ESTROGEN EFFECTS ON CARDIOVASCULAR FUNCTION

By

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ABSTRACT

Cardiovascular disease (CVD) is the number one killer of all-aged women and men in the United States (CDC, 2015). However, women do not develop CVD until 7-10 years later than men. This age gap is correlated with the onset of menopause in women, leading researchers to think that the development of CVD in women is due to the withdrawal of estrogen. The purpose of this study was to explore the cardio-protective role of estrogen in a high-estrogen group (females) and a low estrogen group (males). We hypothesized that females would display lower blood pressures and enhanced vascular function compared to males due to the role of estrogen on the cardiovascular system. Ten male and ten female subjects completed the one-visit study. Females were tested during the early follicular phase of their menstrual cycle to control for estrogen levels. All subjects were tested in the morning after an overnight fast to control for diurnal variations in hormones. Anthropometric, blood pressure, pulse wave analysis, pulse wave velocity, carotid artery compliance and β-stiffness, and carotid artery intima-media thickness measurements were taken. We found females had significantly lower brachial, carotid, and aortic pulse pressures, significantly higher AIx and AIx@75, significantly higher augmented pressure, and significantly lower height and weight. These findings demonstrate differences between healthy males and females in relation to cardiovascular function but suggest further research is needed to determine the effects of estrogen on cardiovascular function.

INTRODUCTION

Cardiovascular disease (CVD) is the number one killer of both men and women in the United States (CDC, 2015). However, CVD has a later onset in women than in men with women developing CVD 7-10 years later in their lifespan (Maas & Appelman, 2010). This delay in CVD

onset is thought to be linked to the menopause transition. Due to the correlation in withdrawal of menarche and onset of CVD, researchers believe that estrogen plays a cardio-protective role, and when estrogen levels drop post-menopause this causes women to develop CVD at rates similar to men of their age. Therefore, the roles of estrogen and how the hormone protects the vasculature is a popular interest in all fields related to public health.

Many biochemical studies have researched the effects of estrogen on the cardiovascular system. There are two estrogen receptors located in the body which are called estrogen receptor α (ER α) and estrogen receptor β (ER β) (Miller et al., 2008). Upon binding to ER α and ER β, estrogen stimulates acute arterial vasodilation by activating endothelial nitric oxide synthase, causing nitric oxide production which then acts on adjacent smooth muscle at the cellular level to induce relaxation (Al Zubair et al., 2005). Estrogen also activates endothelial prostacyclin and endothelium-derived hyperpolarizing factor resulting in the relaxation and vasodilatory effect in the endothelial lining of arterial tissue (Zhou et al., 2013). Estrogen may also binds to the G-protein-coupled estrogen receptor (GPER1) which stimulates vasodilation (Lindsey et al., 2013). Due to these vasodilatory and relaxation effects of estrogen binding, it is proposed that estrogen protects against inflammatory diseases, such as atherosclerosis, in large arteries and the acute vasodilation effects help to reduce total peripheral resistance (TPR) in the vasculature which protects again hypertension (Nicholson et al., 2017). Translated, this means that we propose that females will have lower pressures, including brachial, carotid, and aortic pressures, due to the lowering of TPR caused by the effects of estrogen.

Estrogen levels vary not only between males and females but also in females of different ages, especially before puberty, during pregnancy, and after menopause. At the onset of menarche, levels of plasma estradiol range from 100 pg/mL in the follicular phase to 600 pg/mL

during ovulation. In pregnant women, levels can rise up to 20,000 pg/mL. After menopause, levels drop to what they were pre-menarche, around 5-20 pg/mL (Mendelsohn & Karas, 1999). The significant decrease in estrogen post-menopause along with the significant increase in onset of CVD in women post-menopause is what leads researchers to question the cardio-protective role of estrogen in the body. The effects of estrogen in menstruating females (high estrogen levels) versus males of the same age (low estrogen levels comparably) are what will be investigated in this study.

The measurements of arterial function and blood pressure (BP) in this study were obtained using the noninvasive techniques of ultrasonography (Arietta 70; Aloka, Hitachi, Japan), an automated oscillometric cuff (HEM-907 XL; Omron, Japan), and applanation tonometry (SphygmoCor; AtCor Medical, Sydney, Australia). Carotid measures of intima-media thickness (cIMT), beta stiffness, elastic modulus, and arterial compliance were assessed using ultrasonography. Brachial blood pressure measurements were assessed using an automated oscillometric cuff. Augmentation index, heart rate, augmentation index at 75 bpm, carotid pulse wave velocity, and carotid and aortic blood pressures were assessed using applanation tonometry. These measurements will provide insight into sex differences in vascular function and blood pressure.

cIMT has been shown to increase with age and endothelial dysfunction (Bots et al., 2016). cIMT is also a predictor of myocardial infarction, ischemic stroke, and atherosclerosis in males and females (Loboz-Rudnicka et al., 2016). Atherosclerotic lesions form under the intima layer with age and disease, therefore increasing cIMT, and is representative of the progression of atherosclerosis throughout the entirety of the vasculature. Atherosclerosis is the underlying cause

of cardiovascular disease (Bots et al., 2016) and the differences between young, healthy males and females will be further investigated in this study.

Carotid β stiffness (β stiffness), elastic modulus, and arterial compliance were measured using ultrasonography in this study. All of these variables are measures of the elasticity of the vasculature. High compliance and low stiffness result in lower risks for CVD (Cecelja et al., 2012). High compliancy allows for vessels to reduce the pulsitility of cardiac ejection, thereby providing a dampening effect and lowering blood pressure. The development of atherosclerosis leads to lower compliance, stiffer arteries, higher blood pressure, and development of cardiovascular disease (Atherosclerosis, 2016). In a study by Marlatt et al., gender differences in carotid artery compliancy were observed. Both children and adults were studied with an average age of 28.6 years. The study found that although there were no significant sex differences in compliance in children, adult females had significantly higher compliance in the carotid artery compared to adult males (Marlatt et al., 2012).

Blood pressure is one of the most frequently used measures of arterial function. In clinical settings, patients typically have blood pressure taken at the brachial artery with a sphygmomanometer. This is a quick, easy, noninvasive, and cost-effective way to obtain valuable information on an individual's risk for and progression of CVD. From the blood pressure reading, clinicians obtain measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP). From SBP and DBP, mean arterial pressure (MAP) can be calculated by the equation MAP = [2(DBP) + SBP] / 3, and pulse pressure (PP) can be calculated by subtracting DBP from SBP. MAP provides insight into organ perfusion and elevated MAP may a be an indicator of CVD (Brzezinski, 1990). A PP over 60 is an indicator of increased CVD risk, and PP below 40 may indicate poor heart function (Sheps, 2019). According to the American

Heart Association, normal systolic and diastolic blood pressures are considered to be less than 120 mmHg and 80 mmHg, respectively, when measured at the brachial artery (2017). When blood pressure is equal to or above these numbers at rest, the individual is at a greater risk of developing CVD and the risk increases exponentially the further blood pressures rise above these levels.

Although brachial blood pressure is commonly utilized in a clinical setting, research has shown that central (i.e. carotid and aortic) blood pressures are more indicative of the load on coronary and cerebral arteries, thereby being more clinically relevant for the diagnosis of hypertension (Roman et al., 2007). In the Strong Heart Study, central and brachial pressures were assessed using applanation tonometry in 3520 participants. The study found that central pulse pressures were more directly related to carotid artery hypertrophy and extent of atherosclerosis than were brachial blood pressures (Roman et al., 2007). This is indicative of the clinically relevant role of central pressures in CVD development and progression, and something that should be assessed in addition to brachial blood pressures to provide further insight to disease risk.

Using applanation tonometry, an aortic arterial pressure waveform is created that is composed of the initial wave of blood from the left ventricle and the wave reflected from the aortic bifurcation (Laurent et al., 2006). A stiffer aorta results in this reflected pressure wave returning to the heart faster, during systole. This pressure wave therefore has the ability to "augment" or increase systolic aortic pressure, because the hearts needs to contract against this returning wave, which increases afterload (McEniery et al., 2014). From this central pulse wave which incorporated the reflected wave, we can determine the augmented pressure (AP) which determines if the heart needs to increase pressure for cardiac ejection, and the percentage of

augmentation, which is termed augmentation index (AIx) (Figure 1). Because heart rate alters reflected wave timing, augmentation index is standardized to heart rate of 75 beats per minute (AIx@75). All of these variables provide insight into central arterial stiffness and central blood pressure. In a study by Millasseau et al., the differences in AIx in 778 men and 810 women with a mean age of 46.5 were analyzed and it was discovered that women consistently had higher values of central AIx compared to men (2014).

Pulse wave velocity (PWV) is a measurement of arterial stiffness that is determined by calculating the time it takes blood to travel from the carotid artery to the femoral artery. PWV and arterial stiffness are directly related, with an increased flow velocity as blood travels through stiffer arteries (Millasseau et al., 2005). In a study by Yan et al., carotid PWV was measured in 53 healthy men and 47 healthy women aged 18-37. The study found that men had a higher resting PWV than women, suggesting that men have a higher arterial stiffness than women even before the onset of disease (2013).

The purpose of this study was to examine vascular responses of young-adult males and females to determine the effect of sex on vascular function, arterial stiffness, and blood pressure. Using only healthy subjects between the ages of 18-25 years excludes factors that would affect the vasculature such as various diseases, medications, and age and allows for the observation of sex differences in young adults without these confounding variables. This will give insight to the underlying sex differences prior to the onset of disease and aging that might distort our understanding of the role of estrogen on cardiovascular function.

METHODOLOGY

Subjects

Healthy participants between the ages of 18-25 were recruited and screened via email interview. Twenty participants (ten males, ten females) completed the entirety of the study. The subjects had no history of cardiovascular, renal, pulmonary, or metabolic disease, and were not taking medications with the exception of hormonal birth control. Exclusion criteria included (1) diagnosed with any form of cardiovascular disease including hypertension, valve disease, stroke, myocardial infarction, or cardiac arrhythmia; (2) pulmonary disease; (3) metabolic disease (diabetes mellitus); (4) inflammatory diseases (rheumatoid arthritis and systemic lupus erythematosus); (5) taking medications known to affect inflammation or metabolic function (anti-inflammatories, thyroid medication, statins) in the past 2 weeks; (6) smoking; (7) common cold or influenza and bacterial or viral infection or upper respiratory tract infection 1 month preceding testing; (8) bleeding disorder; (9) anticoagulant therapy; (10) using antioxidant supplementation; (11) obese (BMI>30 kg/m 2); (12) hyperlipidemic (total cholesterol >240 mg/dl); (13) pregnant; (14) gastrointestinal disease (irritable bowel syndrome, ulcerative colitis). This study was approved by the Institutional Review Board of Appalachian State University.

Study Design

Females were tested during the early follicular phase of their menstrual cycle to control for estrogen levels. All subjects were tested in the morning after an overnight fast to control for diurnal variations in hormones. All subjects participated in a single visit. Height and weight were measured using a stadiometer and a digital scale (Healthometer 349KLX Medical Scale) from which BMI was calculated by dividing weight by height^2 (kg/m^2). Subjects rested supine on the table throughout the entire protocol. Following 10 minutes of rest in a temperature controlled, darkened room, measurements of cardiovascular function began.

Resting blood pressure – Resting brachial blood pressure was taken with an automated oscillometric cuff (HEM-907 XL; Omron, Shimane, Japan). BP was taken in duplicate and if the two values were not within 5 mmHg, additional measurements were taken until two values within 5mmHg of each other were obtained. These values were averaged and used for analysis.

Pulse Wave Analysis and Central Blood Pressure – Radial waveforms were obtained using applanation tonometry (SphygmoCor; AtCor Medical, Sydney, Australia) and calibrated with brachial blood pressure. Using a generalized validated transfer function (Holland et al., 2008), a central aortic pressure waveform was reconstructed from the radial artery pressure waveform to obtain central BP. Aortic mean arterial pressure and aortic pulse pressure were determined from the integration of the reconstructed aortic pressure waveform using the SphygmoCor software. This technique has been validated for use in obtaining central pressure (Holland et al., 2008). The augmentation index (AIx) was then calculated. The AIx is calculated from the pulse wave, specifically by taking the ratio of the difference between the early and late systolic peaks of the waveform to the total pulse pressure. Due to AIx being influenced by heart rate, these measurements were normalized to a heart rate of 75 bpm (AIx@75).

Pulse Wave Velocity – Applanation tonometry was used to measure pulse wave velocity (PWV). A single high fidelity transducer was used to measure pressure waveforms at the right common carotid artery and simultaneously measured at the right femoral artery using an inflated blood pressure cuff. The distance between the measurement sites were measured with a tape measure and the PWV was calculated as the difference between measurement sites (in centimeters) and time delay between the proximal and distal wave forms.

Carotid Artery Compliance and β-stiffness – Longitudinal imaging of the right common carotid artery was performed 1-2cm proximal to bifurcation via ultrasound (Arietta 70; Aloka, Tokyo,

Japan) using a 7.5 mHz linear array probe. Measures of arterial compliance, elastic modulus (Ep) and β-stiffness index were calculated using an automated wall detection echo-tracking software. β-stiffness index (β) was calculated as a means of adjusting arterial compliance for changes in distending pressure using the equation:

$$\frac{\log P_1/P_0}{(D_1-D_0/D_0)}$$

where P1 and P0 are the highest (systolic), and lowest (diastolic) diameters. Arterial compliance (AC) was calculated as an absolute change in carotid lumen area for a given increase in pressure. Elastic Modulus (Ep) is determined by the change in carotid artery diameter for a change in arterial BP relative to the average diameter.

Carotid Artery Intima-Media Thickness (cIMT) – Using the same ultrasound and linear array probe, the intima-media thickness of the carotid artery was measured 20mm proximal to the bifurcation. The cIMT was defined as the distance between the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall of the carotid artery. All measurements were made at end diastole and 5 measurements of IMT were averaged to create our final value.

Statistical Analysis

Descriptive statistics are presented as mean ± standard error. Group differences between males and females in anthropometrics were measured using t-tests and all dependent variables (blood pressures, arterial function measures) were tested using a repeated measures analysis of variance (ANOVA). Significance was set at $p < 0.05$. Analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25 (IBM Corp, Armonk, NY).

RESULTS

Ten males and ten females completed the study with an average age of 21.4 ± 0.5 years for males and 21.9 ± 0.6 years for females (Table 1). There was a significant difference between height and weight of males and females, with males being an average of 15.2 cm taller and 16.1 kg heavier than females (Table 1). There was also a significant difference in AIx and AIx@75 between males and females. The AIx on average for males was -10.2 ± 2.0% and the average for females was 5.5 ± 3.1% (Table 2). After standardization at 75 beats/min, the AIx@75 was on average -19.8 ± 2.2% for males and -3.4 ± 3.0% for females (Table 2). There was a significant difference in augmented pressure, with males being on average -3.2 ± 0.7 mmHg and females being on average 1.6 ± 0.9 mmHg (Table 3). Brachial, carotid, and aortic pulse pressures also all had significant differences. Females had an average of 8.6 mmHg lower brachial pulse pressure, 8.7 mmHg lower carotid pulse pressure, and 3.5 mmHg lower aortic pulse pressure than males (Table 3).

DISCUSSION

The present study's aim was to investigate the differences between males and females in cardiovascular function assessed through measurements of arterial function and blood pressures. The significant sex differences were found in brachial, carotid, and aortic pulse pressures; height and weight; and augmented pressure, AIx, and AIx@75.

Augmented pressure was significantly lower in males, which led to the sex differences seen in both standardized and non-standardized augmentation index (AIx). AIx is an estimate of arterial stiffness (Wilkinson, 2000) and is found from the difference of the late systolic minus the early systolic peak in the aortic pressure waveform. AIx is affected by heart rate changes, so

AIx@75 is the standardized AIx at a heart rate of 75 bpm. Heart rate affects AIx because the faster the heart beats, the less time in systole and therefore the reflected wave shifts more towards diastole. So, heart rate and AIx are inversely related and the faster the heart beats, the lower the AIx (Wilkinson, 2000). We found in this study that males have a significantly lower AIx than females both unstandardized and standardized at 75 beats/min. Although this does not agree with our hypothesis that females will display lower pressures and enhanced vascular function compared to males, it is in agreement with previous research. Another study observed the sex differences in aortic augmentation index in adolescents which found that males had a lower AIx compared to females (Barraclough, 2017). Along with heart rate, AIx is also affected by height, age, and MAP (van Trijp, 2004). In this study, we found that males were significantly taller than females (Table 1). Knowing that height is inversely correlated with AIx (Hughes et al., 2012), we can conclude that significant differences in height may contribute to significantly lower AIx in males compared to females. Upon further investigation with a multivariate ANOVA (MANOVA) statistical analysis, it was discovered that upon controlling for height, there were no significant sex differences in AIx and AIx@75. It is also worth noting that AIx is a measurement of the reflected wave which depends both on wave speed and on arterial properties that determine the site and magnitude of reflection (van Trijp, 2004). Therefore AIx is not necessarily indicative of aortic stiffness and may also reflect vascular tone in the surrounding vasculature (van Trijp, 2004).

In this study, brachial, carotid, and aortic blood pressures were all measured. In most clinical settings, solely brachial (peripheral) blood pressure is taken and used to as diagnostic criteria for hypertension. However, brachial blood pressure does not always give the most accurate estimation of the pressure felt at the heart. This is mainly due to the reflected waveform

in the aortic arch and the fact that brachial blood pressure does not measure the effects of the reflected wave (Avolio, 2009). Central pressure is also more strongly related to future cardiovascular events (Roman, 2007; Pini, 2008). Therefore, carotid and aortic (central) blood pressures were important in this study to observe the effects of the reflected wave on blood pressure at the heart. A study on sex differences in the central arterial pressure waveform also found that females have lower central pressures compared to males between the ages of 20-50 years (Hayward, 1997). Due to the increase in central pressure in post-menopausal women, we assume that estrogen plays a role in maintaining vascular function in pre-menopausal females. However, in this study we did not find significant sex differences in brachial, carotid, or aortic SBP and DBP. We found that pulse pressure was significantly lower in females compared to males at all three sites measured (brachial, carotid, and aortic). Pulse pressure is found by subtracting diastolic blood pressure from systolic blood pressure (SBP-DBP). A high pulse pressure is thought to indicate vascular stiffness and dysfunction of the endothelial lining (Sheps, 2019) and are concordant with a previous study on the sex differences in pulse pressure trends with age, in which pulse pressure was found to be lower in young females compared to young males (Skurnick, et al., 2010). These combined results support our hypothesis that females will display lower pressures and improved vascular function.

Although this study did find some important conclusive results, many of the pressures and other indicators of vascular function were not significantly different between males and females. This could be due to many reasons, including our low subject number (n=20) or the fact that level of fitness was not standardized in this study. A higher level of fitness would indicate improved vascular function for either males or females due to the benefits of exercise on the cardiovascular system. Physiological changes occur with increased fitness levels, so this could

skew the data for both males and females (*Physical Activity*, 1996). Future research could include observing age-related changes in healthy males and females. However, with aging, there is an increased risk of disease, which may confound research aiming to study solely the effects of aging and estrogen withdrawal on the cardiovascular system. This study concludes that females exhibited lower pressures in pulse pressures but not brachial, carotid, or aortic SBP or DBP. This research can be further built on in the future to determine the effects of estrogen on cardiovascular function.

TABLES AND FIGURES

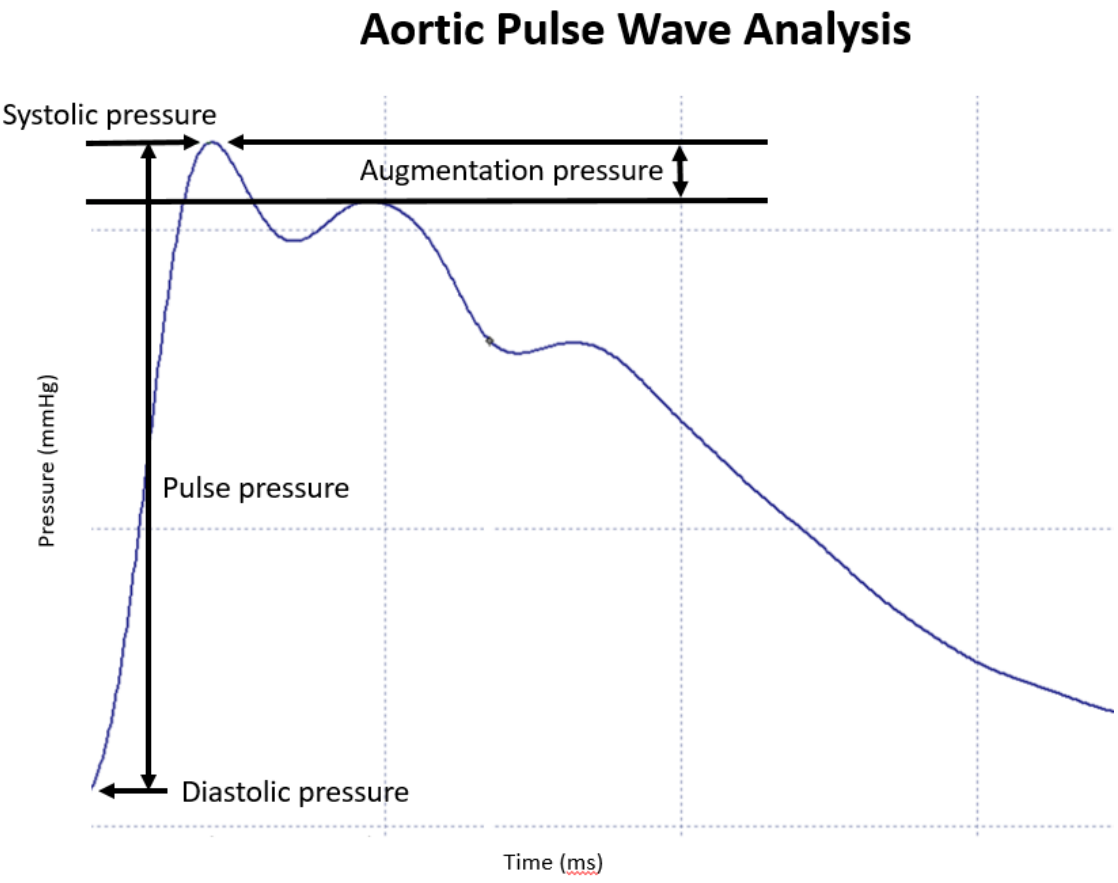

Figure 1 – Aortic Pulse Wave Analysis

	Males	Females
Age (years)	21.4 ± 0.5	21.9 ± 0.6
Height (cm)*	179.1± 1.8	163.9 ± 2.0
Weight (kg)*	78.7 ± 2.4	62.6 ± 1.9
BMI (kg/m^2)	24.6 ± 0.9	23.3 ± 0.6
Carotid IMT (cm)	0.49 ± 0.01	0.49 ± 0.02

Table 1 – Subject Descriptives. Data are presented as mean ± standard error of the mean. *p<0.05 between groups. Cm, centimeters; kg, kilograms; m, meters. Cm, centimeters; kg, kilograms; BMI, body mass index; m, meters; IMT, intima-media thickness.

	Males	Females
Carotid Beta Stiffness (AU)	4.11 ± 0.26	3.70 ± 0.33
Ep (kPa)	44.8 ± 3.0	41.0 ± 3.3
AC (mm^2/kPa)	1.60 ± 0.13	1.64 ± 0.16
AIx* (%)	-10.2 ± 2.0	5.5 ± 3.1
HR (beats/min)	54.9 ± 1.6	55.5 ± 2.8
AIx@75* (%)	-19.8 ± 2.2	-3.4 ± 3.0
carotid PWV (m/s)	4.95 ± 0.18	4.86 ± 0.23

Table 2 – Arterial Function. Data are presented as mean ± standard error of the mean. *p<0.05 between groups. AU, arbitrary units; Ep, elastic modulus; kPa, kilopascals; AC, arterial compliance; AIx, augmentation index; HR, heart rate; AIx@75, augmentation index standardized at a heart rate of 75 beats/min; PWV, pulse wave velocity.

	Males	Females
Brachial SBP (mmHg)	119.6 ± 1.7	116.3 ± 2.9
Brachial DBP (mmHg)	63.0 ± 1.3	68.3 ± 3.4
Brachial MAP (mmHg)	81.9 ± 1.3	84.3 ± 3.1
Brachial PP (mmHg)*	56.6 ± 1.7	48.0 ± 2.2
Carotid SBP (mmHg)	104.6 ± 3.3	101.2 ± 3.4
Carotid DBP (mmHg)	63.0 ± 1.3	68.3 ± 3.4
Carotid MAP (mmHg)	77.8 ± 1.2	82.3 ± 3.3
Carotid PP (mmHg)*	41.6 ± 3.5	32.9 ± 1.6
Aortic SBP (mmHg)	97.1 ± 1.4	98.5 ± 2.7
Aortic DBP (mmHg)	63.6 ± 1.4	68.5 ± 3.5
Aortic MAP (mmHg)	77.8 ± 1.2	82.3 ± 3.3
Aortic PP (mmHg)*	33.5 ± 1.2	30.0 ± 1.1
Augmented Pressure (mmHg)*	-3.2 ± 0.7	1.6 ± 0.9

Table 3 – Pressures. Data are presented as mean ± standard error of the mean. *p<0.05 between groups. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; mmHg, millimeters of Mercury.

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