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Vascular Function in Trained Females

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ABSTRACT

Cardiovascular disease (CVD) is a leading cause of death in women. Early stages of subclinical atherosclerosis cause functional changes to the vasculature manifesting as endothelial dysfunction. These early functional changes give way to structural alterations to the vessel wall that can lead to increased arterial stiffness and irreversible vascular remodeling, (i.e. altered lumen diameter and increased intima media wall thickness (IMT)) which can ultimately lead to a cardiac event. Chronic exercise combined with energy deficiency may lead to a lack of menses or amenorrhea in premenopausal women. This state is associated with endothelial dysfunction and is believed to be attributable to hypoestrogenic mechanisms. Whether there are further detrimental structural changes to the artery in exercise-trained amenorrheic women remains unknown. **PURPOSE:** The primary purpose of this study was to examine indices of endothelial function, arterial stiffness and vascular remodeling in exercise-trained amenorrheic women to gain insight into the extent and severity of subclinical atherosclerotic CVD risk in this population. **HYPOTHESES:** It was hypothesized that, exercise-trained amenorrheic women would have lower endothelial function, higher arterial stiffness, and unfavorable vascular remodeling (greater femoral IMT and smaller femoral artery diameter) compared with exercise-trained eumenorrheic women and similar levels of endothelial dysfunction, arterial stiffness, and vascular remodeling compared to sedentary eumenorrheic women. **METHODS:** Forty-three women participated in this study: 10 exercise-trained amenorrheic women (Age 21 ± 3 years, Body Mass Index (BMI) 21.5 ± 2.0 kg/m^2), 18 exercise-trained eumenorrheic women (Age 22 ± 3 years, BMI 22.9 ± 2.4 kg/m^2), and 15 sedentary eumenorrheic women (Age 23 ± 4 years, BMI 23.9 ± 3.2 kg/m^2). Flow mediated dilation (FMD) of the brachial artery was used to measure endothelial function. Carotid-femoral Pulse Wave Velocity (PWV) was used to measure arterial

stiffness. Diameters and intima media thickness (IMT) of the superficial femoral artery (SFA) were taken as measures of vascular remodeling, and acquired using ultrasonography. Blood lipids, hematocrit, and hemoglobin were measured using a finger stick. A saliva collection was used to measure estradiol. Air displacement plethysmography was used to measure body fat percentage. Maximal oxygen uptake was used to measure maximal aerobic capacity and a 3-day diet record was administered to measure dietary intake. **RESULTS:** FMD in exercise-trained amenorrheic women was not significantly different from exercise-trained and sedentary eumenorrheic women (Amenorrheic: 7.15 ± 4.21 mm vs. Exercise-trained Eumenorrheic: 11.10 ± 4.29 mm vs. Sedentary: 0.25 ± 3.86 mm, $P = 0.07$). There were significant differences between groups in the reactive hyperemia shear rate stimulus (i.e. Area Under the Curve (AUC)), (Amenorrheic: 808 ± 350.1 aU vs. Exercise-trained Eumenorrheic: 862 ± 311.0 aU vs. Sedentary: 1096 ± 311.3 aU, $P = 0.05$). When FMD was normalized for the shear rate stimulus exercise-trained amenorrheic women had a significantly lower FMD response compared with eumenorrheic women (Amenorrheic: 6.91 ± 1.33mm vs. Exercise-trained Eumenorrheic: 10.97 ± 0.98mm vs. Sedentary: 10.57 ± 1.11mm, $P = 0.05$). Aortic PWV in the exercise-trained amenorrheic group was not significantly different from the eumenorrheic groups (Amenorrheic: 5.00 ± 1.00 m/s vs. Exercise-trained Eumenorrheic: 4.60 ± 0.50 m/s vs. Sedentary: 5.40 ± 0.80 m/s, $P = 0.03$). SFA diameters were significantly higher in the exercise-trained groups compared with the sedentary eumenorrheic group (Amenorrheic: 5.70 ± 0.72 mm vs. Exercise-trained Eumenorrheic: 5.68 ± 0.70 mm vs. Sedentary: 5.10 ± 0.63 mm, $P = 0.03$) while IMT was significantly lower in the exercise-trained amenorrheic women compared with the sedentary eumenorrheic women (Amenorrheic: 0.31 ± 0.03 mm vs. Exercise-trained Eumenorrheic: 0.35 ± 0.06 mm vs. Sedentary: 0.38 ± 0.07 mm, $P = 0.01$). 17β-Estradiol levels in exercise-trained

amenorrheic women were similar to levels in eumenorrheic women (Amenorrheic: 1.66 ± 0.84 pg/ml vs. Exercise-trained Eumenorrheic: 2.22 ± 1.17 pg/ml vs. Sedentary: 2.00 ± 0.81 pg/ml, $P = 0.34$). Blood lipid and hematological measures, and 3-day dietary record of nutrients, vitamins and minerals were not significantly different between groups ($P > 0.05$). **CONCLUSIONS:** Exercise-trained amenorrheic women had endothelial dysfunction, but did not have increased arterial stiffness or adverse vascular remodeling when compared to their eumenorrheic counterparts. This study suggests that vascular dysfunction does not extend beyond the endothelium, as arterial stiffness and vascular remodeling are not affected by the amenorrheic state.

VASCULAR FUNCTION IN TRAINED FEMALES

by

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Glossary of Abbreviations:

CVD = Cardiovascular Disease

FMD = Flow-Mediated Dilation

PWV = Pulse-Wave Velocity

SFA = Superficial Femoral Artery

IMT = Intima Media Thickness

NO = Nitric Oxide

eNOS = Endothelial Nitric Oxide Synthase

Synopsis of the Current Study

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality among women in the United States [1]. The prevalence of CVD increases as women age and enter menopause [2]. Atherosclerotic CVD results in changes to the vasculature: endothelial dysfunction, arterial stiffness, and vascular remodeling. Each of these changes is associated with future cardiovascular events, such as myocardial infarction and stroke [3, 4]. Aerobic exercise reduces the risk for CVD [5-7] possibly by improving endothelial function and arterial stiffness while preventing negative vascular remodeling [8]. Chronic aerobic exercise is cardio-protective but when coupled with energy deficiency can be detrimental and lead to exercise-associated amenorrhea or lack of a menstrual cycle in exercise-trained women. Previous studies show that exercise-trained amenorrheic women have lower estrogen concentrations, which contribute to endothelial dysfunction [9-12]. Endothelial dysfunction in exercise-trained amenorrheic women is similar to that observed in postmenopausal women [9-11], which is concerning as the prevalence of CVD is higher in postmenopausal women compared to premenopausal women and men [13]. Since endothelial dysfunction is considered to be an essential first step in the development of atherosclerosis, short or long-term impairment of endothelial function in exercise-trained amenorrheic women might predispose them to further vascular dysfunction, detrimental vascular structural changes and ultimately increased cardiovascular risk. However, few studies have examined the magnitude of subclinical atherosclerotic CVD (i.e. vascular dysfunction beyond the endothelium) in this population.

Chapter I: Introduction

Endothelial dysfunction is proposed as the first step in the atherosclerotic CVD process [15]. The endothelium is a single cell layer responsible for the vasodilator capacity of the artery. Nitric oxide (NO), a potent vasodilator, is produced by endothelial nitric oxide synthase (eNOS) in response to shear stress on the blood vessel wall. Endothelial dysfunction is mainly due to reductions in NO bioavailability [14, 15]. It is well known that chronic endothelial dysfunction can lead to increased arterial stiffness and vascular remodeling [14, 15]. Increased arterial stiffness reduces the vasodilator capacity of the artery and increases blood flow pulsatility [16]. Vascular remodeling is a dynamic structural process that occurs due to changes in hemodynamic stimuli such as increased pulsatile blood pressure or increased blood flow. Chronic endothelial dysfunction and increases in arterial stiffness can aggravate these hemodynamic stimuli and lead to vascular damage and further remodeling (i.e. increased lumen diameter and wall thickness) [17]. Vascular remodeling is marked by structural arterial changes that may be irreversible, ultimately leading to a cardiovascular event (i.e. stroke, myocardial infarction) [22]. Thus, interventions that improve endothelial function and arterial stiffness may reduce the progression of atherosclerotic CVD and subsequent risk for a cardiovascular event [18, 19].

Moderate intensity aerobic exercise may reduce the risk for CVD by improving endothelial function, arterial stiffness and vascular remodeling (i.e. increased lumen diameter and decreased Intima media thickness (IMT)) [5-7, 20-26]. Vascular improvements with exercise training are at least partly due to periodic increases in wall shear stress [7, 27-29] and are mediated by NO [21, 22, 30-33]. However, the positive effects of aerobic exercise on endothelial function and arterial stiffness are intensity dependent. Chronic high intensity exercise can actually negatively impact endothelial function and lead to increased arterial stiffness [34-37].

Chronic high-intensity exercise training in women increases risk for the development of The Female Athlete Triad. Clinical manifestations of this triad include: low energy availability, amenorrhea and osteoporosis [38, 39]. The triad is currently a prevalent medical concern among competitive female athletes [38]. In fact, about 65% of long distance runners and dancers will develop components of The Female Athlete Triad [40]. The occurrence of the triad is higher amongst athletes who participate in sports that require a lower body mass for athletic success, such as gymnastics, running and/or dancing [41]. Of the triad components, amenorrhea (considered an estrogen deficient state) may have the greatest impact on the development of CVD.

Exercise-trained amenorrheic women might be at premature risk for CVD since endothelial dysfunction is observed in these women. Exercise-trained amenorrheic women have lower estrogen concentrations, which have been shown to contribute to endothelial dysfunction in this population [9-12]. It is currently unknown whether vascular dysfunction in premenopausal exercise-trained amenorrheic women extends beyond the endothelium (i.e. increased arterial stiffness, increased IMT, increased lumen diameter). Therefore the severity of subclinical atherosclerosis in this population is undetermined.

This study will explore whether there may be more progressive/advanced vascular dysfunction in exercise-trained amenorrheic women, beyond endothelial dysfunction, manifesting as increases in arterial stiffness and adverse vascular remodeling. Women that engage in high intensity exercise (to the point of losing their menstrual cycle) may have premature vascular dysfunction (i.e. endothelial dysfunction, increased arterial stiffness and adverse vascular remodeling) increasing subclinical atherosclerotic burden and thus future risk for CVD.

Specific Aims and Expected Outcomes of This Thesis

Specific Aim 1: To examine endothelial dysfunction in exercise-trained amenorrheic women.

- *Sub-Aim 1:* To determine whether endothelial dysfunction is related to lower salivary estrogen concentrations in exercise-trained amenorrheics.

Expected Outcome 1: Exercise-trained amenorrheic women will have lower endothelial function compared with exercise-trained eumenorrheic women and similar endothelial function compared to sedentary eumenorrheic women. Lower estrogen concentrations in exercise-trained amenorrheic women will be associated with lower endothelial function.

Specific Aim 2: To examine arterial stiffness in exercise-trained amenorrheic women.

- *Sub-Aim 2:* To determine whether increased arterial stiffness is related to lower salivary estrogen concentrations in exercise-trained amenorrheics.

Expected Outcome 2: Exercise-trained amenorrheic women will have higher arterial stiffness compared with exercise-trained eumenorrheic women and similar arterial stiffness compared to sedentary eumenorrheic women. Lower estrogen concentrations in exercise-trained amenorrheic women will be associated with higher arterial stiffness.

Specific Aim 3: To examine vascular remodeling in exercise-trained amenorrheic women.

- *Sub Aim 3:* To determine whether vascular remodeling is related to lower salivary estrogen concentrations in exercise-trained amenorrheics.

Expected Outcome 3: Exercise-trained amenorrheic women will have smaller artery diameter and larger IMT, compared with exercise-trained eumenorrheics suggesting unfavorable vascular remodeling in this cohort. Lower estrogen concentrations in exercise-trained amenorrheic women will be associated with larger IMT and smaller superficial femoral diameter (SFA) diameter.

Chapter II: Literature Review

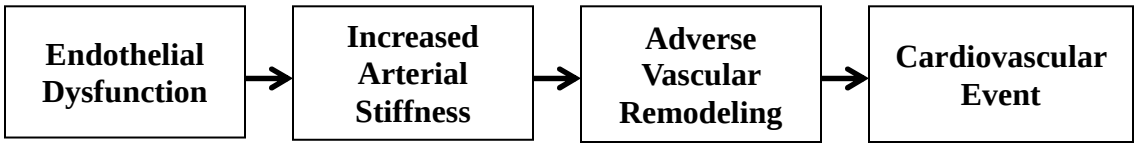
The following literature review will introduce the concepts of: 1) endothelial dysfunction, arterial stiffness and vascular remodeling; 2) the effect of exercise training on vascular function; 3) the effect of estrogen on vascular function; and 4) endothelial dysfunction in exercise-trained amenorrheic women. This review provides the rationale for a study to examine subclinical atherosclerosis in exercise-trained amenorrheics to gain insight into the extent and the severity of subclinical atherosclerosis in this population.

Progression of Subclinical Atherosclerosis

Cardiovascular disease (CVD) is a leading cause of death among women in the United States [1]. Atherosclerotic CVD is a multistage process characterized by structural and functional changes to the artery. Initial functional changes to the endothelium result in increased arterial stiffness and unfavorable vascular remodeling (*Refer to Figure 1*). With increasing age and reductions in estrogen concentrations after menopause, the risk for CVD becomes higher in women than in men [2]. This disparity in risk for CVD may be partly attributable to sex differences in arterial function and structure [42-45].

Endothelial dysfunction is the first detectable event in the atherosclerotic process of CVD [46]. Endothelial dysfunction is largely due to reductions in nitric oxide (NO) bioavailability [47, 48]. NO, a potent vasodilator is produced by the enzyme endothelial nitric oxide synthase (eNOS). As blood flows through an artery it comes in contact with the arterial wall or the endothelium (inner lining of the blood vessel) and creates shear stress. The dragging force stimulates the phosphorylation of eNOS and subsequently, NO production. If left undetected, endothelial dysfunction can lead to detrimental structural changes to the vessel wall. The stiffness of the artery is influenced both by vascular function (i.e. NO and endothelial function)

and vascular structure (i.e. elastin and collagen composition). Increased arterial stiffness decreases the ability of the artery to vasodilate and increases blood flow pulsatility [16]. This is important because chronic pulsatile blood flow can lead to additional vascular endothelial damage [14] and adverse vascular remodeling [15]. Vascular remodeling is an active process that occurs due to changes in hemodynamic conditions, such as increased blood pressure and/or increased pulsatile blood flow [49, 50]. Remodeling is characterized by changes in arterial lumen diameter and clinically relevant changes in wall thickness or intima media thickness (IMT) aimed at maintaining a stable hemodynamic environment and vessel wall tension [49, 50]. The law of LaPlace defines vessel wall tension as (pressure x radius)/ 2 x wall thickness. Increases in hemodynamic forces on the artery wall increase wall tension [49, 50]. Therefore, in order to restore wall tension, the artery will remodel. For example, hypertension is associated with structural changes manifested as a reduction in lumen diameter and/or increase in IMT [51]. These changes maintain optimal wall tension [51]. If undetected, chronic endothelial dysfunction and increased arterial stiffness can lead to permanent structural vascular remodeling and subsequent cardiovascular events (*See Figure 1*).



<u>Figure 1:</u> Chronic endothelial dysfunction can lead to increased arterial stiffness, adverse vascular remodeling and ultimately, risk for a cardiovascular event.

Aerobic Exercise Training and Subclinical Atherosclerosis

Aerobic exercise improves endothelial function [52] as exercise transiently increases blood flow, shear stress and ultimately NO bioavailability [6, 22-25, 28-33, 53-56]. Indeed the

shear stress stimulus is necessary for favorable vascular improvements seen with exercise on the endothelium; experimental abolishment of shear with exercise prevents improvements in endothelial function [20, 21, 27]. In addition to endothelial function, aerobic exercise can lead to improvements/reductions in arterial stiffness [37, 57-61]. Aerobic exercise improves arterial stiffness irrespective of training modality (i.e. walking, swimming, running, cycling) [70, 72, 73]. Interestingly, the benefits of exercise on arterial stiffness might be mediated by NO release. It has been shown that after long-term exercise training, plasma NO concentrations are inversely associated with arterial stiffness [62]. Similarly, animal studies support a NO mediated mechanism for improvements in arterial stiffness with aerobic exercise. In a study examining arterial stiffness in rats, aortic stiffness was lower in exercise-trained rats compared to sedentary rats [63]. A microarray DNA technique determined that aerobic exercise training up-regulated eNOS, and subsequently NO production and this was associated with favorable changes in arterial stiffness [63]. These findings suggest that long term exercise training leads to increased NO release and subsequent improvements in central arterial stiffness [57].

In addition to improvements in endothelial function and arterial stiffness, exercise training is believed to lead to favorable vascular remodeling marked by a larger lumen diameter and smaller IMT. Physical activity levels are inversely associated with IMT, and positively associated with lumen diameter [49, 50, 64-66]. Exercise-trained individuals have substantial arterial structural remodeling [49, 67, 68] as a result of localized or systemic vascular stimuli. In a cross-sectional study it was reported that predominantly lower-limb exercise training leads to a larger diameter of the SFA, while predominantly upper-limb exercise training leads to a larger diameter of the brachial artery [65]. In exercise-trained individuals, the IMT is systematically smaller compared to sedentary individuals [64]. Regular aerobic/endurance exercise results in

regularly sustained elevations in blood flow [34]. Increases in blood flow increase shear stress and vascular NO release, which have been shown to contribute to reductions in IMT and increases in arterial diameters [34]. Vascular remodeling stemming from exercise training might be NO mediated and occur to maintain appropriate levels of shear stress and minimize wall strain that accompanies exercise training [61].

However, the positive effects of aerobic exercise on endothelial function, arterial stiffness and vascular remodeling might be intensity dependent. Previous studies have found that endothelial dysfunction occurs following long distance, repetitive, high intensity exercise sessions such as running a marathon [35, 36, 69]. Also, resting arterial stiffness is elevated in marathon and ultra-marathon runners compared to their sedentary counterparts [61]. Similar mechanisms are proposed for endothelial dysfunction and increased arterial stiffness due to high intensity exercise training: both excessively high shear stress and high levels of oxidative stress that occur with chronic high intensity exercise training reduces NO bioavailability [69-72]. This is supported by previous studies noting similar IMT between exercise-trained individuals and their sedentary counterparts [73, 74]. This suggests that chronic high intensity exercise leads not only to endothelial dysfunction and increased stiffness but might also lead to adverse vascular remodeling.

Exercise-Associated Amenorrhea and Vascular Function

In women, chronic high intensity exercise, when combined with energy deficiency, can lead to exercise-associated amenorrhea. Due to a hypoestrogenic state, exercise-trained amenorrheic women lack a menstrual cycle as occurs in post-menopausal women. As such, it has been demonstrated that exercise-trained amenorrheic women have comparable levels of vascular dysfunction to that witnessed in post-menopausal women [9-11, 71]. Recent studies show that

exercise-trained amenorrheic women have lower endothelial function compared with exercise-trained eumenorrheics (i.e. regular menstruating women) [9-11, 71]. In these studies, endothelial dysfunction observed in exercise-trained amenorrheic women was directly associated with lower levels of serum estrogen (*See Figure 2*) [10, 29, 71, 75].

Amenorrhea, Estrogen and Vascular Function

Estrogen receptors are located on the endothelium and stimulate the production of eNOS, leading to increased production of NO that causes vasodilation [76]. Endothelial function fluctuates across the menstrual cycle in conjunction with estrogen concentrations [76, 77]. Recent studies examined endothelial function at 4 time points in the menstrual cycle: early follicular, late follicular, early luteal and late luteal [72, 73]. Endothelial function, NO and estrogen concentrations are all highest in the late follicular phase, and lowest in the early follicular phase [77, 78]. Arterial stiffness does not fluctuate as strongly across the menstrual cycle, however, estrogen concentrations do impact arterial stiffness [77-80]. Decreases in estrogen concentrations, such as occurs in post-menopausal women, increases arterial stiffness [81]. In animal studies, estrogen therapy significantly increased NO bioavailability [82]. Similarly, estrogen infusion transiently reduces arterial stiffness in postmenopausal women [83].

There is also evidence to suggest that low estrogen in postmenopausal women may contribute to larger IMT, a measurement of adverse structural arterial remodeling [78-80]. Supplemental estrogen via hormone replacement therapy, in postmenopausal women, reduces IMT [83-85]. Specifically, estrogen therapy and selective estrogen receptor modulators significantly reduce femoral IMT [86] and carotid IMT [87] in postmenopausal women, leading to values closer to young premenopausal women. Estrogen may regulate NO by increasing the production of eNOS and the release of NO [76], further improving vascular remodeling. While

estrogen deficiencies and estrogen therapy have been shown to affect indices of remodeling via the IMT, their effect on arterial diameter is largely unknown [88].

Ultimately, female sex hormones may help to explain the disparity in CVD risk between men and women, especially since these differences seem to be only present during reproductive years [89]. Thus, estrogen deficiency may lead to premature endothelial dysfunction, increased arterial stiffness and adverse vascular remodeling (*See Figure 2*) [90].

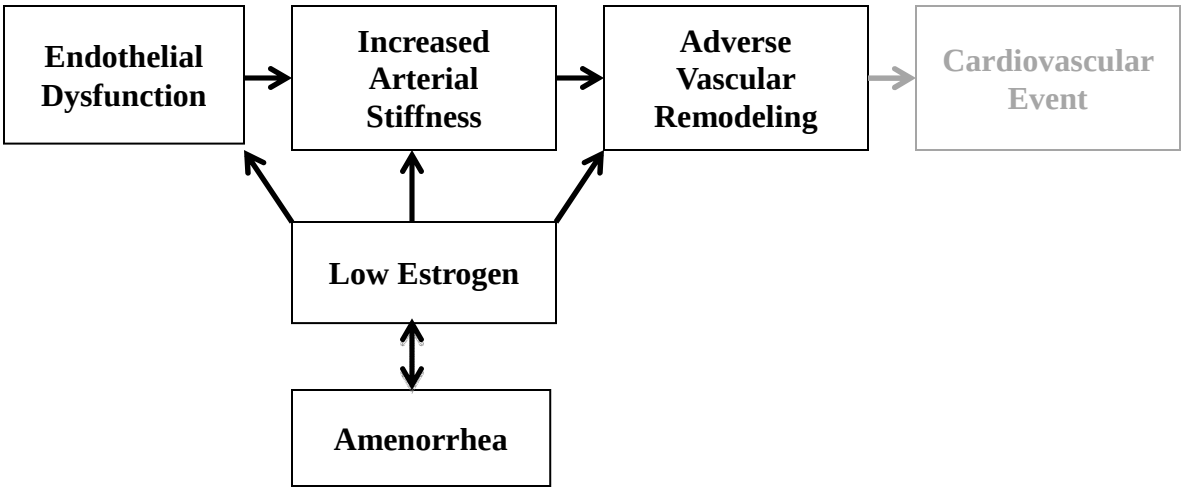


Figure 2: Hypoestrogenism observed in exercise-trained amenorrheic women might contribute to endothelial dysfunction, increased arterial stiffness, adverse vascular remodeling and ultimately, risk for a cardiovascular event.

Diet and Vascular Function

In addition to exercise and disease states, dietary intake has an effect on vascular function. Exercise-trained women are more susceptible to inadequate dietary intake or energy deficiency compared to their male counterparts [91]. The female athlete triad not only includes amenorrhea but also includes disordered eating which can lead to low energy availability and may also contribute to the development of amenorrhea [10]. It is suggested that in addition to the contribution of low estrogen concentrations to vascular dysfunction in exercise-trained amenorrheic women, disordered eating and subsequent energy deficiency may also mediate vascular dysfunction observed in exercise-trained amenorrheic women [38]. Some of the

vascular dysfunction observed in exercise-trained or sedentary women may be due to the lack of or over-consumption of specific nutrients, minerals and vitamins [86]. Therefore, dietary intake is an influential variable to consider when evaluating vascular function in exercise-trained individuals.

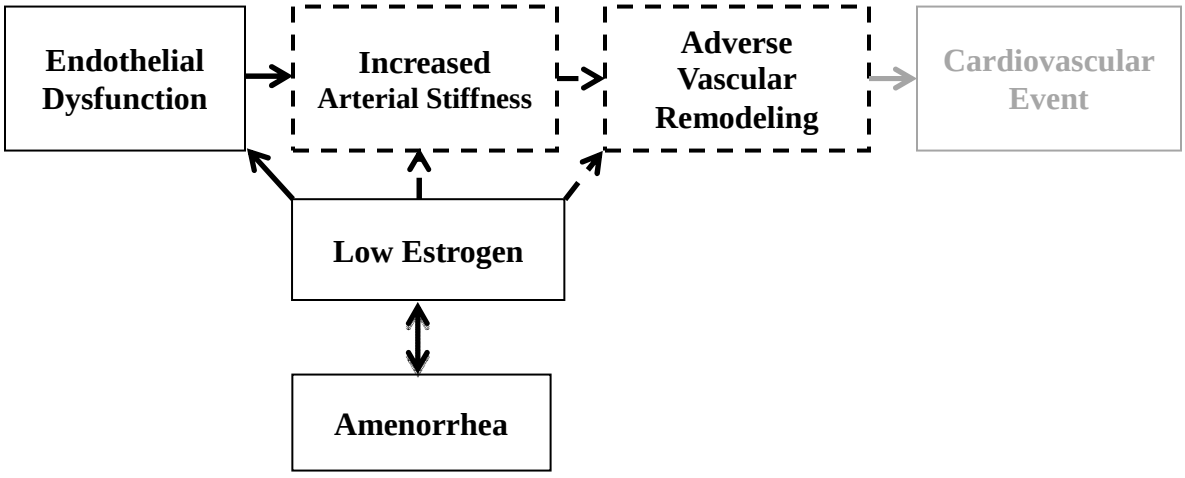
Exercise-trained females are commonly deficient in their dietary fat intake [92]. Although a high fat diet can lead to endothelial dysfunction, increases in arterial stiffness, [93, 94] and a larger IMT [95], deficiencies in dietary fat in female athletes may give greater insight into the causes of menstrual irregularities such as amenorrhea. Normal dietary fat intake has been reported to be necessary to maintain concentrations of sex hormones and may prevent menstrual disturbances in female athletes [91, 96].

Other common nutrient deficiencies in exercise-trained females that may affect endothelial function and arterial stiffness are, antioxidants, B-vitamins, zinc and iron [92]. Antioxidants have a favorable effect on endothelial function and arterial stiffness, as they provide vascular defense against oxidative stress [97]. B-vitamins have antioxidant-like properties and reduce arterial stiffness by inhibiting the breakdown of NO by free radicals [94]. Therefore, antioxidants and B-vitamin deficiencies in female athletes may lead to endothelial dysfunction and increased arterial stiffness. Likewise, zinc deficiencies have been linked to increased blood pressure and impaired NO activity [98] affecting endothelial function and arterial stiffness. Lastly, iron intake can contribute to endothelial dysfunction [99] and arterial stiffness [100]. While maintenance of necessary iron stores is essential for the female athlete, excessive iron stores are linked to increases in oxidative stress and therefore endothelial dysfunction [93]. Iron deficiencies, such as anemia, commonly observed in female athletes, have been shown to affect NO production and therefore, endothelial function [101]. Considering the

effects of specific nutrient intakes and deficiencies on vascular function, dietary intake is an essential component when examining vascular function in exercise-trained women.

Rationale for the Current Study

Exercise-trained amenorrheic women have endothelial dysfunction. It is currently unknown whether these women have higher arterial stiffness and adverse vascular remodeling; more advanced stages of subclinical atherosclerosis (*See Figure 3, Dashed arrows/boxes represent what is unknown, solid arrows/boxes represent what is known*). There is a strong relationship between estrogen, endothelial dysfunction and increased arterial stiffness. Estrogen has a favorable effect on vascular structure and function. Considering the association between estrogen, endothelial function and arterial stiffness, it seems plausible to hypothesize that chronic estrogen deficiency/endothelial dysfunction will lead to increased arterial stiffening and maladaptive vascular remodeling. This study examined endothelial dysfunction, arterial stiffness and vascular remodeling in highly exercise-trained women to gain insight into the effect of exercise-associated amenorrhea on subclinical atherosclerosis and risk for CVD in this cohort.



<u>Figure 3:</u> It is currently unknown whether exercise-trained amenorrheic women have increased arterial stiffness (dashed arrow/box) and adverse vascular remodeling (dashed arrow/box). Thus, premature CVD needs to be examined in this population.

Chapter III: Methods

Participants: Forty-three women participated in this cross-sectional study: 10 exercise-trained amenorrheic women, 18 exercise-trained eumenorrheic women, and 15 sedentary/recreationally active eumenorrheic women. Participants were between the ages of 18-30 years, to avoid the influence of age-related vascular dysfunction that has been shown to occur beyond 30-35 years of age. Participants were recruited from Syracuse University, university collegiate sports teams, local colleges (Upstate Medical University, LeMoyne College) and athletic clubs (Syracuse Chargers, Fleet Feet Sports, Central New York Triathlon Team) in the Central New York Area. Recruitment was via flyers, word of mouth, emails and advertisements. Written informed consent was obtained from all participants. The Syracuse University Institutional Review Board approved this study.

Inclusion Criteria: Healthy exercise-trained and sedentary/recreationally active female participants with and without amenorrhea were included in this study. Participants were stratified into physical activity groups according to self-report via the Paffenbarger Physical Activity Questionnaire (*Appendix 2*). From this validated questionnaire [102], a Physical Activity Index (PAI) was determined from Intensity, Duration, and Frequency of activity (maximal score is 100). A maximal aerobic capacity test was administered to confirm the participants' fitness grouping determined from questionnaire. The amenorrheic group consisted of both primary amenorrhea (absence of menses by age 15) and secondary amenorrhea (absence of 3 consecutive menstrual cycles after menarche). The eumenorrheic group consisted of women with menstrual bleeding every 26 to 32 days for at least 12 months. Menstrual status was determined via a Health History Questionnaire (*Appendix 4*).

Exclusion Criteria: Participants were excluded from the study if they were pregnant (determined using a urine pregnancy test), hypertensive (systolic blood pressure greater than or equal to 140 mmHg; diastolic blood pressure greater than or equal to 90 mmHg), cigarette smokers, had a history of thyroid disorders, diabetes mellitus, or CVD (stroke, heart attack or any past cardiac events), as determined by a health history questionnaire.

Study Design: This was a cross-sectional study examining vascular function across fitness levels and menstrual status. Subjects visited the Human Performance Laboratory on one occasion. Subjects with eumenorrhea completed the study visit during the early follicular stage (within 1-7 days following the start of the menses) of their menstrual cycle. Amenorrheic subjects completed the study visit at any time, unless they were currently taking oral contraceptives, in which case they were asked to come in during the placebo phase of the contraceptive pill. This was done to control for the potential hormonal effects of oral contraceptives on vascular function.

Measures were performed first thing in the morning with the participant in a fasted state (>12 hours post-prandial, no caffeine, no alcohol). Participants were also asked to consume 16 ounces of water the night before their visit and the morning of the testing to moderately control for hydration. Prior to arriving at the laboratory, subjects were asked to complete a 3-day dietary record (two weekdays, one weekend day) to ensure that the dietary intake was not the underlying reason for potential vascular dysfunction and to examine dietary patterns in exercise-trained amenorrheic women. Subjects signed the informed consent form, filled out the questionnaires: health history, physical activity, and the female athlete triad questionnaire. Participants were required to lie in the supine position on the exam table for at least ten minutes in a dimly lit climate controlled room before all hemodynamic measures. At this time, flow-mediated dilation (FMD), pulse wave velocity (PWV), and vascular ultrasonography were performed as measures

of endothelial function, arterial stiffness and remodeling, respectively. Following vascular measurements, blood lipids, hemoglobin, hematocrit and saliva collection for estrogen analysis were performed. Lastly, body fat, using air displacement plethysmography (Bod Pod) was measured and then cardiorespiratory fitness assessed by the maximal oxygen uptake during an exercise test (VO_2 max test) was measured. All consent and testing procedures took approximately 90 minutes.

Blood Pressure: Systolic (SBP) and diastolic (DBP) brachial blood pressure was measured prior to vascular measurements via a validated automated oscillometric cuff (EW3109, Panasonic Electric Works, Secaucus NJ). Pressures were taken in duplicate and averaged. If values were different by more than 5 mmHg a third measure was obtained. Pulse Pressure (PP) was calculated as DBP subtracted from SBP.

Endothelial-dependent Flow Mediated Dilation (FMD): A non-invasive method of assessing endothelial dysfunction is FMD, calculated as the percentage change in arterial diameter due to blood ischemia and reactive hyperemia. The FMD protocol induces reactive hyperemia, which occurs after blood flow to a conduit vessel is occluded. After the release of the occlusion, blood flow is increased compared to baseline flow. This results in increases in shear stress, which leads to release of NO and subsequent vasodilation. A larger change in diameter indicates healthier endothelial function while a smaller change in diameter indicates abnormal endothelial function or endothelial dysfunction. In a recent study, FMD predicted cardiac events in 2000 subjects and a significant inverse relationship between cardiovascular events and FMD exists for the prognosis of atherosclerosis [19].

In the current study, endothelium-dependent vasodilation of the brachial artery was assessed using ultrasound (Prosound α7, Aloka, Tokyo, Japan). The brachial artery was

longitudinally imaged 2-cm distal to the antecubital fossa using a 5.0-13.0 MHz linear array probe. Baseline diameters were measured during end-diastole (measured by ECG R-waves) and end-systole (measured by the end of the ECG T-wave) using ultrasonic calipers. Average brachial diameter was calculated as 1/3systolic diameter + 2/3diastolic diameter. Following baseline brachial artery diameter measurements, a blood pressure cuff was placed around the lower arm and inflated to a supra-systolic pressure (200 mmHg) for 5 minutes (Hokanson, Bellevue, WA). Following this occlusion period, the cuff was released causing reactive hyperemia. Maximum velocity (Vm) was measured continuously for 25-30 seconds following cuff release (time 0 – 30s post cuff release). Following this initial 30s post cuff release measurement, brachial diameters were continuously measured for an additional 2-minutes (time 30s – 150s post cuff release). FMD was expressed as a percentage and calculated as: the baseline diastolic diameter (determined from ECG gating) subtracted from the peak diastolic diameter, divided by the baseline diastolic diameter and taken as a measure of endothelial function. Beat-to-beat mean velocity (MnV) noted during the 25-30 seconds post occlusion were entered into a software program (Graphpad, Prism, 3.0) to calculate the area under the curve (AUC) of the reactive hyperemic response to cuff deflation as an estimate of the shear stress stimulus [103]. The peak Vm within the first 30s immediately following cuff deflation was also recorded as a measure of microvascular reactivity [104].

Pulse Wave Velocity (PWV): A pressure wave is created when the left ventricle contracts and then travels throughout the vascular system. PWV is the rate at which the pressure wave travels through the arterial tree (the higher the PWV, the stiffer the arteries). Arterial stiffness is directly related to risk for cardiovascular events [17, 105, 106] A recent study reported that increased PWV is significantly associated with the *first* stroke and when assessed in conjunction with other

risk factors (such as, age, cholesterol. Diabetes, smoking, blood pressure), central PWV improved risk prediction for CVD [16]. Similarly, PWV assessed in a hypertensive population positively correlated with future stroke death [107].

In the current study, blood pressure waveforms were measured in the carotid, radial, ankle (posterior tibial) and femoral arteries using applanation tonometry (AtCor Medical, SpygmoCor Technology). Aortic stiffness was measured using carotid-femoral PWV. The distance between the super-sternal notch and the carotid pulse-site was measured and the distance between the super-sternal notch and the femoral pulse-site was measured. These distances were subtracted to determine the distance between the carotid and femoral pulse sites. Peripheral stiffness was measured using carotid-radial PWV and femoral-ankle PWV. The distance between the super-sternal notch and the carotid was measured and next the distance between the super-sternal notch and the radial pulse-site were measured. These distances were subtracted to determine the distance between the carotid and radial pulse sites. Lastly, the distance between the super-sternal notch and the femoral were measured and the distance between the super-sternal notch and the posterior-tibial were measured. These distances were subtracted to determine the distance between the femoral and ankle pulse sites. The equation used to determine PWV is: Δ distance (m) / Δ time (s).

Superficial Femoral Artery (SFA) Structure One indication of vascular remodeling is an increase in the intima media wall thickness (IMT). Increased IMT, both centrally and peripherally, is linked with future cardiovascular events [108, 109]. Increases in IMT are due to a combination of intimal hyperplasia/diffusive atherosclerosis and medial smooth muscle hypertrophy in response to increased pulsatility [110, 111]. An increase in femoral (peripheral) IMT is a marker of atherosclerosis and vascular remodeling [68, 108, 112]. In the current study

we assessed vascular remodeling by examining the IMT and lumen diameter of the superficial femoral artery (SFA). The SFA was imaged using Doppler ultrasound (ProSound α7, Aloka, Tokyo, Japan) attached to a 5.0-13.0 MHz linear-array probe. The artery was imaged approximately 8-10 cm distal to the bifurcation of the common femoral artery (CFA). SFA diameters during diastole (R wave) and during systole (T wave) were recorded using ultrasonic calipers. SFA intima-media thickness (IMT) was assessed as the distance from the near wall to far wall lumen-intima interface using a longitudinal view of the artery with both near wall and far wall lumen-IMT boundaries clearly visible. The average SFA diameter was evaluated as 1/3systolic diameter + 2/3diastolic diameter. Both measures of IMT and SFA diameter were used as indices of vascular remodeling.

Biochemical Analyses of 17Beta-Estradiol: 17-Beta estradiol was measured to verify the hormonal status of the women participants. Estradiol was detected in whole saliva samples, using a highly sensitive competitive binding immunoassay (Salimetrics State College, PA). In this particular immunoassay the bound estradiol peroxidase was detected using a spectrophotometer at 450 nm (with a correction of 630nm). The estradiol peroxidase was inversely proportional to the amount of free estradiol present in the saliva samples such that the higher optical density (OD) in the plate represented lower estradiol concentrations, while the lower OD in the plates represented higher estradiol concentrations. All samples were collected in the morning during the early follicular phase of the menstrual cycle.

Aerobic Capacity: A graded exercise test using a treadmill (T150 Med) was used to assess each participant's maximal aerobic capacity. Speed and grade were increased until participants reached volitional fatigue. The highly trained endurance participants started the test at a speed of 3.5 mph, and every two minutes the speed was increased until a speed of 8.5 mph was achieved.

After this stage, the grade was increased by 2.5% every two minutes until the participant reached exhaustion. For the recreationally active/sedentary individuals, the speed started at 3.5 mph and was increased every two minutes until a speed of 7.5 mph was achieved, at which point grade was increased by 2.5% every two minutes until exhaustion was achieved. Heart rate and Rated Perceived Exertion (RPE) were assessed during the stages using a Polar Heart Rate Monitor and an RPE chart, respectively. A metabolic cart was used for breath-by-breath gas analysis of oxygen and carbon dioxide (Parvo Medics). The calibration procedure included the flow meter (using a 3L syringe to mimic normal lung volume) calibration and gas calibration in which the ideal values were between .01-.03%. Peak aerobic capacity was taken as the highest VO_2 obtained. A test was considered valid if: an R-value of 1.15 and a RPE greater than 17 was achieved.

***Menstrual Status*:** A self-administered health history questionnaire was administered to determine the age of menarche (i.e. the age of onset of first menses), gynecological age (i.e. the difference between chronological age and age at menarche), number of menstrual cycles per year, number of days between menses, skipped menses, and use and type of birth control. For the purpose of this study the eumenorrheic women were defined as menstrual bleeding every 26 to 32 days for at least 12 months. Primary amenorrhea was defined as the absence of menarche by the age of 15 and secondary amenorrhea as the cessation of menses for greater than three consecutive cycles after onset of menarche [38].

Body Composition: Participants' body composition was assessed using air displacement phlethysmography (BodPod; COSMED, Concord, CA), to estimate lean body mass and fat mass. Participants' height (cm) and weight (kg) were measured using a wall stadiometer and scale, respectively. Participants were instructed to wear minimal tight fitting clothing (i.e. compression

shorts, a sports bra or a swimsuit, and swim cap). They were asked to sit quietly in the pressure chamber while the total body volume was measured.

3-Day Dietary Record: Participants were instructed to record their dietary intake for two weekdays and one weekend prior to the testing session. The dietary recall was analyzed for percentage of carbohydrate, protein, and fat, kilo-calorie content, and other nutrients, minerals, viatmins using a freely available software (supertracker.usda.gov, My Plate Software).

Blood Lipids: Total cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), triglycerides (TG) and glucose (GLU) levels were measured (Cholestech LDX, Alere). Briefly, a finger lancet was used to obtain a droplet of blood from the index finger of the non-dominant hand. The droplet was transferred to a cartridge to be loaded and read by the automated Cholestech device. This technique has previously been established to be valid and reliable (mean r = .97 for total cholesterol, HDL, LDL, TG, GLU, Alere, Medical).

Hematocrit: The proportion by volume of red blood cells or hematocrit was analyzed using a finger lancet to obtain a droplet of blood from the index finger of the non-dominant hand, which was centrifuged into plasma and red blood cells using CritSpin Micro-hematocrit centrifuge (StatSpin, Inc, Norwood, Mass).

Hemoglobin: Hemoglobin was analyzed using a finger lancet to obtain a droplet of blood from the index finger of the non-dominant hand. With a disposable microcuvette, this droplet was transferred, similar to the blood lipid measurement, to a blood analyzer (The Hemocue Hemoglobin System, Hb201+; Angelholm, Sweden).

Statistical Analyses: All variables were tested for non-normality using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality, before statistical hypothesis tests were performed. Data for continuous variables were analyzed using a one-way ANOVA. Categorical variables from the

Female Athlete Triad Questionnaire were analyzed using chi-square goodness of fit tests. When a significant main effect was detected between groups a Tukey Post Hoc Analysis was performed. Analysis of covariance (ANCOVA) was performed to adjust for the AUC (shear stress stimulus) for FMD responses. Pearson's bivariate correlations were performed between estrogen, body fat, VO_2 max and the following variables: FMD, PWV, IMT and SFA diameter. A significance level was set at $P \leq 0.05$. All data was presented as means ± standard deviation. Statistical Package for the Social Sciences (SPSS 20.0) was used for data analysis.

Chapter IV: Results

Participant Characteristics. Participant descriptive characteristics are shown in Table 1. Participants were well matched across groups for age, height, and weight (Table 1; $p<0.05$). Body fat percentage (%) was significantly different between groups ($p<0.05$). Body fat percentage was significantly lower in the exercise-trained amenorrheic group compared with both eumenorrheic groups ($p<0.05$), while the exercise-trained eumenorrheic group had significantly lower body fat percentage compared with the sedentary eumenorrheic group ($p<0.05$; Table 1). The physical activity index (PAI), calculated from the Paffenbarger Physical Activity Questionnaire was significantly different between groups; specifically the exercise trained eumenorrheic and amenorrheic group had a significantly higher PAI than the sedentary group ($p<0.05$). The exercise-trained amenorrheics had a significantly higher VO_2 Max (ml/kg/min) than the exercise-trained eumenorrheics and the sedentary group ($p<0.05$; Table 1). According to The World Health Organization (WHO, 1997) the gender and age specific guidelines for anemia in women are a hemoglobin value below 12 g/dl and a hematocrit below 36%. Ten women fell below the criteria for hemoglobin and hematocrit and thus, had mild to moderate anemia at the time of the study. Six women had hematocrit values of less than 36% (3 exercise-trained amenorrheic women, 1 exercise-trained eumenorrheic woman, 2 sedentary eumenorrheic women), and 5 women had hemoglobin values less than 12 g/dl (1 exercise-trained amenorrheic woman, 1 exercise-trained eumenorrheic woman, 3 sedentary eumenorrheic women).

Area Under the Curve (AUC) and Peak Velocity. AUC calculated from the recorded blood velocities 25-30 seconds post release of blood pressure cuff occlusion during the FMD protocol, is presented in Table 3. A significant main effect for AUC was detected between groups

($p<0.05$). Post hoc analyses denoted that the exercise-trained amenorrheic group had a significantly lower AUC compared to the sedentary group ($p<0.05$). In contrast, the peak velocity during the reactive hyperemic response was not significantly different between the three groups ($p>0.05$).

Endothelial-dependent Flow Mediated Dilation (FMD). FMD of the brachial artery data are presented in Table 3. No significant main effect was detected for FMD between groups ($p=0.07$). An analysis of covariance (ANCOVA) was performed between FMD to properly adjust for AUC (a measure of the shear stress stimulus), as is customary with this measure [113]. When FMD was adjusted for AUC, the exercise-trained amenorrheics had significantly lower FMD compared to the exercise trained and sedentary eumenorrheics ($p<0.05$; Figure 1).

Pulse-Wave Velocity (PWV). In this cross sectional design, PWV was measured at the carotid-femoral, carotid-radial, and the carotid-ankle pulse sites and is presented in Table 3. Carotid femoral PWV was significantly different between groups. Post-hoc analyses indicated that carotid-femoral PWV was significantly higher in the sedentary group compared to the exercise-trained eumenorrheic group ($p<0.05$) but not significantly different from the exercise-trained amenorrheic group ($p> 0.05$; Figure 2). No significant differences were found in carotid-radial and femoral-ankle PWV ($p>0.05$).

Superficial Femoral Artery (SFA) Structure. Resting SFA diameters and IMT are presented in Table 4. For SFA diastolic diameter and SFA IMT, a significant main effect was detected ($p<0.05$; Figure 3, and Figure 4, respectively). Post hoc analyses determined that the exercise-trained amenorrheics and eumenorrheics had a significantly higher SFA diastolic diameter when compared to the sedentary eumenorrheics ($p<0.05$). However, the exercise-trained amenorrheic women had significantly smaller IMT compared to the sedentary group ($p<0.05$).

Menstrual Status and Biochemical Analyses of 17B-estradiol. Menstrual history/characteristics are presented in Table 2. Age at menarche was significantly different, with the exercise-trained amenorrheics having a significantly higher age of menarche compared to exercise-trained and sedentary eumenorrheics ($p < 0.05$). Likewise, gynecological age was significantly lower in the amenorrheic group compared to the sedentary group ($p <0.05$). In the sedentary group one estradiol concentration had to be excluded due to measurement error ($n = 14$ from $n = 15$). 17B-estradiol was not significantly different between groups ($p > 0.05$). 55.8% of the women were on oral contraceptives (50% of exercise-trained amenorrheic women, 61% of exercise-trained eumenorrheic women, 60% of sedentary eumenorrheic women) and the proportion did not differ across groups ($p>0.05$). According to the female athlete triad questionnaire, the average number of responses, on an 8 point scale on body composition issues across groups was not significantly different (Average of 2.3 responses for exercise-trained amenorrheic women, 3.0 for exercise-trained eumenorrheic women, 2.07 for sedentary eumenorrheic women, $p>0.05$). Also the proportion of women across the three groups that reported a previous stress fracture was not significantly different, 20% of exercise-trained amenorrheic women, 33.3% of exercise-trained eumenorrheic women, 26.7% or sedentary eumenorrheic women ($p>0.05$).

3-Day Dietary Record Characteristics. Based on a three-day dietary record (two weekdays and two weekends), means of dietary information, nutrients, minerals, vitamins are presented in Table 5. *Nutrients.* A significant difference in the protein consumption was detected between groups, in which the exercise-trained amenorrheic women consumed significantly more protein than sedentary eumenorrheic group ($p<0.05$; Table 5). All other nutrients presented in Table 5 were not significantly different when compared across groups ($p>0.05$). *Minerals.* Significant differences were found in potassium, magnesium, phosphorous, and zinc levels ($p<0.05$; Table

5). Post hoc tests revealed that the exercise-trained amenorrheic group consumed a significantly higher level of these minerals than the sedentary group ($p<0.05$). *Vitamins.* Significant differences were found in vitamin B6, Riboflavin, Niacin, and Choline ($p<0.05$; Table 5). Specifically, the exercise-trained amenorrheics consumed significantly more Vitamin B6, and Choline than the sedentary group, and consumed significantly more riboflavin and niacin than both sedentary and exercise-trained eumenorrheic groups ($p<0.05$).

Correlations:

Pearson's bivariate correlations between estrogen and FMD, PWV, SFA IMT, SFA diameter, values were performed to determine associations between vascular dysfunction and estrogen concentrations. No significant correlation was found between estrogen and any vascular parameters assessed (Table 6, $p>0.05$).

Chapter V: Discussion

Using a cross-sectional study design we examined subclinical atherosclerotic cardiovascular disease (CVD) risk in exercise-trained amenorrheic women. Endothelial function was significantly lower in exercise-trained amenorrheic women compared with both exercise-trained eumenorrheic and sedentary eumenorrheic women. However, arterial stiffness in exercise-trained amenorrheic women was not different from their eumenorrheic counterparts, irrespective of training status. Also, exercise-trained amenorrheic women had similar favorable vascular remodeling compared to exercise-trained eumenorrheic women (i.e. larger SFA diameter and smaller IMT compared to sedentary eumenorrheic women). The main findings of this study indicate that exercise-trained amenorrheic women have endothelial dysfunction but may not have increased arterial stiffness and negative vascular remodeling. Thus, vascular dysfunction in exercise-trained amenorrheic women may not extend beyond the endothelium.

Moderate intensity exercise improves vascular function, which has been suggested to reduce risk for CVD [5, 24-27, 30-36, 46-48] but chronic high intensity exercise can lead to vascular dysfunction [54, 67]. In women low energy availability combined with chronic high intensity exercise may lead to exercise-associated amenorrhea. The amenorrheic state has been associated with endothelial dysfunction [9-11, 71, 114]. In support of previous literature we found that exercise-trained amenorrheic women had significant endothelial dysfunction compared to their eumenorrheic counterparts.

It has been suggested that chronic endothelial dysfunction may lead to progressive structural changes to the vessel wall such as increases in arterial stiffness [16]. This is important because increased arterial stiffness is associated with increased risk for CVD and future cardiovascular events [3, 18]. However, we found that arterial stiffness in amenorrheic women

was similar to eumenorrheic women, irrespective of training status. Our finding is novel in that no previous studies have examined arterial stiffness in exercise-trained amenorrheic women. Previous studies have reported increased arterial stiffness in athletes that engage in high intensity aerobic/endurance exercise training (i.e. marathon runners) [61] but this finding is not universal [57]. Our study expands these findings to suggest that chronic high intensity exercise may not increase arterial stiffness in amenorrheic women.

In general, exercise training leads to favorable vascular remodeling marked by larger arterial diameters and smaller IMT, which helps to increase blood flow to the active skeletal muscle while maintaining appropriate vessel wall tension and shear stress [49, 50, 115]. Previous cross sectional studies have found larger conduit artery diameters and smaller IMT in exercise-trained individuals [116-118]. It was hypothesized herein that chronic detrimental, functional changes to the endothelium might ultimately lead to unfavorable vascular remodeling [22]. We found that the exercise-trained amenorrheic and eumenorrheic women both had significantly larger SFA diameters and the amenorrheic group significantly smaller IMT than the sedentary women. This present study is novel in its examination of vascular remodeling of the SFA in exercise-trained amenorrheic women and suggests that amenorrhea does not negatively affect favorable vascular remodeling in response to chronic aerobic/endurance exercise training.

Similar to postmenopausal women, exercise-trained amenorrheic women lack a menstrual cycle and have lower levels of estrogen compared to premenopausal eumenorrheic women. As previously discussed, estrogen plays an important role in vascular function, particularly in the activation of eNOS and the production of NO [119]. Hypoestrogenism contributes to endothelial dysfunction [120-122], and increased arterial stiffness [77-78]. Estrogen therapy is also shown to decrease IMT in postmenopausal women [79-81]. Lower

estrogen concentrations have been linked to endothelial dysfunction in young premenopausal exercise-trained amenorrheic women [9, 11]. Contrary to our hypothesis, we did not find differences between the groups of women in salivary estrogen concentrations. Moreover, there were no associations between vascular parameters and estrogen concentrations.

A potential explanation for similarities in estrogen concentrations between groups might be due to the time of estrogen measurement in the current study. By study design, we measured salivary estrogen concentrations at one time point during the early follicular phase of the eumenorrheic menstrual cycle (i.e. 1-7 days after the onset of the menses phase). During this phase estrogen concentrations and FMD responses have been found to be at their lowest [72, 73]. However, we still hypothesized that there would be differences in estrogen concentrations. It is interesting to note that even at the time point in the eumenorrheic menstrual cycle when FMD and estrogen concentrations are at their lowest, exercise-trained amenorrheic women still had significantly lower FMD (i.e. endothelial function) compared to eumenorrheic women, irrespective of training status [114]. This points to a chronic hypoestrogenic effect rather than an acute response to decreases in estrogen.

An alternative mechanism for endothelial dysfunction in the exercise-trained amenorrheic women might be oxidative stress. Aerobic exercise is beneficial to vascular health but chronic high intensity exercise can lead to increased oxidative stress [72]. It is well known that increased free radical concentrations, which accompany oxidative stress, decrease NO bioavailability, the primary cause for endothelial dysfunction [14, 123]. This could theoretically affect endothelial function without affecting arterial stiffness. Therefore, future studies should examine levels of oxidative stress as a potential mechanism of endothelial dysfunction in exercise-trained amenorrheic women.

Lastly, the female athlete triad not only incorporates amenorrhea but also includes low energy availability [7]. In addition to low estrogen concentrations, low energy availability may contribute to vascular dysfunction in exercise-trained amenorrheic women. Several nutrients deficiencies in exercise-trained women are established to influence vascular function [87]. After examination of the dietary patterns of our participants' nutrient intake was significantly higher in the exercise-trained amenorrheics as compared to the eumenorrheic women. This study suggests that endothelial dysfunction may not be due to nutrient deficiencies in exercise-trained amenorrheic women.

Limitations

Despite our novel findings there are limitations to the current study. The cross sectional nature of the study design does not allow us to adequately explore the mechanism for endothelial dysfunction in exercise-trained amenorrheic women or explain casual relationships. Thus, a longitudinal study design might provide insight into cyclic hormonal fluctuations, chronic estrogen load and endothelial dysfunction in exercise-trained amenorrheic women. Additionally, nutritional intake might be inaccurate due to self-report and motivation of subjects to record their diet. This may contribute to the inability to accurately address the potential of disordered eating and/or specific nutrients/minerals/vitamins in amenorrhea. A 24-hour diet recall and/or food frequency questionnaire could be administered in future studies to improve the accuracy of these measures. Although none of the women self-reported disordered eating in our study, The Female Athlete Triad Questionnaire used to assess and screen for eating disorders was not an ideal measurement of eating disorders and body image. There are other questionnaires such as the Eating Disorder Examination Questionnaire (EDE-Q) that more accurately project whether an individual is at risk or suffers from an eating disorder.

In the current study we did not exclude for anemia. It was reported in our study that approximately a quarter of the participants had mild anemia, defined as lower than normal hemoglobin concentrations or hematocrit values. Anemia is common among young women, thus it is not unexpected to find anemic women in this study population [124, 125]. Anemia may reduce NO bioavailability as low hemoglobin concentrations may impair NO release and therefore, reduce endothelial function [101, 126-129]. Also, anemia can limit oxygen transport and negatively affect aerobic performance (i.e. VO_2 Max) [125, 130]. When we compared anemic women to non-anemic women (when matched for training status and menstrual status) both endothelial function and VO_2 max were not different. Moreover, there were no associations between anemic markers (e.g. low hemoglobin and low hematocrit) and FMD ($r = .312$, $r = -.027$) or VO_2 Max ($r = -.163$, $r = .053$). Thus, the presence of anemia may not have affected our findings on endothelial function and aerobic capacity. Future studies that examine female athletes should consider screening for anemia due to its potential effect on NO bioavailability, endothelial function and aerobic performance.

In the current study we did not exclude for oral contraceptive use. Oral contraceptives may positively affect endothelial function in exercise-trained amenorrheic women [12]. The effects of oral contraceptives on arterial stiffness in exercise-trained amenorrheic women are unknown, however, oral contraceptive use might increase arterial stiffness in young women [131]. In an attempt to minimize the effects of and group differences in oral contraceptive use, menstrual cycle and oral contraceptive use were tightly controlled. Groups were well matched for oral contraceptive use, and women were consistently measured during the placebo phase of their oral contraceptive and during the early follicular phase of the menstrual cycle. When we compared vascular function between women who used contraception and those who did not use

oral contraceptives (in the group as a whole and within each group) we found that FMD, PWV, IMT and arterial diameter did not differ.

Future Directions

Our findings suggest that vascular dysfunction in exercise-trained amenorrheic women may not extend beyond the endothelium. It is necessary to discern potential reasons for endothelial dysfunction in amenorrheic women since endothelial dysfunction is not only associated with subclinical atherosclerosis but it is also associated with other essential physiological responses that are of importance to the female athlete [108-110]. For instance, endothelial dysfunction might lead to reductions in exercise capacity [132] because reductions in NO can lead to decreased perfusion to the working skeletal muscle [133]. Also reductions in endothelial function might reduce muscle force and peak power output [134]. Finally, endothelial dysfunction has also recently been linked to repetitive stress/strain injuries, which may be common in runners [135].

Future investigations should measure estradiol concentrations at different time points of the menstrual cycle in order to truly capture the hormonal profile of amenorrhea. Moreover, estrogen at the receptor level should be examined. Studies show that estrogen receptor alpha expressed in endothelial cells might influence endothelial function via eNOS activation [136]. If circulating estrogen levels cannot explain the decrease in FMD in amenorrhea, it could be that estrogen receptors are dysfunctional.

Additionally, future studies should look into impaired NO cofactors that might be responsible for endothelial dysfunction in exercise-trained amenorrheic women. Moreau et al suggest that reduced tetrahydrobiopterin (BH_4) plays a role in vascular dysfunction in postmenopausal women based on the examination that postmenopausal women exhibit reduced

FMD [137]. BH_4 is an eNOS cofactor that synthesizes NO. A decrease in BH_4 can lead to increases in oxidative stress and subsequently stiffening of the arteries. There may be potential in this mechanism for amenorrheics who exhibit a similar vascular profile to postmenopausal women.

Conclusion

In the current study we examined subclinical atherosclerotic CVD in exercise-trained amenorrheic women. We found that exercise-trained amenorrheic women had significant endothelial dysfunction when compared to their exercise-trained and untrained eumenorrheic counterparts. The novel findings of this study are: (1) aortic stiffness is similar in exercise-trained amenorrheic women and eumenorrheic women, irrespective of training status and (2) exercise-trained amenorrheic women had similar favorable vascular remodeling compared to exercise-trained eumenorrheic women, manifesting as a larger SFA diameter and smaller IMT compared to sedentary eumenorrheic women. The main findings of this study imply that vascular dysfunction is not systemic and is restricted to the endothelium. Exercise-associated amenorrhea may not alter arterial structure/remodeling in response to chronic/habitual exercise training. It should be underscored that amenorrhea and endothelial dysfunction are associated with other medical concerns (i.e. decreased bone health, increased risk for stress fractures). In the current study the women were young and otherwise healthy individuals so we do not currently know the long-term effects of endothelial dysfunction in exercise-trained amenorrheic. Future investigations are necessary to examine the mechanisms of endothelial dysfunction in this population and long-term clinical consequences with advancing age.

Figure and Table Abbreviations

SBP = Systolic Blood Pressure

DBP = Diastolic Blood Pressure

FMD = Flow Mediated Dilation

PWV = Pulse Wave Velocity

AUC = Area Under the Curve

IMT = Intima Media Thickness

SFA = Superficial Femoral Artery

Table 1: Descriptive Characteristics and Blood Measures

	Trained Amenorrheic (n = 10)	Trained Eumenorrheic (n = 18)	Sedentary Eumenorrheic (n = 15)	Probability (Main Effect)
Age (yrs)	21 ± 3.0	22 ± 3.0	23 ± 4.0	0.26
Total Cholesterol (mg/dL)	161 ± 19.0	166 ± 27.0	175 ± 36.0	0.50
Triglycerides (mg/dL)	64 ± 17.0	69 ± 29.0	77 ± 40.0	0.57
Low Density Lipoprotein (mg/dL)	89 ± 18.0	89 ± 22.0	99 ± 30.0	0.52
High Density Lipoprotein (mg/dL)	66 ± 10.0	64 ± 10.0	65 ± 18.0	0.96
Glucose (mg/dL)	82 ± 4.0	82 ± 10.0	82 ± 5.0	0.97
Hematocrit (%)	36 ± 7.0	40 ± 3.0	39 ± 5.0	0.06
Hemoglobin (g/dL)	13.3 ± 1.0	13.3 ± 0.7	12.7 ± 1.5	0.32
Brachial SBP (mmHg)	112 ± 6.0	112 ± 8.0	112 ± 4.0	0.99
Brachial DBP (mmHg)	69 ± 8.0	72 ± 8.0	72 ± 4.0	0.45
Resting HR (bpm)	51 ± 11.0	56 ± 9.0	66 ± 9.0*^	0.00
Body Fat (%)	18.8 ± 3.9	21.3 ± 4.8*	29.2 ± 4.9*^	0.00
Height (cm)	168 ± 6.0	165 ± 6.0	166 ± 9.0	0.39
Weight (kg)	61.0 ± 7.6	62.2 ± 8.7	65.9 ± 9.6	0.32
Body Mass Index (BMI) (kg/m^2)	21.5 ± 2.0	22.9 ± 2.4	23.9 ± 3.2	0.10
Lung Volume (L)	3.3 ± 0.2	3.2 ± 0.2	3.2 ± 0.4	0.45
Lean Body Mass (kg)	49.5 ± 6.1	48.7 ± 5.1	46.4 ± 5.4	0.32
VO_2 Max (ml/kg/min)	50.3 ± 6.7	49.8 ± 3.4*	37.8 ± 5.30*^	0.00
Physical Activity Index (PAI)	88.8 ± 17.8	84.7 ± 18.4*	27.9 ± 13.9*^	0.00

Table 1: A one way ANOVA indicates significant differences between groups on: resting HR, body fat percentage, VO_2 max test and PAI. Mean ± standard deviations, *^ $p < 0.05$ significance. *Versus exercise-trained amenorrheic women, ^Versus exercise-trained eumenorrheic women

Table 2: Menstrual History

	Trained Amenorrheics (n=10)	Trained Eumenorrheics (n = 18)	Sedentary Eumenorrheics (n= 15)	Probability (Main Effect)
Age of Menarche (yrs)	13 ± 2.00	12 ± 1.00*	12 ± 1.00*	0.04
17β-Estradiol (pg/ml)	1.66 ± .84	2.22 ± 1.20	2.00 ± .81	0.34
Gynecological Age (yrs)	7 ± 3.00	9 ± 3.00	11 ± 4.00*	0.05

Table 2: A one-way ANOVA indicated significance in the three groups in age of menarche and gynecological age. Mean ± standard deviation, *$p < 0.05$ significance. *Versus exercise-trained amenorrheic women.

Table 3: Arm Hemodynamics

	Trained Amenorrheic (n = 10)	Trained Eumenorrheic (n = 18)	Sedentary Eumenorrheic (n = 15)	Probability (Main Effect)
FMD (%)	7.15 ± 4.21	11.10 ± 4.29	10.25 ± 3.86	0.07
PWV-Carotid-Radial (m/s)	7.2 ± 1.18	7.6 ± .76	7.9 ± 1.23	0.20
PWV-Carotid-Femoral (m/s)	5.0 ± 1.00	4.6 ± .51	5.4 ± .76^	0.03
PWV-Femoral-Ankle (m/s)	9.1 ± 1.60	9.1 ± 1.50	8.4 ± 1.30	0.36
Area Under the Curve (aU)	808 ± 350.10	862 ± 311.00	1096 ± 311.30*	0.05
FMD_{AUC}	6.91 ± 1.33	10.97 ± .98*	10.57 ± 1.11*	0.05
Mean Brachial Diameter (mm)	3.27 ± .33	3.20 ± .37	3.02 ± .29	0.15

Table 3: A one-way ANOVA indicated that groups were significantly different on carotid-femoral-PWV, FMD_{AUC}, Brachial Shear Rate. Mean ± standard deviations, *^$p < 0.05$ significance. *Versus exercise-trained amenorrheic women, ^versus exercise-trained eumenorrheic women

Table 4: Leg Hemodynamics

	Trained Amenorrheic (n = 10)	Trained Eumenorrheic (n = 18)	Sedentary Eumenorrheic (n = 15)	Probability (Main Effect)
Systolic SFA Diameter (mm)	5.46 ± .74	5.33 ± .76	4.90 ± .59	0.08
Diastolic SFA Diameter (mm)	5.70 ± .72	5.68 ± .70	5.10 ± .63^*	0.03
SFA Diameter (mm)	5.61± .71	5.60 ± .70	5.03 ± .61^*	0.04
Diastolic SFA IMT (mm)	.31 ± .03	.35 ± .06	.38 ± .07*	0.01

Table 4: A one-way ANOVA indicated that groups were significantly different in SFA diameter, average SFA diameter, and diastolic SFA IMT. Mean ± standard deviations, *^$p < 0.05$ significance, *Versus exercise-trained amenorrheic women, ^Versus exercise-trained eumenorrheic women.

Table 5: Dietary Record Information

	Trained Amenorrheic (n= 10)	Trained Eumenorrheic (n=18)	Sedentary Eumenorrheic (n =15)	P-Value (Main Effect)
Total Calories	1900.40 ± 497.81	1596.67 ± 411.74	1483.13 ± 452.74	0.08
Protein (g/d)	87.50 ± 22.61	72.33 ± 16.54	62.13 ± 13.99*	0.00
Carbohydrate (g/d)	239.00 ± 75.22	203.22 ± 70.68	176.20 ± 71.72	0.10
Dietary Fiber (g/d)	23.80 ± 8.15#	21.17 ± 9.61#	18.67 ± 7.62#	0.35
Total Fat (g/d)	34.00 ± 3.62	32.89 ± 5.99	35.93 ± 5.82	0.29
Saturated fat (g/d)	11.00 ± 2.31	11.00 ± 3.91	11.20 ± 2.68	0.98
Monounsaturated Fat (g/d)	13.00 ± 2.49	12.28 ± 2.80	13.73 ± 2.79	0.32
Polyunsaturated Fat (g/d)	7.50 ± 1.51	6.67 ± 2.22	8.00 ± 2.33	0.20
Linoleic Acid (g/d)	14.40 ± 5.27	10.83 ± 5.04#	11.93 ± 5.12#	0.21
Alpha Linoleic Acid (g/d)	1.21 ± .57	1.03 ± .63#	1.25 ± .45	0.50
Omega-3 EPA (mg/d)	36.60 ± 75.71	11.06 ± 11.64	12.07 ± 10.93	0.19
Omega-3 DHA (mg/d)	83.70 ± 131.52	32.94 ± 28.51	36.33 ± 31.86	0.14
Cholesterol (mg/d)	249.70 ± 110.21	260.28 ± 154.14	195.00 ± 81.363	0.30
Calcium (mg/d)	913.30 ± 234.48#	787.78 ± 373.25#	687.93 ± 217.49#	0.19
Potassium (mg/d)	2891.40 ± 688.42#	2298.83 ± 785.63#	2149.80 ± 739.33*#	0.05
Sodium (mg/d)	2471.90 ± 495.45	2365.17 ± 689.21	2313.07 ± 836.95	0.86
Copper (µg/d)	1555.20 ± 591.80	1373.56 ± 851.96	1053.20 ± 406.97	0.17
Iron (mg/d)	15.10 ± 4.41#	14.22 ± 5.43#	12.00 ± 2.85#	0.19
Magnesium (mg/d)	373.20 ± 140.75	291.17 ± 102.51#	255.73 ± 100.74*#	0.04
Phosphorous (mg/d)	1410.20 ± 320.88	1132.72 ± 281.31*	1020.40 ± 240.20*	0.01
Selenium (µg/d)	112.50 ± 28.87	96.06 ± 26.81	92.2 ± 26.73	0.18
Zinc (mg/d)	11.20 ± 3.68	9.78 ± 2.51	8.00 ± 2.42*	0.02
Vitamin A (µg RAE)	822.30 ± 378.17	894.89 ± 899.46	585.60 ± 242.33#	0.37

Vitamin B6 (mg/d)	3.06 ± 1.92	2.09 ± .92	1.77 ± .62*	0.03
Vitamin B12 (µg/d)	4.48 ± 3.11	3.89 ± 1.88	3.14 ± 2.33	0.37
Vitamin C (mg/d)	99.80 ± 41.10	103.67 ± 74.41	89.53 ± 58.53	0.81
Vitamin D (µg/d)	3.80 ± 3.16[#]	2.39 ± 1.85[#]	2.47 ± 1.64[#]	0.22
Vitamin E (mg AT)	12.30 ± 8.50[#]	9.17 ± 6.11[#]	8.60 ± 4.95[#]	0.34
Vitamin K (µg/d)	113.80 ± 64.91	285.44 ± 720.04	149.60 ± 138.64	0.59
Folate (µg DFE)	556.40 ± 263.86	504.22 ± 222.66	510.00 ± 192.68	0.83
Thiamin (mg/d)	1.50 ± .31	1.35 ± .50	1.28 ± .34	0.42
Riboflavin (mg/d)	2.34 ± .79	1.75 ± .51*	1.49 ± .36*	0.00
Niacin (mg/d)	27.00 ± 7.45	20.50 ± 6.62*	19.07 ± 4.25*	0.01
Choline (mg/d)	316.30 ± 84.55[#]	273.61 ± 78.08[#]	250.16 ± 55.85*[#]	0.01

Table 6: A one-way ANOVA indicated that protein, potassium, magnesium, phosphorous, zinc, vitamin B6, Riboflavin, Niacin, Choline were significantly different between groups. Mean ± standard deviation, *$p < 0.05$ significance; *versus exercise-trained amenorrheic women, [#]Nutrient, mineral or vitamin deficiency based on Dietary Guideline for Americans, 2010.

Table 7: Pearson's Bivariate Correlations

	Estrogen	FMD	PWV	SFA Diameter	SFA IMT	Body Fat
FMD	-.001					
PWV	-.109	.025				
SFA Diameter	.013	-.127	-.203			
SFA IMT	.034*	.283	.186	-.113		
Body Fat	-.264	.242	-281	-.205	-.264	
VO_2 Max	.228	-.208	-.417*	-.303	.228	-.779*

Table 7: Pearson's Bivariate Correlations between Estrogen, FMD, PWV, Mean SFA Diameter, SFA IMT, Body Fat, VO_2 Max. *$p < 0.05$ significance.

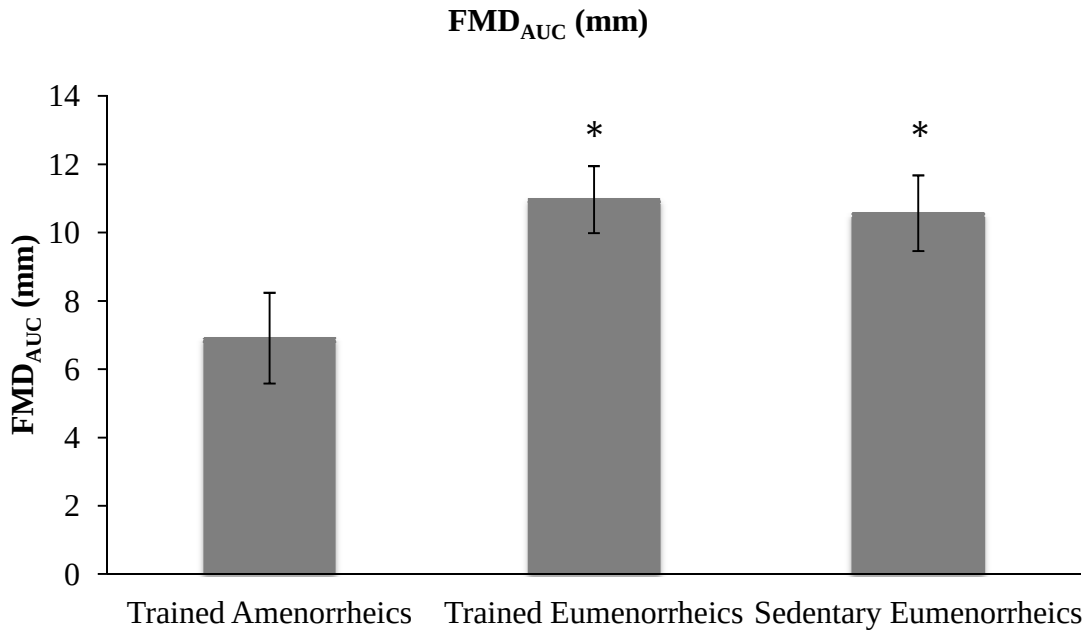


Figure 1: A one way ANOVA indicated that FMD_{AUC} (mm) was significantly lower in exercise-trained amenorrheic women compared with the eumenorrheic counterparts, Mean ± standard deviations, *$p < 0.05$ significance. *Versus exercise-trained amenorrheic.

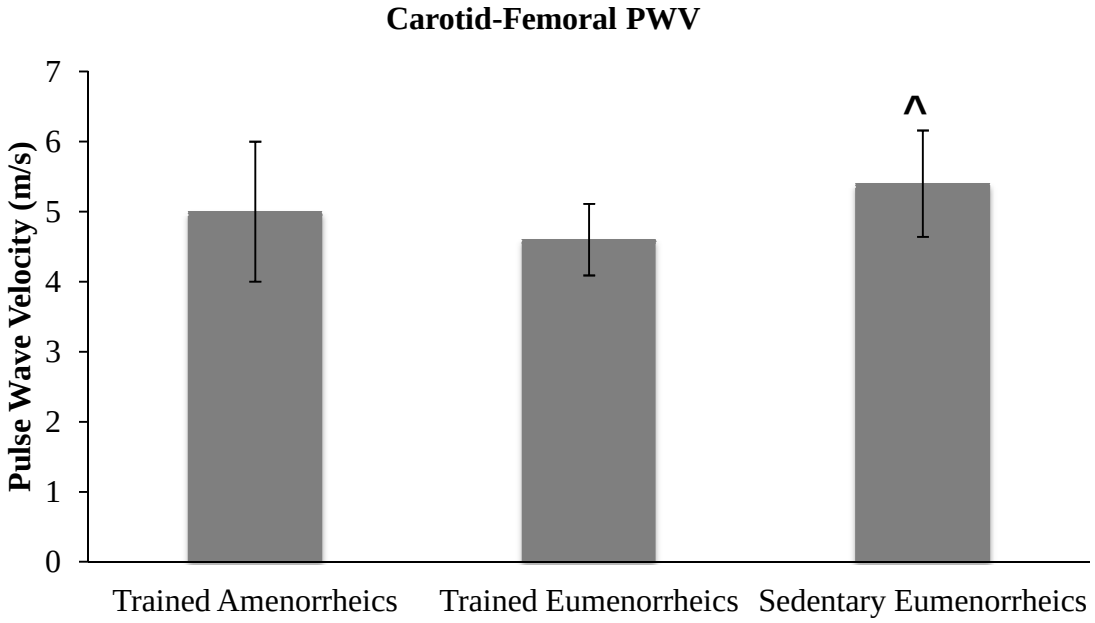


Figure 2: A one way ANOVA indicated that PWV-Carotid Femoral was significantly lower in exercise-trained eumenorrheic women compared with sedentary eumenorrheic women across groups, Mean ± Standard Deviations**,** ^$p < 0.05$ significance. ^Versus exercise-trained Eumenorrheic.

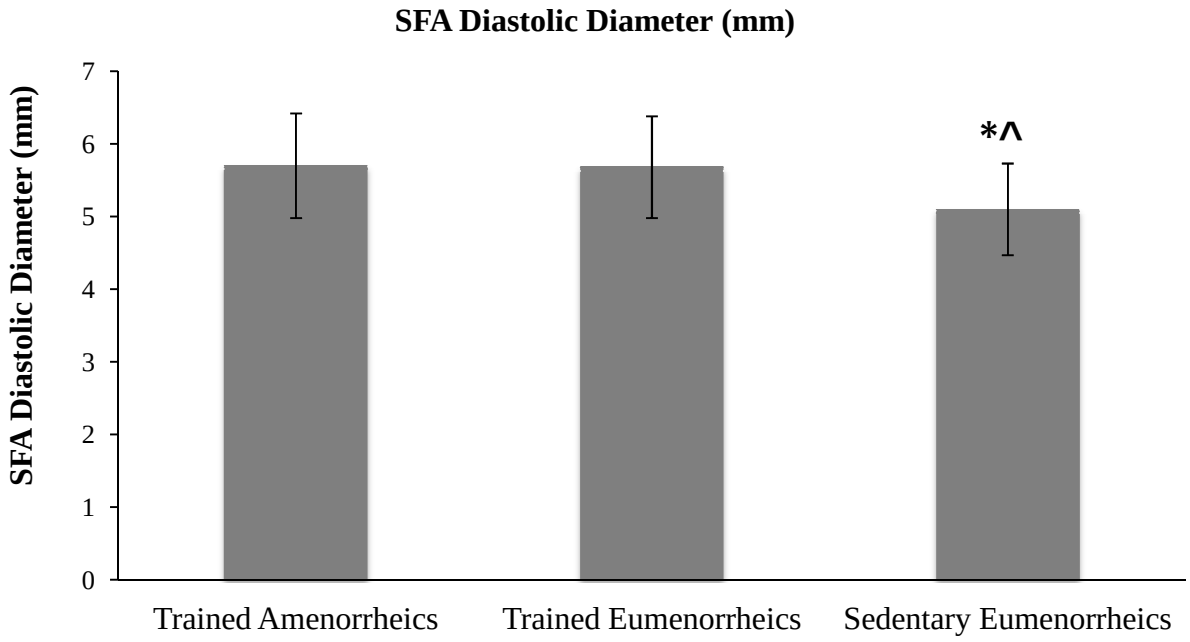


Figure 3: A one-way ANOVA indicated that exercise-trained amenorrheic and eumenorrheic women had a significantly larger diastolic diameter (mm) compared with sedentary eumenorrheic women. Mean ± Standard Deviations, *^$p < 0.05$ significance; *Versus exercise-trained Amenorrheic;^Versus exercise-trained Eumenorrheic.

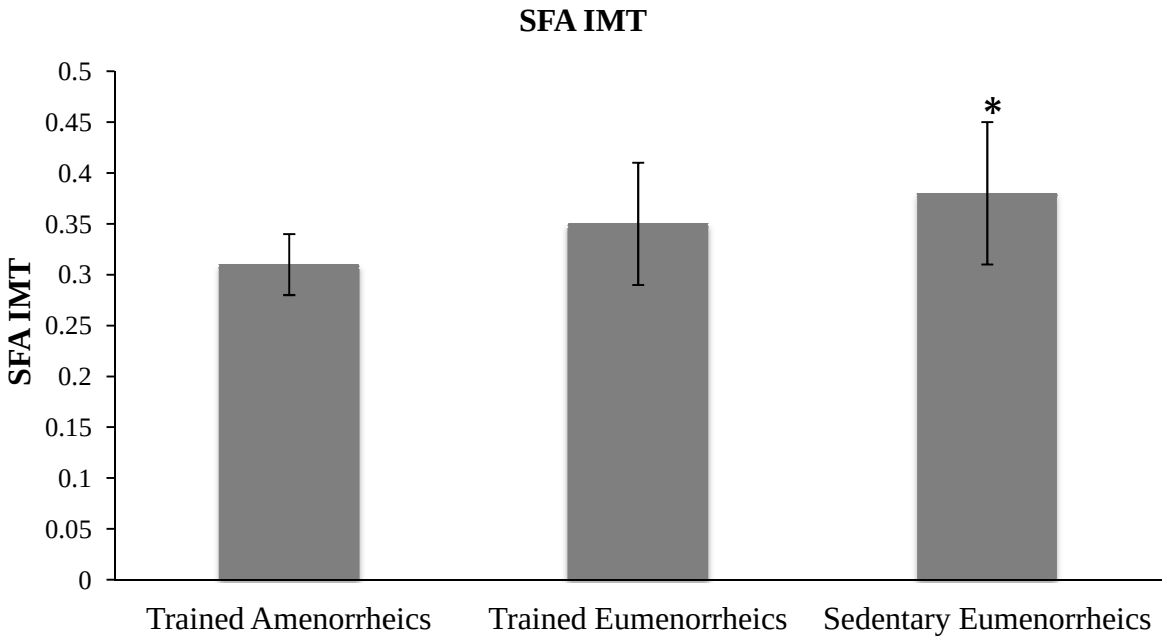


Figure 4: A one-way ANOVA indicated that the SFA IMT was significantly smaller in exercise-trained amenorrheic women compared with sedentary eumenorrheics, Mean ± Standard Deviations, *$p < 0.05$ significance. *Versus exercise-trained Amenorrheic.

APPENDIX 1

EXERCISE SCIENCE
820 COMSTOCK AVENUE
201 WOMEN'S BUILDING
SYRACUSE, NY 13210
(315)-443-2114

EXERCISE ASSOCIATED AMENORRHEA AND ARTERIAL STIFFNESS IN ENDURANCE-TRAINED FEMALES

Principal Investigator: Kevin Heffernan, Ph.D.
Telephone: 315-443-9801
Email: ksheffer@syr.edu
IRB Protocol #: 12-316

My name is Jacqueline Augustine and I am a Graduate Student at Syracuse University working with Dr. Kevin Heffernan. We are inviting you to participate in a research study. Involvement in the study is voluntary, so you may choose to participate or not to participate. This sheet will explain the study to you and please feel free to ask questions about the research if you have any. I will be happy to explain anything in detail if you wish.

Purpose

We are interested in learning more about the artery health in females across fitness levels and menstrual statuses. Exercise is widely beneficial to the arteries and has been shown to prevent the risk of cardiovascular disease. Many women who regularly exercise experience menstrual cycle irregularities and therefore it is of interest to us if this affects their risk for cardiovascular disease. Therefore, we are also interested in how the menstrual cycle influences arterial health. Amenorrhea is defined as a lack of menstrual cycle and often occurs in women who regularly exercise and/or are endurance trained. We wish to examine if endurance trained amenorrheic and eumenorrheic females have altered vascular function.

Who can participate?

- Women between the ages of 18-30 without a history of high blood pressure, thyroid disorder, heart disease, diabetes, are not pregnant and currently do not smoke.

Do I have to participate?

- Your participation in this study is voluntary, which means you get to decide whether or not you want to participate

- Make sure that you read this entire form before making a decision and take as much time as you need.
- Feel free to ask as many questions about the study as you want. If you do not understand a term in the form, ask, and a researcher will explain it for you.
- If you decide to participate in the study you will be asked to sign a consent form.
- Do not sign the consent form until all of your questions have been answered and you understand what will happen in the study.
- Your signature means that you agree to participate in this study.
- You can ask for a copy of this form whether or not you agree to take part in the study.
- Your decision not to be in this research study will not result in any loss of benefits to which you are otherwise entitled.

Can I Withdraw From The Study Once It Has Started?

- At any time you may remove yourself from the study without giving any reason.
- If you are a student, withdrawing from the study will not affect your grade in courses in any way.

What Can I Expect From Participating?

For this study, you will need to visit the Human Performance Laboratory located in the Women's Building at Syracuse University once. When you visit the lab, we will ask you to come first thing in the morning on an empty stomach (so no eating since the previous night). You will be asked to fill out and sign this consent form, Health History Questionnaire, Female Athlete Triad Questionnaire. You may be asked to perform a pregnancy test (over the counter urine test). You will bring with you your dietary 3-day diary and we will discuss this and do a 24 hour recall in which you will recall all you have had to eat in the last 24 hours. You will then be asked to lie down and rest for 10 minutes. Following rest, we will check your cholesterol and blood sugar, hemoglobin and hematocrit by obtaining a very small drop of blood from your fingertip. We will measure your blood pressure using a machine that inflates/deflates a small cuff around your wrist. Following this we will check your artery stiffness. To do this, we will use a very sensitive blood pressure machine that looks like a pen with a little watch battery at the end. We will place this pen on top of your skin over your wrist, neck and upper leg. From these measurements, we can determine how stiff your arteries are. This measurement is non-invasive (no needles/no blood) and will only take a few minutes. We will then use the ultrasound to look at your brachial arm artery and femoral/upper leg artery. The arm artery will be examined before and after a blood pressure cuff is inflated for 5 minutes on your forearm (non-invasive, no needles/no blood). This might be uncomfortable, as it will feel tight, like when the doctor takes your blood pressure at the doctor's office, but does not pose any risk and will not be done on your leg artery. This is meant to examine how well your arteries respond to the stress of increased blood flow (endothelial function). The ultrasound probe placed on your leg artery will feel cold and the measurement itself will be very quick. This will give us important information on how your

arteries may also change in a trained or not trained state. You will then give us a salivary sample to measure hormones (used only for research purpose not for clinical purposes), have your body fat percentage measured in the BodPod and perform a Maximal fitness level test, also known as a VO_{2Max} testing protocol on the treadmill. In which you will run until exhaustion to determine your fitness level.

Visit 1 Outline:

- You will be asked to sign a consent form
- You will fill out a health history, physical activity questionnaire, female athlete triad questionnaire
- Prior to this visit upon scheduling you will be sent ample information regarding a 3-day dietary analysis in which you will fill out your diet over the course of 3 days. You will be asked to bring this with you and this will be discussed at the time of your visit, with a 24-hour recall. You will be given interesting information regarding your diet.
- Female participants will be asked the following questions (which will be asked and detailed on the health history questionnaire): When was your last menstrual cycle? Are you asexually active? If so, do you use a method of birth control? Which type and how do you use this type of birth control? [i.e. hormonal form (Depo-Provera) and method (continuous birth control)]. If you take the oral contraceptive pill do you skip the placebo/withdrawal pills? (All detailed on the health history questionnaire). If their last menstrual cycle was greater than 28 days then a pregnancy test will be administered. In addition to these questions all female subjects will be asked to provide a urine sample to be tested via an over the counter pregnancy test. Any subjects with a positive result will also be disqualified and deemed ineligible from study participation. If present a positive pregnancy result participants will also be suggested to see their health care provider
- Your arterial stiffness and endothelial function will be measured.
- Blood Lipids, Hematocrit, Hemoglobin will be measured via a finger stick.
- We will measure your hormones (estrogen) via a salivary drool sample. This will take approximately 10 minutes at the most. This measurement of estrogen levels is only for research purposes not for clinical and/or diagnostic purposes.
- You will have your body fat measured using the Bod Pod. For this measurement, you will be required to wear a bathing suit (but don't worry, there's no water involved; you will not get wet). You will be asked to sit in a chamber that resembles a giant egg for 10 minutes. This machine measures your volume to calculate body fat.
- Aerobic capacity (Maximal Fitness Level) VO_{2Max} test. You will be supervised during all exercise testing.
- This session will take approximately 2-3 hours

What Benefits Can I Expect From Participating?

- A benefit from this study is helping us understand the potential health benefits of endurance exercise to improve artery health
- You may feel good about helping others by participating
- You will receive information on your body fat, cholesterol, blood pressure, hematocrit, hemoglobin, fitness level (VO_{2Max}) and diet.
- These tests are not being used to diagnose a problem. But if you do have high blood pressure, high cholesterol or are close to anemia values in hemoglobin content we will inform you to go the university health center or go see your family physician.

Are There Any Potential Risks From Participating In This Study?

- There are no more than minimal risks (risks encountered in everyday life) associated with this study.
- There is possible discomfort with the 5-minute inflation of the blood pressure cuff to measure endothelial function.
- You may experience discomfort from the finger stick to test your blood lipids, hematocrit, and hemoglobin. This will only be done twice or three times and no more than that and we will use different fingers each time to reduce discomfort. If desired, we can also place ice on the finger prior to the finger stick to reduce discomfort from the pinch.
- There is a small risk of infection associated with the finger stick. However we will reduce this risk by ensuring that everything we use is clean and sterile.
- There is also discomfort associated with arterial stiffness or leg blood blow measurements. Ultrasound gel may seem cool and tingly on the skin but that is it.
- There may be abnormal changes in heart rate and blood pressure associated with exercise. These abnormal changes are very rare and you will be asked to report any abnormal feelings throughout the exercise sessions. The research team will constantly be monitoring heart rate and blood pressure. We will also instruct you on proper breathing to help reduce this risk.
- There is also risk of injury and muscle soreness from the exercise protocol. All exercise sessions will be directly supervised to reduce risk for injury. Ensuring that you communicate with the researcher throughout the protocol will reduce risks. A proper cool down is also set it place for each participant. If at any point you are uncomfortable or feel

pain anywhere, please tell us immediately. If this occurs you will stop the protocol and not have to continue.

- A risk to participation in this study is the uncovering of a potential eating disorder, presence of amenorrhea, and/or the presence of stress. Information about your health may be revealed to you that you were not already aware of. The Health History Questionnaire includes a detailed Menstrual Status Questionnaire that might define you as being amenorrheic. Likewise, the Female Athlete Triad Questionnaire might screen you positive for an eating disorder or stress. In either scenario you will be given ample resources that are provided to you at the end of this consent form. However, we are not diagnosing you and all of our methods are only being used for research purposes.

Are There Any Costs?

- There will be no costs to you for participating in this study.

Who Can See Information About This Study?

- The research records from this study will be confidential. Confidentiality means that it is our responsibility to keep any information you provide private and safe.
- Only members of the trained research staff for this study may look over your research records.
- The paperwork, results and records will be kept in a locked filing cabinet that only the researchers will have access to.
- All information stored on computers requires a password access it. Only members of the research team will have this password.
- The data and research record will be stored for up to 10 years.
- Your individual results will not be used in any way (we will average all results and display group averages only when presenting findings in papers and presentations)

What Are My Rights In This Study?

- Your participation in this study is voluntary.
- If at any point you wish to withdraw yourself from the study you may.
- You do not give up any of your legal rights by participating in this study.

In Case Of Injury:

- In the event of illness or physical injury resulting from taking part in this research study, medical treatment will not be compensated for and you will be responsible for any costs not paid by your insurance company. No other compensation is offered by Syracuse University. However, treatment can be sought at Syracuse University or University Hospital. Syracuse University has no plans to give you money if you are injured. You have not waived any of your legal rights by signing this form.

Resources:

- For Syracuse University students, employees and faculty, these resources will be: Syracuse University Health Services (315-443-9005), Syracuse University Academic Services (315-443-2506), Syracuse University Counseling Services (315-443-4715) and their Health Care Provider. For those participants that may not be Syracuse students, faculty or employees and are not comfortable with these resources provided will be referred to see their Health Care Provider if they are not already seeing them.

Who Can I Contact For Questions Or More Information?

- If you have any questions, concerns, exercise related injuries or complaints about this study at any time, please feel free to contact:
- Jacqueline Augustine at Jaimse@syr.edu or call her cell-phone at 860-508-8996.
- Dr. Kevin Heffernan at ksheffer@syr.edu or call his office at 315-443-9801.
- If you cannot reach Dr. Heffernan or Jacqueline or you would like to speak to someone else about any questions, concerns, complaints, or your rights as a participant in this study, you can call the Syracuse University Institutional Review Board (IRB) at 315-443-3013

If you do not want to take part in the protocol, you have the right to refuse to take part, without penalty. If you decide to take part and later no longer wish to continue, you have the right to withdraw from the study at any time, without penalty.

By signing below you indicate that you have read and fully understood this informed consent form. You are fully aware of the purpose and procedures of this study as well as the risks, discomforts, and benefits associated with the experimental protocol and that you sign this document freely and voluntarily.

All of my questions have been answered, I am 18 years of age or older, and I wish to participate in this research study. I have received a copy of this consent form.

_____ _____

Signature of participant Date

_____

Printed name of participant

_____ _____

Signature of researcher Date

_____

Printed name of researcher

Paffenbarger Physical Activity Questionnaire

SUBJECT ID: _____ Date: _____

Birth date: _____ Male/Female_____ Age: _____

PLEASE ANSWER THE FOLLOWING QUESTIONS BASED ON YOUR AVERAGE DAILY PHYSICAL ACTIVITY HABITS FOR THE PAST YEAR

1. How many stairs did you climb up on an average day during the past year?
_____ stairs per day (1 flight or floor=10 stairs)

2. How many city blocks or their equivalent did you walk on an average day during the past year?
_____ blocks per day (12 blocks = 1 mile)

3. Do you run or cycle? (circle one). If so how many miles a week
?_____

4. List any sports, leisure, or recreational activities you have participated in on a regular basis during the past year. Enter the average number of times per week you took part in these activities and the average duration of these sessions. Include only time you were physically active (that is, actual playing or activity time).

Sport or Recreation	Times per Week	Hours per day	Minutes per day

5. On a usual weekday and a weekend day, how much time do you spend on the following activities?

Sport or Recreation	Usual weekday Hours/Day	Usual weekend Hours/Day
a. Vigorous activity (digging in the garden, strenuous sports, jogging, aerobic dancing, sustained swimming, brisk walking, heavy carpentry, bicycling on hills, etc.)		
b. Moderate activity (housework, light sports, regular walking, golf, yard work, lawn mowing, painting, repairing, light carpentry, ballroom dancing, bicycling on level ground. Etc.)		
c. Light activity (office work, driving car, strolling, personal care, standing with little motion, etc.)		
d. Sitting activity (eating, reading, desk work, watching TV, listening to radio, etc.)		
e. Sleeping or reclining		

APPENDIX 3

PHYSICAL ACTIVITY INDEX

Evaluate your current exercise program by selecting your score for each category.

	Score	Activity
Intensity		
	5	Sustained heavy breathing and perspiration
	4	Intermittent heavy breathing and perspiration, as in tennis
	3	Moderately heavy, as in cycling and other recreational sports
	2	Moderate, as in volleyball, softball
	1	Light, as in fishing
Duration		
	4	Over 30 minutes
	3	20 to 30 minutes
	2	10 to 20 minutes
	1	Less than 10 minutes
Frequency		
	5	6 to 7 times per week
	4	3 to 5 times per week
	3	1 to 2 times per week
	2	A few times per month
	1	Less than once a month

Intensity X Duration X Frequency = Score Total

Your Score: _____ x _____ x _____ = _____

Evaluation of Activity Score		
Score	**Evaluation**	**Activity Category**
81 to 100	Very active lifestyle	High
60 to 80	Active and healthy	Very good
40 to 59	Acceptable but could be better	Fair
20 to 39	Not good enough	Poor
Under 20	Sedentary	

APPENDIX 4

SELF-ADMINISTRATED PRE-EXERCISE MEDICAL HISTORY FORM

All information given is confidential. It will enable us to better understand you and your health and fitness habits.
To ensure that you do not meet any of the exclusion criteria, you must answer all the questions included here.

SUBJECT ID._____Date:_____

Birth date:_____

Male/Female _____ Age: _____

Health History Questionnaire

	Yes	No
1. Do you ever get chest pains while at rest and / or during exertion?	____	____
2. If the answer to question 1 is "yes", has a physician diagnosed these pains?		
3. Have you ever had a heart attack or been told of any problems with your heart?	____	____
4. If the answer to question 3 is "yes", was your heart attack within the last year?	____	____
5. Have you ever had an echocardiogram? If yes, when?	____	____
6. Do you have high blood pressure (i.e., a reading of more than 140 / 90) ?	____	____
7. If the answer to question 4 is "yes" is your high blood pressure currently being treated by medication (e.g., "water pills") ?	____	____
8. Have you ever been diagnosed with high cholesterol?	____	____
9. Do you lose your balance because of dizziness or do you ever lose consciousness?	____	____
10. Has your physician ever specifically told you not to do "heavy" or "hard" exercise?	____	____
11. Have you been diagnosed with any of the following:		

- Diabetes Mellitus ____ ____
- Rheumatoid Arthritis ____ ____
- Bleeding disorder ____ ____

Medical History

Do you have or have you ever had: (check if yes)

____ heart murmur
____ extra/skipped heart beats
____ chest pain or pressure
____ high blood pressure
____ heart attack
____ stroke
_____ arthritis
_____ asthma
_____ bronchitis
_____ cancer
_____ diabetes
_____ emphysema

Medical History *(continued)*

Do you have or have you ever had: (check if yes)

____ leg cramps
____ varicose veins
____ dizziness/fainting
____ back pain
____ shortness of breath
____ injuries to back, knees, ankles
_____ epilepsy
_____ pneumonia
_____ rheumatic fever
_____ scarlet fever
_____ surgery

Explanations/Commments/Descriptions:_____

_____

Other diseases/injuries/medical problems: _____

_____

Medicines/Drugs you are now taking(please list dosages):_____

_____

Family History

Please indicate the <u>number</u> of blood relative (mother, father, grandparents, siblings who have or have had the following:

Heart attack or stroke before age 50 _____
Heart attack or stoke after age 50 _____
Congenital heart disease _____
Heart operations _____
High blood pressure _____
Diabetes _____
Substantially overweight _____
High cholesterol levels _____

Remarks:_____

Present Symptoms Review

Do you ever experience any of the following during exercise?

_____ Chest pain
_____ Shortness of breath
_____ Heart palpitations
_____ Cough on exertion

Health Inventory

Smoking Habits

Do you smoke cigarettes at present? Yes _____ No _____
If yes, how many per day? <1/2 pack _____ 1/2 to 1 pack _____
1 - 2 packs _____ >2 packs _____

Did you smoke cigarettes in the past and quit permanently? Yes _____ No _____

How may years has it been since you quit? _____
How many packs per day were you smoking before you quit? _____
How many years did you smoke before you quit? _____

_Menstrual Status

At what age did you have your first menstrual period? _____

What was the date of your last menstrual period?_____

Have you ever been amenorrheic (only 1-2 periods in a year)? _____
If yes, for how long? _____

If your last menstrual cycle was greater than 28 days ago and/or you have a history of amenorrhea are you currently under the care of a health care provider? _____

Do you currently experience a menstrual cycle? _____

Have you ever had any skipped menses, if yes how many? _____

Do you use oral contraceptives? _____Which kind? _____ What dose?_____ If yes, for how long? _____
Do you take the withdrawal/Placebo pills?_____

Do you use Depo-Provera for birth control? _____If yes, for how long have you used this?_____

How many periods in a year do you have? _____

About how many days are there between periods? _____

APPENDIX 5

The Female Athlete Triad Questionnaire
Circle Yes or No

1. Do you worry about your weight or body composition?

Yes No

2. Do you limit or carefully control the foods that you eat?

Yes No

3. Do you try to lose weight to meet weight or image/appearance requirements in your sport?

Yes No

4. Does your weight affect the way you feel about yourself?

Yes No

5. Do you worry that you have lost control over how much you eat? Yes No

6. Do you make yourself vomit, use diuretics or laxatives after you eat?

Yes No

7. Do you currently or have you ever suffered from an eating disorder?

Yes No

8. Do you ever eat in secret?

Yes No

9. What age was your first menstrual period?

Yes No

10.Do you have monthly menstrual cycles?

Yes No

11.How many menstrual cycles have you had in the last year?

Yes No

12.Have you ever had a stress fracture?

Yes No

Please circle the response that best matches your situation.
Never= 1 Rarely=2 Occasionally=3 More often than not=4
Regularly= 5 Always=6

1. Do you want to weigh more or less than you do? 1 2 3 4 5 6

2. Do you lose weight regularly to meet weight requirements for your sport? 1 2 3 4 5 6

How do you do it?_____

3. Is weight/body composition an issue for you? 1 2 3 4 5 6

4. Are you satisfied with your eating habits? 1 2 3 4 5 6

5. Do you think your performance is directly affected by your weight? 1 2 3 4 5 6

If so how?_____

6. Do you have forbidden foods? 1 2 3 4 5 6

7. Are you a vegetarian? 1 2 3 4 5 6

Since what age?_____

8. Do you miss meals? 1 2 3 4 5 6

If so, how often? For what reason?_____

9. Do you have rapid increases of decreases in your body weight? 1 2 3 4 5 6 10. What do you consider your ideal competitive weight? 1 2 3 4 5 6

11. Has anyone ever suggested you lose weight or change your eating habits?
1 2 3 4 5 6

12. Has a coach, judge, or family member ever called you fat? 1 2 3 4 5 6

13. What do you do to control your weight?_____

_____

14. Do you worry if you have missed a workout? 1 2 3 4 5 6

15. Do you exercise or are you physically active as well as training for your sport?
1 2 3 4 5 6

16. Do you have stress in your life outside of sport? 1 2 3 4 5 6

What are these stresses?_____

17. Are you able to cope with stress? 1 2 3 4 5 6

How?_____

18. What is your family structure?_____

19. Do you use or have you use(d) these ways to lose weight?

a. laxatives 1 2 3 4 5 6

b. diuretics 1 2 3 4 5 6

c. vomiting 1 2 3 4 5 6

d. diet pills 1 2 3 4 5 6

e. saunas 1 2 3 4 5 6

f. plastic bags or wrap during training 1 2 3 4 5 6

g. other methods(please state)_____ 1 2 3 4 5 6

APPENDIX 6

Three Day Food Log

1. Please write down everything you eat and drink for 3 typical days. Try to include at least one weekend day - Saturday or Sunday.

2. Record this in the column marked FOOD and BEVERAGES.

3. Record only amounts EATEN, not amount served.

4. Record the brand name and method of cooking in the "METHOD OF PREPARATION / BRAND NAME" column.

5. Under 'AMOUNT', record in 'teaspoons', 'cups', or fractions of these. You may use 'slices' or 'pieces' when necessary. If something eaten has a specific measurement on the label, record that amount. For example: Coke - 12 ounce can, Hershey bar 1.45 ounces.

6. It is important to remember the following while recording different types of food:

 Milk: State if whole, skim, fortified, powdered, liquid, evaporated, or chocolate

 Liquids: Record amount of milk and all beverages in 'cups' or 'ounces'.

 Bread: Specify white, rye, whole wheat, raisin, etc.

 Meats: Give the length, width and thickness of the portion, or its weight in 'ounces' after cooking.

 Cereals, rice, and pasta: Record amount of cereals, rice, and pasta in 'cups' or fractions of cup. Do not record in 'BOWLS'. List anything added e.g. fruit, sugar

 Fruits and Vegetables: Specify, fresh, frozen, canned, dried, or freeze dried.

 Condiments: Record any jelly, butter, ketchup, mayonnaise or seasonings added.

 Canned foods: Record what food is packed in – oil, water, syrup, etc

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EDUCATION

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- B.A. in Psychology, Pre-Medicine
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TEACHING EXPERIENCE

Graduate Teaching Assistant 2012-present

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  - PPE 497 Exercise Physiology, PPE 295 Introduction to Exercise Science
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Future Professoriate Program (FPP) 2012-present

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AWARDS AND GRANTS

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Graduate Student Organization (GSO) Travel Grant 2013-2014
School of Education (SOE) Travel Grant 2012-2013
Graduate Student Organization (GSO) Travel Grant 2012-2013
Mid-Atlantic Regional American College of Sports Medicine Conference Award 2013

- Top 5 abstract out of 100

Phi Kappa Phi Honor Society Inductee, Syracuse University 2013

Mid-Atlantic Regional American College of Sports Medicine Conference Award 2012
- Top 5 abstract out of 100

Joan N Burstyn Syracuse University, School of Education Grant 2011-2012
- Grant for collaborative research in education

Syracuse University School of Education Dean Scholarship 2011-2012
Crusader in the News named by the Holy Cross Magazine 2009
- For outstanding individual achievement during the Cross Country season.

PROFESSIONAL MEMBERSHIPS

American College of Sports Medicine (ACSM) 2012-present
Mid-Atlantic Regional Chapter of American College of Sports Medicine (ACSM) 2012-present
National Strength and Conditioning Association (NSCA) 2013-present
National Science Teacher's Association (NSTA) 2013-present
Phi Kappa Phi Honor's Society 2013-present

COURSES

Writing in the Sciences, Stanford University (Online course) 2013

COMMUNITY INVOLVEMENT

American Heart Association Walk, Syracuse, NY 2012-present
Regular Consultant for Syracuse University, NCAA Division 1 Women's Soccer Team 2012-present

SERVICE RELATED ACTIVITIES

Syracuse University Physical Education and Exercise Science, Search Committee 2013
- Physical Education Urban Inclusive Position, Assistant Professor

Syracuse University School of Education Board of Visitors Meeting 2013

PROFESSIONAL CERTIFICATIONS

American Red Cross, Adult and Pediatric CPR/AED/First Aid 2013-present
NSCA Certified Strength and Conditioning Specialist (CSCS) in progress 2014 Expected

INTERCOLLEGIATE ATHLETICS

NCAA Division I Athletics, College of the Holy Cross 2007-2011
- Varsity Cross Country, Indoor, Outdoor Track and Field

PUBLICATIONS

Augustine, J. Tarzia, B. Kasprowicz, A. Heffernan, K. Effect of a Single Bout of Resistance Exercise on Arterial Stiffness Following a High Fat Meal, The International Journal of Sport Medicine, 2013, 34; 1-6.

Spartano NL, ***Augustine JA***, Lefferts WK, Hughes WE, Redmond JG, Martin ED, Kuvin JT, Gump BB, Heffernan KS. Arterial stiffness as a non-invasive tissue biomarker of cardiac target organ damage. Current Biomarker Findings, 2013, 4, 23-34.

Lefferts, WL, ***Augustine, JA,*** Heffernan, KS. Resistance exercise-mediated increases in carotid artery stiffness do not affect cerebral blood flow pulsatility, Frontiers in Integrative Physiology, 2014, 5:101.

Heffernan KS, Lefferts, WL, ***Augustine, JA***. Hemodynamic Correlates of Late Systolic Flow Velocity Augmentation. International Journal of Hypertension, 2013.

PUBLICATIONS IN REVIEW

Nicole L. Spartano, ***Jacqueline A. Augustine***, Wesley K. Lefferts, Brooks B. Gump, Kevin S. Heffernan. The relationship between carotid blood pressure reactivity to mental stress and carotid intima-media thickness, *Atherosclerosis, In Review*.

PRESENTATIONS

Augustine, J., (2013). Mid-Atlantic Regional American College of Sports Medicine (ACSM), Abstract Graduate Student Finalist and Slide Presentation, Harrisburg, PA. *Vascular Function in Exercise-Trained Females,* Free Communication Slide Session.

Augustine, J., (2013). National American College of Sports Medicine (ACSM), Indianapolis, IN, 2013. *Vascular Function Following a High Fat Meal And Resistance Exercise.* Thematic Poster Presentation.

Augustine, J., (2012). Mid-Atlantic Regional Conference (MARC), American College of Sports Medicine (ACSM) Abstract Graduate Student Finalist and Presentation, Harrisburg, PA. *Vascular Function Following a High Fat Meal And Resistance Exercise,* Communication Slide Session.

ABSTRACTS

Augustine, J., Tarzia, B., Kasprowicz, A., Heffernan, K**.** Vascular Function Following a High-Fat Meal with Resistance Exercise, International Journal of Exercise Science: Conference Proceedings, 2012, (Slide Presentation Mid-Atlantic Regional American College of Sports Medicine Conference, November, 2012).

Augustine, J., Lefferts, W., Martin, E., Spartano, N., Heffernan, K. Vascular Function in Exercise-Trained Women, International Journal of Exercise Science: Conference Proceedings, 2013, (Slide Presentation Mid-Atlantic Regional American College of Sports Medicine Conference, November 2013).

Lefferts, W., ***Augustine, J***., Heffernan, K. Resistance Exercise, Carotid Artery Stiffness, and Cerebral Blood Flow Pulsatility, International Journal of Exercise Science: Conference Proceedings, 2013 (Slide presentation Mid-Atlantic Regional American College of Sports Medicine Conference, November 2013).

Martin, E., ***Augustine, J***., Spartano, N., Lefferts, W., Heffernan, K. No Association Between Body Fat and Arterial Stiffness in Non-obese Women, International Journal of Exercise Science:

Conference Proceedings, 2013, (Slide Presentation Mid-Atlantic Regional American College of Sports Medicine Conference, Harrisburg, PA, November, 2013).

Spartano, N., ***Augustine, J***., Lefferts, W., Hughes, W., Morse, B., Martin, E., Bill, K., Gump B., Heffernan K. Carotid Blood Pressure Reactivity is Associated with Carotid Intima-Media Thickness Independent of Central Adiposity**,** International Journal of Exercise Science: Conference Proceedings, 2013, (Slide Presentation Mid-Atlantic Regional American College of Sports Medicine Conference, Harrisburg, PA, November, 2013).

Heffernan KS, Lefferts WL, ***Augustine JA***. Resistance Exercise-Induced Increases in Carotid Artery Stiffness Do Not Affect Cerebral Blood Flow Pulsatility, (National Artery Society Conference, Chicago, IL, September, 2013).

Statz C, Rai M, Ras A, ***Imse J***, Zaeem, F, Mulamalla R, Wencker D. Novel Findings of Upregulation of Neutrophil Gelatinase-B Associated Lipocalin (NGAL) in Myocytes of Advanced Heart Failure Patients. Journal of Cardiac Heart Failure, 2011 (National Heart and Lung Transplant Conference, 2011).

Hughes, W.E., Spartano, NL., Lefferts, WK., ***Augustine, JA***., Heffernan, KS**.** Sex Differences in Arterial Stiffness and Left Ventricular Pressure Energetics, International Journal of Exercise Science: Conference Proceedings, 2013, (Slide Presentation Mid-Atlantic Regional American College of Sports Medicine Conference, Harrisburg, PA, 2013).