individuals respectively, were required to reach significance. Thus, sample size may have contributed to the non-significant relationship between cvBRS and arterial distensibility in this study.

In addition to sample size, three methodological factors may have resulted in the non-significant relationship between cvBRS and arterial distensibility in this study. First, whereas the current study employed cross-spectral analysis in the LF domain to estimate cvBRS, Lenard et al.[15] measured cvBRS via a sequence technique in which only increases in RRI and SBP were included. Although spectral and sequence methods are highly correlated,[65] the method employed by Lenard et al. excluded down sequences (i.e. decreases in RRI and SBP) and therefore cvBRS measurement was limited to half of the cardiovagal baroreflex responses to changes in SBP. The observation that cvBRS is greater in response to pressor than depressor stimuli (i.e. hysteresis)[44] may account for

discrepant findings as our spectral method measures SBP and RRI changes in both directions.

Second, the current study calculated distensibility with local CCA and CS PP's using a hand held tonometer and AA PP using a transfer function algorithm of brachial PP. Acquisition of local PP for calculation of distensibility is recommended, given that PP increases towards the periphery, leading to an underestimation of distensibility at central sites.[11] Lenard et al. utilized PP obtained at the brachial artery in their calculation of both CS and AA distensibility. Thus, the relationship between distensibility and spontaneous cvBRS found by Lenard et al. may not be valid as their arterial distensibility does not reflect the true elastic properties of the CS and AA.

Third, although it is not explicitly stated in their report, Lenard et al. may have adjusted finger BP to brachial artery values, a method that is commonly practiced in spontaneous cvBRS assessment.[58] Consequently, the same BP may have been used in the measurement of both cvBRS and arterial distensibility, leading to an overestimated association between these measures. Indeed this is observed in the current study by examining the relationship between cvBRS and arterial distensibility when calculated using brachial PP (CCA: $r = 0.55$, $p < 0.01$; CS: $r = 0.45$, $p = 0.01$; AA: $r = 0.41$, $p = 0.10$).

We failed to detect a significant negative relationship between cvBRS and cfPWV due to the small effect size ($\eta^2_{parial} = 0.002$, power = 0.058) in the association. Analysis revealed that a sample size of 3550 was required to identify a significant relationship. Both Mattace-Rasso et al.[190] and Michas et al.[191] examined the cfPWV-cvBRS relationship in large samples ($n = 2083$ and 160, respectively) of older adults with

comorbidities such as HT, atherosclerosis and hyperlipidemia. Therefore, our results suggest that the relationship between cvBRS and cfPWV is very weak in young, healthy individuals. It is speculated that the relationship may have been stronger in previous studies due to the stiffening of proximal elastic aorta, causing stiffness to be relatively more homogeneous throughout the entire aorta of elderly individuals.[155] Thus, cfPWV may have more accurately reflected proximal aortic stiffness, which could be responsible for the significant relationship between cfPWV and cvBRS in these studies.

5.4.1 The Influence of Sex on the Arterial Mechanical Properties-cvBRS Relationship

Our second hypothesis was that compared to females, males will demonstrate a stronger relationship between arterial mechanical properties and cvBRS. This was based on the findings that males often have greater cvBRS than females,[19-21] while females have demonstrated greater large artery elasticity.[22, 24, 179] These findings contradict one another. Given that arterial deformation of baroreceptor harbouring vessels is required to elicit the baroreflex cascade and given the finding that arterial elasticity is positively correlated with cvBRS,[13, 15] it is assumed that the sex with greater arterial elasticity would have greater cvBRS. However, no study to date has examined whether the relationship between arterial mechanical properties and cvBRS is the same in both sexes nor has a study examined whether differences in arterial mechanical properties explain cvBRS disparities in males and females. Although there were no significant relationships between cvBRS and sex by distensibility (CCA, CS, AA) and sex by cfPWV interaction terms, this may be owing to sample size issues and inadequate statistical power. Thus, we cannot conclude which sex had a stronger relationship between arterial mechanical

properties and cvBRS. Despite this, the current study is the first to explain sex differences in cvBRS with differences in arterial mechanical properties.

5.5 Multivariate Correlates of cvBRS

Multivariate regression was used to examine whether sex differences in cvBRS could be explained by other physiological factors measured in the present study. Several studies have demonstrated that cvBRS is diminished with age.[21, 42] In this study, males were slightly older than females. Although the age difference was not large (2 years), it may have confounded our finding that females had greater cvBRS than males. However, after controlling for age the effect of sex on cvBRS remained significant. Moreover, findings indicate that both body fat and central body fat distribution are associated with decreased cvBRS.[90] In line with research, females in our sample demonstrated a greater proportion of body fat while males had a greater accumulation of central fat mass (greater WHR).[229] After controlling for both proportion of body fat and WHR, the effect of sex on cvBRS remained. Furthermore, research indicates a negative linear relationship between SBP and cvBRS.[42] In line with previous research,[19] males in our sample had greater SBP than females. With the addition of SBP to the multivariate regression, the effect of sex on cvBRS was non-significant. When controlling for AA distensibility the effect of sex was no longer significant, suggesting that AA distensibility may be responsible for the observed sex differences in cvBRS. Analysis was limited as only 17 individuals had both cvBRS and AA distensibility data. Nonetheless, this is the first study to demonstrate that sex differences in AA distensibility may contribute to sex differences in cvBRS. Although our findings suggest that either SBP or AA distensibility may have contributed

to sex differences in cvBRS, these variables were strongly associated in our sample ($r = -0.75$, $p < 0.01$; Appendix A-2). Indeed the literature suggests a strong relationship between arterial distensibility and BP.[165, 168] Given that arterial deformation in response to changes in SBP is required to initiate the baroreflex cascade,[230] it is postulated that AA distensibility rather than SBP may be a determinant of cvBRS sex differences in this study. Therefore, a given change in SBP elicits a greater relative deformation in AA diameter, eliciting greater baroreflex-mediated changes in HR in females than males. However, this conclusion is made cautiously as it assumes that neural activity was similar in males and females and given the cross-sectional study design.

This study found that a greater cvBRS in females was associated with sex differences in AA distensibility. As there were no sex differences in CS distensibility in our sample, it appears that sex-related variance in AA distensibility may be sufficient to observe dissimilarities in cvBRS. From this, one may infer that AA baroreflex afferent fibres have a relatively greater contribution to baroreflex HR regulation than CS baroreflex afferent fibres. This study did not test this hypothesis, though previous research in both clinical and animal studies support this claim.[231-233] In order to examine the contribution of CS and AA baroreflexes, Ferguson et al. employed elaborate techniques involving bolus administration of a pressor drug alone and in combination with the application of neck pressure. The former stimulates both CS and AA baroreceptors to measure integrated cvBRS and the latter only stimulates the AA baroreceptors to measure the AA contribution to cvBRS. Upon elimination of CS afferent contribution, cvBRS was reduced by 30%, which suggests that the AA has a greater contribution to integrated cvBRS.[231] Similarly, Mancia and colleagues compared cvBRS

during changes in neck pressure versus vasoactive drug injections. Herein, cvBRS was three times greater in response to vasoactive drug administration compared to neck pressure modulation.[232] Collectively, these findings suggest a dominant role of AA baroreceptors in baroreflex-mediated regulation of HR.

Findings from studies in animal models provide additional support to human findings. Using arterial perfused, decerebrate rats, Pickering et al. found cvBRS to be reduced by 85% when one AA baroreflex afferent was cut and abolished when both AA afferents were cut (with preserved CS innervation), while no significant changes in cvBRS occurred when CS afferents were cut (with preserved AA innervation).[233] These findings further those in humans by suggesting that AA afferents are critical for cvBRS. However, observations by Pickering et al. imply that CS afferents are not involved in baroreflex-mediated HR regulation. This is unlikely in humans, as previous studies[231, 232] have shown that CS baroreceptors contribute to baroreflex-mediated HR regulation.

5.6 Strengths and Limitations

The current study has several strengths. First, distensibilities of the CCA and CS were measured with local PP's using a hand held tonometer and AA distensibility was calculated using estimated central PP obtained via transfer function of brachial PP.[202] Applanation tonometry of the CCA and CS yields waveforms similar to those obtained intra-arterially;[234] thus allowing investigators to measure PP at the same location that arterial diameters were collected. This approach enables accurate calculation of distensibility compared to using brachial PP. Distensibilities calculated using brachial PP are underestimated because of peripheral PP amplification.[11] Furthermore, this study

provided a comprehensive understanding of arterial mechanical properties, particularly those innervated by barosensitive afferent fibres. Limited research has examined the relationship between cvBRS and both CS (unpublished studies in our laboratory and others[14]) and AA mechanical properties via ultrasonography.[15] Moreover, no study to date has assessed the relationship between cfPWV and cvBRS in young individuals. Additionally, we were able to ensure inter-participant reliability as testing occured from 0:900-11:00 hours after a fasted rest to normalize for circadian rhythm[235] and the influence of metabolic or nutritional factors.[236] To normalize ovarian hormone concentrations,[127] females were tested during menstruation. Moreover, this study controlled for confounding factors in order to provide an accurate assessment of the effect of sex on cvBRS and the arterial mechanics-cvBRS relationship. This was accomplished both *a priori,* by including only young, healthy, normotensive individuals and statistically, via multivariate regression.

The current study may have been limited by the following. First, this study may have been subject to self-report bias. Questionnaires were used to assess menstrual phase and health status. Serum sex hormones, follicle and luteinizing hormones, neurohormones (e.g. norepinephrine), fluid regulatory hormones (e.g. arginine vasopressin) or serum lipid profiles were not measured. Second, of our females, 12 were taking OC's. To date, no study has demonstrated a modulatory effect of OC on cvBRS. In fact, Wilczak et al. found no association between OC use and spontaneous cvBRS.[139] Given previous findings, it is assumed that during menstruation, ovarian hormone concentrations were low in both OC and non-OC users.[128] However, we cannot confirm that OC exogenous hormones were adequately metabolized and excreted, nor can we rule out the occurrence

of withdrawal induced endogenous production of estrogen in OC users.[212] Considering that a large proportion of young females have taken OC's,[138] their inclusion improves the generalizability and clinical applicability of this study. In contrast, our findings are only generalizable to females during their low hormone phase, which accounts for one fourth of their reproductive life. Third, although this study excluded endurance athletes, it did not control for fitness level or physical activity. However, it is unlikely that these variables contributed to cvBRS sex differences as a previous study found sex differences in cvBRS independent of fitness level.[19] In addition, given that young males are generally more active than females[237] and that exercise improves cvBRS,[104] it is hypothesized that males would have greater cvBRS; however, this was not observed. Fourth, although the average male proportion of body fat in this study (11.8 ±4.7%) was similar to others,[229] several individuals had values below 10% with relatively high BMI, suggesting a high proportion of lean mass, possibly attributed to resistance training. Resistance training may have been responsible for both the lower aortic distensibility observed in males of our sample; given the inverse relationship between resistance training and arterial elasticity[238, 239] as well as cvBRS.[240] Fifth, AA ultrasonography was attempted in all participants; however, the sample size was restricted to 17 participants with at least two high quality AA images. The anatomical variability of the AA and its branches has been reported[241] and may have limited identification of the region between the brachiocephalic and left CCA braches; thus hindering image acquisition. Moreover, in some cases the AA could not be properly visualized due to increased chest mass or a small sternal notch area. Last, because a cross-sectional design was employed, we emphasize caution when

interpreting the results regarding the contribution of AA mechanics to sex differences in cvBRS.

5.7 Perspectives

Given the current findings, future research should examine the following topics. To confirm the current findings, an experimental study employing bolus injections of vasoactives to elicit pressor and depressor stimuli while simultaneously assessing cvBRS and the arterial mechanical properties of the CS and AA should be conducted. This approach would allow investigators to assess the causal relationship between cvBRS and CS as well as AA mechanics. Additionally, it would be possible to identify if AA mechanics were responsible for sex differences in cvBRS. Moreover, this design would provide insight to the contribution of AA and CS to cvBRS during large changes in BP, such as those that occur during orthostasis. To further expand knowledge of BP regulation, the abovementioned design could be employed with the addition of microneurography to assess the influence of sex on the relationship between arterial mechanics and sympathetic BRS.

As mentioned, this study did not evaluate the contribution of neural pathways to sex differences in cvBRS. The neural component of the baroreflex consists of baroreceptor nerve terminals, afferent nerves, medullary centres, efferent nerves and the synaptic clefts at the heart.[1] Additionally, higher cortical centres are involved in baroreflex control of HR.[242] Currently, investigators (including our laboratory) are utilizing continuous ultrasonography of CCA diameters to gain an understanding of the neural contribution to integrated cvBRS.[6] This technique is promising, though it does not

enable one to pinpoint precise neural structures contributing to sex differences. For example, using magnetic resonance imaging Kimmerly et al.[243] identified that differences in forebrain activity may contribute to sex differences in cvBRS responses to lower body negative pressure. Hence, further research (perhaps using magnetic resonance imaging) targeting individual neural regions of the cardiovagal baroreflex, especially those of the medulla (e.g. NTS, nucleus ambiguus etc.) may provide greater insight regarding sex differences in cvBRS.

In addition to generating important knowledge, this study has also raised questions regarding the importance of AA mechanics in BP regulation. Given the limited number of studies to have examined the AA,[15, 22] there are many avenues for researchers to explore. To elucidate mechanisms responsible for sex differences in AA mechanics, investigators may opt to examine the effect of menstrual stage and the accompanying sex hormone concentrations on AA mechanical properties. Moreover, given the prognostic significance of proximal aortic mechanics, future research should investigate the effect of exercise, ageing, inactivity and nutrition on AA elastic properties.

CHAPTER VI: CONCLUSIONS

This study aimed to examine the influence of sex on the relationship between arterial mechanical properties and cvBRS. Previous research indicates that the efficiency of HR regulation via the cardiovagal baroreflex varies by sex.[19, 20] Investigators have hypothesized that sex differences in the arterial components of the cardiovagal baroreflex (i.e. barosensory vessels; CS and AA) and/or the neural components (i.e. neural pathways; afferent/efferent nerves and medulla) contribute to differences in cvBRS. Due to their involvement in the cardiovagal baroreflex,[1, 5] arterial mechanical properties demonstrate a strong association with cvBRS.[12, 13] However, no study to date has examined whether the relationship between arterial mechanical properties and cvBRS is similar in males and females. Thus, whether differences in arterial mechanics contribute to sex differences in cvBRS remains unknown. Although we did not examine neural pathways, this was the first study to assess sex differences in both arterial mechanical properties and cvBRS. Moreover, rather than exclusively examining the properties of the CCA (as in previous studies), this study comprehensively examined arterial mechanics by measuring CCA, CS and AA distensibility as well as cfPWV. The AA has only been investigated once previously using sonography,[15] and scarcely using more elaborate techniques such as magnetic resonance imaging.[22, 178] Additionally, the CS has only been examined in a limited number of studies (in unpublished studies from our laboratory and others[14]). Thus, this study is novel both in its ability to explain sex differences in cvBRS and its ability to employ imaging techniques requiring high technical skill.

The arterial mechanical properties-cvBRS relationship did not reach statistical significance in this study. The novel finding in this study was that both cvBRS and AA

distensibility were greater in females than males. However, no sex differences were found for CCA or CS distensibility as well as cfPWV. Controlling for AA distensibility in multivariate analysis abolished sex differences in cvBRS, suggesting that AA mechanics may contribute to sex differences in cvBRS. These findings are important as they implicate AA mechanical properties as a potential mechanism contributing to sex differences in the cardiovagal arm of BP regulation. Disparities in the rates of syncope[120] and the prevalence of CVD in males and females[119] further underscore the significance of these findings, as the cardiovagal baroreflex is a principle modulator of short-term BP and both aortic elasticity as well as cvBRS have prognostic capacity.[10, 160] Future research aimed at assessing the contribution of neural pathways to sex differences in cvBRS will provide a comprehensive understanding of short-term BP regulation in both sexes. Furthermore, given the advancements in imaging technology, future research should aim to examine factors influencing AA mechanics to gain a greater understanding of beat-by-beat BP regulation via both the cardiovagal and sympathetic baroreflexes.

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APPENDIX A

Additional results

A-1. The Effect of Sex on both cvBRS and AA Distensibility

To assess the effect of sex on cvBRS and AA distensibility, a one-way MANOVA was conducted. This model was restricted to those with both cvBRS and AA distensibility data (n = 17). The analysis indicated a significant effect of sex on cvBRS and AA distensibility ($F(14,2)= 4.33$, $p = 0.03$, $\eta^2_{partial} = 0.38$, power = 0.65).

A-2. Univariate Correlates of Arterial Mechanical Properties

Univariate correlation analyses were performed. CCA distensibility was correlated with fat mass and proportion of fat mass ($r = -0.40, -0.43$, respectively, $p < 0.05$). AA distensibility was correlated with height, body mass, BMI, lean mass, SBP and MAP ($r = -0.46, -0.60, -0.55, -0.63, -0.75, -0.59$, respectively, $p < 0.05$). cfPWV was correlated with SBP ($r = 0.53$, $p < 0.01$) and when stratified by sex, cfPWV was correlated with SBP ($r_{spearman} = 0.67$, $p < 0.01$) and MAP ($r = 0.47$, $p < 0.05$) in males, but not females ($p > 0.05$). CS distensibility did not demonstrate any significant correlations with hemodynamic or anthropometric variables.

A-3. Comparing CCA, CS and AA Mechanical Properties

A repeated measures ANOVA was employed to assess arterial mechanical properties across the CCA, CS and AA (Figure A-1). Fourteen individuals had distensibility, while 15 had distension and 32 individuals had PP at all three sites. Bonferroni post-hoc analyses indicated that distensibility was greatest at the AA compared to both the CCA

(13.9 ±5.9 vs. 6.91 ±1.8 $mmHg^{-1}$ $x10^{-3}$; $p < 0.01$) and CS (13.9 ±5.9 vs. 4.27 ±1.1 $mmHg^{-1}$ $x10^{-3}$, $p < 0.01$), while no difference existed between CCA and CS distensibilities ($p = 0.19$). Furthermore, AA distension was greater than both CCA (0.35 ±0.07 vs. 0.057 ± 0.01 cm; $p < 0.001$) and CS distension (0.35 ±0.07 vs. 0.058 ±0.02 cm; $p < 0.01$). No difference existed between CCA and CS distension ($p = 1.00$). PP was greater at the CS than both the CCA (37 ±8 vs. 27 ±7 mmHg, $p < 0.01$) and AA (37 ±8 vs. 26 ±7 mmHg, $p < 0.01$) whereas no difference existed in CCA and AA PP's ($p = 1.00$).

A-4. Comparing CCA and CS Mechanical Properties

A paired sample *t* test was conducted to compare arterial mechanical properties between the CCA and CS. Twenty-eight individuals had distensibility, 32 had PP and 31 had distension at both sites. Distensibility was greater in the CCA than the CS (7.09 ±1.9 vs. 5.03 ±1.9 $mmHg^{-1}$ $x10^{-3}$, $p < 0.01$). PP was greater at the CS than the CCA (37 ±8 vs. 27 ±7 mmHg, $p < 0.01$). No difference in distension was observed (CCA: 0.058 ±0.01 vs. CS: 0.062 ±0.02 cm, $p = 0.13$).

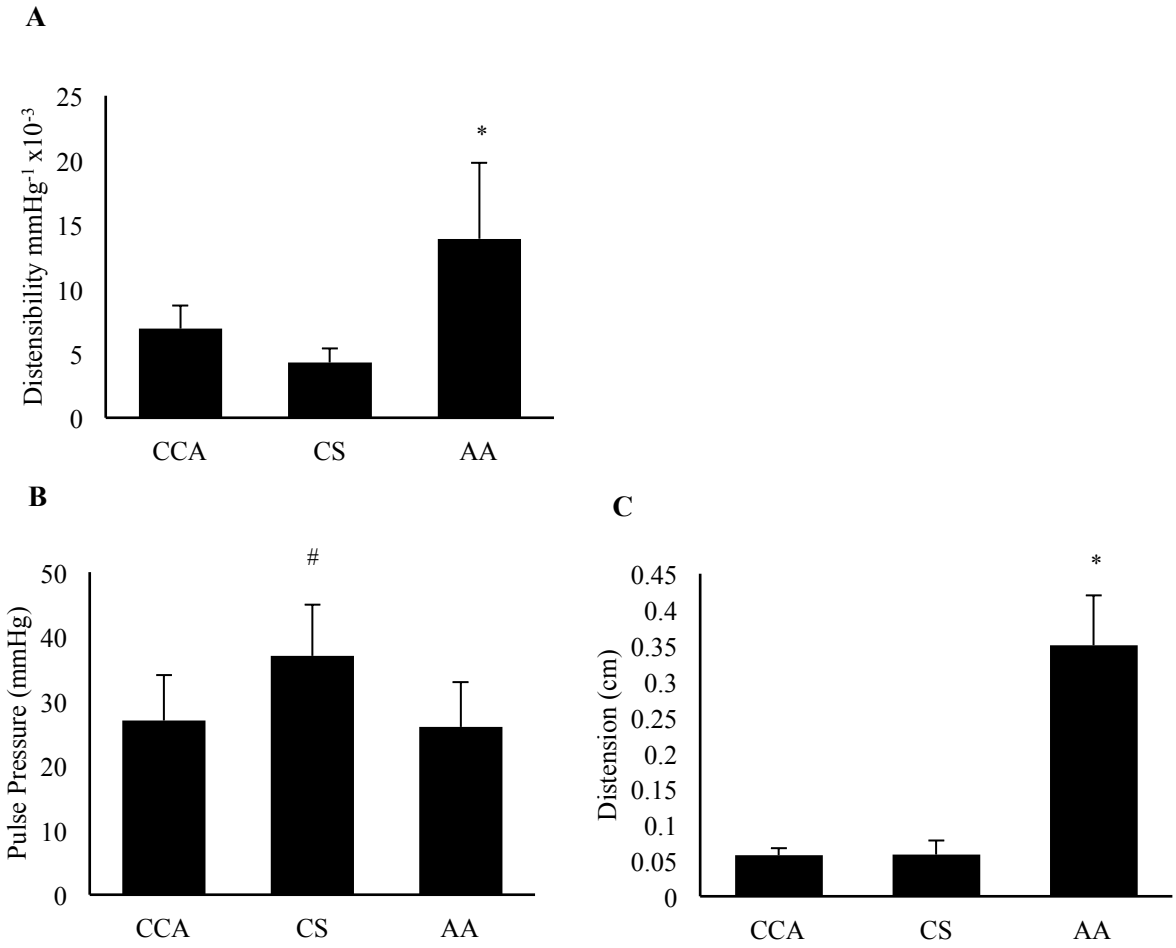

Figure A-1. Examining distensibility (A, *n* = 13), pulse pressure (B, *n* = 32) and distension (C, *n* = 15) by measurement site (CCA, common carotid artery; CS, carotid sinus; AA, aortic arch). Bars represent group means. Error bars represent SD.
* indicates $p < 0.05$ vs. CCA and CS.
# indicates $p < 0.05$ vs. CCA and AA.

APPENDIX B

Ultrasound images of the CCA, CS and AA

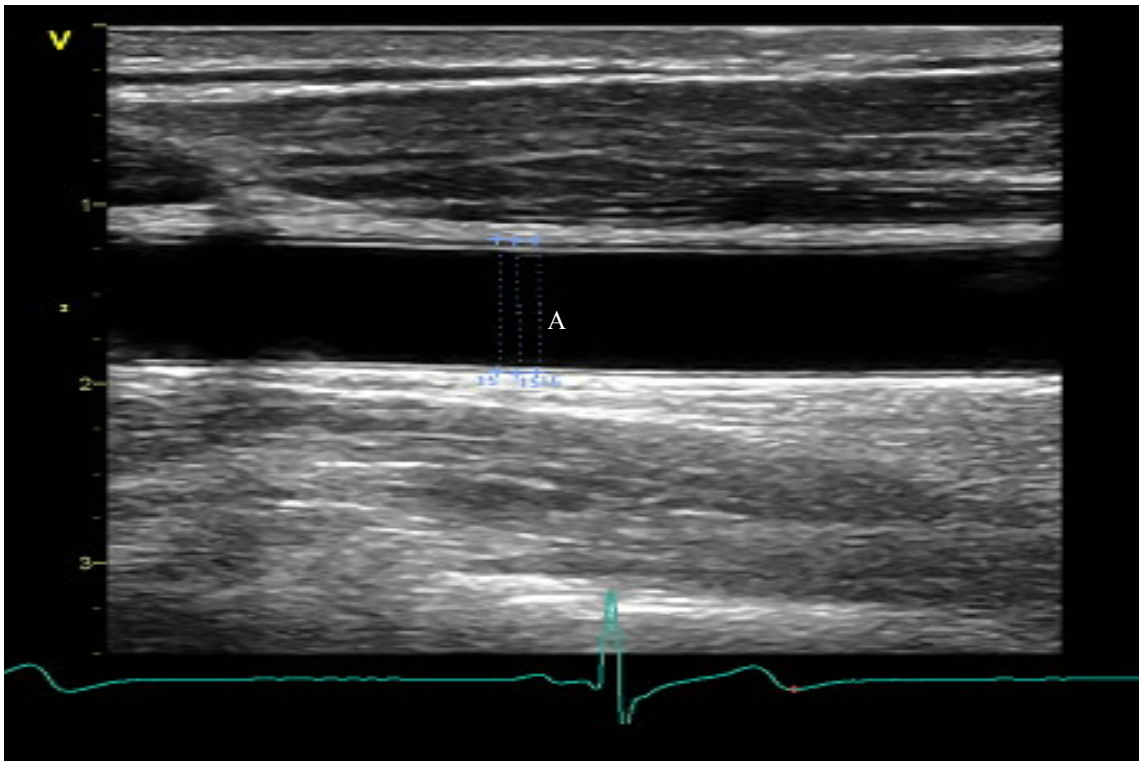

Figure B-1. Longitudinal view of the right CCA (common carotid artery) using B-mode ultrasonography. Note: diameter measurements were taken using EchoPAC calipers (A).

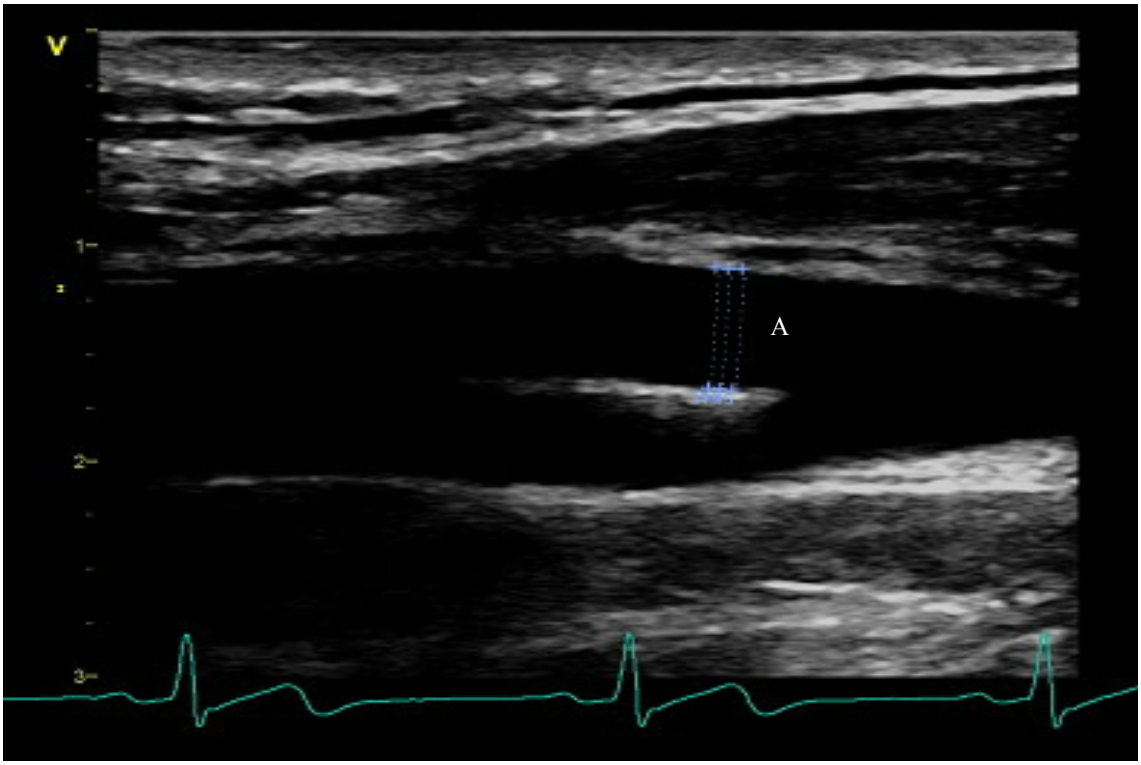

Figure B-2. Longitudinal view of the CCA (common carotid artery) bifurcating into the internal and external carotid arteries using B-mode ultrasonography. Note: diameter measurements were taken at the proximal internal carotid artery using EchoPAC calipers (A).

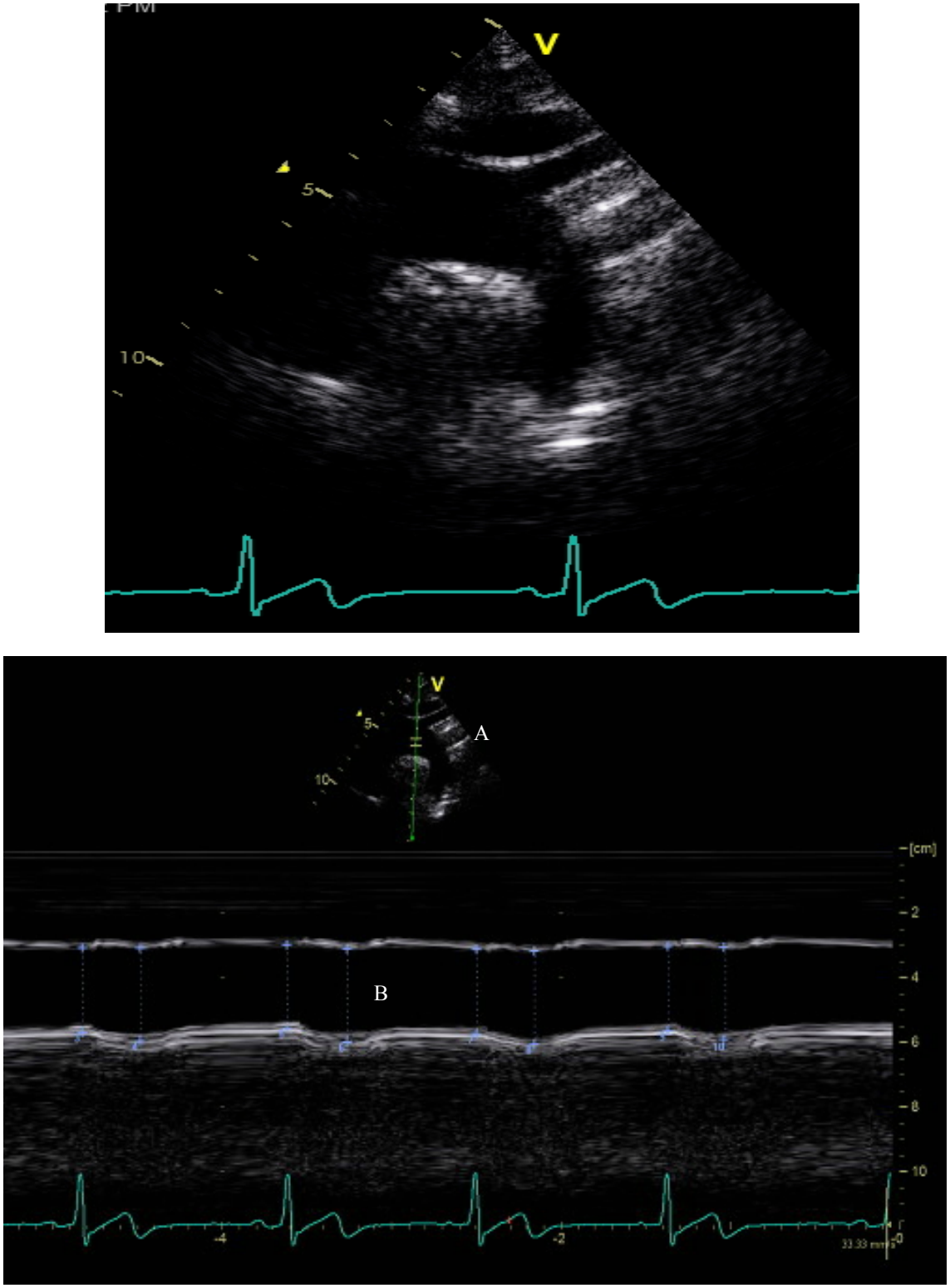

Figure B-3. Longitudinal view of the AA (aortic arch) using B-mode (top) and M-mode (bottom) ultrasonography. Note: the M-line (A) was positioned between the brachiocephalic and left CCA (common carotid artery) branches. Diameter measurements were taken in M-mode over time using EchoPAC calipers (B).

APPENDIX C

Brock University Research Ethics Board Approval Forms

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: 8/13/2014

PRINCIPAL INVESTIGATOR: O'LEARY, Deborah - Community Health Sciences

FILE: 14-017 - O'LEARY

TYPE: Masters Thesis/Project STUDENT: Stephen Klassen
SUPERVISOR: Deborah O'Leary

TITLE: Investigating the influence of sex on arterial stiffness and baroreflex sensitivity.

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 8/29/2015

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **8/13/2014** to **8/29/2015**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 8/29/2015. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: [redacted]

Brian Roy, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	March 30, 2015		
PRINCIPAL INVESTIGATOR:	O'LEARY, Deborah - Community Health Sciences		
FILE:	14-017 - O'LEARY		
TYPE:	Masters Thesis/Project	STUDENT:	Stephen Klassen
		SUPERVISOR:	Deborah O'Leary

TITLE: Investigating the influence of sex on arterial stiffness and baroreflex sensitivity.

ETHICS CLEARANCE GRANTED

Type of Clearance: MODIFICATION Expiry Date: 8/31/2015

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

Modification: Measurement of radial pulse wave contours

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **8/31/2015**. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: [redacted]

Brian Roy, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.