EFFECTS OF THE MENSTRUAL CYCLE ON VASCULAR FUNCTION

By

ERIC JOSIAH ADKISSON

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2008

To my wife, Lisa; and to my parents, Van and Mary Kay Adkisson, who continually support me and push me to succeed in all of my scholastic and professional endeavors.

ACKNOWLEDGMENTS

I thank Dr. Randy Braith for the opportunity to pursue this degree in his laboratory at the University of Florida. His guidance on this project and all other matters has been invaluable. I also thank Darren Casey and Darren Beck for their friendship and advice. Lastly, I thank the other members of my committee, Dr. Steve Dodd and Dr. Scott Powers, for their suggestions and guidance.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

EFFECTS OF THE MENSTRUAL CYCLE ON VASCULAR FUNCTION

By

Eric Josiah Adkisson

December 2008

Chair: Randy Braith
Major: Applied Physiology and Kinesiology

Few studies of arterial compliance and endothelial function have been performed in pre-menopausal females. Fluctuations in naturally occurring female sex hormones may alter vascular function. We studied changes in vascular reactivity during the menstrual cycle of 23 healthy pre-menopausal women (age, 19.69 years). Subjects were examined during the four major phases of the menstrual cycle; Early Follicular (EF), Late Follicular (LF), Early Luteal (EL), and Late Luteal (LL). Four non-invasive measures were performed during each of the menstrual cycle phases: augmentation index (AI), central and peripheral pulse wave velocity (PWV), venous occlusion plethysmography (VOP), and brachial artery flow-mediated dilation (FMD). Nitric oxide (NO) levels were also measured during these times.

Vascular reactivity was greatest during the LF phase, compared to EF and LL. This is evidenced by increases in brachial artery flow-mediated dilation (EF, 3.35%; LF, 5.12%; LL, 3.71%; P=0.05), as well as, peak forearm (EF, 21.58; LF, 25.86; LL, 21.50 ml/min/100ml tissue; P=0.05) and total calf (EF, 7.26; LF, 8.87; LL, 7.62ml/min/100ml tissue; P=0.05) hyperemic blood flow. Furthermore, there was a reduction in augmentation index (AI) corrected for a heart rate of 75 (EF, 9.17%; LF, 1.35%; LL, 7.04%; P=0.05), an increase in round trip travel time (Δt_p) of the reflected wave (EF, 104.57; LF, 119.04; LL, 107.74 ms; P=0.05) and a reduction in wasted

left ventricular (LV) energy (EF, 10.22; LF, 5.67; LL, 9.91 dynes/cm^2/sec; P=0.05). Also, plasma nitrite/nitrate (NOx) increased during the Late Follicular phase (EF, 25.93; LF, 47.97; EL, 26.10; LL, 27.73 μmol/L; P=0.05). The temporal pattern of NO was paralleled by plasma levels of estrogen at the 4 measurement phases (EF, 78.85; LF, 158.62; EL, 91.52; LL, 68.32 pg/ml; P=0.05).

This is the first study to comprehensively assess systemic arterial function, menstrual cycle hormones and vaso-active mechanisms throughout the menstrual cycle in the same cohort of women. We found that the greatest changes in vascular reactivity occurred during the Late Follicular phase of the menstrual cycle. The mechanism responsible for this phenomenon, in part, is increased NO bioavailability, secondary to an estrogen-mediated response. Due to these variations between phases, laboratory vascular testing in pre-menopausal females must be standardized by menstrual cycle phase to avoid variability.

CHAPTER 1
INTRODUCTION

In recent years, due to the increasing risk for cardiovascular disease (CVD) in the years after menopause, research concerning women and menstrual cycle hormones has been primarily focused on post-menopausal women. Arterial compliance (inverse of arterial stiffness) and endothelial function, measures of cardiovascular function, decrease in men and women with age,[1-8] but evidence supports postmenopausal women having a steeper age related decline than age-matched men.[1-9] In comparison to their age matched male counterparts, women have lower systolic blood pressure (SBP), pulse pressure (PP) and mean arterial pressure (MAP) before menopause, but this sex difference begins to narrow in the fifth and six decade, disappears by the seventh and is reversed by the eighth for all 3 measures, respectively.[10, 11] CVD and CVD related mortality show a similar trend with respect to men and women. Pre-menopausal females account for less documented cases of CVD than men of similar age. However, after menopause this trend is reversed with women now accounting for the majority of cases.[12] This evidence suggests that the menstrual cycle plays an active role on the cardiovascular system, with estrogen protecting pre-menopausal females from CVD.

As a measure of prevention against CVD for post-menopausal women, hormone replacement therapy (HRT) has been used extensively. However, the effects of HRT are conflicting. Estrogen alone has been shown to attenuate the age-related decrease in arterial compliance and endothelial function.[13-16] In contrast, there are reports suggesting that estrogen alone has no effect on vascular function.[17, 18] Several reports did not identify any changes with combination replacement (estrogen + progesterone),[14, 18, 19] which may suggest a possible antagonistic effect of progesterone on the vasculature. Regardless of the effects of HRT on the cardiovascular system, recent evidence suggests that HRT does not protect against CVD.[20, 21]

Few studies of arterial compliance and endothelial function have been performed in pre-menopausal females. A thorough understanding of variations in vascular function during the menstrual cycle is important. Fluctuations in naturally occurring female sex hormones may alter vascular function. A careful characterization of arterial function during the 4 stages of the female menstrual cycle will be beneficial for 2 reasons. First a systematic analysis of variation in arterial reactivity throughout the phases of the menstrual cycle may provide information on the underlying sex differences in CVD risk. Secondly, a systematic characterization of cyclical variability in arterial function will help to develop standardization guidelines for laboratory testing in pre-menopausal women. To our knowledge there are no standard guidelines for such testing.

Accordingly, the purpose of this investigation was to determine the effects of the menstrual cycle on arterial compliance and endothelial function in pre-menopausal females. This is the first study to examine the entire vascular tree (central elastic, peripheral muscular and micro-vascular resistance arteries) in the same cohort of women throughout their menstrual cycles. Also, this is the first study to assess nitric oxide (NO) production during all phases of the female menstrual cycle.

- **Specific Aim 1:** To measure vascular function in central elastic, peripheral muscular and micro- vascular resistance arteries throughout 4 time-points of the normal female menstrual cycle.
- **Hypothesis 1:** Cyclic production of estrogen and progesterone will alter vascular function throughout the menstrual cycle.
- **Specific Aim 2:** To determine if laboratory testing of vascular function in females needs to be standardize to the same phase of the menstrual cycle
- **Hypothesis 2:** Standardization guidelines are essential to minimize laboratory variability when performing vascular tests in pre-menopausal females.
- **Specific Aim 3:** To investigate the effect the menstrual cycle has on NO production.

- **Hypothesis 3:** Production of NO will be altered during the 4 phases of the menstrual cycle.

CHAPTER 2
LITERATURE REVIEW

The arterial system is composed of arteries which vary in size and function and work together to maintain blood flow throughout the body. Large elastic arteries of the central circulation, such as the aorta, serve to "cushion" the intermittent output of the left ventricle during systole. In turn, the elastic recoil during diastole stabilizes the fluctuations in arterial pressure and allows for blood flow in the peripheral circulation. Blood flow pushed into the peripheral circulation is transported through muscular conduit arteries, such as the femoral and brachial arteries. Muscular conduit arteries have the ability to alter smooth muscle tone and therefore, modify the speed of the pressure waves, commonly referred to as pulse wave velocity. Lastly, the small arterioles, or resistance vessels, transport and control blood flow into tissues. These vessels are thought to control mean arterial pressure by altering their diameter. Overall, the various types and regions of arteries function to convert pulsatile load from the heart into steady flow at the capillary level and maintain a relatively constant perfusion pressure.

Overview of Arterial Compliance

Arterial compliance is defined as a change in arterial blood volume (ΔV) for a given change in arterial blood pressure (ΔP) (i.e. $\Delta V / \Delta P$).[22] In general, a compliant artery is able to expand and recoil in response to the cardiac cycle, stabilizing fluctuations in arterial pressure. Reductions in large artery compliance is an important indicator of cardiovascular risk[23, 24] and has recently been linked to CVD related mortality.[25] There is an age associated decrease in arterial compliance.[2-9] O'Rourke and Nichols[26] reported that aortic compliance, measured by PWV, is decreased by approximately 100% between 20 and 80 years of age. Interestingly, it seems that with aging compliance is not affected in peripheral arteries, but is limited to the central elastic arteries.[26-28]

An explanation for this phenomenon may be found in the morphology of the arteries. When compared to peripheral arteries, central elastic arteries, such as the aorta, have a higher composition of elastic fibers. With age there are 3 morphologic changes in the aorta. First, elastin begins to thin, split, fray and fragment. Secondly, collagen, an extremely stiffer fiber than elastin, doubles in content from age 20 to 70 years.[22] Thirdly, arterial calcification occurs in some people. The associated decrease in elastic fibers and increase in stiff fibers with calcification causes profound changes in central compliance. This age-related histological transformation is not seen in peripheral muscular arteries. Changes in compliance in these muscular arteries are primarily due to changes in smooth muscle tone.

A decrease in compliance or increase in stiffness in either elastic or muscular vessels can lead to altered blood flow. Additionally, decreased compliance increases the transmission velocity of pressure waves in the arterial system. It is important to understand that pressure waves can and do travel forward and retrograde in the arterial system. A reflected wave occurs when the primary forward traveling wave encounters sudden changes in vessel stiffness or diameter, such as bifurcations or at the interface with resistance vessels, and a portion of the wave is reflected back toward the heart. In a properly functioning system a summation of reflected waves from many sites arrive back in the ascending aorta post-systole augmenting coronary perfusion pressure. However, with decreased compliance, the reflected wave returns to the ascending aorta during late systole. The consequence due to the overlap of the reflected wave into systole is increased arterial pressures due to higher SBP and lower DBP, thereby causing increased LV afterload and altering coronary perfusion.[22] Higher SBP and PP, lower DBP, and LV hypertrophy have all been identified as independent risk factors of cardiovascular related morbidity and mortality.[29-31] Measuring compliance using PWV by applanation tonometry

provides information on large artery stiffness, small artery tone and endothelial function[32, 33] and may act as a marker for the development of future CVD.

Overview of Endothelial Function and Nitric Oxide

Vascular endothelium was once thought of as a quiescent single layer of cells lining the lumen of blood vessels. However, we now know the endothelium to be an extremely important biologically active organ, producing a myriad of mediators that regulate vascular smooth muscle tone and prevent against the development of CVD. One such important mediator is the vasodilator nitric oxide (NO). NO is produced by the reaction of L-Arginine, oxygen, and endothelial nitric oxide synthase (eNOS) with citrulline as a byproduct.[34] Upon synthesis, NO diffuses across the endothelial cell membrane and into vascular smooth muscle cells. Once in the muscle cell, NO stimulates the movement of intra-cellular calcium out of the cell, thus causing muscle relaxation.[35]

Proper functioning endothelium continuously produces NO, which is important in maintaining resting vascular tone.[36] Increased production above basal levels is stimulated in 2 ways: shear stress-mediated and agonist-mediated. Mechanical shear stress or pulsatile laminar flow along the endothelial wall causes the synthesis and release of NO and is an important local mechanism for the maintenance of vascular tone.[37] Agonists such as acetylcholine and bradykinin bind to receptors on endothelial cells causing the release of NO.[36, 38, 39] Both sheer stress-mediated and agonist-mediated production of NO function by increasing endothelium intra-cellular calcium, which binds to an inactive eNOS complex releasing the active eNOS molecule.

Endothelial dysfunction is often a term used to define a decrease in NO bio-availability. As well as being an important vasodilator, NO also plays a role in preventing plaque formation by suppressing leukocyte and platelet activity and inhibiting smooth muscle proliferation.

Decreased levels of NO would promote a vascular environment more prone to plaque formation as well as vasoconstriction. Endothelial dysfunction is seen in patients with coronary and peripheral vascular disease as well as people with conventional cardiovascular risk factors[40, 41] and is an independent predictor of cardiac events.[41]

Menstrual Cycle Hormones

Estrogen

Estrogen is a general term used to define a group of important steroid hormones synthesized from cholesterol that regulate the female menstrual cycle. Multiple estrogens have been identified in plasma of human females, the most abundant being beta (β)-estradiol. Estrogens are produced primarily by developing follicles in the ovaries and the corpus luteum. During the early follicular (EF) phase follicle-stimulating hormone (FSH) and lutenizing hormone (LH) secreted from the anterior pituitary initiate and sustain follicular growth. Estrogen concentration rapidly increases during the early and late follicular (LF) phases. In the LF phase, just prior to ovulation, there is a surge of LH that triggers ovulation and the formation of the corpus luteum. Estrogen levels are at their highest before ovulation and quickly fall in the beginning of the early luteal (EL) phase. Estrogen production begins to slightly increase between the early and late luteal (LL) phases due to a completely functional corpus luteum. Levels then decline at the onset of menstruation.

Estrogen plays an essential role in regulating a woman's reproductive process and preparing the uterus for pregnancy. Along with estrogen's important role in the menstrual cycle, estrogen is an active modulator of arterial function. For example, oral administration of estrogen decreases low-density lipoprotein (LDL) cholesterol and increases high-density lipoprotein (HDL) cholesterol in both pre-menopausal and post-menopausal females.[20, 42] Also, endogenous sources of estrogen modulate lipid throughout the menstrual cycle. LDL levels decrease and

HDL levels increase when estrogen is high and the reverse is seen when estrogen is low.[43-45] These changes are associated with a decrease risk in CVD,[46] but account for only approximately one third of the observed clinical benefits of estrogen.[47]

In addition to the affect on lipoproteins, estrogen has direct actions on arterial wall physiology. Endothelial cells contain two estrogen receptors; estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ).[48, 49] Activation of these receptors by estrogen rapidly elicits non-genomic activation of intracellular signaling, as well as, classical genomic pathways involving regulation of gene transcription. The rapid vasodilating effect of estrogen in endothelial cells is related to its ability to increase NO by stimulating eNOS activity[48, 50] through the activation of a subgroup of ERα.[51] ERβ is not associate with rapid non-genomic vasodilation,[52] but both ERα and ERβ act as transcription factors increasing eNOS expression,[53, 54] thus increasing NO availability in the long-term.

In vascular SMC's, estrogen has been shown to produce a rapid dilating response associated with the regulation of intracellular calcium. Estrogen receptors have been identified on SMCs[55-57] and it is clear that estrogen lowers the concentration of intra-cellular calcium. Even though one study has indicated depressed sarcoplasmic reticulum calcium release due to estrogen,[58] other studies suggest attenuating calcium influx[59] and/or stimulating calcium efflux.[60] Another important means of regulating calcium influx has been identified and works indirectly. Estrogen stimulates the activity of the large-conductance, calcium and voltage-activated potassium (BK_{Ca}) channels causing a efflux of potassium that repolarizes membrane potential and closes voltage-dependent calcium channels.[61] In aggregate, a decreased concentration of calcium inside the cell would cause a subsequent acute relaxation of the blood vessels.

Progesterone

Progesterone is another steroid hormone produced during the menstrual cycle. The primary production site of progesterone during the menstrual cycle is the corpus luteum, but is also produced by the adrenal glands, brain, and, during pregnancy, by the placenta. During the follicular phases progesterone production is minimal, but slightly rises just prior to ovulation. After ovulation and the formation of the corpus luteum, progesterone levels rise sharply and peak approximately one week after ovulation during the luteal phases. Along with estrogen, progesterone is important for preparing the uterus for pregnancy and serve extremely important functions during pregnancy.

Progesterone's effect on the cardiovascular system remains unclear and controversial. It is frequently administered along with estrogen in HRT and is thought to counteract some beneficial features of estrogens. As stated previously, supplementation of estrogen alone has been shown to attenuate the age-related decrease in arterial compliance and endothelial function,[13-16] but when progesterone is combined with estrogen, these benefits are blunted.[14, 18, 19] However, not all studies have shown that progesterone blunts the beneficial effects of estrogen.[62-64] One primary concern involves progesterone's adverse effects on lipoprotein levels. Progesterone opposes estrogen by lowering the level of HDL.[65, 66] In contrast to these findings, there are reports of progesterone having no effect on estrogen associated lipoprotein changes.[63, 64] These differences have been attributed to the type of progesterone used. Progesterones with lower levels of androgenic activity have only small effects[66-68] or no effects[69] on lipoprotein levels when used in combination with estrogen[67] or when used alone.[68]

Similar to estrogen, progesterone interacts directly on vascular wall physiology, but with possible deleterious effects. Progesterone receptors (PR) have been localized on vascular SMC,[70] endothelial cells[71] and two subtypes, PR-A and PR-B, have been identified in human

aorta SMC.[72] Both receptors have been previously demonstrated to repress transcriptional activities of estrogen receptors.[73] In vascular SMC, progesterone has been shown to up-regulate gene expression of angiotensin II type 1 (AT1) receptors[74] that mediate the effects of the renin-angiotensin system (RAS) and induce vasoconstriction.[75] In contrast, estrogen down regulates AT1 receptors through a NO dependant mechanism.[76] In addition to increasing gene expression, progesterone's effect on AT1 receptors may be partly due to the repression of estrogen receptor activity, thus regulating NO production and creating an environment more prone to vasoconstriction.

Previous Investigations

The effects of the 4 phases of the menstrual cycle on vascular function are inconclusive. We hypothesize that inconsistencies in the literature exist for two reasons. First, state-of-the-art noninvasive techniques were not utilized. Secondly, measurements were cross-sectional and not carefully standardized in the same cohort of women.

Studies Reporting Significant Change

Williams *et al.*[77] measured pre-menopausal females at 4 time points during the menstrual cycle (EF, LF, EL, & LL). They showed that brachial FMD increased from EF to the LF phase, fell in the EL phase, and increased again in the LL phase. Hashimoto *et al.*[78] also saw a increase from EF to LF in brachial FMD, but FMD remained elevated during the luteal phase. In this study, females were only measured once during the luteal phase (5 to 7 days after ovulation), which coincides with the LL phase of the Williams study. If another measurement was taken closer to ovulation when estrogen levels were low, Hashimoto *et al.*[78] may have seen an associate fall in brachial FMD as did Williams. In addition, Hashimoto *et al.*[78] measured brachial FMD on age-matched male counterparts and showed that pre-menopausal females compared to males only during EF.

Williams *et al* [77] also measured whole body arterial compliance (WBAC), a measurement based on a two-element Windkessel model of the circulation and area method of Liu *et al.*[79] by simultaneous measurement of ascending aortic blood flow and carotid blood pressure. WBAC gives a measure of both central and peripheral compliance and was shown to increase from the EF phase to the LF phase and fell in both luteal phases. Changes in radial artery compliance were similar Giannattasio *et al.*[80]. Measurement of central PWV (carotid-femoral) showed no changes between all phases. Significant changes in WBAC and brachial FMD with no changes in central PWV suggested that peripheral, not central, compliance changed throughout the menstrual cycle

However, Hayashi *et al.*[81] showed no change in peripheral PWV (femoral-dorsalis pedis) during 5 time points of the menstrual cycle (EF, LF, O (Ovulation), EL & LL). Interestingly, central arterial compliance, measured by ultrasound imaging of the common carotid artery, increased from the EF phase and the LF phase and into the O phase and decreased in the EL and LL phases making this study the first to suggest that central, not peripheral, compliance changes in response to the menstrual cycle. This directly contradicts the suggestion inferred from the data of Williams.

Studies Reporting No Significant Change

Ounis-Skali *et at.*[82] saw no change in central PWV (carotid-femoral), peripheral PWV (carotid-radial) or AI_a. In this study, females were measured on only two occasions, EF and mid luteal. This study's lack of significant change may be due to the small number of measurements and when they were conducted. Early follicular is associated with low estrogen and low progesterone production, whereas in the mid-luteal phase estrogen and progesterone production are increased. Assuming progesterone counter acts estrogen's effects, changes in vascular function may not be seen between EF and mid-luteal. In addition, Willlekes *et al.*[83] tracked

arterial wall properties using ultrasound in the aorta and femoral arteries in 12 females. Examinations were administered six to eight times during each cycle, which sufficiently encompasses all important time points and hormone fluctuations. Although an adequate amount of examinations were taken, other factors may have contributed to this study's inability to show significant change. First, a sample size of 12 subjects may be too small to see any significant fluctuations. Second, subjects were required to refrain from alcohol, smoking, and caffeine only 3 hours before measurements. Finally, ultrasound measurements of the carotid and femoral arteries cannot substitute for a complete battery of vascular tests and therefore cannot definitively suggest that the female menstrual cycle has no influence on the cardiovascular system.

CHAPTER 3
METHODS AND MATERIALS

Our study investigated the effects of the menstrual cycle on vascular function. We used a prospective cohort research design to assess the changes in vascular function during four time points during a normal menstrual cycle.

Subjects

All subjects were recruited using verbal announcements in classes held within the College of Health and Human Performance. All subjects signed a University of Florida Health Science Center Institutional Review Board (IRB) approved informed consent form prior to participating in the study. We performed a complete battery of vascular tests on 23 pre-menopausal females.

Inclusion Criteria

- Clinically healthy females
- Between 18 and 40 years of age

Exclusion Criteria

- Diabetic
- Receiving any type of contraceptives
- Smoking
- Resting blood pressure over 135/85

Specific Measurements

Subjects were required to make a total of 4 laboratory visits during one menstrual cycle. Each subject was required to track and document their menstrual phases for one full menstrual cycle prior to the laboratory visits. This information determined the proper timing for each visit. All testing took place in the Clinical Exercise Physiology (CEP) Laboratory in the Center for Exercise Science, University of Florida, Gainesville, FL.

Arterial Stiffness Testing

Pulse wave analysis (PWA)

Following a 15 minute rest period in a supine position, brachial systolic, diastolic, and pulse blood pressure measurements were performed in triplicate in the left arm using an automated noninvasive blood pressure (BP) cuff (VSM MedTech, Ltd.). An average of three BP measurements were used for resting BP values. The assessment of arterial wave reflection characteristics are performed noninvasively using the SphygmoCor system (AtCor Medical, Sydney, Australia). High-fidelity radial artery pressure waveforms are recorded by applanation tonometry of the radial pulse using a "pencil type" micromanometer (Millar Instruments, Houston, Texas). Optimal recording of the pressure wave is obtained when the hold-down force of the transducer on the artery is such that the resulting waveform has a stable baseline for at least 10 beats. Using a validated generalized mathematical transfer function the SphygmoCor system synthesizes a central aortic pressure waveform.[84, 85] This technique has been shown to be reproducible.[86, 87] The reliability of aortic pressure waveform measurements in our laboratory was established by sequential measurements on 7 young healthy men on 3 separate days. The mean coefficient of variation for these measurements was 6.5%.[88]

The central aortic pressure wave (P_s-P_d) is composed of a forward traveling wave with amplitude (P_i-P_d), generated by left ventricular ejection and a reflected wave with amplitude (P_s-P_i) that is returning to the ascending aorta from the periphery (Figure 2-1).[89] The contribution or amplitude of the reflected wave to ascending aortic pulse pressure can be estimated by the aortic augmentation index (AI_a). AI_a is defined as reflected wave amplitude divided by pulse pressure and expressed as a percentage [$AI_a = (P_s-P_i)/(P_s-P_d) \times 100$].[89, 90] P_s indicates peak systolic pressure, P_i is an inflection point that indicates the beginning upstroke of the reflected pressure wave, and P_d is the minimum diastolic pressure. The forward and reflected waves travel in

opposite directions along the artery at the same velocity. The round-trip travel time (Δt_p) of the forward traveling wave from the ascending aorta to the major reflection site and back is measured from the foot of the forward traveling pressure wave to P_i. Δt_p is inversely related to arterial pulse wave velocity and arterial stiffness, and directly related to the distance to the reflecting site.[89]

Pulse wave velocity (PWV)

With the subject supine, tonometry transit distances from the supra-sternal notch to the radial (SSN-R), femoral (SSN-F) and carotid (SSN-C) and from the femoral to the dorsalis pedis (F-DP) recording site were measured with a tape measure (Figure 2-2). Arterial applanation tonometry (SphygmoCor) were then obtained from the carotid, radial, femoral, and dorsalis pedis arteries in that order in rapid succession. High-fidelity pressure waveforms were recorded non-invasively using a "pencil type" micromanometer. Foot-to-foot pulse wave velocity to each peripheral site (dorsalis pedis, radial and femoral) were calculated by determining the delay between the appearance of the pressure waveform foot in the carotid and peripheral sites.[91] The distance between recording sites was adjusted for parallel transmission in the aorta and carotid by subtracting SSN-C from SSN-R and SSN-F. The corrected distances will be divided by the respective foot-to-foot transmission delays (Carotid-radial, Carotid-femoral) to give pulse wave velocity. Central pulse wave velocity (in the mostly elastic aorta) was evaluated using the carotid-femoral (C-F) data and peripheral pulse wave velocity (in the more muscular conduits) using the femoral-dorsalis pedis (F-DP) and carotid-radial (C-R) data. Pulse wave velocity between the various measuring sites were used as an indirect measure of regional arterial stiffness. Reliability of pulse wave velocity among the different regions was established by sequential measurements on 7 young men on 3 separate days. The mean coefficient of variation for C-R, C-F, and F-DP were 4.5, 2.1, and 5.3%, respectively.[88]

Endothelial Function Testing

Brachial artery flow-mediated dilation

Brachial artery reactivity testing was performed using a high-resolution ultrasound (HDI 3000, ATL, Inc). Brachial artery reactivity measurements were made with the subjects in a supine position following a fast of at least 4 hours and abstaining from caffeine and alcohol for at least 12 hours prior to the measurements. After lying quietly for 15 minutes, a 10.5 MHz linear phase array ultrasound transducer was used to image the right brachial artery longitudinally and was recorded digitally using Pinnacle Studio Plus software (Pinnacle Systems, Inc.). After obtaining resting baseline end diastolic diameters and blood flow velocity, a blood pressure cuff placed on the upper forearm, 1-2 cm below the elbow was inflated to 200 mmHg for 5 minutes. The transducer was held in the same position for the duration of cuff inflation. Using a cuff position that is distal to the site of the ultrasound measurement has been shown to produce less variability than proximal placement of the cuff[92] and has been suggested to serve as a more accurate bio assay of endothelial nitric oxide (NO) availability.[93] Immediately following cuff release, brachial artery blood flow velocity was measured for 20 seconds. Brachial artery diameter was then imaged and recorded for an additional 2 minutes.

Reactive hyperemia blood flow results in flow-mediated dialation (FMD) of the brachial artery due to shear stress-induced NO release from the endothelial wall. About 60 seconds after cuff deflation, peak brachial artery diameter has been reported to occur and is a valid measure of endothelial-mediated artery reactivity.[94] Brachial FMD is calculated as absolute (Δmm) and relative change (%) in brachial artery diameter in response to the forearm hyperemic stimulus. Because the main stimulus for FMD is an acute increase in vascular shear stress or blood flow, peak FMD values will be normalized for the magnitude of the hyperemic stimulus.[95] Shear rate (velocity/diameter) is used to quantify the hyperemic stimulus.[93]

Brachial artery diameters are determined during end-diastole by measuring the distance between the near and far wall of the intima using image analysis software (Image Pro, Data Translation, Inc.). Five anterior to posterior point measurements within a 2-3 cm segment are made and the average distances are recorded as the diameter.

Forearm and calf flow-mediated dilation

Patients were tested while in a supine position in a quiet, temperature-controlled room, approximately 21-22°C and relative humidity of approximately 40-50%. Venous occlusion plethysmography (EC-6, D.E. Hokanson, Inc.) using calibrated mercury strain gauges were used to determine forearm blood flow (FBF) and calf blood flow (CBF) as previously described by others.[96, 97] Briefly, the forearm and lower leg was positioned above heart level and a strain gauge was placed around the widest part of the non-dominant forearm (~5 cm below anticubital fossa) or calf (~10 cm below patella). To measure FBF, an upper arm cuff (Hokanson SC-10) was placed approximately 5 cm above the anticubital fossa and inflated to 50 mmHg for 5 seconds every 15 seconds using a rapid cuff inflator (EC-20, Hokanson, Inc.) to prevent venous outflow.[97] To measure CBF, an upper thigh cuff was placed approximately 5 cm above the patella and inflated to 50 mmHg for 5 seconds every 15 seconds. To prevent blood flow to the hand and foot during plethysmography, a cuff (Hokanson, Inc.) was placed around the wrist and ankle, respectively, and inflated to 220mmHg 1 minute before each measurement. The FBF or CBF output signal will transmit to a NIVP3 software program (Hokanson, Inc.) on a PC computer and expressed as milliliters (mL) per minute per 100 mL of tissue ($ml \cdot min^{-1}$ per 100 mL tissue). Absolute blood flow is determined by the rate of change of limb circumference (e.g. slope) during the 5 second venous occlusion, which correlates highly to arterial blood inflow into the limb.[96, 98] The average of 8 plethysmographic measurements over a 2-minute period will be recorded as the resting FBF or CBF.

After baseline FBF is confirmed to be stable for 2 minutes and recorded, the upper arm cuff was inflated to 200 mmHg for 5 minutes. The cuff was then released and endothelium-dependent FBF was measured during reactive hyperemia blood flow. FBF was measured every 15 seconds for 3 mintues. Peak FBF is recorded as the area under the *time x blood flow* curve after baseline FBF is subtracted using the trapezium rule.[99] Reactive hyperemia induced peak FBF has been shown to correlate highly with acetylcholine-induced FBF in patients with essential hypertension.[100] Thus, indicating that reactive hyperemia serves as a good noninvasive measurement of endothelium dependent dilation diameter in resistance arteries of the forearm.

Endothelium-dependent CBF is measured using the same techniques and methods as described above for endothelium-dependent FBF. The reliability of peak FBF and CBF using venous occlusion plethysmography is validated in our lab by a pilot study of 9 young healthy males who had peak FBF and CBF during reactive hyperemia performed 3 visits separated by 1 week.[101] Mean CV% of resting and peak FBF was 17% and 6.6%, respectively, and resting and peak CBF was 15.2% and 8.4%, respectively.

Blood Collection

Venous blood samples for serum were collected in tubes containing no additive, allowed to clot at room temperature for 15 minutes, and immediately centrifuged at 3,000 rpm from 15 minutes at 4ºC. Venous blood samples for plasma were collected in tubes containing ethylenediaminetetraacetic acid (EDTA), placed on ice for 15 minutes, and centrifuged immediately using the same protocol as the serum tubes. All serum and plasma samples were separated into 1.5 ml eppendorf tubes and immediately stored at -80ºC until batch analysis at the end of the study.

Biochemical Analysis

Menstrual cycle hormones

Estradiol is the major estrogen secreted during the female menstrual cycle. Plasma Estradiol were measured using a commercially available kit (Cayman Chemical, Inc.), which is based on the competition between Estradiol and Estradiol-acetylcholinesterase (Estradiol Tracer) conjugate for Estradiol Antiserum. The amount of Estradiol Tracer that is able to bind to Estradiol Antiserum is determined spectrophotometrically and is inversely proportional to the concentration of estrogen.

Levels of progesterone begin to rise during the luteal phase of the menstrual cycle to help prepare the uterine lining for implantation. Plasma Progesterone were measured using a commercially available kit (Cayman Chemical, Inc.), which is based on the competition between Progesterone and Progesterone -acetylcholinesterase (Progesterone Tracer) conjugate for Progesterone-specific rabbit antiserum. The amount of Progesterone Tracer that is able to bind Progesterone-specific rabbit antiserum is determined spectrophotometrically and is inversely proportional to the concentration of progesterone.

Vasodilator production

Because NO is rapidly converted to nitrate and nitrite (NOx) in plasma, NOx was used to estimate NO production. Since plasma NOx levels can be influenced by dietary nitrate, subjects were asked to follow the National Institutes of Health low nitrate diet guidelines 24 hours prior to each blood draw[102]. Plasma NOx were measured using a commercially available kit (Cayman Chemical, Inc.), which converts all nitrate to nitrite using nitrate reductase. Spectrophotometric analysis of total nitrite will be performed using Greiss reagent and the absorbance measured at 540 nm.

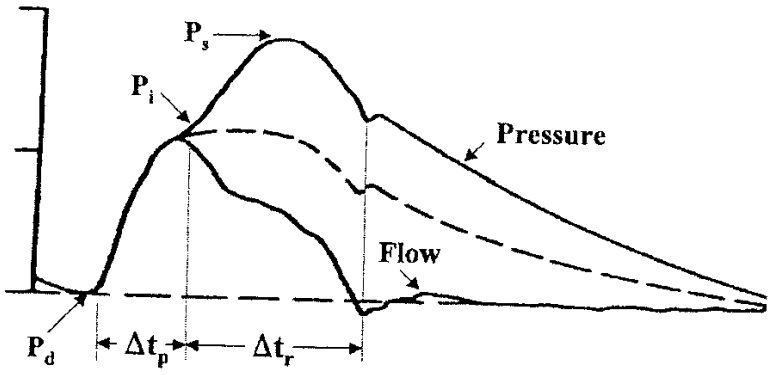

Figure 3-1. Ascending aortic pressure waveform. P_s = Peak aortic systolic pressure; P_d = minimum aortic diastolic pressure; P_i = inflection point of the reflected wave ; Δt_p = duration of reflected wave from the heart to the periphery and back; Δt_r = systolic duration of the reflected pressure wave [89].

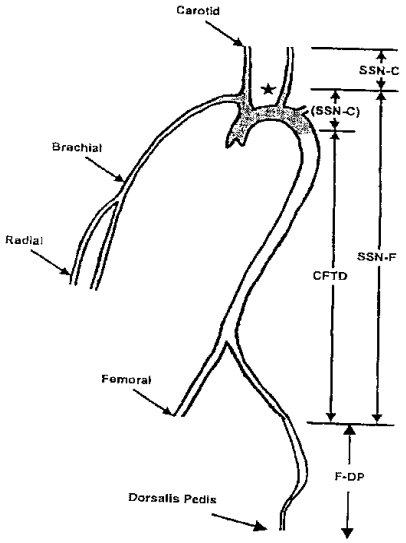

Figure 3-2. Determination of the pulse wave transit distances from body surface measurements. The star denotes the suprasternal notch. SSN-C = suprasternal notch-carotid distance; SSN-F = suprasternal notch-femoral distance; CFTD = carotid femoral transit distance; F-DP = femoral-dorsalis pedis distance.

CHAPTER 4
RESULTS

A total of twenty-three (n=23) subjects were recruited and tested during the 4 phases of a normal menstrual cycle. The study was approved by the University of Florida Health Science Institutional Review Board and all subjects signed written informed consent to participate in the study.

Subject Characteristics

Descriptive characteristics were measured at all time points (EF, LF, EL and LL) and results are shown in Table 4-1. There were no statistically significant ($p \geq 0.05$) differences in descriptive characteristics across the 4 measurement time points.

Blood Pressure and Pulse Wave Analysis

Blood pressure components and pulse wave analysis results are presented in Table 4-2 and Figures 4-1 – 4-10. There was no significant change in heart rate, peripheral pulse pressure, and aortic pulse pressure ($p \geq 0.05$) during the four phases of the menstrual cycle. LF values were significantly lower than EF values in peripheral systolic blood pressure (105.61±6.53 vs. 110.91±7.76, $P<0.05$), peripheral diastolic blood pressure (68.17±4.55 vs. 72.87±4.90, $P<0.05$), aortic diastolic blood pressure (69.22±4.58 vs. 73.78±5.88, $P<0.05$) and mean arterial blood pressure (80.57±5.10 vs. 85.00±5.82, $P<0.05$). There were significantly lower LF values than LL values in peripheral systolic blood pressure (105.61±6.53 vs. 110.39±7.51, $P<0.05$), peripheral diastolic blood pressure (68.17±4.55 vs. 71.69±6.39, $P<0.05$) and mean arterial blood pressure (80.57±5.10 vs. 84.43±6.39, $P<0.05$). There was a trend toward decreased aortic systolic blood pressure in LF compared to EF (93.61±5.43 vs. 98.48±6.81, $P=0.072$).

Pulse wave analysis results demonstrated a significant decrease in aortic augmentation index (AI) (14.04±8.75 vs. 6.96±8.75, P<0.05) and aortic augmentation index normalized for a heart rate of 75 bpm (AI@75 bpm) (9.17±8.87 vs. 1.35±8.84, P<0.05) between EF and LF. Round trip travel time of the reflected wave (Δt_p) significantly increased (104.57±15.14 vs. 119.04±19.08, P<0.05) during LF compared to EF. There was a significant decrease in augmented pressure (3.48±2.27 vs. 1.57±2.15, P<0.05) and wasted left ventricular energy (10.22±5.65 vs. 5.67±4.56, P<0.05) from EF to LF. There were significantly lower LF values than LL values in aortic augmentation index (AI) (6.96±8.75 vs.12.52±9.24, P<0.05) and aortic augmentation index normalized for a heart rate of 75 bpm (AI@75 bpm) (1.35±8.84 vs. 7.04±11.00, P<0.05). Round trip travel time of the reflected wave (Δt_p) significantly decreased (119.04±19.08 vs. 107.74±10.28, P<0.05) during LL compared to LF. There was a significant increase in augmented pressure (1.57±2.15 vs. 3.09±2.94, P<0.05) and wasted left ventricular energy (5.67±4.56 vs. 9.91±6.99, P<0.05) from LF to LL.

Central and Peripheral Arterial Stiffness

Central and peripheral pulse wave velocity (PWV) results are presented in Table 4-3. No significant change was observed in carotid-radial, carotid-femoral or femoral-distal PWV throughout all phases.

Forearm and Calf Resistance Arterial Blood Flow

Forearm and calf resistance arterial blood flow values during reactive hyperemia are presented in Table 4-4 and Figures 4-11 and 4-12. No significant change was observed in resting FBF, total FBF AUC_{3min}, resting CBF and peak CBF. There was a significant increase in peak FBF (21.58±4.37 vs. 25.86±5.91, P<0.05) and total CBF AUC_{3min} (7.26±1.30 vs. 8.87±2.55, P<0.05) from EF to LF. There was a trend towards increased resting CBF (1.74±0.47 vs.

2.04±0.60, P=0.13) and total FBF AUC_{3min} (6.12±1.96 vs. 7.56±2.98, P=0.12) from EF to LF. There were significantly higher LF values than LL values in peak FBF (25.86±5.91 vs. 21.50±5.76, P<0.05) and total CBF AUC_{3min} (8.87±2.55 vs. 7.62±1.72, P<0.05).

Brachial Artery Endothelial Function

Brachial artery flow-mediated dilation (FMD) results are shown in Table 4-5 and Figures 4-13, 4-14 and 4-15. There were no significant changes in baseline diameter (mm), resting SR and peak SR. Significant increases were observed in absolute dilation (mm) (0.16±0.07 vs. 0.23±0.11, P<0.05), brachial FMD (%) (3.35±1.88 vs. 5.12±2.36, P<0.05) and normalized FMD (s^{-1}) (0.44±0.29 vs. 0.64±0.28, P<0.05) during LF compared to EF. Significant decreases were observed in absolute dilation (mm) (0.23±0.11 vs. 0.16±0.08, P<0.05), brachial FMD (%) (5.12±2.36 vs. 3.38±1.37, P<0.05) and normalized FMD (s^{-1}) (0.64±0.28 vs. 0.43±0.22, P<0.05) during LF compared to EL. Significant decreases were observed in absolute dilation (mm) (0.23±0.11 vs. 0.17±0.11, P<0.05), brachial FMD (%) (5.12±2.36 vs. 3.71±2.31, P<0.05) and normalized FMD (s^{-1}) (0.64±0.28 vs. 0.44±0.23, P<0.05) during LF compared to LL.

Menstrual Cycle Hormones and Nitric Oxide

Menstrual cycle hormones and nitric oxide results are presented in table 4-6 and Figures 4-16 and 4-17. There were significant increases in estradiol (78.85±0.30 vs. 158.62±0.59, P<0.05) and NOx (25.93±21.25 vs. 47.97±40.53, P<0.05) during LF compared to EF. No significant changes were observed in progesterone. LF values were significantly higher than EL and LL values in estradiol (158.62±0.59 vs. 91.52±0.54 & 68.32±0.35, P<0.05) and NOx (47.97±40.53 vs. 26.10±21.86 & 27.73±21.05, P<0.05).

Table 4-1. Descriptive Characteristics

	EF	LF	EL	LL
Age (yr)	19.69±1.29	19.69±1.29	19.69±1.29	19.69±1.29
Height (cm)	165.61±5.78	165.61±5.78	165.61±5.78	165.61±5.78
Weight (kg)	61.89±12.10	61.69±11.88	61.69±11.94	62.03±11.96
Body Mass Index (kg/m^2)	22.47±3.70	22.41±3.67	22.39±3.64	22.54±3.67

Values are mean ± SD; EF=Early Follicular phase; LF=Late Follicular phase; EL=Early Luteal phase; LL=Late Luteal phase

Table 4-2. Blood pressure components and pulse wave analysis

	EF	LF	EL	LL
HR (bpm)	64.30±7.16	64.30±9.10	64.00±7.66	62.96±8.48
PSBP (mmHg)	110.91±7.76	105.61±6.53*	108.35±5.64	110.39±7.51$^{\#}$
PDBP (mmHg)	72.87±4.90	68.17±4.55*	69.52±5.55	71.69±6.39$^{\#}$
PPP (mmHg)	38.09±5.38	38.09±6.62	38.87±4.71	37.96±5.82
ASBP (mmHg)	98.48±6.81	93.61±5.43	96.70±6.33	96.52±6.21
ADBP (mmHg)	73.78±5.88	69.22±4.58*	70.57±5.62	71.39±5.78
APP (mmHg)	25.04±3.50	25.04±3.72	25.17±3.31	25.13±3.68
MAP (mmHg)	85.00±5.82	80.57±5.10*	82.57±5.50	84.43±6.39$^{\#}$
AgBP (mmHg)	3.48±2.27	1.57±2.15*	2.83±1.99	3.09±2.94$^{\#}$
AI (%)	14.04±8.75	6.96±8.75*	11.09±7.56	12.52±9.24$^{\#}$
AI @ HR = 75 BPM (%)	9.17±8.87	1.35±8.84*	5.21±7.77	7.04±11.00$^{\#}$
Δt_p (ms)	104.57±15.14	119.04±19.08*	116.61±20.81	107.74±10.28$^{\#}$
Wasted LV energy ($dynes/cm^2/sec$)	10.22±5.65	5.67±4.56*	8.13±6.11	9.91±6.99$^{\#}$

Values are mean ± SD; *P<0.05 vs. EF. $^{\#}$P<0.05 vs. LF. HR = heart rate; PSBP = peripheral systolic blood pressure; PDBP = peripheral diastolic blood pressure; PPP = peripheral pulse pressure; ASBP = aortic systolic blood pressure; ADBP = aortic diastolic blood pressure; APP = aortic pulse pressure; MAP = mean arterial blood pressure; AgBP = augmented blood pressure; AI = augmentation index; Δtp = round trip travel time of reflected pressure wave from ascending aorta to peripheral reflecting sites and back; LV = left ventricular.

Table 4-3. Pulse Wave Velocity

	EF	LF	EL	LL
CFPWV (m/sec)	6.48±0.58	6.12±0.58	6.37±0.49	6.35±0.45
CRPWV (m/sec)	9.52±1.08	8.87±1.10	9.17±1.21	9.30±1.21
FDPWV (m/sec)	8.67±1.47	8.19±1.38	8.54±1.28	8.59±1.24

Values are mean ± SD; CFPWV = carotid-femoral pulse wave velocity; CRPWV = carotid-radial pulse wave velocity; FDPWV = femoral-distal pulse wave velocity.

Table 4-4. Forearm and calf flow-mediated dilation

	EF	LF	EL	LL
Resting FBF	1.64±0.58	1.84±0.55	1.67±0.55	1.72±0.63
Peak FBF	21.58±4.37	25.86±5.91*	22.38±6.91	21.50±5.76#
Total FBF AUC_{3min}	6.12±1.96	7.56±2.98	6.40±2.07	6.13±1.87
Resting CBF	1.74±0.47	2.04±0.60	1.92±0.51	1.75±0.35
Peak CBF	27.32±4.25	28.98±5.42	26.97±5.63	26.21±4.29
Total CBF AUC_{3min}	7.26±1.30	8.87±2.55*	8.01±1.90	7.62±1.72#

Values are mean ± SD; *P<0.05 vs. EF. #P<0.05 vs. LF. Units are ml/min/100ml tissue; FBF = forearm blood flow; CBF = calf blood flow; AUC = area under flow x time curve.

Table 4-5. Brachial artery flow-mediated dilation

	EF	LF	EL	LL
Baseline diameter (mm)	4.46±0.29	4.64±0.29	4.51±0.33	4.53±0.37
Absolute dilation (mm)	0.16±0.07	0.23±0.11*	0.16±0.08†	0.17±0.11#
Brachial FMD (%)	3.35±1.88	5.12±2.36*	3.38±1.37†	3.71±2.31#
Resting SR	0.76±0.47	0.63±0.29	0.68±0.28	0.85±0.73
Peak SR	9.48±2.36	9.04±1.54	9.52±2.81	10.15±2.58
Normalized FMD (s^{-1})	0.44±0.29	0.64±0.28*	0.43±0.22†	0.44±0.23#

Values are mean ± SD; *P<0.05 vs. EF. † P<0.05 vs. LF. #P<0.05 vs. LF. FMD = flow-mediated dilation; SR = shear rate

Table 4-6. Menstrual cycle hormones and nitric oxide

	EF	LF	EL	LL
Estradiol (pg/ml)	78.85±0.30	158.62±0.59*	91.52±0.54†	68.32±0.35#
Progesterone (pg/ml)	14.46±9.01	17.42±13.40	18.93±13.36	11.70±6.41
NOx (μmol/L)	25.93±21.25	47.97±40.53*	26.10±21.86†	27.73±21.05#

Values are mean ± SD; *P<0.05 vs. EF. † P<0.05 vs. LF. #P<0.05 vs. LF. NOx = nitrite/nitrate

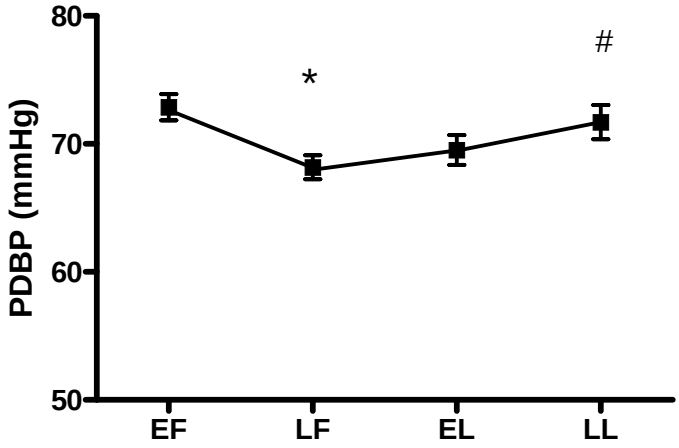

Figure 4-1. Peripheral systolic blood pressure (PSBP) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

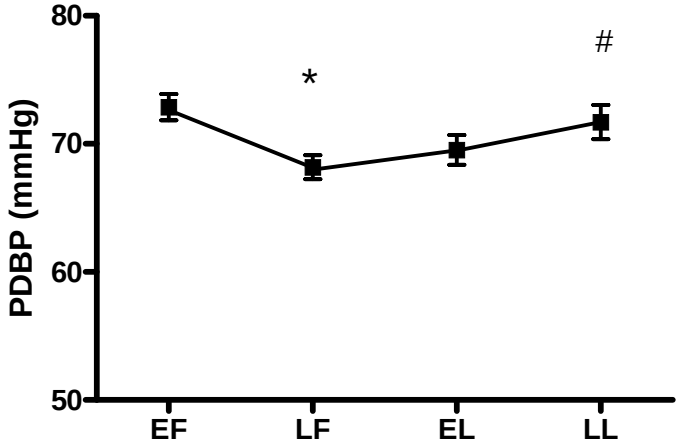

Figure 4-2. Peripheral diastolic blood pressure (PDBP)during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

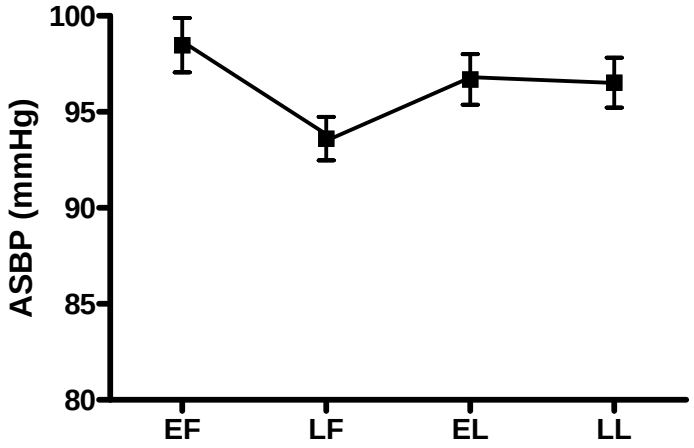

Figure 4-3. Aortic systolic blood pressure (ASBP) during the 4 phases of the menstrual cycle.

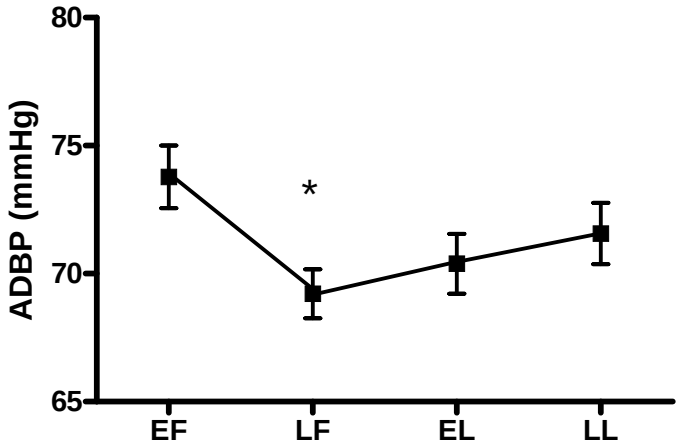

Figure 4-4. Aortic diastolic blood pressure (ADBP) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF.

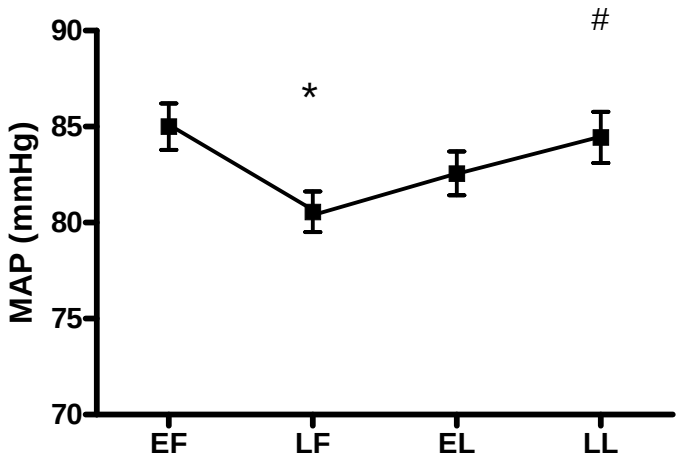

Figure 4-5. Mean arterial blood pressure (MAP) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

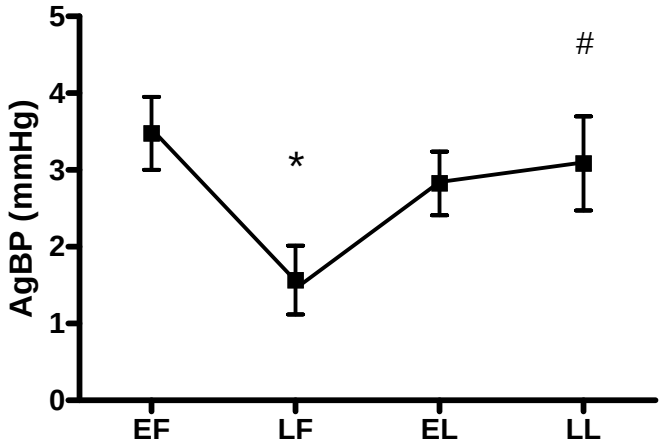

Figure 4-6. Augmented blood pressure (AgBP) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

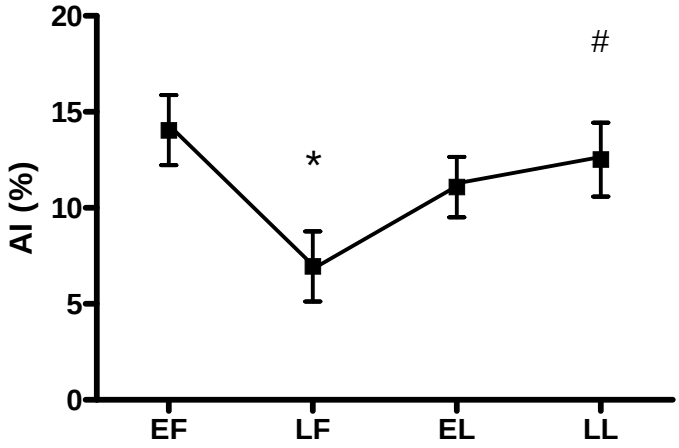

Figure 4-7. Aortic augmentation index (AI) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

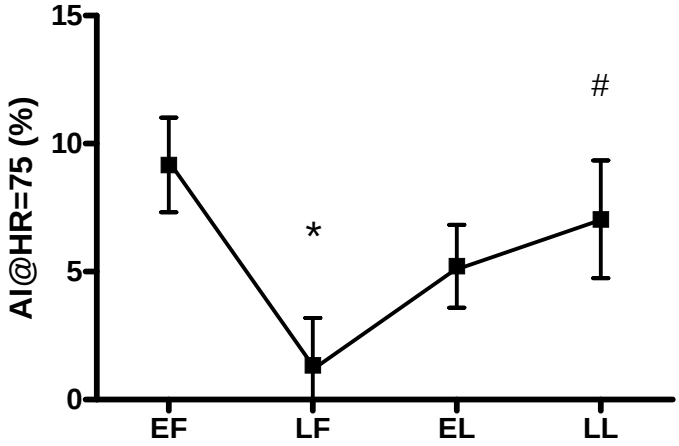

Figure 4-8. Aortic augmentation index normalized for heart rate at 75 bpm (AI@HR=75) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

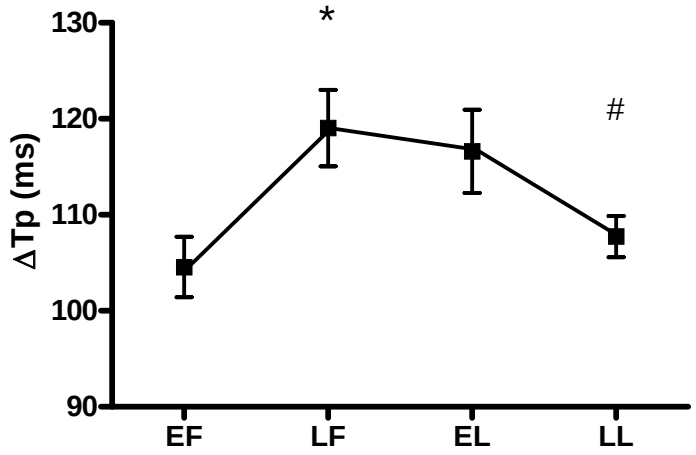

Figure 4-9. Roundtrip travel time of reflected wave (Δt_p) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

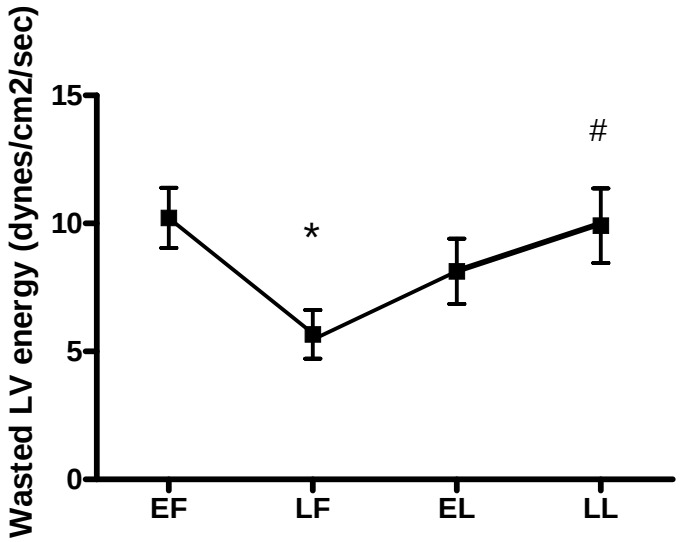

Figure 4-10. Wasted left ventricular (LV) energy during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

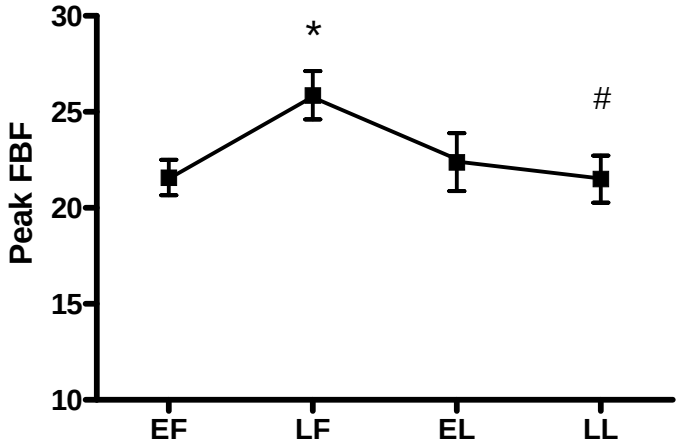

Figure 4-11. Peak forearm blood flow (FBF) during the 4 phases of the menstrual cycle. Units are ml/min/100ml tissue. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

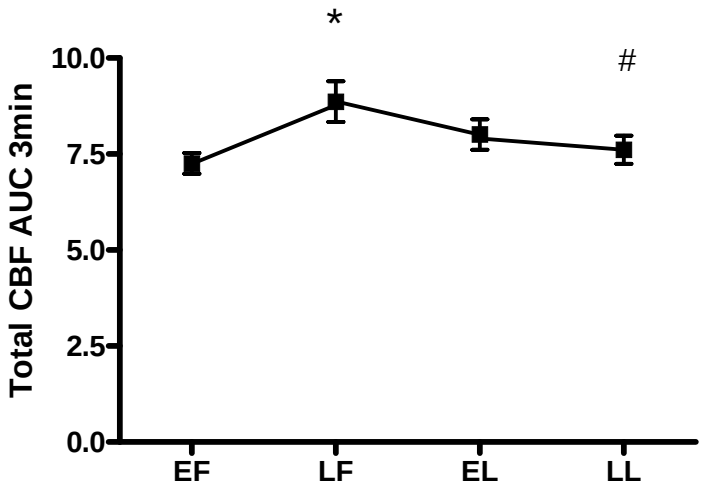

Figure 4-12. Total calf blood flow (CBF) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. #P<0.05 vs. LF.

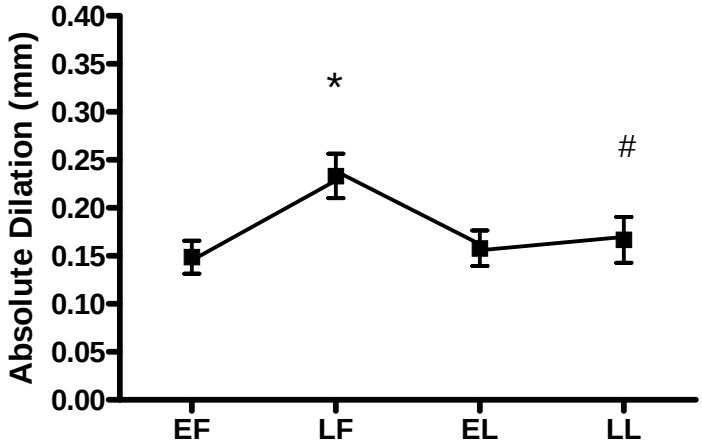

Figure 4-13. Absolute dilation of the brachial artery during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF. † P<0.05 vs. LF. #P<0.05 vs. LF.

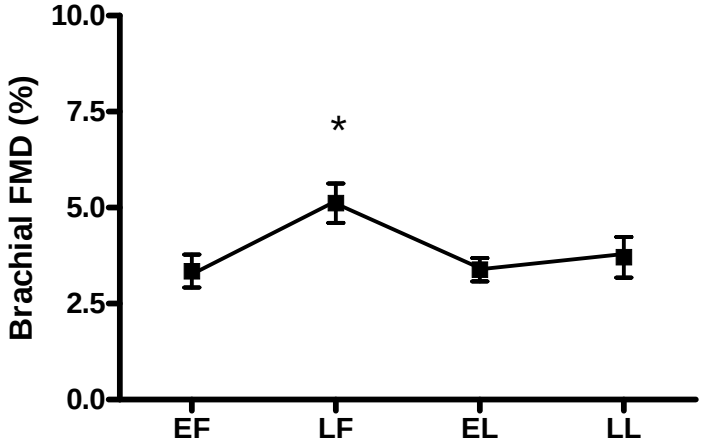

Figure 4-14. Brachial artery flow-mediated dilation (FMD) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF, EL, & LL.

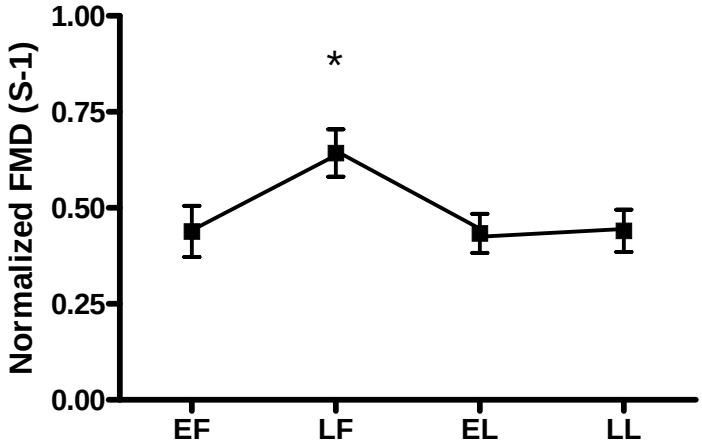

Figure 4-15. Normalized brachial artery flow-mediated dilation (FMD) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF, EL, & LL.

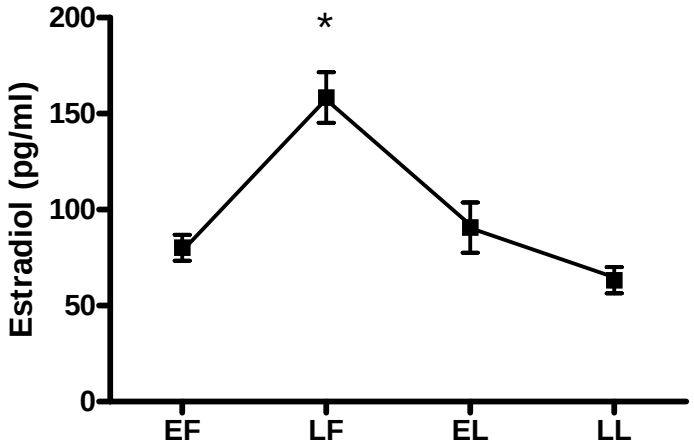

Figure 4-16. Estradiol during the 4 phases of the menstrual cycle.Data are mean ±SEM. *P<0.05 vs. EF, EL, & LL.

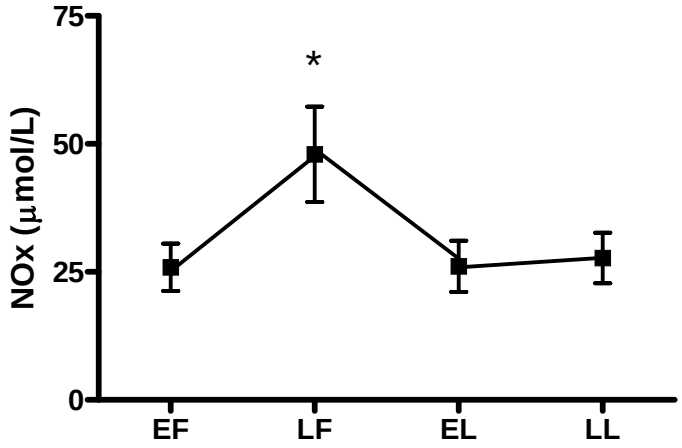

Figure 4-17. Nitrite/nitrate (NOx) during the 4 phases of the menstrual cycle. Data are mean ±SEM. *P<0.05 vs. EF, EL, & LL.

CHAPTER 5
DISCUSSION

The purpose of this study was to assess variability in arterial function during the female menstrual cycle. Secondarily, we sought to determine if investigators need to standardize, according to menstrual phase, laboratory vascular function testing in pre-menopausal females. Our data consistently show that the temporal pattern of vascular function was significantly different across the 4 phases of the menstrual cycle. Due to this variability, our data argue that vascular testing in pre-menopausal females must be standardized according to menstrual phase.

This is the first study to comprehensively assess arterial function, menstrual cycle hormones and vaso-active mechanisms throughout the menstrual cycle. The present study demonstrated that the entire vascular system was affected by the 4 temporal phases of the menstrual cycle. Vascular reactivity in central, peripheral and micro-vascular arteries increased toward the end of the follicular phase (Phase 2) and fell during the luteal phase (Phase 3-4). Changes in vascular reactivity perfectly mirrored the cyclic production of estrogen during the menstrual cycle. This was the first study to measure NO throughout the 4 phases of the menstrual cycle in the same cohort of women. Our data suggest that NO production may be a primary mechanism driving fluctuations in arterial reactivity. As shown in figure 4-17, plasma levels of NO fluctuated in parallel with estrogen by increasing by approximately 85% from the early to late follicular (Phase 2) and falling during the luteal phases (Phases 3 & 4). Both endothelial and vascular smooth muscle cells contain estrogen receptors.[48, 49, 55-57] Binding of estrogen to these receptors activates pathways that subsequently increase NO bio-availability.[48, 50, 53, 54] We assume that the increased production of estrogen during the late follicular phase results in a greater opportunity for estrogen + estrogen receptor binding, thereby causing an increase in NO bioavailability. NO is an important modulator of vascular tone[36] and vascular

reactivity[94, 103] and this estrogen-mediated increase in NO is likely the mechanism responsible for increased vascular reactivity during the late follicular phase.

This study demonstrated that during the late follicular phase of the menstrual cycle, females experience a dramatic improvement in peripheral endothelial function. This is evidenced by a 53% increase in brachial flow mediated dilation (FMD) between the early follicular (Phase 1) and the late follicular phase (Phase 2) (See Figure 4-14). Brachial FMD returned to baseline values during the luteal phases (Phases 3 & 4). Previous studies measuring brachial FMD have produced conflicting findings. Consistent with results of the present study, Williams *et at.*[77] and Hashimoto *et at.*[78] both reported an increase in brachial FMD from early to late follicular. However both studies also reported a second rise in brachial FMD during the luteal phase (Phase 4).[77, 78] The explanation for their disparate FMD results is unclear. We speculate that their luteal FMD data were collected too near the late follicular phase. Indeed, both studies reported estrogen levels during the luteal phase that were not significantly different from values recorded at the late follicular phase.[77, 78]

Endothelial function in resistance arteries also demonstrated a temporal pattern of improvement during the late follicular phase. Peak forearm blood flow (FBF), measured by venous occlusion plethysmography, increased 20% from early follicular (Phase 1) to late follicular (Phase 2) (See Figure 4-11) as well as total calf blood flow (CBF) (22%).

Central hemodynamics also showed significant improvement in the late follicular phase. Pulse wave analysis demonstrated an 85% reduction in aortic augmentation index, when corrected for a heart rate of 75 (AI@HR=75), and a 14% increase in round trip travel time of the reflected wave (Δt_p) from the early follicular (Phase 1) to the late follicular phase(Phase 2) (Figures 4-8, 4-9). Reduction in reflected pressure amplitude led to a 44% decrease in wasted

left ventricular (LV) energy (Figure 4-10). This reduction is mainly due to a decrease in augmented pressure (55%) (Figure 4-6). To date, one study has examined central pulse wave characteristics and reported no significant variations between menstrual phases.[82] This study measured subjects once during the early follicular phase (Phase 1) and once during the early luteal phase (Phase 3). Our findings also show no significant difference between early follicular and early luteal. We speculate that central hemodynamics data collected by Ounis-Skali *et al.*[82] was obtained too early in the follicular phase or too late in the luteal phase to see any associated vascular variations.

Our study showed no changes in central or peripheral arterial stiffness, when measured by pulse wave velocity (PWV). This is in agreement with all previous studies that have measured PWV throughout the menstrual cycle.[77, 81, 82]

Our study was the first to report significant change in systemic blood pressure across the 4 phases of the menstrual cycle. Peripheral systolic blood pressure (PSBP), peripheral diastolic blood pressure (PDBP), and aortic diastolic blood pressure dropped by approximately 4 mmHg from early follicular (Phase 1) to the late follicular (Phase 2) (See Figures 4-1, 4-2 & 4-4). Blood pressure began to increase at the onset of the luteal phase (Phase 3) and reached levels equal to early follicular by the end of the luteal phase (Phase 4). Our blood pressure data have clinical relevance and indicate that clinicians must take into account menstrual cycle phase in pre-menopausal females when measuring blood pressure and/or prescribing antihypertensive therapeutic strategies.

Cardiovascular disease (CVD) in pre-menopausal females is conspicuously less than in men of similar age[12]. Cyclical elevations in circulation estrogen are cited as one mechanism responsible for this phenomenon. Our data suggests that late follicular (Phase 2) spikes in NO

may confer some degree of cardiovascular 'protection' in females. Besides being an important regulator of vascular tone, NO inhibits leukocyte adhesion, platelet aggregation, expression of adhesion molecules, endothelin-1 (ET-1), vascular growth and inflammation.[104] A decrease in NO bio-availability is a direct indication of endothelial dysfunction and is an independent predictor of cardiac events.[41] The surge of NO experienced in the late follicular phase would occur approximately every 3 weeks throughout the menstrual years of a female and may provide a lasting protecting affect against CVD.

In conclusion, this study shows that central, peripheral and micro-vascular reactivity increases in the late follicular phase prior to ovulation and falls during the luteal phase. The mechanism responsible, in part, for improved phase 2 vascular reactivity is increased NO bioavailability, secondary to an estrogen mediated response. The data also highlights the importance of standardizing vascular testing in pre-menopausal females. Unless laboratory testing is standardized, the temporal pattern of vascular reactivity will introduce significant measurement variability in pre-menopausal females. Lastly, our data indicate that the temporal pattern of vascular function in pre-menopausal females should be taken into account when measuring blood pressure in the clinical setting.

LIST OF REFERENCES

1. Benjamin EJ, Larson MG, Keyes MJ, et al. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation.* Feb 10 2004;109(5):613-619.

2. Bulpitt CJ, Cameron JD, Rajkumar C, et al. The effect of age on vascular compliance in man: which are the appropriate measures? *J Hum Hypertens.* Nov 1999;13(11):753-758.

3. Resnick LM, Militianu D, Cunnings AJ, et al. Pulse waveform analysis of arterial compliance: relation to other techniques, age, and metabolic variables. *Am J Hypertens.* Dec 2000;13(12):1243-1249.

4. Weinberger MH, Fineberg NS, Fineberg SE. Effects of age, race, gender, blood pressure, and estrogen on arterial compliance. *Am J Hypertens.* Apr 2002;15(4 Pt 1):358-363.

5. Haluska BA, Jeffriess L, Downey M, et al. Influence of Cardiovascular Risk Factors on Total Arterial Compliance. *J Am Soc Echocardiogr.* Aug 7 2007.

6. Waddell TK, Dart AM, Gatzka CD, et al. Women exhibit a greater age-related increase in proximal aortic stiffness than men. *J Hypertens.* Dec 2001;19(12):2205-2212.

7. Karpanou EA, Vyssoulis GP, Papakyriakou SA, et al. Effects of menopause on aortic root function in hypertensive women. *J Am Coll Cardiol.* Nov 15 1996;28(6):1562-1566.

8. McEniery CM, Yasmin, Hall IR, et al. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol.* Nov 1 2005;46(9):1753-1760.

9. Van der Heijden-Spek JJ, Staessen JA, Fagard RH, et al. Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. *Hypertension.* Feb 2000;35(2):637-642.

10. Franklin SS, Gustin Wt, Wong ND, et al. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation.* Jul 1 1997;96(1):308-315.

11. Burt VL, Whelton P, Roccella EJ, et al. Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988-1991. *Hypertension.* Mar 1995;25(3):305-313.

12. Thom T, Haase N, Rosamond W, et al. Heart disease and stroke statistics--2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation.* Feb 14 2006;113(6):e85-151.

13. McGrath BP, Liang YL, Teede H, et al. Age-related deterioration in arterial structure and function in postmenopausal women: impact of hormone replacement therapy. *Arterioscler Thromb Vasc Biol.* Jul 1998;18(7):1149-1156.

14. Scuteri A, Lakatta EG, Bos AJ, et al. Effect of estrogen and progestin replacement on arterial stiffness indices in postmenopausal women. *Aging (Milano).* Apr 2001;13(2):122-130.

15. Miura S, Tanaka E, Mori A, et al. Hormone replacement therapy improves arterial stiffness in normotensive postmenopausal women. *Maturitas.* Aug 20 2003;45(4):293-298.

16. Reis SE, Gloth ST, Blumenthal RS, et al. Ethinyl estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. *Circulation.* Jan 1994;89(1):52-60.

17. Cagnacci A, Modena MG, Malmusi S, et al. Effect of prolonged administration of transdermal estradiol on flow-mediated endothelium-dependent and endothelium-independent vasodilation in healthy postmenopausal women. *Am J Cardiol.* Aug 1 1999;84(3):367-370, A369-310.

18. Teede HJ, Liang YL, Kotsopoulos D, et al. A placebo-controlled trial of long-term oral combined continuous hormone replacement therapy in postmenopausal women: effects on arterial compliance and endothelial function. *Clin Endocrinol (Oxf).* Nov 2001;55(5):673-682.

19. Sorensen KE, Dorup I, Hermann AP, et al. Combined hormone replacement therapy does not protect women against the age-related decline in endothelium-dependent vasomotor function. *Circulation.* Apr 7 1998;97(13):1234-1238.

20. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *Jama.* Aug 19 1998;280(7):605-613.

21. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *Jama.* Jul 17 2002;288(3):321-333.

22. Nichols W, O'Rourke M. *McDonalds's Blood Flow in Arteries: Theoretical, Experimental and Clinical Practices*. 5th ed. London: Arnold; 2005.

23. Blacher J, Asmar R, Djane S, et al. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension.* May 1999;33(5):1111-1117.

24. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol.* May 2005;25(5):932-943.

25. Laurent S, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension.* May 2001;37(5):1236-1241.

26. O'Rourke MF, Nichols WW. Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. *Hypertension.* Apr 2005;45(4):652-658.

27. Seals DR, Tanaka H, Clevenger CM, et al. Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic pressure: role of arterial stiffness. *J Am Coll Cardiol.* Aug 2001;38(2):506-513.

28. Bortolotto LA, Hanon O, Franconi G, et al. The aging process modifies the distensibility of elastic but not muscular arteries. *Hypertension.* Oct 1999;34(4 Pt 2):889-892.

29. Darne B, Girerd X, Safar M, et al. Pulsatile versus steady component of blood pressure: a cross-sectional analysis and a prospective analysis on cardiovascular mortality. *Hypertension.* Apr 1989;13(4):392-400.

30. Madhavan S, Ooi WL, Cohen H, et al. Relation of pulse pressure and blood pressure reduction to the incidence of myocardial infarction. *Hypertension.* Mar 1994;23(3):395-401.

31. Witteman JC, Grobbee DE, Hofman A. Relation between aortic atherosclerosis and blood pressure. *Lancet.* Jun 25 1994;343(8913):1649.

32. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J.* Nov 2006;27(21):2588-2605.

33. Wilkinson IB, Hall IR, MacCallum H, et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. *Arterioscler Thromb Vasc Biol.* Jan 2002;22(1):147-152.

34. Hunt BJ PL, Schachter M, and Halliday A. *An Introduction to Vascular Biology: From Science to Clinical Practice*: Cambridge University Press; 2002.

35. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med.* Dec 30 1993;329(27):2002-2012.

36. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet.* Oct 28 1989;2(8670):997-1000.

37. Vallance P, Chan N. Endothelial function and nitric oxide: clinical relevance. *Heart.* Mar 2001;85(3):342-350.

38. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* Nov 27 1980;288(5789):373-376.

39. Groves P, Kurz S, Just H, et al. Role of endogenous bradykinin in human coronary vasomotor control. *Circulation.* Dec 15 1995;92(12):3424-3430.

40. Drexler H. Endothelial dysfunction: clinical implications. *Prog Cardiovasc Dis.* Jan-Feb 1997;39(4):287-324.

41. Heitzer T, Schlinzig T, Krohn K, et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation.* Nov 27 2001;104(22):2673-2678.

42. Bradley DD, Wingerd J, Petitti DB, et al. Serum high-density-lipoprotein cholesterol in women using oral contraceptives, estrogens and progestins. *N Engl J Med.* Jul 6 1978;299(1):17-20.

43. Muesing RA, Forman MR, Graubard BI, et al. Cyclic changes in lipoprotein and apolipoprotein levels during the menstrual cycle in healthy premenopausal women on a controlled diet. *J Clin Endocrinol Metab.* Oct 1996;81(10):3599-3603.

44. Kim HJ, Kalkhoff RK. Changes in lipoprotein composition during the menstrual cycle. *Metabolism.* Jun 1979;28(6):663-668.

45. Jones DY, Judd JT, Taylor PR, et al. Menstrual cycle effect on plasma lipids. *Metabolism.* Jan 1988;37(1):1-2.

46. Castelli WP, Garrison RJ, Wilson PW, et al. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *Jama.* Nov 28 1986;256(20):2835-2838.

47. Mendelsohn ME, Karas RH. Estrogen and the blood vessel wall. *Curr Opin Cardiol.* Sep 1994;9(5):619-626.

48. Caulin-Glaser T, Garcia-Cardena G, Sarrel P, et al. 17 beta-estradiol regulation of human endothelial cell basal nitric oxide release, independent of cytosolic Ca2+ mobilization. *Circ Res.* Nov 1997;81(5):885-892.

49. Venkov CD, Rankin AB, Vaughan DE. Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function. *Circulation.* Aug 15 1996;94(4):727-733.

50. Chen Z, Yuhanna IS, Galcheva-Gargova Z, et al. Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest.* Feb 1999;103(3):401-406.

51. Chambliss KL, Yuhanna IS, Mineo C, et al. Estrogen receptor alpha and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. *Circ Res.* Nov 24 2000;87(11):E44-52.

52. Traupe T, Stettler CD, Li H, et al. Distinct roles of estrogen receptors alpha and beta mediating acute vasodilation of epicardial coronary arteries. *Hypertension.* Jun 2007;49(6):1364-1370.

53. Kleinert H, Wallerath T, Euchenhofer C, et al. Estrogens increase transcription of the human endothelial NO synthase gene: analysis of the transcription factors involved. *Hypertension.* Feb 1998;31(2):582-588.

54. Hayashi T, Yamada K, Esaki T, et al. Estrogen increases endothelial nitric oxide by a receptor-mediated system. *Biochem Biophys Res Commun.* Sep 25 1995;214(3):847-855.

55. Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. *Circulation.* May 1994;89(5):1943-1950.

56. Hodges YK, Tung L, Yan XD, et al. Estrogen receptors alpha and beta: prevalence of estrogen receptor beta mRNA in human vascular smooth muscle and transcriptional effects. *Circulation.* Apr 18 2000;101(15):1792-1798.

57. Register TC, Adams MR. Coronary artery and cultured aortic smooth muscle cells express mRNA for both the classical estrogen receptor and the newly described estrogen receptor beta. *J Steroid Biochem Mol Biol.* Feb 1998;64(3-4):187-191.

58. Han SZ, Karaki H, Ouchi Y, et al. 17 beta-Estradiol inhibits Ca2+ influx and Ca2+ release induced by thromboxane A2 in porcine coronary artery. *Circulation.* May 15 1995;91(10):2619-2626.

59. Stice SL, Ford SP, Rosazza JP, et al. Role of 4-hydroxylated estradiol in reducing Ca2+ uptake of uterine arterial smooth muscle cells through potential-sensitive channels. *Biol Reprod.* Mar 1987;36(2):361-368.

60. Prakash YS, Togaibayeva AA, Kannan MS, et al. Estrogen increases Ca2+ efflux from female porcine coronary arterial smooth muscle. *Am J Physiol.* Mar 1999;276(3 Pt 2):H926-934.

61. White RE, Darkow DJ, Lang JL. Estrogen relaxes coronary arteries by opening BKCa channels through a cGMP-dependent mechanism. *Circ Res.* Nov 1995;77(5):936-942.

62. Wenger NK, Speroff L, Packard B. Cardiovascular health and disease in women. *N Engl J Med.* Jul 22 1993;329(4):247-256.

63. Nabulsi AA, Folsom AR, White A, et al. Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. The Atherosclerosis Risk in Communities Study Investigators. *N Engl J Med.* Apr 15 1993;328(15):1069-1075.

64. Honisett SY, Pang B, Stojanovska L, et al. Progesterone does not influence vascular function in postmenopausal women. *J Hypertens.* Jun 2003;21(6):1145-1149.

65. Walsh BW, Schiff I, Rosner B, et al. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med.* Oct 24 1991;325(17):1196-1204.

66. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial. *Jama.* Jan 18 1995;273(3):199-208.

67. Tikkanen MJ, Nikkila EA, Kuusi T, et al. High density lipoprotein-2 and hepatic lipase: reciprocal changes produced by estrogen and norgestrel. *J Clin Endocrinol Metab.* Jun 1982;54(6):1113-1117.

68. Silfverstolpe G, Gustafson A, Samsioe G, et al. Lipid metabolic studies in oophorectomised women: effects on serum lipids and lipoproteins of three synthetic progestogens. *Maturitas.* Aug 1982;4(2):103-111.

69. Weinstein L. Efficacy of a continuous estrogen-progestin regimen in the menopausal patient. *Obstet Gynecol.* Jun 1987;69(6):929-932.

70. Lee WS, Harder JA, Yoshizumi M, et al. Progesterone inhibits arterial smooth muscle cell proliferation. *Nat Med.* Sep 1997;3(9):1005-1008.

71. Vazquez F, Rodriguez-Manzaneque JC, Lydon JP, et al. Progesterone regulates proliferation of endothelial cells. *J Biol Chem.* Jan 22 1999;274(4):2185-2192.

72. Nakamura Y, Suzuki T, Inoue T, et al. Progesterone receptor subtypes in vascular smooth muscle cells of human aorta. *Endocr J.* Apr 2005;52(2):245-252.

73. Kraus WL, Weis KE, Katzenellenbogen BS. Inhibitory cross-talk between steroid hormone receptors: differential targeting of estrogen receptor in the repression of its transcriptional activity by agonist- and antagonist-occupied progestin receptors. *Mol Cell Biol.* Apr 1995;15(4):1847-1857.

74. Nickenig G, Strehlow K, Wassmann S, et al. Differential effects of estrogen and progesterone on AT(1) receptor gene expression in vascular smooth muscle cells. *Circulation.* Oct 10 2000;102(15):1828-1833.

75. Griendling KK, Murphy TJ, Alexander RW. Molecular biology of the renin-angiotensin system. *Circulation.* Jun 1993;87(6):1816-1828.

76. Ichiki T, Usui M, Kato M, et al. Downregulation of angiotensin II type 1 receptor gene transcription by nitric oxide. *Hypertension.* Jan 1998;31(1 Pt 2):342-348.

77. Williams MR, Westerman RA, Kingwell BA, et al. Variations in endothelial function and arterial compliance during the menstrual cycle. *J Clin Endocrinol Metab.* Nov 2001;86(11):5389-5395.

78. Hashimoto M, Akishita M, Eto M, et al. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation.* Dec 15 1995;92(12):3431-3435.

79. Liu Z, Brin KP, Yin FC. Estimation of total arterial compliance: an improved method and evaluation of current methods. *Am J Physiol.* Sep 1986;251(3 Pt 2):H588-600.

80. Giannattasio C, Failla M, Grappiolo A, et al. Fluctuations of radial artery distensibility throughout the menstrual cycle. *Arterioscler Thromb Vasc Biol.* Aug 1999;19(8):1925-1929.

81. Hayashi K, Miyachi M, Seno N, et al. Variations in carotid arterial compliance during the menstrual cycle in young women. *Exp Physiol.* Mar 2006;91(2):465-472.

82. Ounis-Skali N, Mitchell GF, Solomon CG, et al. Changes in central arterial pressure waveforms during the normal menstrual cycle. *J Investig Med.* Sep 2006;54(6):321-326.

83. Willekes C, Hoogland HJ, Keizer HA, et al. Female sex hormones do not influence arterial wall properties during the normal menstrual cycle. *Clin Sci (Lond).* May 1997;92(5):487-491.

84. Sharman JE, Lim R, Qasem AM, et al. Validation of a generalized transfer function to noninvasively derive central blood pressure during exercise. *Hypertension.* Jun 2006;47(6):1203-1208.

85. Chen CH, Nevo E, Fetics B, et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation.* Apr 1 1997;95(7):1827-1836.

86. Siebenhofer A, Kemp C, Sutton A, et al. The reproducibility of central aortic blood pressure measurements in healthy subjects using applanation tonometry and sphygmocardiography. *J Hum Hypertens.* Sep 1999;13(9):625-629.

87. Wilkinson IB, Fuchs SA, Jansen IM, et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *J Hypertens.* Dec 1998;16(12 Pt 2):2079-2084.

88. Casey DP PG, Nichols WW, and Braith RW. Measurement of Pulse Wave Velocity and Augmentation Index is Reproducible in Young, Healthy Men. *Med Sci Sport Exer.* 2006;38(5):S185.

89. Nichols WW, Singh BM. Augmentation index as a measure of peripheral vascular disease state. *Curr Opin Cardiol.* Sep 2002;17(5):543-551.

90. Murgo JP, Westerhof N, Giolma JP, et al. Aortic input impedance in normal man: relationship to pressure wave forms. *Circulation.* Jul 1980;62(1):105-116.

91. Mitchell GF, Izzo JL, Jr., Lacourciere Y, et al. Omapatrilat reduces pulse pressure and proximal aortic stiffness in patients with systolic hypertension: results of the conduit hemodynamics of omapatrilat international research study. *Circulation.* Jun 25 2002;105(25):2955-2961.

92. Doshi SN, Naka KK, Payne N, et al. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci (Lond).* Dec 2001;101(6):629-635.

93. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol.* Oct 15 2005;568(Pt 2):357-369.

94. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* Jan 16 2002;39(2):257-265.

95. Eskurza I, Monahan KD, Robinson JA, et al. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. *J Physiol.* Apr 1 2004;556(Pt 1):315-324.

96. Hokanson DE, Sumner DS, Strandness DE, Jr. An electrically calibrated plethysmograph for direct measurement of limb blood flow. *IEEE Trans Biomed Eng.* Jan 1975;22(1):25-29.

97. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol.* Dec 2001;52(6):631-646.

98. Greenfield AD, Whitney RJ, Mowbray JF. Methods for the investigation of peripheral blood flow. *Br Med Bull.* May 1963;19:101-109.

99. Matthews JN, Altman DG, Campbell MJ, et al. Analysis of serial measurements in medical research. *Bmj.* Jan 27 1990;300(6719):230-235.

100. Higashi Y, Sasaki S, Nakagawa K, et al. A noninvasive measurement of reactive hyperemia that can be used to assess resistance artery endothelial function in humans. *Am J Cardiol.* Jan 1 2001;87(1):121-125, A129.

101. Pierce G. Reproducibility of forearm and calf blood flow during reactive hyperemia in young, healthy subjects. *Med Sci Sport Exer.* 2004;36:abstract.

102. Pannala AS, Mani AR, Spencer JP, et al. The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. *Free Radic Biol Med.* Mar 1 2003;34(5):576-584.

103. Lieberman EH, Gerhard MD, Uehata A, et al. Flow-induced vasodilation of the human brachial artery is impaired in patients <40 years of age with coronary artery disease. *Am J Cardiol.* Dec 1 1996;78(11):1210-1214.

104. Hornig B, Drexler H. Reversal of endothelial dysfunction in humans. *Coron Artery Dis.* Sep 2001;12(6):463-473.

BIOGRAPHICAL SKETCH

Eric Josiah Adkisson completed his undergraduate degree in exercise physiology at the University of Florida (UF) in August 2006. Immediately afterward he began pursuing a Master of Science degree in applied physiology and kinesiology under Dr. Randy Braith. While at UF, he served as a graduate assistant in the Alan C. Moore Sport and Fitness department, teaching undergraduate conditioning, jogging, weight training, and backpacking. Eric has been selected for a position at the Marine Corp Officer Candidate School (OCS) in Quantico, VA.