# THE EFFECTS OF PREVIOUS AMENORRHEA ON VASCULAR FUNCTION

A Thesis

by

KATHERINE T WILLIFORD

Submitted to the School of Graduate Studies
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTERS OF SCIENCE

May 2020

Department of Health and Exercise Science

THE EFFECTS OF PREVIOUS AMENORRHEA ON VASCULAR FUNCTION

A Thesis

By

KATHERINE T. WILLIFORD

May 2020

APPROVED BY:

_____

Dr. Rebecca Kappus

Chairperson, Thesis Committee

_____

Dr. Denise Martz

Member, Committee

_____

Dr. Erin Bouldin

Member, Committee

_____

Mike McKenzie, Ph.D.

Dean, Cratis D. Williams School of Graduate Studies

**Abstract**

Purpose: Young premenopausal women are susceptible to amenorrhea, which contributes to negative vascular remodeling and endothelial dysfunction. It is unknown whether these vascular changes are permanent or reversible with the restoration of estrogen levels and the regaining of a consistent menstrual cycle.

Methods: This study examined subclinical cardiovascular disease risk factors and the vascular function of 10 eumenorrheic women, and 6 previously amenorrhoeic women. Anthropometric measurements, radial pulse wave analysis, carotid-femoral pulse wave velocity, carotid intima media thickness, beta stiffness, bone mineral density, brachial flow mediated dilation, and handgrip exercise blood flow measurements were taken and analyzed.

Results: Previously amenorrhoeic women had significantly ($p<0.05$) lower systolic blood pressure, mean arterial pressure, aortic systolic blood pressure, aortic diastolic blood pressure, and aortic mean arterial pressure, and a higher amount of physical activity during the week than eumenorrheic women. There were no differences between groups in average flow velocity, forearm blood flow, brachial diameter, and forearm vascular conductance for flow mediated dilation and dynamic handgrip exercise.

Conclusion: There were no significant vascular structure remodeling differences between groups, and the amenorrhoeic group displayed lower peripheral and central blood pressure, thus suggesting that there are no long term detrimental cardiovascular effects of previous amenorrhea.

## Acknowledgements

This research would not have been possible without the help and support of my Thesis Committee; Dr. Rebecca Kappus, Dr. Denis Martz, and Dr. Erin Bouldin. I am also very grateful for the aid my lab assistants provided during data collection. Without them much of the data collection would have been extremely difficult. Thank you, Emma Frye, Cameron Jowers, Emily Heier, Anna Mills, and Dylan Richard, for your help and work ethic. Last but not least I would like to thank my family, friends, and the Graduate Exercise Science faculty for your support, patients, and compassion to help propel me through this project. I will be forever grateful to those who have helped me conclude this project.

Table of Contents

1. **Introduction**

Cardiovascular disease (CVD) is the leading cause of death in America for both men and women, however, it manifests 7 to 10 years later in women compared to men. This is thought to be due to the hormone estrogen, which has been shown to be beneficial in maintaining healthy levels of blood pressure and arterial function. Therefore, between the ages of 20 and 50, males are more susceptible to developing cardiovascular disease but following the menopause transition, females develop a significantly higher risk. When a woman goes through menopause her body stops producing high levels of estrogen, leaving her vessels susceptible to endothelial dysfunction, like plaque buildup or a decrease in the elastin to collagen ratio, resulting in elevated risk of cardiovascular disease, as well as depleting her bone mineral density, increasing risk of osteopenia or osteoporosis (Taddei et al., 1996).

In a female's life, it is common to miss a menstrual cycle unrelated to purposeful birth control methods. There are a wide range of reasons why this might be: low body fat percentage, an eating disorder, excessive exercise, stress, and abnormal hormone regulation. When a woman's body does not experience a menstrual cycle for several months, called amenorrhea, her body is not producing adequate estrogen. Because of the known link between estrogen and cardiovascular health, it is possible this long-term reduction of estrogen may be detrimental on the heart and blood vessels.

Research has shown females who experience amenorrhea due to estrogen deficiency are at a higher risk of developing low bone mineral density (osteoporosis) and premature cardiovascular disease (Rothman & Wierman, 2013). Using brachial flow mediated dilation, a

measure of endothelial function, scientists demonstrated a significant reduction in arterial dilation in women with amenorrhea, which is a risk factor to CVD. This research could be vital in determining early onset of cardiovascular disease risk factors and future overall health for women.

*Problem Statement*

This study determined whether women between the ages of 18-30, who previously experienced amenorrhea for at least six months, demonstrated impaired vascular function at the time of the study despite regaining a regular menstrual cycle.

*Hypothesis*

Women who have previously suffered from amenorrhea will exhibit lower endothelial function, stiffer vessels, and higher pressures compared to women without a history of amenorrhea.

*Summary*

Two groups of women between the ages of 18-30 were recruited. The amenorrheic group will consist of women who have previously been without their menstrual cycle for at least six consecutive months, not due to factors such as birth control, medication usage, thyroid disease, pregnancy, lactation, or menopause. The eumenorrheic group will be women who have consistently had a menstrual cycle (have not missed one for more than three consecutive months). Flow mediated dilation will be utilized to determine endothelial function and carotid beta stiffness and central (aortic) pulse wave velocity will be used to measure the stiffness of the vessels. The results from both groups will be compared to determine if there is impaired vascular function due to amenorrhea.

## 2. <u>Literature Review</u>

**Importance of Estrogen**

Estrogen is the main female reproductive hormone that is primarily produced in the ovaries, but can also be produced by adipose tissue and the adrenal glands. Estrogen is crucial during the pre-pubertal stage of female secondary sex characteristics, like widening of the hips and development of breast tissue, pubic hair, and armpit hair (Tanner & Whitehouse, 1976). Once a young woman has started her menarche, estrogen is essential in regulating the growth of the uterine lining and regularity of menstruation (Tanner & Whitehouse, 1976; Treloar, Boynton, Behn, & Brown, 1967).

The 3 types of hormones that fall under the estrogen family are estrone (E1), estradiol (E2) and estriol (E3). E1 is not a very strong form of estrogen and is mostly found in small amounts in adipose tissue and muscle. E2 is a steroid that is produced by the ovaries and is the most dominant form of estrogen that can be found in both men and women. E3 is a waste product created after the body uses E2 and is only found in the body at significant amounts during pregnancy (Miller & Duckles, 2008).

Estrogen is crucial not only to the reproductive system, but also other organs such as the brain, skin, bones, heart and liver. Estrogen helps maintain body temperature and regulates parts of the brain for sexual development as well as chemicals like endorphins, oxytocin, serotonin, and dopamine. When estrogen production begins, the collagen content in the skin increases improving the thickness and quality of the skin. Estrogen helps preserve bone strength and prevent mineral loss and lowers risk of osteopenia and osteoporosis. The hormone also regulates cholesterol production in the liver and is a vasodilator for our vascular system (White, 2002).

The average age for an adolescent girl to have her menarche is between the ages of 12-14. A menstrual cycle every 28 to 30 days is termed eumenorrhea, or a healthy cycle. If a woman's cycle occurs with intervals of greater than 35 days, then her menstruation would be classified as oligomenorrhea. Primary amenorrhea is defined as the absence of menarche by age 15 and secondary amenorrhea is the cessation of menses for 3 consecutive cycles after onset of menarche (Anne Z Hoch et al., 2011). Secondary amenorrhea can be caused by medication, low body fat, stress, female athlete triad, disordered eating, deficiency in estrogen levels, and low levels of follicular stimulating hormone (Rothman & Wierman, 2013).

**Estrogen and Cardiovascular System**

Estrogen aids the cardiovascular system in vasodilation, to allow more nutrients (proteins, carbohydrates, fats, vitamins, minerals, hormones, and $H_2O$) to be circulated without high pressures (Kelley & Bendich, 1996). The endothelium is a single cell inner most layer that is in direct contact with the blood and circulating nutrients. The endothelium plays a critical role in vascular homeostasis by regulating vessel tone, diameter, coagulation factors, vascular inflammation, platelet and leukocytes, and cell proliferation (Shechter et al., 2009).

The binding of estrogen to estrogen receptors α and β, stimulate the endothelium to vasodilate via synthesis of nitic oxide (NO) regulating peripheral blood flow. Estrogen receptors (ERs) are located in all of our cells, involving many different mechanisms of action. There are different pathways and mechanisms of action for the interaction of estrogen and ERs. The Genomic pathway aids in vasodilation of the vessels through embedded ERs in the endothelial cell membrane (Ueda & Karas, 2013). When estrogen is in contact with theses ERs, this initiates gene regulation of Endothelial Nitric Oxide Synthase (eNOS), enhancing NO synthesis (Li et al., 2007; Miller & Duckles, 2008; Stirone, Boroujerdi, Duckles, & Krause, 2005; Williams, Adams,

4

Herrington, & Clarkson, 1992). Once NO is released, it then diffuses into the smooth muscle cell. In the smooth muscle cell, NO interacts with GTP releasing GMP initiating relaxation of the smooth muscle and vasodilation(Ueda & Karas, 2013). This encourages elasticity, homeostasis of the vessel wall, prevention of vascular inflammation, coagulation, cell proliferation, and increasing vessel diameter, reducing pressures allowing the blood to circulate without turbulence or low sheer stress (Tousoulis, Kampoli, Tentolouris, Papageorgiou, & Stefanadis, 2012).

Estrogen receptors are vital to the cardiovascular health of an individual. A mutation in ERα can cause early onset of atherosclerosis in the coronary arteries and endothelial dysfunction (Miller & Duckles, 2008). Estrogen and ERα has also been linked to promoting angiogenesis, preventing injury along the endothelial lining, and initiating the cascade of NO for vasodilation (Chakrabarti, Morton, & Davidge, 2014).  ERα has also been linked to positively influencing the calmodulin network in the endothelium, that encourages proper vasodilators factors (Tran et al., 2016).  Estrogen and ERs are vital to the cardiovascular system because they upregulate eNOS, increasing the synthesis NO, encouraging vasodilation of the vessels (Chakrabarti et al., 2014; Chen et al., 1999).

**Estrogen Loss and Cardiovascular Dysfunction**

Statistically, women have a longer life expectancy than the average male (Taddei et al., 1996). There are many factors that affect this such as environment, culture, genetics, and physiology. Women are at a significantly lower risk of developing cardiovascular disease during their childbearing years compared to men of similar age (Taddei et al., 1996; White, 2002). When a woman begins  the menopause transition, estrogen stores begin to deplete, resulting in vascular dysfunction (Chakrabarti et al., 2014; Mendelsohn & Karas, 1999). After a woman goes through menopause, their susceptibility to cardiovascular disease surpasses men (Gavin, Seals,

Silver, & Moreau, 2009). Over time, vascular dysfunction leads to stiffer arteries, higher blood pressures and progression of plaque buildup. Atherosclerosis is one of the leading causes of death in western society, and estrogen has been recognized to inhibit its progression (T. J. Anderson et al., 1995; Mendelsohn & Karas, 1999; Taddei et al., 1996). Several studies have provided evidence that show estrogen decreases levels of low-density lipoproteins and triglycerides, and increases high-density lipoproteins by affecting the expression of hepatic apoprotein genes (Mendelsohn & Karas, 1999). Estrogen possesses anti-atherogenic characteristics, but the molecular mechanism of this response is still unknown (Grosman-Rimon et al., 2019).

In 2009, researchers sought to determine whether the expression of ERα is influenced by the amount of estrogen in the body and how that relates to endothelial function. Premenopausal women were tested during their early follicular phase (EF) and late follicular (LF) stage of their menstrual cycle and compared the values to postmenopausal women. Researchers found that the EF (low estrogen) stage of the menstrual cycle experienced equivalent values to postmenopausal women in vascular tone, which was significantly lower than the LF phase (high estrogen), which demonstrated higher ERα expression. ERα abundance is positively associated with eNOS expression to promote vasodilation of vessels (Gavin et al., 2009). With the depletion of estrogen, ERα and other estrogen receptors are not able to efficiently interact with the endothelial lining and prevent injury (Chakrabarti et al., 2014). Over time, areas of the endothelium begin to deteriorate, leaving it very susceptible to unstable plaque build-up eventually leading to atherosclerosis and coronary artery disease (T. J. Anderson et al., 1995).

Multiple studies have been done analyzing previous amenorrhea in female athletes and its association with endothelial dysfunction. Researchers found that amenorrheic athletes

experience significant reduction in dilation diameter of their vessels and well as loss of bone

mineral density (Anne Z Hoch et al., 2011; Rickenlund, Eriksson, Schenck-Gustafsson, &

Hirschberg, 2005; Zeni Hoch et al., 2003). Endothelial dysfunction is recognized to be a major

risk factor in the development of atherosclerosis, hypertension, and heart failure (Shechter et al.,

2009). Important measurements to access the onset of CVD is central and peripheral blood

pressures.  Blood pressure values are used to examine the pressure load experienced by the heart

with each contraction. Central blood pressure reflects the pressure vital organs such as the heart,

brain, and kidneys are exposed to (Sharman & Laurent, 2013). BP can be a major clinical

implication for risk of CVD, ventricular hypertrophy, and other diseases (Sharman & Laurent,

2013). There is a positive association between systolic and diastolic BP and risk of CVD (Sesso

et al., 2000). Studies have shown that individuals with CVD have had a higher central BP than

peripheral BP, compared to healthy individuals (Sharman & Laurent, 2013). This is important to

look at when accessing endothelial dysfunction, and arterial stiffness in amenorrheic females.

## 3. <u>Methods</u>

**Study Design**

Sixteen subjects ranging in age from 18-30 years were recruited from the community and university. Ten had not experienced amenorrhea and six subjects had experienced amenorrhea (had not had a menstrual cycle for at least 6 months at some point in their life). Exclusion criteria included history of cardiovascular disease, metabolic disease, cancer, chromosomal disorder, pituitary tumor, current pregnancy, and anomalies of the reproductive system. Subjects on medications known to interfere with blood flow, blood pressure, vasodilation, or cardiovascular function were excluded. There was a lot of interest in this study, unfortunately some had to be turned away due to being currently amenorrheic. All subjects reported to the lab for one visit, which consisted of signing an informed consent, filling out a health history questionnaire, and completing assessments of anthropometric measures including a dual-energy X-ray absorptiometry (DEXA) scan. Following these measures, the participant rested supine in a temperature controlled, darkened room for the cardiovascular measurements, which consisted of central and peripheral blood pressures, pulse-wave analysis and velocity, flow mediated dilation, carotid beta-stiffness and carotid intima-media thickness.

Figure 1. Methodolgy



| Informed consent Health history questionnaire Height, weight | 5 minutes supine rest in darkened room Brachial BP in duplicate | Applanation tonometry (PWA, PWV) | Carotid IMT Carotid beta stiffness | Flow mediated dilation | Dynamic handgrip exercises | DEXA scan |

Sequence of events and measurements that occured during a visit

**Anthropometric Measurements**

Height was measured in centimeters using a stadiometer and weight was assessed using a digital scale (Healthometer 349KLX Medical Scale) in kilograms. Participants underwent a DEXA scan (GE Lunar iDXA; GE Healthcare, Madison, Wisconsin,USA) for assessment of body composition, including body fat percentage (BF%) and whole-body bone mineral density (BMD).

**Brachial artery blood pressure (BP) assessment**

Resting systolic BP and diastolic BP was measured at the brachial artery using 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 2 values within 5 mmHg of each other were obtained. These averaged values were used for analysis.

**Pulse-Wave Velocity (PWV)**

Applanation tonomtetry (AtCor Medical, SphygmoCor Technology, Sydney, Australia) was used to measure pulse wave velocity (PWV). A single high-fidelity pressure transducer was used to measure pressure waveforms at the right common carotid artery while a blood pressure cuff simultaneously measured pressure waveforms at the right femoral artery (central stiffness). The distance between the measurement sites was measured with a tape measure in cm. The PWV is the difference between measurement sites and time delay between the proximal and distal wave forms. This calculation determines the velocity at which the blood is traveling from the heart to the femoral artery and is indicative of the stiffness of the central vasculature (aortic stiffness). A higher PWV indicates greater stiffness within the vasculature.

**Central Blood Pressure**

Radial artery pressure waveforms were obtained using applanation tonometry (Millar Instruments, Houston, TX) and calibrated with the brachial BP obtained previously. Using a generalized validated transfer function(D. J. Holland, J. W. Sacre, S. J. McFarlane, J. S. Coombes, & J. E. Sharman, 2008; Thijssen et al., 2011), a central aortic pressure waveform is reconstructed from the radial artery pressure waveform (SphygmoCor; AtCor Medical, Sydney, Australia) to obtain central BP. Aortic mean arterial pressure and aortic pulse pressure was 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 (David J Holland, Julian W Sacre, Sarah J McFarlane, Jeffrey S Coombes, & James E Sharman, 2008).

**Carotid Intima-Media Thickness**

The right common carotid artery was imaged with ultrasound (Arietta 70, Aloka, Tokyo, Japan) using a 7.5 MHz linear-array probe (SSD-5500; Aloka, Tokyo, Japan) 20 mm proximal to the carotid bifurcation. The intima–media thickness of the common carotid artery, 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, was measured at end of diastole.

**Carotid Artery Stiffness**

Using the same ultrasound and linear-array probe, the carotid artery was imaged again to determine the β-stiffness index (β), which adjusts arterial compliance for changes in distending pressure. Carotid β-stiffness was calculated as a means of adjusting arterial compliance for changes in distending pressure using the equation:

$$Carotid\ \beta-\text{stiffness}\ \frac{log\ P1/P0}{(D1-D0/D0)}$$

where P1 and P0 are the highest (systolic) and lowest (diastolic) carotid pressures and D1 and D0 are the maximum (systolic) and minimum (diastolic) diameters.

**Flow Mediated Vasodilation (FMD)**

Flow-mediated vasodilation (FMD) was assessed using ultrasonography. A rapid release cuff was placed below the elbow joint on the widest part of the elbow. The subject laid supine with the right arm stabilized using an immobilizer cushion. The brachial artery was captured in longitudinal image sections, 5-10 cm proximal to placement of a blood-pressure cuff, using high frequency (5-15Mhz) linear-array probe (Thijssen et al., 2011). In B-mode, split mode used to simultaneously measure the arterial diameter and Doppler velocity. The flow signals were corrected at an insolation angle of 60 degrees. The sample volume was placed in the middle of the artery, with a large sampling area. Gated images were recorded at 5 frames per second using Vascular Tools (Medical Imaging Applications, Coralville, IA, USA). Baseline measurements of resting brachial flow velocity and diameter were assessed for 60 seconds. The blood pressure cuff was inflated with an ischemic stimulus maintained for 5 min. Diameter and flow velocity was recorded 30 seconds prior to cuff release, and deflation diameter and flow velocity were recorded for 3 minutes post deflation. A single technician collected and analyzed all FMD data. These measurements are directly related to endothelial health, and can assess for possible clinical markers for risk of cardiovascular disease.

**Dynamic Handgrip Exercise**

Brachial blood flow was measured during dynamic hand grip exercise. In a supine

position, the subjects performed 3 maximal contractions, squeezing a dynamometer (TSD121C hand dynamometer, Biopac Systems, Inc., Goleta, CA). The best attempt of the 3 contractions was used as the subject's maximal voluntary contraction (MVC). Following a 5 min rest period, subjects performed rhythmic handgrip exercise at 10% and 20% of MVC. Following auditory prompts from a metronome, the subjects contracted their dominant hand for 1 second and relaxed their hand for 2 seconds for a total of 5 minutes at each exercise intensity. There was a 10-minute rest between the 10% MVC and 20% MVC exercise bouts. Brachial artery diameter and flow velocity were measured using ultrasonography (Arietta 70; Aloka) with a high-frequency (7.5 MHz) linear-array probe. Images were recorded using Vascular Tools (Medical Imaging Applications, Coralville, IA, USA). Resting brachial diameter and flow velocity were recorded for 60 seconds prior to the start of exercise. Exercise diameter and flow velocities were recorded and averaged during the final 60 seconds of each exercise bout (10% and 20% MVC). Average brachial diameter (in cm) and average brachial flow velocity (in m/s) were used to calculate brachial blood flow using the equation:

$$Flow\ velocity\ \pi\ (\frac{brachial\ diameter^2}{4}) \times 60$$

Brachial blood pressures were measured at rest and during each exercise bout using an automated oscillometric cuff (HEM-907 XL; Omron, Shimane, Japan). The average mean arterial blood pressure (in mmHg) and average brachial blood flow (mL/min) over the final 60 seconds was used to calculate forearm vascular conductance (FVC) using the following equation:

$$FVC = \frac{brachial\ blood\ flow}{mean\ arterial\ pressure} \times 100$$

**Statistical Analysis**

Data were analyzed using the SPSS software (version 20.0, SPSS, Inc., Chicago, IL). A one way Anova was run comparing the eumenorrhea and amenorrheic group with all of the other variables. A repeated measures ANOVA was used to compare the eumenorrheic and previously amenorrheic women with the three different hand grip exercise intensities (Rest, MVC_10%, MVC_20%). When significance was detected between groups, a Bonferroni Post Hoc Analysis was performed. An ANCOVA was performed to control for variance and confounding factor that may affect significance.

## 4. <u>Results:</u>

Participant characteristics are provided in Table 1. There were no statistically significant differences between groups in age, body mass index, bone mineral density, or body fat percentage. Amenorrhoeic women performed more weekly physical activity, an average of 280 mins/wk versus the eumenorrheic group at 156 mins/wk. There were no significant differences between menarche age in the eumenorrheic and amenorrhoeic women. Of the 16 participants, 6 were taking birth control (2 in the eumenorrheic group and 4 in the amenorrhoeic group). The women who previously experienced amenorrhea had a loss of menses between 9 to 15 months.

PWV, IMT, beta stiffness, and endothelial function measures are presented in Table 2. There were no significant differences in any of these measurements between eumenorrheic and amenorrheic women

Refer to Tables 3 & 4 and Figure 2. There were significant differences between groups in brachial systolic blood pressure, brachial mean arterial pressure, aortic systolic pressure, aortic diastolic pressure, and aortic mean arterial pressure. There were no significant differences between the eumenorrheic and amenorrhoeic group for FBF, average flow velocity, systolic and diastolic BPs, mean arterial pressure, and FVC during dynamic handgrip exercise. There was a significant difference in FBF and FVC between the three different intensities (Rest, MVC_10, and MVC_20).

Table 1. Descriptive characteristics and menstrual history

| Descriptive | Eumenorrheic (n=10) | Amenorrheic (n=6) | ANOVA p-value | F Value |
|---|---|---|---|---|
| Age | 23 ± 2.98 | 22 ± 1.37 | 0.78 | 0.08 |
| Height (cm) | 166.45 ± 5.48 | 166.17 ± 5.59 | 0.92 | 0.1 |
| Weight (kg) | 61.87 ± 9 | 62.81 ± 6.24 | 0.83 | 0.5 |
| BMI | 22.26 ± 2.55 | 22.77 ± 2.38 | 0.70 | 0.16 |
| BF% | 28.74 ± 4.29 | 27.81 ± 2.80 | 0.65 | 0.21 |
| PA (min)* | 156 ± 71.21 | 280 ± 30.332 | 0.001 | 16.07 |
| Age of Menarche | 12.1 ± 1.4 | 12.7 ± 1.4 | 0.44 | 0.64 |
| MVC | 14.8 ± 1.6 | 14.5 ± 2.9 | 0.84 | 0.4 |
| BMD | 1.16 ± 0.08 | 1.17 ± 0.08 | 0.87 | 0.03 |

Data are presented as mean ± standard deviation. $*p < 0.05$. BMI, body mass index; BF%, body fat

percentage; PA, Physical activity; MVC, maximum voluntary contraction; BMD, bone mineral density

Table 2. Endothelial Function

| | Eumenorrheic (n=10) | Amenorrheic (n=6) | ANOVA p-value | F -value |
|---|---|---|---|---|
| IMT | 0.49 ± 0.047 | 0.49 ± 0.036 | 0.86 | 0.03 |
| Bstiff | 4.19 ± 0.97 | 4.48 ± 0.68 | 0.53 | 0.41 |
| FBF | 84.3 ± 27.8 | 69.87 ± 21.2 | 0.30 | 1.18 |
| MaxBD (mm) | 3.52 ± 0.4 | 3.77 ± 0.2 | 0.20 | 2.1 |
| FMD% | 10.9 ± 4.4 | 15.3 ± 5.9 | 0.10 | 2.9 |
| HR | 60 ± 6 | 52 ± 9 | 0.07 | 3.8 |
| Brachial Diameter | 3.17 ± 0.27 | 3.27 ± 0.24 | 0.50 | 0.6 |
| Avg. Flow | 17.6 ± 4.3 | 13.5 2.6 | 0.06 | 4.2 |
| PWV (m/s) | 17.6 ± 4.3 | 13.5 ± 2.6 | 0.18 | 1.9 |

Data are presented as mean ± standard deviation.
IMT, carotid intima media thickness; Bstiff, carotid beta stiffness; FBF, forearm blood flow; MaxBD, maximum brachial diameter; FMD%, flow mediated dilation percentage; HR, heart rate; Avg. Flow, average flow velocity; PWV, pulse wave velocity.

Table 3. Blood pressures

| Pressures | Eumenorrhea | Amenorrhea | ANOVA p-value | F-value |
|---|---|---|---|---|
| SBP* | 116.1 ± 6.8 | 106.1 ± 10.3 | 0.03 | 5.49 |
| DPB | 66.3 ± 6.4 | 60.0 ± 7.2 | 0.09 | 3.32 |
| MAP* | 82.9 ± 5.2 | 75.4 ± 6.4 | 0.02 | 6.57 |
| PP | 49.8 ± 8.2 | 46.2 ± 11.4 | 0.47 | 0.54 |
| aorSBP* | 100 ± 6.7 | 89 ± 5.9 | 0.005 | 10.9 |
| aorDBP* | 67.2 ± 6.5 | 59.1 ± 5.9 | 0.03 | 6.1 |
| aorMAP* | 82.3 ± 5.8 | 72.5 ± 3.5 | 0.002 | 13.7 |
| aorPP | 32.8 ± 7.3 | 29.8 ± 6.3 | 0.40 | 0.7 |

Data are presented as mean ± standard deviation.
*p<0.05.
MAP= Mean arterial pressure; *PP= Pulse pressure;  SBP: Systolic blood pressure; DBP: Diastolic blood pressure;  aorSBP= Aortic systolic blood pressure;  aorDBP= Aortic diastolic blood pressure; aorMAP= Aortic mean arterial  pressure; aorPP= Aortic Pulse Pressure


Table 4. Adjusted means after controlling for physical activity

| Values: | Eumenorrhea | Amenorrhea | p-value |
|---|---|---|---|
| aorDBP | 65.9 ± 2.4 | 61.5 ± 3.4 | 0.344 |
| aorSBP* | 100.4 ± 2.5 | 88.4 ± 3.5 | 0.033 |
| aorMAP* | 81.9 ± 2 | 73.2 ± 2.8 | 0.050 |
| SBP | 117.4 ± 3 | 104 ± 4.5 | 0.055 |
| MAP | 82.4 ± 2.2 | 76.2 ± 3.1 | 0.188 |

Data presented
*p<0.05
aorDBP= Aortic diastolic pressure; aorSBP= Aortic systolic blood pressure; aorMAP= Aortic mean arterial pressure; SBP= Systolic blood pressure; MAP= Mean arterial pressure

Figure 2. Group differences in brachial diameter and flow responses to dynamic handgrip exercise with rest.
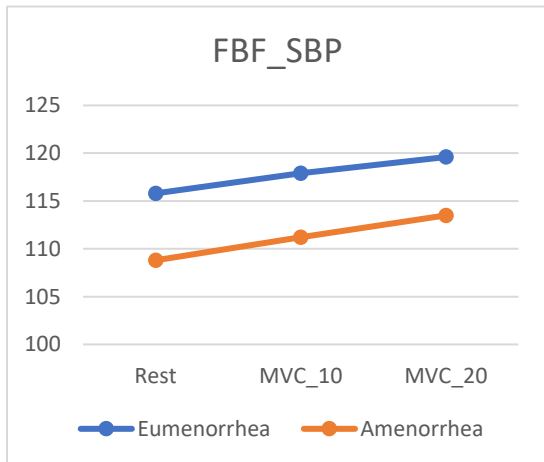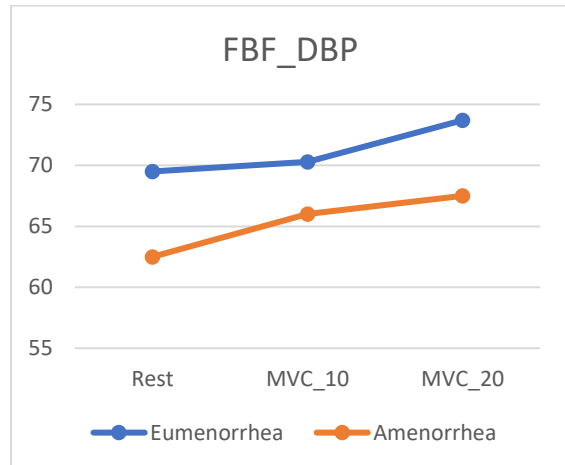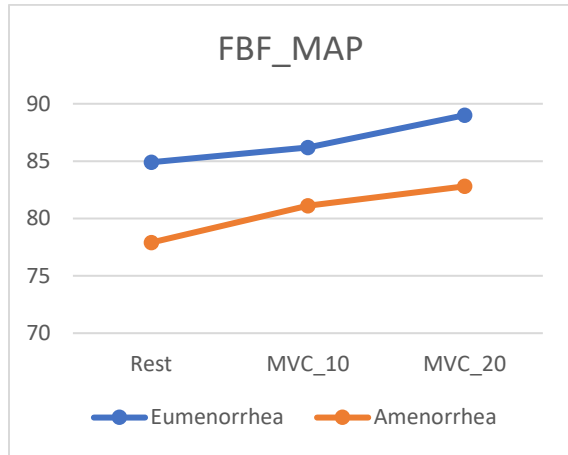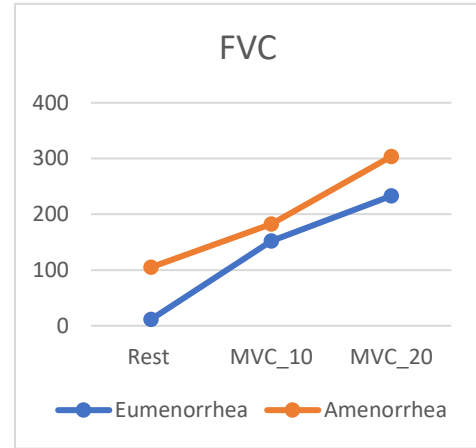
A.
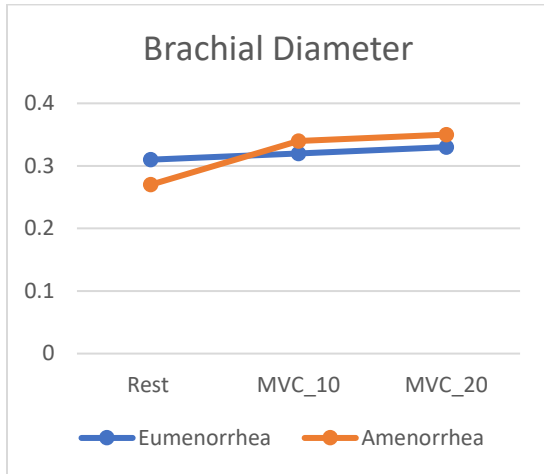


B.
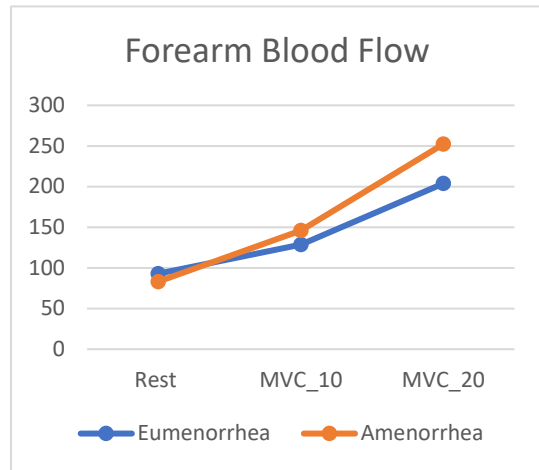


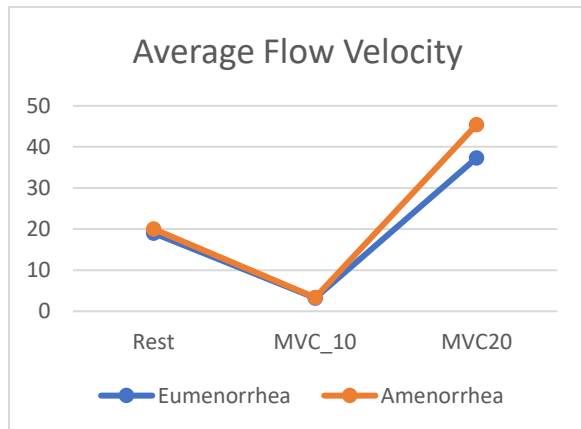C.



D.

E.



F.



G.



**Figure 1.** MVC_10= Max voluntary contraction at 10%; MVC_20= Max voluntary contraction at 20%;

FBF_SBP= forearm blood flow systolic blood pressure; FBF_DBP= forearm blood flow diastolic blood pressure;

FBF_MAP= forearm blood flow mean arterial pressure

## 5. <u>Discussion:</u>

This study assessed systemic vascular function and subclinical markers for cardiovascular disease in eumenorrheic and previously amenorrhoeic women. There were no significant differences between groups in IMT, beta stiffness, FMD average flow velocity, and pulse wave velocity. Amenorrhoeic women displayed lower SBP, MAP, aorSBP, aorMAP, and aorDBP. Collectively, these findings suggest that despite a significant period of amenorrhea, females do not display long-lasting negative remodeling of their vasculature after regaining a regular menstrual cycle. Thus, while amenorrhea and hypoestrogenic milieu is associated with endothelial dysfunction, this appears to be reversible assuming a menstrual cycle is regained.

### 4.1 Endothelial Function

From previous literature, researchers have found that athletes that were currently amenorrhoeic at the time of testing had significantly lower brachial FMD, compared to eumenorrheic athletes (Todd J Anderson et al., 2011; Augustine et al., 2016). Our findings demonstrated no difference in FMD when comparing women who previously had amenorrhea with consistently eumenorrheic women. The peak flow velocity is a measurement of reactive hyperemia after 5 mins of occlusion of the brachial artery. This measurement assesses microvascular reactivity and is associated with future CVD risk (Todd J Anderson et al., 2011; Lee, Martin, Fung, & Anderson, 2012). The data shows there is no difference between groups, indicating that the women who previously experienced amenorrhea did not show impaired vascular reactivity in the resistance vessels (Adkisson et al., 2010). Increases in vascular diameter due to shear stress is largely indicative of nitric oxide in a healthy endothelial single cell layer of the vessel wall (Augustine et al., 2016; Bank, Shammas, Mullen, & Chuang, 1998; ANNE ZENI HOCH et al., 2003). Because there were

no differences observed in FMD%, this suggests that endothelial dysfunction does not persist and/or can be reversable in young women who regain a menstrual cycle following amenorrhea.

**4.2 Arterial Stiffness**

Research shows that chronic endothelial dysfunction may lead to changes in vascular structure, increasing the risk of CVD (Mitchell et al., 2005). Examples are damage or plaque build-up in the vessel wall or an increase in the collagen/elastin ratio. These are all important factors increasing risk of cardiovascular disease (Ben-Shlomo et al., 2014; van Sloten et al., 2014). According to our data there was no difference between groups in carotid intima-media thickness or beta stiffness, which is consistent with previous research demonstrating that with proper estrogen levels and a menstrual cycle continuously maintained for 2 years, reversal of negative remodeling of the vasculature and endothelial function can occur (Grosman-Rimon et al., 2019).

**4.3 Dynamic Hand Grip Exercise**

Blood vessels dilate in response to physiological stimulus or changes like acute and chronic shear stress when exercising. Vessel dilation due to shear stress is endothelial dependent and is the result of an increase in nitric oxide release from the endothelial cells (Anton et al., 2006; Ben-Shlomo et al., 2014). There were no significant differences detected between the groups in brachial diameter, average flow velocity, FBF, exercising systolic blood pressure, exercising diastolic blood pressure, and FVC. However, there was significance between intensities for FBF, and FVC (Barbosa et al., 2018). This is expected with an increase in intensity, creating more work on the working muscle. This increases fuel demand and encourages vasodilation to increase blood profusion. Since there was an increase in FBF and FVC, this suggests low vascular resistance and

efficient dilation mechanisms of the endothelial layer. This further encourages the theory that an increase in estrogen, and the regaining of a regular menstrual cycle compounded with regular exercise, can mitigate any negative vascular remodeling in young, healthy, and active women.

**4.4 Central and Peripheral Blood Pressures & Exercise**

Central blood pressure reflects the pressure vital organs such as the heart, brain, and kidneys are exposed to (Sharman & Laurent, 2013). Studies have developed a strong rationale that cardiovascular events are closely related to central BP (McEniery, Cockcroft, Roman, Franklin, & Wilkinson, 2014). This study found the previously amenorrhoeic group displayed lower central BP and peripheral BP when compared to the eumenorrheic group. Previous studies looking at amenorrheic and eumenorrheic women, have not found differences in central and peripheral BPs (De Souza & Williams, 2004; ANNE ZENI HOCH et al., 2003). There is an inverse relationship between exercise and blood pressure. It is known that endurance exercise has a positive effect on the cardiovascular system by reducing blood pressure (Shephard & Balady, 1999). During exercise, there is an increase in shear stress on the vessel wall, initiating vasodilation that persists into the post-exercise period, resulting in post exercise hypotension and an overall  lower blood pressure set point (Swain et al., 2003). Estrogen is also thought to help increase the bioavailability of nitric oxide also resulting in vasodilation, and when compounded with exercise is thought to encourage angiogenesis and the regulation of blood flow and pressures (Swain et al., 2003). According to our data, there were differences between groups in physical activity, with the previously amenorrhoeic group exercising more than the eumenorrheic group. Because the amenorrhoeic group displayed lower central and peripheral blood pressures, the significantly higher physical activity level may be the reason for the discrepancy. We followed up with a one-way ANCOVA to determine the effect of physical activity on BP and we were able to determine

that PA in minutes per week did not impact the significant difference between groups in aortic SBP (p=0.03) and aortic MAP (p=0.05), but did eliminate significance seen in aorSBP, brachial SBP and brachial MAP, although brachial SBP was trending (p=0.055) (adjusted means reported in Table 3). Since blood pressures that were significant are not longer with physical activity being controlled for. This suggests that physical activity may have a substantial effect on the cardiovascular health of young women, even despite the previous presence of amenorrhea. It is unknown why the aorSBP and aorMAP were significant once physical activity was controlled for, since this was an unexpected find, it would be beneficial to look at how many years the participants had exercises, to see if this had any effect on these values.

**4.6 Limitations**

Since there is a significant difference in physical activity between the two groups, it would have been ideal to match physical activity levels between groups. Future research should assess groups with similar activity levels in order to appropriately elucidate the role of previous amenorrhea on vascular function.

We also did not directly measure estrogen or confirm ovulation status, therefore it is plausible that some participants may have experienced early ovulatory bleeding or breakthrough that can sometimes occur, impacting vascular measures and estradiol concentrations (Augustine et al., 2016). In addition, women taking oral contraceptives were not excluded from this study. However, all participants were tested during their early follicular stage of their menstrual cycle, or during the placebo pill phase, in order to control for hormonal levels during testing.

Another limitation to consider is that the vascular health of the previously amenorrheic women was unknown while they did not experience a menstrual cycle. While unlikely, it is

possible there was not any negative vascular remodeling, causing no significant difference to be found.

**4.7 Future Studies**

It would be beneficial in the future to organize a longitudinal study, that follows women who are currently amenorrhea, and perform similar measurements as this study. Once the participants have regained a consistent menstrual cycle, it would be beneficial to perform the same measurements and compare the results to a eumenorrheic group matched for physical activity.

Another parameter that needs to be looked at is women who are/were amenorrheic and not physically active. Since our study found results suggesting the benefits of physical activity despite menstrual status, it would be beneficial to look at the effects of amenorrhea on women who are not physically active and see if there are long term effects on cardiovascular health.

## 5. Conclusion

In this study we found that there were no significant differences in endothelial function between previously amenorrhoeic and eumenorrheic women. In addition, groups displayed no differences in aortic or carotid arterial stiffness. Significance was observed in the minutes of physical activity per week, as well as SBP, MAP, aorSBP, aorDBP, and aorMAP. This research highlights that previous amenorrhea experienced early in life, may not have long-term negative cardiovascular repercussions as long as regular menstruation resumes.

References

Adkisson, E. J., Casey, D. P., Beck, D. T., Gurovich, A. N., Martin, J. S., & Braith, R. W. (2010). Central, peripheral and resistance arterial reactivity: fluctuates during the phases of the menstrual cycle. *Experimental Biology and Medicine, 235*(1), 111-118.

Anderson, T. J., Charbonneau, F., Title, L. M., Buithieu, J., Rose, M. S., Conradson, H., . . . Lonn, E. M. (2011). Microvascular function predicts cardiovascular events in primary prevention: long-term results from the Firefighters and Their Endothelium (FATE) study. *Circulation, 123*(2), 163-169.

Anderson, T. J., Uehata, A., Gerhard, M. D., Meredith, I. T., Knab, S., Delagrange, D., . . . et al. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol, 26*(5), 1235-1241.

Anton, M. M., Cortez-Cooper, M. Y., DeVan, A. E., Neidre, D. B., Cook, J. N., & Tanaka, H. (2006). Resistance training increases basal limb blood flow and vascular conductance in aging humans. *Journal of Applied Physiology, 101*(5), 1351-1355.

Augustine, J. A., Lefferts, W. K., Dowthwaite, J. N., Brann, L. S., Brutsaert, T. D., & Heffernan, K. S. (2016). Subclinical atherosclerotic risk in endurance-trained premenopausal amenorrheic women. *Atherosclerosis, 244*, 157-164.

Bank, A. J., Shammas, R. A., Mullen, K., & Chuang, P. P. (1998). Effects of short-term forearm exercise training on resistance vessel endothelial function in normal subjects and patients with heart failure. *Journal of cardiac failure, 4*(3), 193-201.

Barbosa, T. C., Kaur, J., Stephens, B. Y., Akins, J. D., Keller, D. M., Brothers, R. M., & Fadel, P. J. (2018). Attenuated forearm vascular conductance responses to rhythmic handgrip in

young African-American compared with Caucasian-American men. *American Journal of Physiology-Heart and Circulatory Physiology, 315*(5), H1316-H1321.

Ben-Shlomo, Y., Spears, M., Boustred, C., May, M., Anderson, S. G., Benjamin, E. J., . . . Cruickshank, J. K. (2014). Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol, 63*(7), 636-646.

Chakrabarti, S., Morton, J. S., & Davidge, S. T. (2014). Mechanisms of estrogen effects on the endothelium: an overview. *Can J Cardiol, 30*(7), 705-712. doi:10.1016/j.cjca.2013.08.006

Chen, Z., Yuhanna, I. S., Galcheva-Gargova, Z., Karas, R. H., Mendelsohn, M. E., & Shaul, P. W. (1999). Estrogen receptor α mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest, 103*(3), 401-406.

De Souza, M. J., & Williams, N. I. (2004). Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women. *Human reproduction update, 10*(5), 433-448.

Gavin, K. M., Seals, D. R., Silver, A. E., & Moreau, K. L. (2009). Vascular endothelial estrogen receptor alpha is modulated by estrogen status and related to endothelial function and endothelial nitric oxide synthase in healthy women. *J Clin Endocrinol Metab, 94*(9), 3513-3520. doi:10.1210/jc.2009-0278

Grosman-Rimon, L., Wright, E., Freedman, D., Kachel, E., Hui, S., Epstein, I., . . . Eilat-Adar, S. (2019). Can improvement in hormonal and energy balance reverse cardiovascular risk factors in athletes with amenorrhea? *American Journal of Physiology-Heart and Circulatory Physiology, 317*(3), H487-H495.

HOCH, A. Z., DEMPSEY, R. L., CARRERA, G. F., WILSON, C. R., CHEN, E. H.,

BARNABEI, V. M., . . . GUTTERMAN, D. D. (2003). Is there an association between

athletic amenorrhea and endothelial cell dysfunction? *Medicine & Science in Sports &

Exercise, 35*(3), 377-383.

Hoch, A. Z., Papanek, P., Szabo, A., Widlansky, M. E., Schimke, J. E., & Gutterman, D. D.

(2011). Association between the female athlete triad and endothelial dysfunction in

dancers. *Clinical journal of sport medicine: official journal of the Canadian Academy of

Sport Medicine, 21*(2), 119.

Holland, D. J., Sacre, J. W., McFarlane, S. J., Coombes, J. S., & Sharman, J. E. (2008). Pulse

wave analysis is a reproducible technique for measuring central blood pressure during

hemodynamic perturbations induced by exercise. *Am J Hypertens, 21*(10), 1100-1106.

doi:10.1038/ajh.2008.253

Holland, D. J., Sacre, J. W., McFarlane, S. J., Coombes, J. S., & Sharman, J. E. (2008). Pulse

wave analysis is a reproducible technique for measuring central blood pressure during

hemodynamic perturbations induced by exercise. *American journal of hypertension,

21*(10), 1100-1106.

Kelley, D. S., & Bendich, A. (1996). Essential nutrients and immunologic functions. *The

American journal of clinical nutrition, 63*(6), 994S-996S.

Lee, V., Martin, B.-J., Fung, M., & Anderson, T. J. (2012). The optimal measure of

microvascular function with velocity time integral for cardiovascular risk prediction.

*Vascular Medicine, 17*(5), 287-293.

Li, L., Hisamoto, K., Kim, K. H., Haynes, M. P., Bauer, P. M., Sanjay, A., . . . Bender, J. R.

(2007). Variant estrogen receptor-c-Src molecular interdependence and c-Src structural

requirements for endothelial NO synthase activation. *Proc Natl Acad Sci U S A, 104*(42), 16468-16473. doi:10.1073/pnas.0704315104

McEniery, C. M., Cockcroft, J. R., Roman, M. J., Franklin, S. S., & Wilkinson, I. B. (2014). Central blood pressure: current evidence and clinical importance. *European heart journal, 35*(26), 1719-1725.

Mendelsohn, M. E., & Karas, R. H. (1999). The protective effects of estrogen on the cardiovascular system. *N Engl J Med, 340*(23), 1801-1811. doi:10.1056/nejm199906103402306

Miller, V. M., & Duckles, S. P. (2008). Vascular actions of estrogens: functional implications. *Pharmacological reviews, 60*(2), 210-241.

Mitchell, G. F., Vita, J. A., Larson, M. G., Parise, H., Keyes, M. J., Warner, E., . . . Benjamin, E. J. (2005). Cross-sectional relations of peripheral microvascular function, cardiovascular disease risk factors, and aortic stiffness: the Framingham Heart Study. *Circulation, 112*(24), 3722-3728.

Rickenlund, A., Eriksson, M. J., Schenck-Gustafsson, K., & Hirschberg, A. L. (2005). Amenorrhea in female athletes is associated with endothelial dysfunction and unfavorable lipid profile. *J Clin Endocrinol Metab, 90*(3), 1354-1359. doi:10.1210/jc.2004-1286

Rothman, M. S., & Wierman, M. E. (2013). Amenorrhea *Endocrine Secrets* (pp. 394-400): Elsevier.

Sesso, H. D., Stampfer, M. J., Rosner, B., Hennekens, C. H., Gaziano, J. M., Manson, J. E., & Glynn, R. J. (2000). Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in men. *Hypertension, 36*(5), 801-807.

Sharman, J. E., & Laurent, S. (2013). Central blood pressure in the management of hypertension: soon reaching the goal? *J Hum Hypertens, 27*(7), 405-411. doi:10.1038/jhh.2013.23

Shechter, M., Issachar, A., Marai, I., Koren-Morag, N., Freinark, D., Shahar, Y., . . . Feinberg, M. S. (2009). Long-term association of brachial artery flow-mediated vasodilation and cardiovascular events in middle-aged subjects with no apparent heart disease. *Int J Cardiol, 134*(1), 52-58. doi:10.1016/j.ijcard.2008.01.021

Shephard, R. J., & Balady, G. J. (1999). Exercise as cardiovascular therapy. *Circulation, 99*(7), 963-972.

Stirone, C., Boroujerdi, A., Duckles, S. P., & Krause, D. N. (2005). Estrogen receptor activation of phosphoinositide-3 kinase, akt, and nitric oxide signaling in cerebral blood vessels: rapid and long-term effects. *Mol Pharmacol, 67*(1), 105-113. doi:10.1124/mol.104.004465

Swain, R. A., Harris, A. B., Wiener, E. C., Dutka, M. V., Morris, H. D., Theien, B. E., . . . Greenough, W. T. (2003). Prolonged exercise induces angiogenesis and increases cerebral blood volume in primary motor cortex of the rat. *Neuroscience, 117*(4), 1037-1046.

Taddei, S., Virdis, A., Ghiadoni, L., Mattei, P., Sudano, I., Bernini, G., . . . Salvetti, A. (1996). Menopause is associated with endothelial dysfunction in women. *Hypertension, 28*(4), 576-582.

Tanner, J. M., & Whitehouse, R. H. (1976). Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Archives of disease in childhood, 51*(3), 170-179.

Thijssen, D. H., Black, M. A., Pyke, K. E., Padilla, J., Atkinson, G., Harris, R. A., . . . Green, D.
J. (2011). Assessment of flow-mediated dilation in humans: a methodological and
physiological guideline. *Am J Physiol Heart Circ Physiol, 300*(1), H2-12.
doi:10.1152/ajpheart.00471.2010

Tousoulis, D., Kampoli, A. M., Tentolouris, C., Papageorgiou, N., & Stefanadis, C. (2012). The
role of nitric oxide on endothelial function. *Curr Vasc Pharmacol, 10*(1), 4-18.

Tran, Q.-K., Firkins, R., Giles, J., Francis, S., Matnishian, V., Tran, P., . . . Gebert-Oberle, B.
(2016). Estrogen enhances linkage in the vascular endothelial calmodulin network via a
feedforward mechanism at the G protein-coupled estrogen receptor 1. *Journal of
Biological Chemistry, 291*(20), 10805-10823.

Treloar, A. E., Boynton, R. E., Behn, B. G., & Brown, B. W. (1967). Variation of the human
menstrual cycle through reproductive life. *International journal of fertility, 12*(1 Pt 2),
77-126.

Ueda, K., & Karas, R. H. (2013). Emerging evidence of the importance of rapid, non-nuclear
estrogen receptor signaling in the cardiovascular system. *Steroids, 78*(6), 589-596.
doi:10.1016/j.steroids.2012.12.006

van Sloten, T. T., Schram, M. T., van den Hurk, K., Dekker, J. M., Nijpels, G., Henry, R. M., &
Stehouwer, C. D. (2014). Local stiffness of the carotid and femoral artery is associated
with incident cardiovascular events and all-cause mortality: the Hoorn study. *J Am Coll
Cardiol, 63*(17), 1739-1747.

White, R. E. (2002). Estrogen and vascular function. *Vascul Pharmacol, 38*(2), 73-80.

Williams, J. K., Adams, M. R., Herrington, D. M., & Clarkson, T. B. (1992). Short-term administration of estrogen and vascular responses of atherosclerotic coronary arteries. *J Am Coll Cardiol, 20*(2), 452-457.

Zeni Hoch, A., Dempsey, R. L., Carrera, G. F., Wilson, C. R., Chen, E. H., Barnabei, V. M., . . . Gutterman, D. D. (2003). Is there an association between athletic amenorrhea and endothelial cell dysfunction? *Med Sci Sports Exerc, 35*(3), 377-383. doi:10.1249/01.mss.0000053661.27992.75

**Vita**


      Katherine T Williford was born in Chapel Hill, NC, to Billy and Linda Williford. She graduated from Appalachian State University in Boone, NC in December 2018, with a Bachelor's of Science in Exercise Science. The following spring, she entered Appalachian States Graduate School to study Clinical Exercise Science, and in May 2020 he was awarded the Masters in Clinical Exercise Science. Following graduation Katherine is currently working within the health and rehabilitation field and will be applying to Physician Assistant school in the fall.