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MENOPAUSAL TRANSITION STATUS AND VASCULAR RESPONSES TO EXERCISE

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ABSTRACT

Objective: To determine if menopausal transition status and fitness influence peripheral vascular responses to dynamic leg exercise.

Design: Healthy women were grouped according to early perimenopausal (n=16, 48±4 yrs), late perimenopausal (n=12, 50±3yrs), and early postmenopausal (n=12, 54±3 yrs) status based on self-reported menstrual bleeding history and follicle-stimulating hormone (FSH) concentration. Common femoral artery blood flow (FBF, Doppler ultrasound), mean arterial pressure (MAP, Finometer) and femoral vascular conductance (FVC = FBF/MAP) were measured during graded single leg knee extensor exercise. Body composition (DEXA), pulse wave velocity (PWV), cardiorespiratory fitness (treadmill VO_{2max}) and daily physical activity (accelerometer) were also measured.

Results: Peri- and post-menopausal group averages for vascular responses to exercise did not differ. However, among perimenopausal women, differences in submaximal FBF and FVC responses to knee extensor exercise were observed between early and late stages (early > late, $p \leq 0.05$). Multivariate stepwise regression identified FSH as the best predictor of the differences in FVC (r=-0.44, $p \leq 0.05$) and FBF (r=-0.45, $p \leq 0.05$) during submaximal exercise in early and late perimenopausal women. Also, there were differences in peak FBF and FVC when normalized to peak power. Multivariate stepwise regression identified systemic cardiorespiratory fitness as the best predictor of peak leg exercise vasodilation.

Conclusions: These results suggest that ovarian hormone dynamics, cardiorespiratory fitness, and inflammatory status can influence the rise in blood flow to exercising skeletal muscles in mid-life women. Menopausal women who maintain an active lifestyle and

high fitness level may prevent perimenopausal reductions in peak leg vasodilation during exercise but another intervention may be needed to prevent attenuation of leg hemodynamic responses during submaximal leg exercise.

Key Words*:* menopause, exercise, limb blood flow, vasodilation, accelerometer

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LITERATURE REVIEW

Overview

One of the most significant life changes a woman goes through is the menopausal transition. Many factors influence a woman's health during this midlife transition. While many studies have examined the peripheral circulation in young women and older, post-menopausal women, few studies have focused on midlife women undergoing the menopausal transition. This review will summarize the literature pertaining to the physiology of normal, healthy aging, including natural menopause in women and its associated increases in cardiovascular risk. Additionally, there will be a description of how increased vascular risk can impact womens' lives. The relationships between arterial stiffness, physical activity, and aging will also be addressed. To conclude, there will be a discussion on what remains unknown about menopause and what merits further research.

Arterial Aging

Aging is a complex process. It cannot be described solely in terms of years but rather must be viewed as the accumulation of physiological changes in multiple systems. The changes can reduce the capacity to cope with stress and maintain homeostasis. Normal aging is associated with structural and functional changes in most physiological systems. Loss of skeletal muscle mass and decline in power, alterations in body composition (reduced bone density, increased body fat, etc.), and reductions in cardiovascular function each have significant implications for age-related declines in health and normal daily functioning.[40] This review concentrates on vascular changes that

occur with age in women, focusing on the perimenopausal and early post menopausal phases during which hormonal changes occur.

Structurally, arteries tend to increase in lumen size and wall thickness with age while also increasing in stiffness which is demonstrated by increased pulse wave velocity.[39] Additionally, many of the substances involved in the inflammatory response and/or atherosclerosis are more abundant in aging arteries. Functionally, the largest change that occurs with age is impaired distensibility. [39] Decreased distensibility, or increased stiffness, results from several factors including structural changes, increased endothelial permeability, and a reduced nitric oxide-dependent vasodilator response to acetylcholine.[39] Older individuals also have significantly attenuated resting leg blood flow and greater leg vascular resistance.[41] In addition to normal vascular changes associated with aging, the risk of atherosclerosis increases with age. Atherosclerotic vessels are stiffer and have stenosis, plaques, and lesions.[39] There is functional evidence to show that lifestyle interventions such as exercise or sodium restriction can have favorable effects on arterial structure and function in humans,[39] which may help delay cardiovascular events in healthy elderly individuals.

Natural Menopause

Menopause is an influential period in the aging process of women which is characterized by hormonal changes. One of the most noteworthy characteristics of perimenopause is significant variability in the sex hormones. FSH and estrogen tend to fluctuate throughout perimenopause while also showing steady trends. Estrogen progressively decreases during menopause while FSH progressively increases throughout menopause.[42]

This period is characterized by profound effects on multiple physiological systems. These include accelerated loss of muscle mass and strength,[33] an increase in weight gain with significant increases in central body fat,[34,35] increases in total cholesterol and LDL,[14] a decrease in bone density and bone mass, and increased stiffness of central arteries.

Cardiovascular/Vascular Risk Increases during Menopause

Prior to menopause, women have a lower risk for CVD than do men of similar age. Due to decreased estrogen as well as advancing age, women undergoing the menopausal transition become at increased risk for cardiovascular disease (CVD). CVD risk can lead to several pathologies including hypertension, myocardial infarctions, and stroke. During midlife, women catch up to men in terms of risk for CVD. After the menopausal transition, however, a woman's risk for CVD increases to the point that women have a higher risk for CVD than men.[43] The significant increase in CVD risk illustrates that the menopausal transition is a vulnerable period of a woman's life from a cardiovascular health progression standpoint.

Vascular Function and Structure during Midlife

Changes in vascular function can dramatically affect vascular heath, CVD risk, and the ability to perform activities of daily living. Estrogen causes vasodilation of vessels through several different pathways.[36] The significant reduction in circulating estrogen that occurs during menopause results in the loss of a potent vasodilator in a woman's circulation. This reduction results in decreased endothelial vasodilator function, a risk factor known to be a key early sign of the atherosclerotic process.[36]

Understanding how vascular structure and function change during the menopausal transition, particularly in healthy women, is important for understanding why CVD risk increases dramatically during midlife in women. Additionally, it is important to understand how lifestyle factors such as exercise and nutrition modulate these vascular changes. If these changes and influences can be understood, it is reasonable to presume that pharmaceuticals and lifestyle changes can be prescribed that can make a positive impact by decreasing the cardiovascular risk of women during and after menopause.

Changes in Arterial Stiffness/Structure Across Menopause

Several studies have reported that arterial stiffness increased with advanced age in women. The studies, however, showed that increased arterial stiffness could be reversed with exercise training.[27] With regards to the menopausal transition, a recent study showed that postmenopausal women exhibited larger arterial diameters than pre-/early perimenopausal women.[45] Another study showed that while there is an accelerated arterial diameter (carotid intima-medial thickness) increase in peri/post-menopausal women as compared to premenopausal women, a dietary and exercise intervention can slow this progression.[18]

Exercise and Vasodilatory Reponses Across Menopause

Several studies have been conducted on vascular function and menopause. Our laboratory has previously shown that healthy older women (>60 yrs) exhibit attenuated leg vasodilation during graded and peak exercise as compared to younger women.[37,38] Additionally, estrogen deficient women (premenopausal hypoestrogenic or postmenopausal) show reduced lower limb blood flow responses including decreased

local vascular conductance, compared with women having clinically normal levels of estrogen,[11] indicating the effect estrogen has on vasodilation.

According to past research, reduced availability of nitric oxide (NO) as a result of increased oxidative stress is one of the key mechanisms mediating reduced endothelium-dependent dilation (EDD) with aging in both sexes.[29] Vascular oxidative stress increases with age as a consequence of greater production of reactive oxygen species without a compensatory increase in antioxidant defenses.[29] Increased activity of the potent vasoconstrictor endothelin-1, the development of vascular inflammation, and reduced expression of oestrogen receptor α in postmenopausal females also probably contributes to impaired EDD with aging.[29]

With regard to physical activity, a study showed that a single bout of aerobic exercise increased EDD in postmenopausal women but not premenopausal women indicating the importance of aerobic exercise in healthy post-menopausal women.[38] Additionally, it has been shown that healthy postmenopausal women exhibit blunted sympathetic vasoconstriction in exercising muscle as compared to premenopausal women and postmenopausal women undergoing one month of transdermal oestrogen replacement therapy.[6] Collectively, these studies indicate that vasodilator and vasoconstrictor responses in healthy women can be influenced by physical activity. In summary, menopausal status appears to influence peripheral endothelial function and vascular responsiveness in healthy women.

Focus of Present Thesis

While there has been a tremendous amount of research conducted on vascular structure and function in older women and young women, few studies have focused on

women across the menopausal transition. More research is required to understand vascular structure and function to characterize the menopausal transition, with careful consideration of any covariates.[44] Studies need to examine the variability of vascular measurements such as conductance and arterial stiffness and look at the impact of fitness across the menopausal transition. The present research focused on determining if menopausal status influences specific vascular responses and if cardiorespiratory fitness has a modulatory role on those vascular responses. These findings should have important implications for prevention (primary and secondary) of vascular impairments for women, helping to indicate to what extent exercise can slow or reverse vascular problems in women at different stages of their lives.[46]

INTRODUCTION

The menopause transition is generally associated with diminished health status including increased weight gain, vasomotor symptoms, elevated blood lipid concentrations, and increased risk for cardiovascular disease after menopause.[1] The likely culprit underlying these changes is the fluctuating level of female reproductive hormones, particularly estrogen deficiency. Estrogen has multiple biological effects that are long-lasting (genomic) such as reducing plasma levels of low-density lipoprotein cholesterol[2] or fast-acting (non-genomic) such as activation of nitric oxide synthase and subsequent endothelial cell release of nitric oxide.[3] Vascular function is particularly susceptible to estrogen deficiency since estrogen is demonstrated to decrease synthesis of vasoconstrictive metabolites from the cyclooxygenase pathway, increase production of endothelium-derived vasodilators (nitric oxide and prostaglandins), and indirectly induce smooth muscle relaxation.[4]

Observational studies report decreased vasodilator capacity[5] and increased vasoconstrictor responsiveness[6,7] in the peripheral vasculature of postmenopausal women as compared to premenopausal women. Acute replacement of estrogen to plasma levels similar to premenopausal women potentiates forearm vascular responses to acetylcholine in postmenopausal women[8] suggesting that reduced limb vasodilator responsiveness in postmenopausal women is due in part to decreased nitric oxide bioavailability.[3] Interestingly, acute aerobic exercise increases brachial artery flow-mediated dilation in postmenopausal women to premenopausal levels and by a similar magnitude as acute estrogen supplementation.[9] Moreover, regular aerobic exercise in postmenopausal women is associated with enhanced brachial artery flow-mediated dilation[10], increased peak calf

muscle ischemic vascular conductance[11], and increased systemic vasodilation (*i.e.*, reduced total peripheral resistance) during acute exercise.[12,13] These findings highlight the negative impact of menopause on arterial function and the beneficial vasodilatory effects of regular aerobic exercise in postmenopausal women. However, relatively little information is available on changes in peripheral vascular responses in women undergoing the menopause transition (*i.e.*, perimenopause). Perimenopause is characterized by increased body fat[14] that can lead to increased inflammation[15] which has the potential to impair vasodilator responses in the resistance arteries of both coronary[16] and skeletal muscle vascular beds.[17] Additionally, studies report accelerated changes in common carotid artery intima-media thickness[18] and adventitial diameter[19] in perimenopausal women, and postmenopausal acceleration of arterial stiffening[20] which collectively suggest possible menopause-stage dependent alterations in vascular structure.

With this information as a background, the primary objectives of the present study were 1) to determine if menopausal status (*i.e.*, early perimenopausal vs. late perimenopausal vs. early postmenopausal) influences vascular responses to graded leg exercise or regional arterial stiffness in healthy women, and 2) to investigate the modulatory role that cardiorespiratory fitness may have on these responses at any of the three menopausal stages. Given that estrogen concentrations are reported to be chronically reduced by one year post-menopause (*i.e.*, 12 months after the final episode of spontaneous vaginal bleeding)[21], we hypothesized that exercise-induced vasodilator responses in the legs and estimates of arterial compliance of early postmenopausal women would, on average, be attenuated in comparison to perimenopausal women. We further hypothesized that cardiorespiratory fitness level, due to its well established

influence on adiposity and metabolic risk factors in women at midlife, would exhibit a favorable modulatory influence on leg exercise hemodynamics and arterial compliance stiffness estimates, particularly during the perimenopausal period when such metabolic and body composition alterations are reported to be most prominent.[14]

METHODS

Participants

Forty middle-aged women (40-60 yrs) were recruited for this study. Women were divided into early perimenopausal (n=16), late perimenopausal (n=12) and postmenopausal (n=12) groups based on self-reported bleeding history. Perimenopausal women reported experiencing irregular menstrual cycles during the previous 6 months not associated with pathology. Postmenopausal women reported not experiencing a menstrual cycle during the previous 12 months. All participants were healthy as determined by medical history questionnaire, physical examination, resting electrocardiogram, blood tests for plasma lipid levels, and resting blood pressure below 140/90 mmHg. Five women who met these criteria (two perimenopausal and three postmenopausal) were taking blood pressure or lipid lowering medications, but analysis showed exclusion of these women did not alter our results. Consequently, these women are included in the present analysis. No participants were taking any form of hormone therapy at the time or within 6 months of the study and were asked to refrain from caffeine for $\geq$ 12 hours prior to exercise testing. Participants provided written consent to participate in the study after receiving an explanation of the experimental procedures and possible risks associated with participation. This study was approved by the Office for Research Protections at The Pennsylvania State University in agreement with the guidelines set forth by the Declaration of Helsinki.

Hormone Assessment

To further document menopause status, levels of reproductive hormones were measured from ten-hour fasted blood samples (collected between 7:30 and 10:00am) and, in perimenopausal women, between day 3 and day 5 (early follicular phase) of their menstrual cycle. Blood samples were allowed to clot for 30 min at room temperature, and centrifuged at 3000 rpm for 15 min at 4°C. All samples were analyzed by Quest Diagnostics. Estradiol-17β was analyzed using liquid chromatography tandem mass spectrometry. Analytical sensitivity for the estradiol assay is 2 pg mL^{-1}. Follicle stimulating hormone and luteinizing hormone were analyzed using immune-chemiluminometric assay with analytical sensitivity of 0.07 MIU mL^{-1} and 0.7 MIU mL^{-1} for follicle stimulating hormone and luteinizing hormone, respectively.

Body Composition

Whole body composition was assessed using dual x-ray absorptiometry (model QDR 4500W, Hologic, Waltham, MA) while participants were in the supine position. Total body fat and lean (fat-free) mass were measured with standard cut lines. Abdominal adiposity was calculated from manually defined specific regions of interest, specifically from the upper edge of the first lumbar vertebra to the anterior superior iliac spine.

Arterial Stiffness

Women were in the supine position for 10 min prior to measurement of segmental pulse wave velocity (PWV) using a vascular testing device (VP2000, Colin Medical, Kyoto, Japan) that calculates PWV by dividing the distance between two arterial

recording sites by the transit time. Brachial and ankle waveforms were simultaneously measured using plethysmographic sensors and oscillometric pressure sensors located in extremity cuffs. Carotid and femoral arterial pressure waveforms were measured by applanation tonometry sensors manually held in place above the left common carotid artery and left common femoral artery. Carotid-femoral PWV is considered an index of central arterial stiffness, femoral-ankle PWV is an index of peripheral arterial stiffness, and brachial-ankle PWV reflects both central and peripheral arterial stiffness. Carotid-femoral and femoral-ankle PWV were not collected for one perimenopausal woman and one postmenopausal woman due to difficulty obtaining an arterial pressure waveform signal from the femoral artery.

Physical Activity

Daily physical activity was objectively measured using a uniaxial accelerometer (Actigraph model GT1M, Pensacola, FL) that was positioned over the participants' left hip with an adjustable elastic belt. Participants wore the accelerometer for 15 days as part of a daily diary study and were asked to take off the accelerometer during bathing, swimming, and/or sexual activity. Data from four representative days (one day was a weekend day) were used to provide an estimate of daily physical activity levels. The accelerometer collected data in 1 minute epochs and the data was processed and analyzed using the ActiLife data analysis software from Actigraph (version 5.1.5). The following outcome measures were used: total physical activity counts; total energy expenditure; percent time spent in sedentary (0-259 counts), light (260-759 counts), moderate (760-5724 counts), and vigorous (5725+ counts) physical activity computed using the default ActiLife criteria which combine the standard "work-energy theorem"

and the Freedson equation.[22,30,31] Accelerometer data was not collected in one perimenopausal woman due to participant error in following instructions. Also, percent vigorous physical activity data from one postmenopausal woman was removed from the group data since it was an extreme outlier (this subjects value was more than 3 standard deviations above the group mean).

VO_{2max} Testing

Each participant performed a modified Balke treadmill test to maximal effort.[23] This graded test consisted of a two minute warm-up at 2.5 mph followed by adjustment of the speed to elicit 70-75% of age-predicted maximal heart rate after which point the speed remained the same throughout the exercise test. The intensity of exercise increased every 2 minutes by increasing the elevation by 2.5% until the participant reached volitional fatigue. Pulmonary oxygen uptake was measured using indirect calorimetry (Parvomedics, Sandy, Utah). On average, women achieved maximal heart rates >95% age-predicted (perimenopausal: 101 ± 5%, postmenonpausal: 104 ± 8%) and a respiratory exchange ratio >1.09 (perimenopausal: 1.14 ± 0.03, postmenopausal: 1.15 ± 0.04) indicating maximal effort during treadmill exercise. Women were divided into fitness groups based on treadmill maximal oxygen uptake (VO_{2max}) normative percentile values specific for age and sex.[24] Women below the 50^{th} percentile were considered lower fit while women above the 50^{th} percentile were considered higher fit.

Knee Extensor Exercise

On a separate day from the maximal treadmill test, women performed submaximal single leg knee extensor exercise. Women were seated in a semi-reclined

position with knees flexed at an angle of 90º and thighs strapped to the chair to avoid extraneous movement during exercise. The left foot was placed in a medical boot that was connected to the pedal arm of a Monark cycle ergometer that was placed behind the participant. Knee extensions were performed at 40 kicks per minute with the left leg which moved through a nearly full range of motion (90–150º). The exercise protocol consisted of three minute stages. The first stage consisted of quiet rest, followed by knee extensions against no resistance (0 watts), and finally knee extensions as resistance increased by 5 watts every three minutes until the subject could no longer maintain the cadence.

During exercise, heart rate was measured using a modified 3-lead electrocardiogram. Blood pressure (systolic, diastolic, and calculated mean arterial pressure) was measured continuously from a finger on the right hand using a beat-by-beat blood pressure system (Finometer MIDI, Finapress Medical Systems, Netherlands). Heart rate and blood pressure was collected on-line at a sampling frequency of 400 Hz and stored using a data acquisition system (Powerlab, ADInstruments, Castle Hill, Australia). For each subject, heart rate and blood pressure were averaged over the last 30 seconds of each workload during steady-state conditions.

Vascular Responses to Exercise

Diameter and blood flow velocity of the left common femoral artery were measured using Doppler ultrasound (HDI 5000, Philips; Bothell, Washington). Velocity measurements were sampled in real time (400 Hz) using a data acquisition system (Powerlab, AD Instruments; Castle Hill, Australia). Mean blood velocity was calculated from the first 30 seconds during the last minute of each work rate. High-resolution

diameter measurements were taken during the last 30 s of each work rate, images were recorded directly to DVD, and diameter was measured across the cardiac cycle using edge-detection software (Brachial Analyzer Software, Medical Imaging Applications; Iowa City, IA). Femoral artery blood flow for each work rate was calculated by multiplying the cross-sectional area (πr^2) of the femoral artery with mean blood velocity. Femoral vascular conductance was calculated by dividing blood flow into mean arterial blood pressure. Peak femoral vascular conductance was the highest 30 second average achieved during exercise.

Statistical Analysis

Independent sample t-tests were used to test for differences in variables between menopausal groups. Analysis of covariance was used to test for differences between menopausal groups after controlling for age. Pearson correlation coefficients were calculated to estimate the relation between pairs of variables. Partial correlation analysis was performed to test for independent relations after controlling for covariates. Stepwise multiple regression ($P \leq 0.10$ for inclusion and exclusion) was applied to determine the best predictors of arterial stiffness and the femoral vascular conductance responses to exercise. Repeated measures two-way analysis of variance (group x stage) was used to compare blood flow, mean arterial pressure, and femoral vascular conductance within menopausal and fitness groups across work rate. Minitab software (version 15) was used for all statistical analyses. Statistical significance was set at $p \leq 0.05$. All data are expressed as mean ± SD, unless otherwise indicated.

RESULTS

Group Characteristics (Table 1)

Perimenopausal versus Postmenopausal Women

Consistent with the design of this study, estradiol concentrations were lower and FSH and LH concentrations were higher in the early postmenopausal (vs. perimenopausal) group. On average, these postmenopausal women were a few years older and had higher ($p \leq 0.05$) adiposity (BMI, % fat, abdominal fat), cholesterol, low-density lipoprotein (LDL), resting diastolic blood pressure (DBP), and pulse-wave velocities than their perimenopausal counterparts. Early postmenopausal women were also less fit (lower treadmill VO_2max per kg of FFM; table 2) and spent less time ($p \leq 0.05$) in vigorous activity as compared to perimenopausal women.

Early Perimenopausal versus Late Perimenopausal Women

Compared to early perimenopausal women, late perimenopausal women had lower estradiol concentrations and higher FSH and LH concentrations. Late perimenopausal women also had a higher ($p \leq 0.05$) percentage of lymphocytes in their blood as compared to early perimenopausal women. All measures of body composition, cardiovascular risk profile, arterial stiffness, and physical activity levels were similar between early and late perimenopausal women.

Vascular Responses to Exercise

Perimenopausal versus Postmenopausal Women

Femoral blood flow (FBF), mean arterial pressure (MAP), and femoral vascular conductance (FVC) at baseline rest and during all knee extensor exercise work rates were

similar in the combined perimenopausal group as compared to the postmenopausal group (Figure 1). Perimenopausal and postmenopausal women also achieved similar peak work rates during this mode of exercise.

Early Perimenopausal versus Late Perimenopausal Women

Baseline resting measurements of FBF, MAP, and FVC were similar between early and late perimenopausal women as were the peak work rates achieved during graded knee extensor testing. However, perimenopausal status appeared to influence the hemodynamic profile/responses to exercise, both during absolute submaximal levels and at peak exertion. Late perimenopausal women had lower ($p \leq 0.05$) FVC values at 10, 15, and 20 watts as compared to early perimenopausal women (Figure 2). This was primarily a function of the attenuated leg blood flow responses at these submaximal work rates, because MAP responses did not significantly differ between the early and late perimenopausal women. Peak FBF and FVC per watt as well as the change in MAP from baseline to 20 watts were lower ($p \leq 0.05$) in late versus early perimenopausal women. VO_{2max} (ml/kg/FFM/min) was similar between early and late perimenopausal women.

Multiple Regression Models

Based on Pearson product-moment correlation coefficients, it was determined that a measure of cardiorespiratory fitness (VO_{2max} (ml/kg/FFM/min)), a measure of adiposity (percent body fat), a measure of blood lipids (LDL cholesterol), blood pro-inflammatory markers (absolute monocytes and absolute basophils), and concentrations of a reproductive hormone (FSH) were consistently correlated with most or all outcome variables. These correlations were used to determine which variables were entered into

the regression models (Table 3, Table 4). Two multiple regression models were constructed. Model A was constructed for perimenopausal women (n=26) and model B was constructed for the entire sample (*i.e.*, perimenopausal and early postmenopausal women combined; n=37). In order to determine if reproductive hormonal status influenced the hemodynamics, a second test for each model was run to determine if FSH explained variance above and beyond the other independent variables.

Perimenopausal Women

When controlling for fitness, adiposity, blood lipid and inflammatory markers, FSH predicted additional variance in FVC and FBF ($p \leq 0.05$) at the 15 watt workload (a representative absolute submaximal workload). LDL, absolute monocytes, and absolute basophils predicted variance in the slope of MAP ($p \leq 0.05$). When statistically controlling for fitness, adiposity, blood lipid and pro-inflammatory levels, FSH predicted variance in the rise of femoral blood flow ($p \leq 0.05$). Percent body fat predicted variance in the rise of MAP ($p \leq 0.05$).

In Model A, VO_{2max} (ml/kg/FFM/min) predicted variance ($p \leq 0.05$) in peak FVC and FBF. Absolute monocyte counts also explained variance in peak FVC ($p \leq 0.05$). Percent of time spent in vigorous exercise strongly correlated with peak FVC ($R^2 = .66$, $p \leq 0.05$) and peak FBF ($R^2 = .68$, $p \leq 0.05$). No significant variance was explained by this set of independent variables on baseline resting measurements, MAP responses (peak or absolute), or the slope of the FBF response (Table 5).

Perimenopausal and Postmenopausal Women

When controlling for cardiorespiratory fitness, adiposity, blood lipid and pro-inflammatory factors in the entire sample (*i.e.*, Model B), FSH predicted additional unique variance in FVC ($p \leq 0.05$) at the 15 watt work rate. Absolute monocytes also predicted variance in FVC ($p \leq 0.05$) at the 15 watt work rate (a representative submaximal absolute workload) and in the slope and rise of FVC ($p \leq 0.05$).

In Model B, cardiorespiratory fitness predicted variance ($p \leq 0.05$) in peak FVC and FBF. Absolute monocyte levels also explained variance in peak FVC ($p \leq 0.05$). Percent of time spent in vigorous exercise correlated with peak FVC ($R^2 = .66$, $p \leq 0.05$) and peak FBF ($R^2 = .68$, $p \leq 0.05$). No significant variance was explained by the independent variables on baseline resting measurements, MAP measurements, or FBF responses (absolute, rise, or slope of FBF response; Table 6).

TABLES AND FIGURES

TABLE 1. Baseline characteristics of healthy early perimenopausal, late perimenopausal, and early postmenopausal women

Characteristics	**Combined Peri (n=28)**	**Early Peri (n=16)**	**Late Peri (n=12)**	**Early Post (n=12)**
Age (y)	49 ± 3	48 ± 4	50 ± 3	54 ± 3[a,c,d]
Body Composition				
BMI (kg m^2)	23 ± 3	23 ± 3	23 ± 3	25 ± 2[a,c,d]
Body fat (%)	28 ± 6	28 ± 7	29 ± 6	34 ± 5[a,c,d]
Abdominal Fat (g)	1307 ± 543	1228 ± 553	1413 ± 563	1877 ± 803[a,c]
Fat Free Mass (kg)	43 ± 5	43 ± 5	44 ± 6	43 ± 5
Cardiovascular Risk Profile				
Triglycerides (mg dL^{-1})	67 ± 25	67 ± 27	68 ± 21	79 ± 25[c]
Cholesterol (mg dL^{-1})	173 ± 26	167 ± 22	181 ± 28	188 ± 17[a]
LDL (mg dL^{-1})	91 ± 21	86 ± 16	97 ± 24	105 ± 19[a,c]
HDL (mg dL^{-1})	68 ± 15	68 ± 17	70 ± 13	68 ± 18
Resting SBP (mmHg)	112 ± 11	113 ± 10	110 ± 12	118 ± 11[d]
Resting DBP (mmHg)	66 ± 9	66 ± 10	66 ± 9	73 ± 11[a,c,d]
Arterial Stiffness				
Brachial-ankle PWV (cm/s)	1220 ± 135	1224 ± 112	1214 ± 169	1305 ± 102[a,c]
Femoral-ankle PWV (cm/s)	928 ± 87	937 ± 88	917 ± 90	953 ± 76
Hormone Levels				
FSH (mIU/mL)	37 ± 34	11 ± 7	74 ± 20[b]	82 ± 33[a,c]
LH (mIU/mL)	23 ± 20	9 ± 7	41 ± 17[b]	48 ± 20[a,c]
Estradiol (MIU)	107 ± 156	162 ± 188	31 ± 15[b]	25 ± 13[a,c]
Pro-Inflammatory markers				
Neutrophils (%)	59 ± 9	61 ± 10	55.5 ± 8	55 ± 7[c]
Lymphocytes (%)	30 ± 8	27 ± 8	34 ± 7[b]	34 ± 6[c]
Basophils (%)	.4 ± .5	.3 ± .4	.5 ± .5	.4 ± .5
Physical Activity				
Sedentary (%)	83 ± 4	83 ± 5	84 ± 3	84 ± 4
Light (%)	8 ± 2	9 ± 2	8 ± 2	8 ± 2
Moderate (%)	8 ± 2	8 ± 3	8 ± 2	8 ± 3
Vigorous (%)	.4 ± .5	.3 ± .5	.5 ± .6	.06 ± .12[a,c,d]
Counts Per Minute	237 ± 80	229 ± 87	247 ± 74	209 ± 63

Values are mean ± SD. BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse-wave velocity; FFM, fat-free mass; FSH, follicle stimulating hormone; LH, luteinizing hormone.

[a] Peri vs. Post ($p \leq 0.05$), [b] Early Peri vs. Late Peri ($p \leq 0.05$) , [c] Early Peri vs. Post ($p \leq 0.05$), [d] Late Peri vs. Post ($p \leq 0.05$)

FIGURE 1. Femoral blood flow, mean arterial pressure, and femoral vascular conductance response (± SE) to single-leg knee extensor exercise (Rest to Peak Power) in perimenopausal and early postmenopausal women. There were no significant differences between perimenopausal and postmenopausal women (p>0.05).

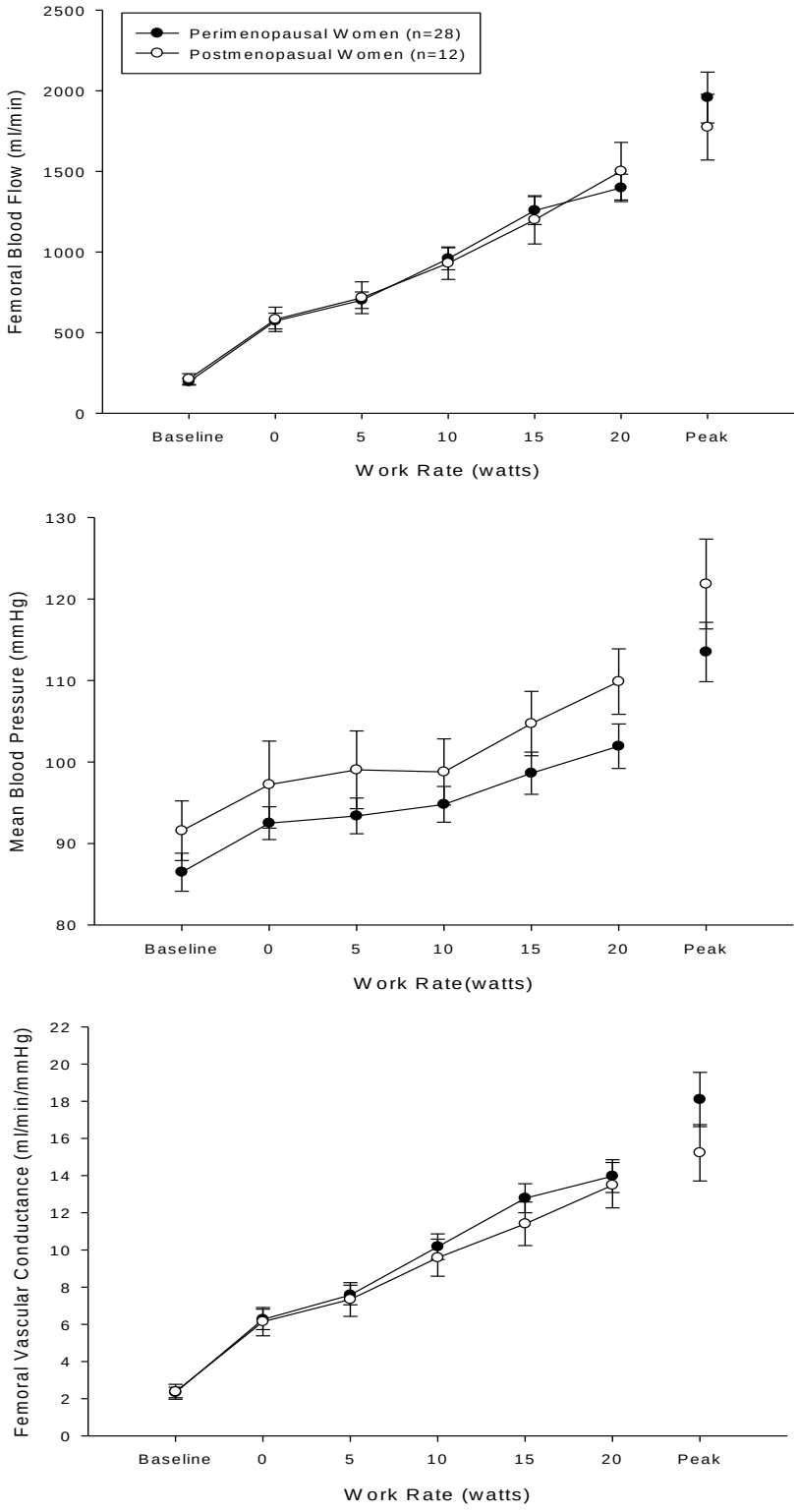

FIGURE 2. Femoral blood flow, mean arterial pressure, and femoral vascular conductance responses (± SE) to single-leg knee extensor exercise (Rest to Peak Power) in early and late perimenopausal women.

* significant difference between early and late group ($p \leq 0.05$).

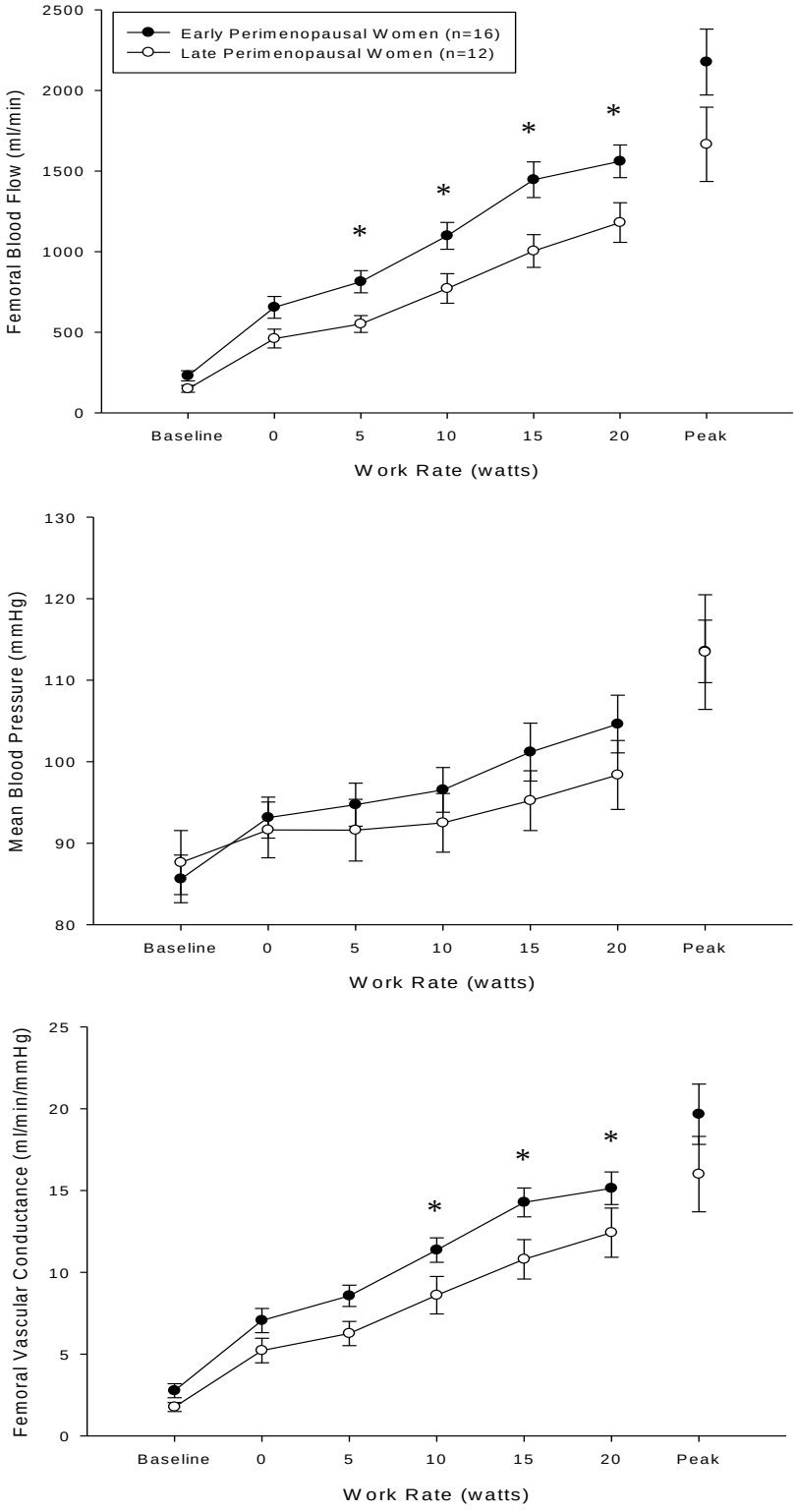

TABLE 2. Peak exercise comparisons of healthy early perimenopausal, late perimenopausal, and early postmenopausal women

Characteristics	**Combined Peri (n=28)**	**Early Peri (n=16)**	**Late Peri (n=12)**	**Early Post (n=12)**
Treadmill Responses				
VO_{2max} (ml kg^{-1} min^{-1})	36 ± 7	36 ± 7	36 ± 6	29 ± 3[a,c,d]
VO_{2max} (ml kg FFM^{-1} min^{-1})	52 ± 7	52 ± 7	51 ± 6	46 ± 3[a,c,d]
Knee Kick Responses				
Watts	32 ± 8	31 ± 8	34 ± 9	28 ± 4[d]
FBF (ml/min)	1957 ± 835	2176 ± 817	1666 ± 799	1774 ± 706
FBF/Watt (ml/min W)	60 ± 19	70 ± 15	47 ± 15[b]	63 ± 27[d]
MAP (mmHg)	113 ± 19	114 ± 15	113 ± 24	122 ± 19
ΔMAP (Baseline - 20 W)	15 ± 14	21 ± 10	11 ± 6[b]	18 ± 14[d]
FVC (ml/min/mmHg)	18 ± 8	20 ± 7	16 ± 8	15 ± 5[c]

Values are mean ± SD. FBF, femoral blood flow; MAP, mean arterial pressure; FVC, femoral vascular conductance; VO_{2max}, maximal oxygen uptake; FFM, fat-free mass.

[a] Peri vs. Post ($p \leq 0.05$), [b] Early Peri vs. Late Peri ($p \leq 0.05$), [c] Early Peri vs. Early Post ($p \leq 0.05$), [d] Late Peri vs. Early Post ($p \leq 0.05$)

TABLE 3. Associations of leg hemodynamic variables with fitness, fatness, and selected blood measurements (all 3 groups; n=40)

Hemodynamics	**VO_{2max} (ml/kg FFM/ min)**	**Body Fat (%)**	**LDL**	**Absolute Basophils**	**FSH**	**Counts Per Minute**	**% in Vigorous**
FBF							
Resting FBF	.19	-.09	-.14	-.05	-.22	.03	.11
FBF at 15W	.41*	-.12	-.31	-.36*	-.41*	.23	.26
Peak FBF	.65*	-.40*	-.42*	-.42*	-.35*	.45*	.57*
Slope (0-20W)	.33*	-.24	-.25	-.24	-.18	.22	.31
MAP							
Resting MAP	-.16	-.02	.22	.22	.35*	.09	-.03
MAP at 15W	-.14	.22	-.02	.14	.11	.04	-.09
Peak MAP	-.17	.18	.02	.08	.21	-.11	-.07
FVC							
Resting FVC	.21	-.04	-.21	-.12	-.28	-.01	.13
FVC at 15W	.50*	-.25	-.34*	-.45*	-.50*	.22	.33*
Peak FVC	.69*	-.48*	-.43*	-.41*	-.38*	.47*	.61*
Slope (0-20W)	.44*	-.44*	-.22	-.24	-.22	.27	.45*

Values are Pearson correlation coefficients. *****Significant correlation (p<0.05)
FBF, femoral blood flow; FVC, femoral vascular conductance; LDL, low-density lipoprotein; FFM, fat-free mass; FSH, follicle stimulating hormone.

TABLE 4. Associations of leg hemodynamic variables with fitness, fatness, and selected blood measurements (perimenopausal women only; n=28)

Hemodynamics	**VO_{2max} (ml/kg FFM/ min)**	**Body Fat (%)**	**LDL**	**Absolute Basophils**	**FSH**	**Counts Per Minute**	**% in Vigorous**
FBF							
Resting FBF	.22	-.03	-.25	.02	-.24	.13	.20
FBF at 15W	.41*	-.09	-.44*	-.28	-.51*	.23	.34
Peak FBF	.73*	-.44*	-.51*	-.41*	-.33	.59*	.68*
Slope (0-20W)	.37	-.33	-.38*	-.19	-.32	.32	.46*
MAP							
Resting MAP	-.06	-.23	.06	.09	.12	.29	.09
MAP at 15W	-.08	.21	-.21	.08	-.26	.13	.02
Peak MAP	-.08	.14	-.16	-.08	.08	.08	.05
FVC							
Resting FVC	.22	.09	-.28	-.06	-.25	.05	.18
FVC at 15W	.46*	-.23	-.39*	-.34	-.43*	.20	.36
Peak FVC	.73*	-.50*	-.45*	-.38*	-.27	.57*	.66*
Slope (0-20W)	.41*	-.52*	-.25	-.19	-.17	.34	.48*

Values are Pearson correlation coefficients. *****Significant correlation (p<0.05)
FBF, femoral blood flow; FVC, femoral vascular conductance; LDL, low-density lipoprotein; FFM, fat-free mass; FSH, follicle stimulating hormone.

TABLE 5. Multiple regression results of model a for early and late perimenopausal women

	Full Model	**VO_{2max} (ml/kg/ FFM/min)**	**% Body Fat**	**LDL**	**Absolute Monocytes**	**Absolute Basophils**	**FSH^**
Peak							
FVC	.000*	.005*	.183	.270	.041*	.558	.072
FBF	.000*	.019*	.404	.058	.444	.125	.078
Absolute (15W)							
FVC	.067	NS	NS	NS	NS	NS	.018*
FBF	.069	NS	NS	NS	NS	NS	.015*
Slope							
FVC	.038*	.323	.051	.955	.142	.545	.257
FBF	.276	NS	NS	NS	NS	NS	.177
MAP	.014*	.062	.122	.016*	.040*	.035*	.355
Rise							
FVC	.034*	.714	.084	.570	.112	.541	.106
FBF	.097	NS	NS	NS	NS	NS	.039*
MAP	.021*	.368	.008*	.053	.303	.076	.113

Values are p-values. FVC, femoral vascular conductance; FBF, femoral blood flow; FSH, follicle stimulating hormone; FFM, fat-free mass; NS, not significant.

^ controlling for VO_{2max}, percent body fat, LDL, absolute monocytes and basophils

* significant p-value ($p \leq 0.05$)

TABLE 6. Multiple regression results of model B for early perimenopausal, late perimenopausal, and early postmenopausal women

	Full Model	**VO_{2max} (ml/kg/ FFM/min)**	**% Body Fat**	**LDL**	**Absolute Monocytes**	**Absolute Basophils**	**FSH^**
Peak							
FVC	.000*	.003*	.285	.373	.049*	.525	.359
FBF	.000*	.014*	.656	.299	.404	.254	.563
Absolute (15W)							
FVC	.004*	.130	.608	.453	.031*	.199	.014*
FBF	.052	NS	NS	NS	NS	NS	.067
Slope							
FVC	.009*	.171	.161	.754	.039*	.792	.577
FBF	.267	NS	NS	NS	NS	NS	.707
MAP	.158	NS	NS	NS	NS	NS	.442
Rise							
FVC	.004*	.459	.181	.845	.012*	.339	.379
FBF	.162	NS	NS	NS	NS	NS	.577
MAP	.110	NS	NS	NS	NS	NS	.205

Values are p-values. FVC, femoral vascular conductance; FBF, femoral blood flow; FSH, follicle stimulating hormone; FFM, fat-free mass; NS, not significant.

^ controlling for VO_{2max}, percent body fat, LDL, absolute monocytes and basophils

* significant p-value ($p \leq 0.05$)

DISCUSSION

Relatively little is known about peripheral circulatory responses to exercise in women undergoing the menopause transition. In the present study, vasodilator responses to acute leg exercise were compared 1) between perimenopausal and early postmenopausal women, and 2) between early perimenopausal and late perimenopausal women. The primary new finding is that perimenopausal status (*i.e.*, early vs. late) appears to influence the magnitude of the increase in blood flow to exercising muscles; this was most clearly demonstrated by significantly attenuated blood flow and conductance responses across most submaximal work rates in the late compared to early perimenopausal group. Perimenopausal influences on submaximal leg exercise hemodynamics were further suggested by the variance in these responses that were explained by FSH, a primary hormonal signal of approaching ovarian failure. Collectively, these findings suggest that approaching ovarian failure in healthy mid-life women attenuates the rise in blood flow to active muscles during exercise.

Menopausal transition status and submaximal exercise hemodynamics

Contrary to our original hypothesis, there were no significant differences in hyperemic or vasodilatory responses to graded knee extensor exercise between the peri- and post-menopausal women of this study; group averages for femoral blood flow and vascular conductance at most submaximal work rates were very similar (Figure 1). The similarity of these leg vascular responses between peri- and post-menopausal women was unexpected considering our previous reports of attenuated leg blood flow and vascular conductance responses in post- menopausal women.[38,46] However, this was likely attributable to the disparate blood flow and conductance responses (*i.e.*, large variability)

observed in the perimenopausal group as a whole, a finding that is discussed further below. The "younger" age of the present group of postmenopausal women (~54 yrs) compared to our prior studies (60 to 70 yrs) might also explain smaller pre- vs. post-menopausal hemodynamic group differences in the present study; such a postulate is less likely, however, given that these early postmenopausal women were on average less fit, had more body fat, and had stiffer arteries than the perimenopausal groups (Table 1), factors which would tend to attenuate rather than preserve exercise hyperemia relative to their peri-menopausal counterparts.

Perimenopausal status and submaximal exercise hemodynamics

While baseline measurements of FBF, mean arterial pressure (MAP), and FVC were similar between early and late perimenopausal women, FBF and FVC were significantly lower at most submaximal work rates in late compared to early perimenopausal women (Figure 2). Stepwise multiple regression analysis indicated that the reproductive hormone FSH was the strongest predictor of FBF and FVC at an absolute submaximal workload (15 watts). This novel finding indicates that menopausal status, particularly between early and late perimenopause, influences leg vasodilatory responses to exercise.

Perimenopausal status and peak exercise hemodynamics

At peak knee extensor work rates, FBF tended to be lower in the late peri (vs. early peri) group ($p=0.055$) and was significantly lower when normalized to group differences in peak watts (70 vs. 47 ml/watt; $p < 0.05$). Similar to other studies in healthy young and healthy older, postmenopausal women, there were correlations between peak systemic aerobic capacity (VO_{2max} (ml/kg FFM/min)) and peak flow capacity of the legs

(Table 3).[38] Additionally, VO_{2max} (ml/kg FFM/min) and physical activity measurements of counts per minute and percent of time spent in vigorous activity strongly correlated with both peak FBF and FVC indicating that habitual activity and aerobic exercise may increase peak hemodynamic responses. These results, plus the high degree of variability observed in the maximal cardiorespiratory response to exercise training among women at mid-life [25], underscore the importance of objectively examining both fitness and habitual activity in studies of vascular structure and function around the time of menopause.

Potential modulatory influences on leg exercise hemodynamics in mid-life women

VO_{2max} (ml kg FFM^{-1} min^{-1}), a body fat-independent measure of cardiorespiratory fitness, positively correlated with FBF and FVC at an absolute workload (15 watts) while LDL and FSH were negatively correlated with these submaximal leg exercise hemodynamics. These findings suggest that fitness, blood lipid levels, and shifts in ovarian function/hormonal status appear to be having a modulatory effect on local hemodynamic responses to exercise. Our multiple regression analysis further indicated that when the influences of cardiorespiratory fitness, pro-inflammatory markers, and other independent variables were held constant in the model, FSH added unique (additional) variance to the prediction of leg exercise hemodynamics at a given submaximal work rate.

The correlation and regression analysis indicated that peak systemic aerobic capacity, VO_{2max} (ml/kg FFM/min), was the strongest predictor of peak FBF and FVC, indicating the role fitness plays in modulating the peak hemodynamic responses in women across mid-life. These results agree with past studies, showing that increased exercise and aerobic capacity often result in increased peak hemodynamic responses[47].

There was some evidence that systemic markers of inflammation (absolute monocytes and basophils) may influence the hemodynamic response even in the present groups of women who do not have overt cardiovascular risk factors (Table 3, 5). Monocytes explained variance in peak FVC and the slope of MAP indicating the possibility that pro-inflammatory mediators may be involved. Further analysis of pro-inflammatory markers in model B (including perimenopausal and early postmenopausal women) showed that monocytes explained significant variance in peak FVC, absolute FVC at 15 watts, the slope of FVC, and the rise of FVC indicating that pro-inflammatory mediators may become more involved as the menopause transition progresses. Additional support for such a postulate comes from studies implicating C-reactive protein (a pro-inflammatory marker) with impaired dilator capacity specifically within skeletal muscle resistance arteries[17] and a recent report linking elevated white blood cell counts with impaired nitric oxide-mediated, endothelium-dependent vasodilator responses in mid-life and older healthy women.[26] Clearly, future research is warranted to investigate possible links between physical activity/inactivity, inflammation, and skeletal muscle vascular function during menopause.

Possible explanations for the influence of menopausal status effects on leg exercise hemodynamics include decreased estrogen concentration and increased inflammation. Estrogen can cause vasodilation of vessels through several different pathways.[36] The significant drop in estrogen that occurs during menopause results in the loss of a potent vasodilator in a woman's circulation. This drop may result in decreased endothelial function leading to decreases in blood flow.

Experimental considerations

There are several factors that influence the interpretation of the present findings and potentially limit the conclusions that can be made. First, the cross-sectional design of this study did not allow for examination of the impact of increasing or decreasing cardiorespiratory fitness or physical activity on peripheral vascular function within the same individuals or as a function of fluctuating hormone levels during the menopause transition.

A second experimental consideration was the limited sample size. Due to the timing-dependent restrictions imposed by menopausal classification and the nature of extensive physiological testing, the sample size was limited to approximately 40 subjects. While this is a relatively low sample size, we found that the subjects, on average, were representative of the larger menopausal transition group. The three subgroups (early peri, late peri, and early post) had similar FSH values when compared with larger, epidemiological studies.[19]

A final consideration concerns how generalizable our results are given the relatively low cardiovascular and metabolic disease risk status of the present participants, which differs markedly from the average menopausal woman in today's society. Additionally, many of these women (~75% of each menopausal group) were experiencing varying menopausal symptoms including hot flashes, sweating, and/or rapid heart rate based on an initial screening questionnaire. We are currently studying vascular responses and arterial stiffness in higher risk menopausal women (including a subgroup comparison of symptomatic vs. asymptomatic women) to examine the modulatory role of fitness/activity and fatness on these outcomes in women with elevated cardiovascular risk.

CONCLUSIONS AND SIGNIFICANCE

Although we observed no overall group differences in vasodilator responses to submaximal leg exercise or peripheral vascular reserve (peak leg exercise conductance) between peri- and early post-menopausal women, there were substantial hemodynamic differences during both submaximal and peak exercise between the perimenopausal sub-groups (early vs. late). Additionally, there was an independent influence of FSH on submaximal hemodynamics during submaximal exercise across menopausal stages. Potential modulatory influences of cardiorespiratory fitness and pro-inflammatory status (monocyte and basophil counts) on peak exercise hemodynamics were also identified. Collectively, these results suggest that ovarian hormone dynamics, cardiorespiratory fitness, and inflammatory status influence the rise in blood flow to the leg muscles during exercise in healthy mid-life women. The associative observations of the current study provide a rationale for conducting exercise training +/- anti-inflammatory supplementation studies to determine if such interventions can counteract the perimenopausal attenuation of blood flow to exercising muscles.

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APPENDIX – INFORMED CONSENT FORM

Informed Consent Form for Biomedical Research
The Pennsylvania State University

TITLE OF STUDY: Psychological and Physiological Effects of Exercise in Menopausal Women

INVESTIGATORS: *Steriani Elavsky, Ph.D.* (Principal Investigator)
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WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH?
You are a woman between the age of 40 and 60 years who shows signs of menopause and has expressed an interest in participating in our research. Our research will examine psychological responses to exercise in women of different menopausal status. We are also looking at how the stages of menopause impact various measures of vascular health and the rise in muscle blood flow that accompanies exercise.

WHO IS CONDUCTING THIS STUDY?
This research is a collaborative study between *Steriani Elavsky, Ph.D.* (Principal investigator) and *David Proctor* (Co-investigator) in the department of Kinesiology at Pennsylvania State University. Members of Dr. Elavsky and Dr. Proctor's research teams will be assisting with this study, as will medical professionals in Penn State's General Clinical Research Center (GCRC).

WHAT IS THE PURPOSE OF THIS STUDY?
There is considerable between-person variability in the occurrence and severity of menopausal symptoms (hot flashes, night sweats, psychological distress, etc.) during menopause. To what extent this variability may be influenced by exercise, both acutely and chronically, remains unclear. The menopausal transition is also a period of accelerated progression of cardiovascular and metabolic disease risk, particularly factors influencing blood pressure (e.g., blood vessel stiffening, changes in body composition). The purpose of this study is to determine how psychological and physiological responses to a single bout of exercise, as well as markers of vascular aging, compare between symptomatic vs. asymptomatic menopausal women.

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST?
This research study will take place in the General Clinical Research Center, Noll Laboratory (Exercise & Vascular Aging Lab), and Recreation Building (Aging & Psychology Lab) at Penn State University. Your part of this study will involve 2 screening visits and as many as 3 study visits. You will be also asked to answer questions on a personal digital assistant (or PDA) on a daily basis for a period of 15 days. The entire study will be completed within approximately 4-6 weeks.

WHAT WILL I BE ASKED TO DO?
A member of the research team will fully explain each procedure that applies to your participation. The study and its procedures are outlined below:

- **Screening visits**
  Visit 1 – Your height and weight will be measured using standard medical equipment along with vital signs (heart rate and blood pressure) at the GCRC. Resting ECG will be monitored for the presence of arrhythmias. Blood samples will be collected from an arm vein to measure cholesterol and hormone levels in your blood, oxygen carrying capacity, and to perform standard blood chemistry tests. (1 hour) _____ **(your initials)**

  Visit 2 – A physical exam will be conducted by a GCRC medical staff to assess your general health status. Following this exam, you will be asked to lie on a padded table for assessment of your body composition using DEXA technology. You may have heard about this test referred to as a "bone scan". You will be scanned on the DEXA machine to assess your bone mass, bone density and body fatness which involves lying still on your back for approximately 10 minutes while the scanner moves above you. Your spine and hip will also be scanned which involves lying still for another 3-4 minutes and placing your legs onto a padded block which keeps your spine close to the scanning table or slightly rotating your left leg ("pigeon toed" position) and securing the leg with Velcro strap to a positioning block to obtain a good view of your hip. (30min) _____ **(your initials)**

  Lastly, you will have ECG electrodes attached to your chest so that heart rate and cardiac rhythm can be monitored by GCRC medical staff during a graded treadmill test. The graded treadmill test will measure your fitness level. For this test, you will be asked to walk/jog on a motorized treadmill as the incline of the treadmill belt is increased every 2-3 minutes (i.e., the exercise will become harder) until you cannot continue. During the test, you wear a nose clip and breathe into a tube to measure the oxygen and carbon dioxide you breathe out. You will also rate how you feel and how hard you are working by using a numbered scale matched to short phrases (rating of perceived exertion or RPE scale). The test is most accurate if you do your best to exercise as long as you can. However, you can stop whenever you want to stop. The test itself is 10-20 minutes long. Additionally, you will be asked to complete a brief battery of psychological measures before and after the test.

  (1 hour 15 min) _____
  (your initials)

  In the event that abnormal test results are obtained during screening, you will be made aware of the results in 7 days and recommended to contact your private medical provider for follow-up.

- **Familiarization visit**
  You will be asked to visit Noll Laboratory where you will be familiarized with an *Actigraph* activity monitor, PDA device (i.e., a small handheld computer the size of a deck of cards), and a *Biolog* monitor for assessing hot flashes should you experience them. The activity monitor, which is a small non-invasive device (the size of a small box of matches), is worn on a belt on your hip at all times except when bathing, showering, swimming, or sexual activity. The monitor does not require any manipulation or operation. The *Biolog* monitor measures sweating response at the sternum (breastbone) located on your chest. If you are currently experiencing symptoms such as hot flashes and night sweats, you will be asked to wear the monitor for 24 hours during the day and overnight at home. The *Biolog* monitor is a light weight, portable device that is attached to the skin on the upper chest using two electrodes

affixed to either side of the sternum. The electrodes are attached to the monitor using two lead wires which can be hidden beneath a woman's clothes. The monitor itself is similar in size to the PDA (but slightly thicker) and it is worn in a small bag around the shoulder. The monitor will be removed upon waking and will be returned to the GCRC or may be picked up by study staff depending on your preference.

(20 min) _____ **(your initials)**

You will then be familiarized with the knee-kick machine that you will be tested on during study visit 2. Briefly, you will be assisted onto a reclined padded chair where your left foot will be strapped into a boot attached to the kicking machine. You will be instructed to extend and relax your upper leg as you kick against minimal resistance at a rate of 40 kicks per minute. (10 min) _____ **(your initials)**

- **Exercise visits**
  Study Visit 1 – Treadmill walking at a moderate intensity (total visit = 1.5 hour)

  The following procedures are associated with this visit:

  - **Psychological Assessment.** You will be asked to complete a battery of questionnaires before treadmill exercise. This assessment will be repeated at the end of the exercise and again 20 and 40 minutes later. Additionally, every minute during exercise you will be asked questions. _____ **(your initials)**

  - **Heart Rate and Blood Pressure.** Heart rate will be measured by Polar heart rate monitors worn around your chest. Resting blood pressure will be measured with an automated machine that requires the placement of a blood pressure cuff on your upper arm (bicep) and a small cuff on your wrist that will periodically inflate on its own to measure pulse pressure. _____ **(your initials)**

  - **Oxygen Uptake.** At rest and during exercise oxygen uptake (VO_2) will be measured using a mouthpiece, nose clip, and a machine that analyzes your expired air for oxygen and carbon dioxide. Measurements will be made at rest while you are seated and intermittently during the exercise test. _____ **(your initials)**

  - **Submaximal Treadmill Exercise.** You will be assisted onto a treadmill where you will be asked to warm-up for 5 minutes at a slow walking speed followed by 20 minutes of exercise at a self-selected pace equivalent to the ratings of 12-16 on the Ratings of Perceived Exertion (RPE) scale. During exercise, measurements of perceived exertion, enjoyment, and feelings state responses will be measured every minute along with heart rate. _____ **(your initials)**

  Study Visit 2 – Vascular function and graded knee kick exercise (total visit = 1.5 hour)

  The following procedures are associated with this visit:

  - **Vascular Health Measurements.** You will lay flat on a bed with blood pressure cuffs on your ankles and arms. Plastic cube-shaped sensors will be placed on your chest, wrists, neck, and upper thigh to measure how fast each pulse of blood travels through your blood vessels. The information collected from this procedure will allow us to

estimate the stiffness of the arteries in your upper and lower body as well as the adequacy of blood flow in your leg arteries.

Following this test, a blood pressure cuff will be placed around your forearm and will be inflated for 5 minutes at a pressure that stops blood flow through your forearm. We will place a Doppler probe (plastic device) on the surface of your skin just above your elbow to measure artery size and the speed of blood in this artery before and after the cuff is deflated. _____ **(your initials)**

- **Knee Kick Exercise.** You will extend your leg in a kicking motion (40 kicks per minute) with your foot strapped into a boot. The intensity of exercise will increase every 3 minutes in a stepwise fashion until you cannot effectively maintain the 40 kick per minute frequency. _____ **(your initials)**

- **Doppler Ultrasound.** The blood flow entering your leg will be measured using an ultrasound machine that produces sound waves to measure blood vessel size and the speed of your blood. This machine will be used when you are at rest, during exercise, and during the vascular health measurement in the forearm.
_____ **(your initials)**

- **Heart Rate and Blood Pressure.** Heart rate will be measured by placing three sticky electrodes on your chest and reading the electrocardiogram (ECG) signal. Blood pressure will be measured with an automated machine that requires the placement of a blood pressure cuff on your upper arm (bicep) and a small cuff on your wrist that will periodically inflate on its own to measure pulse pressure. _____ **(your initials)**

- **Leg Muscle Oxygenation.** Tissue oxygenation during knee kicking exercise will be measured by placing a sticky electrode about the size of a small envelope on your thigh. A near infrared machine will measure levels of oxygen in your contracting thigh muscles by sending an infrared light into the muscle.
_____ **(your initials)**

ARE THERE REASONS WHY I SHOULD <u>NOT</u> TAKE PART IN THIS STUDY?
The following criteria would make you ineligible for this study:

- You are younger than 40 or older than 60 years of age
- You are currently using or have used any form of hormone therapy or hormonal contraception in the last 6 months
- Your body mass index is lower than 18 (underweight) or higher than 40 (significant obesity)
- You regularly use herbal supplements or other alternative therapies directly marketed for reduction of symptoms such as hot flashes
- You have functional limitations that prevent you from being able to walk on a motorized treadmill or extend your knees while kicking against resistance

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?
It is not possible to identify all potential risks associated with these research procedures, but the researcher(s) have taken reasonable safeguards to minimize the known risks associated with this study.

- **Blood Pressure/Vascular Health Measurements.** There is a risk of temporary discomfort at the sites where blood pressure cuffs are inflated (i.e. wrists, ankles, upper arm). The discomfort might be greater the longer the cuffs are inflated. In addition, you may feel a tingling sensation in the fingers or toes while the cuff is inflated; however, this feeling goes away quickly after the cuff is deflated. _____ **(your initials)**

- **Doppler Ultrasound.** There is a minimal risk that the ultrasound probe will irritate your skin.

  _____
  (your initials)

- **Heart Rate.** There is a minimal risk that an allergic reaction could occur from the adhesive on the ECG electrodes.

  _____ **(your initials)**

- **Submaximal Treadmill Exercise.** There is a minimal risk of muscle soreness, muscle strain, temporary discomfort, and falling during walking. There also exists a minor risk of an acute cardiac event (e.g. heart attack).

  _____ **(your initials)**

- **Maximal Graded Treadmill Test.** There is discomfort associated with exercise testing to maximum effort, including temporary muscle fatigue and shortness of breath. These feelings go away very quickly after exercise is stopped. It is possible that you may also experience lightheadedness, chest discomfort, cramping in the legs, irregular heartbeats, and irregular blood pressures during this test. The risk of life-threatening problems (such as a heart attack) is very rare (1 in 2500 tests). Other potential risks, including fainting, nausea, muscle strain, muscle soreness, or falling, will be minimized by proper warm-up, familiarization procedures, and cool-down. GCRC staff will closely watch you throughout exercise and recovery.

  _____ **(your initials)**

- **Knee Kick Exercise.** There is a slight risk of thigh muscle strain and muscle soreness resulting from this exercise. Muscle soreness may be present the following day.

  _____ **(your initials)**

- **Leg Muscle Oxygenation.** There is a slight risk of bruising and tissue numbness/tingling that may be caused by the blood pressure cuff used to calibrate the machine. The seriousness of these risk and discomforts are low since they quickly go away following cuff deflation. _____ **(your initials)**

DEXA Scan. The Dual Energy X-ray Absorptiometry (DEXA) bone density procedure exposes an individual to a small amount of radiation where the X-ray beam crosses the body. This radiation exposure is not necessary for your medical care and is for research purposes only. This protocol calls for a total body scan that may be repeated several times over the course of this protocol. The dose for one total body scan is equivalent to a whole body radiation dose of about 4.5 millirem.

A millirem is a unit of whole-body radiation dose. For comparison purposes, the average person in the United States receives a radiation exposure of 300 mrem per

year from natural background sources, such as from the sun, outer space, and from radioactive materials that are found naturally in the earth's air and soil. 4.5 mrem is less than you would receive from 6 days of natural background radiation. All women of child-bearing age (regardless of sexual activity and contraceptive usage) must also submit to a pregnancy test (urine test) to rule out pregnancy. _____ **(your initials)**

- **Blood Sampling.** The risks involved with taking blood from you include some local pain and bruising where the blood is taken. Well-trained, experienced clinical staff at the GCRC will draw your blood sample. Blood sampling may cause light-headedness and diziness and if this occurs, your symptoms will be alleviated by having you lie flat with your feet raised. As with any procedure involving taking blood, infection is possible. Standard hospital procedures will be used to avoid infections.

  _____

  (your initials)

- **Other Risks.** Study questionnaires may contain questions you may find to be personal or uncomfortable, however, you may skip any questions you wish not to answer. You may also be incovenienced by the burden of daily data collection, including the wear of the activity and the Biolog monitors.

  _____ **(your initials)**

WILL I BENEFIT FROM TAKING PART IN THIS STUDY?
There are no direct benefits for you in participating in this study.

DO I HAVE TO TAKE PART IN THE STUDY?
Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits, clinical care, or treatment to which you are otherwise entitled.

WHAT WILL IT COST ME TO PARTICIPATE?
There is no cost to you for participating except that associated with your transportation to our research facilities at Noll Laboratory.

WHO WILL SEE THE INFORMATION THAT I GIVE?
We will keep private all research records that identify you, to the extent allowed by law. Your information will be combined with information from other participants taking part in the study. When we write about the study to share it with other researchers, we will write about the combined information we have gathered. You will not be identified in these written materials. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records (e.g., such as records maintained by physicians, hospitals, etc.). In the event of any scientific publication resulting from the research, no personally identifiable information will be disclosed.

We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records which will be given a code number. Your name and the associated code number will be stored in different places under lock and key. You should know, however, that there are some circumstances in which we may have to show your information to other people. Penn State's Office for Research Protections, the Biomedical Institutional Review

Board, and the Office for Human Research Protections in the U.S. Dept. of Health and Human Services may review records related to this research study.

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THE STUDY?

You will receive compensation for your time spent in three study visits ($20 per visit for a total of $60). There is no compensation associated with the completion of the screening visits, the daily PDA data collection or wearing of the monitors. However, you will receive copies of results of all screening tests performed for your records.

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH?

Please be aware that in the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against Pennsylvania State University for injury resulting from negligence of the University or its investigators.

WHAT IF I HAVE QUESTIONS?

Please contact *Steriani Elavsky, Ph.D.* (Principal Investigator) at 865-7851 or *David N. Proctor, Ph.D.* at 863-0724 with questions, complaints, or concerns about the study. You can also call this number if you feel this study has harmed you. Questions about your rights as a research participant may be directed to Penn State University's Office for Research Protections at (814) 865-1775. *If you find that any of the questions posed during the research caused psychological feelings of distress beyond normal daily living, please call* 1-800-273-8255 (24-hour, toll free National Suicide Prevention Lifeline) or 814-235-8222 (Central Pennsylvania Community Help Center) or go to Community Help Center (http://www.communityhelpcentre.com) for immediate assistance.

Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 6 pages.

_____ _____

Participant Signature Date

_____

Printed name of person agreeing to take part in the study

_____ _____

Name of person providing information to participant Date

ACADEMIC VITA

Name:

Steven Howard Tucker

Address:

126 Peggy Lane
Johnstown, PA 15904

E-Mail ID:

sht5004

Education:

The Pennsylvania State University (Fall 2007-Spring 2011)
Eberly College of Science
Schreyer Honors College
Premedicine, B.S.
Honors in Kinesiology

Thesis Title:

Menopausal Transition Status and Vascular Responses to Exercise

Thesis Supervisor:

David N. Proctor, Ph.D.

Medical and Research Experience:

Penn State Vascular Aging & Exercise Laboratory under Dr. Proctor - Researcher
University Park, PA Summer 2008 – Spring 2011

- Recruit, educate, and clinically test research subjects with individual ages ranging from 18 to 85
- Conduct and analyze clinical research on blood flow to improve understanding of healthy aging
- Completing undergraduate honors thesis titled Menopause Transition Status and Vascular Responses to Exercise

Conemaugh Health System Mentors in Medicine Program - Intern and Clinical Assistant
Johnstown, PA Summer 2009

- Shadowed, interviewed, and assisted 16 different medical specialists for one week each

Penn State Health Policy Smart Spaces Center under Dr. Scieaj - Researcher
University Park, PA Spring 2009 – Spring 2010

- Researched and analyzed India's healthcare system and the aging population of the country
- Presented findings at Penn State Health Policy meeting and at the *International Symposium on Quality of Life Technology* at Carnegie Mellon University

Penn State College of Medicine Early Admittance Program - Participant
University Park, PA and Hershey, PA Summer 2009 – Spring 2011

- Applied, interviewed, and was accepted to the College of Medicine during my sophomore year in the Eberly College of Science

Penn State College of Medicine Primary Care Scholars Program - Participant
Hershey, PA Summer 2009

- Spent two weeks learning about problems facing our nation's primary care workforce, learning about the different fields within primary care, and shadowing primary care physicians

John P. Murtha Neuroscience and Pain Institute - Research Intern and Clinical Assistant
Johnstown, PA Summer 2008

- Assisted in clinical data collection and analysis of research protocols on Traumatic Brain Injury, Post-Polio Syndrome, Parkinson's Disease, Stroke, and Epilepsy
- Spent time at Hershey Neurological Outpatient Center for comparative analysis of ALS clinic

University of Pittsburgh Heart, Lung, and Esophageal Surgery Institute - Extern
Pittsburgh, PA Spring 2008

- Met with director Dr. Gleason and learned about the research in the thoracic aortic laboratory

Penn State Biochemistry and Molecular Biology Department - Researcher
University Park, PA Fall 2007 – Spring 2008

- Conducted experiments to better understand gene regulation by working with the PAD4 protein with a focus on clone and purification work

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Leadership and Service Experience:

Penn State Eberly College of Science Biology Department – Teaching Assistant
University Park, PA Fall 2010 – Spring 2011

- Instruct and lead biology labs for a class of 25 college students
- Design quizzes and complete all grading accounting for 30% of the students' total course grade

Penn State Eberly College of Science Student Council - Treasurer
University Park, PA Spring 2008 – Spring 2011

- Responsible for a $3000 annual budget used for college-wide events
- Participate in a wide-range of recruitment events for the College of Science

Penn State Eberly College of Science Summer Camps – Curriculum Mentor
University Park, PA Summer 2010, Summer 2011

- Assisted in teaching and promoting science to students in grades 2-12

Penn State Fresh START - Director of Team Leader Training
University Park, PA Fall 2008 – Spring 2010

- Plan and implement Penn State's largest annual day of service for incoming students
- Recruit, select, and train over 100 individuals to lead more than 1,000 volunteers

Academic Committee for Penn State Schreyer Honors College Orientation - Chairman
University Park, PA Summer 2008-2009

- Implemented all academic events for the annual 3-day orientation for freshmen Scholars
- Worked with Honors college faculty and staff to make interactive academic presentations

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Other Activities and Involvement:

Volunteer at Conemaugh Hospital (2006-2010), Schreyer Honors College (SHC) Student Council (2007 - Present), SHC Student Blogger (2007-2008), Adult/Infant/Child CPR and First Aid Certification (2008 - Present), Penn State Summer Special Olympics Volunteer (Summer 2008, 2009), Penn State Urban Service Experience (Fall 2008), SHC Day of Service (Spring 2009), Springfield THON (Spring 2010 – Fall 2011), Mount Nittany Medical Center Shadowing Experiences (Fall 2010 – Spring 2011)

Awards:

Ritchie & Wiedhahn $14000 Academic Excellence Scholarship (2007 - Present), The President's Freshman Award (2008), 'A+' Chem 113 Award (2008), National Society of Collegiate Scholars (2008), Phi Kappa Phi (2009), Golden Key International (2008), President Sparks Award(2009), Alpha Epsilon Delta (2009), Evan Pugh Scholar Junior Award (2010), Schreyer Honors College Summer Research Grant (2010), Ruth E. Duffy Premedicine Endowment (2010), Phi Beta Kappa (2011), Evan Pugh Scholar Senior Award (2011), Eberly College of Science Student Marshal (2011)