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Effects of the Follicular Menstrual Phase on Forearm Vascular Conductance in Abdominal Obese Premenopausal Women During Graded Handgrip Exercise

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Abstract

Purpose: Previous studies have reported a sympatholytic action of estrogen on the vasculature in response to increased sympathetic outflow, an effect most notable during exercise, providing for necessary increases in blood flow to working muscle. In contrast, elevated concentrations of progesterone can inhibit this action of estrogen, impairing increases in blood flow. We hypothesize that the peak concentration of estrogen during the proliferative portion of the follicular phase of the menstrual cycle in female humans will increase vascular conductance during exercise when the effects of progesterone are negligible. In addition, we hypothesize that overweight abdominally obese females will have an attenuated conductance response to dynamic exercise during the same menstrual phase.

Methods: Participants engaged in graded forearm exercise using an isotonic handgrip dynometer with sequential increases in resistance at a cadence of 30 contractions/minute until task failure. They performed exercise at time points of the menstrual cycle corresponding to low concentrations of both sex hormones and elevated estrogen, while progesterone remained low. Blood flow and vascular conductance were measured using Doppler ultrasound.

Results: This revealed a trend that abdominal obese women during a phase of low estrogen had a lower overall blood flow and vascular conductance response than healthy controls at matching resistance stages during rest and exercise. This group difference was attenuated during the proliferative phase with elevated circulating estrogen. There is not a statistically significant interaction between Ovarian Phase and Weight group ($P = 0.778$).

Conclusion: The results indicate that overweight women are at a disadvantage during exercise in increasing blood flow to working muscles, which can be detrimental to overall fitness improvement during the early and potentially late follicular phase of the menstrual cycle.

Keywords: Abdominal obese, Pre-menopausal females, Brachial artery exercise, Blood flow, Menstrual cycle

1 Introduction

Estrogen provides a protective role in maintaining proper endothelial function in pre-menopausal women [28]. During exercise, estrogen can facilitate increased blood flow to working muscles through endothelial-dependent dilation via activation of endothelial nitric oxide synthase (eNOS). This action of estrogen proves to be sympatholytic as it occurs with increased sympathetic outflow via activation of the exercise pressor reflex. In contrast, progesterone can act to block this sympatholytic mechanism of estrogen limiting increases in blood

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flow during exercise [4]. In healthy women, this balance in hemodynamic effects of sex hormones serves to regulate increases in blood flow and vascular conductance. Overweight and abdominal obese conditions, however, correlate to disturbances in the menstrual cycle. These menstrual disturbances could potentially lead to decreases in estrogen production and an increased level of androgens in young women who are overweight [21]. Potentially, the hormonal imbalance could leave overweight women at a disadvantage during exercise due to decreased blood flow and conductance to working muscle.

Excessive body weight in young women can potentially intensify the advancement of peripheral vascular dysfunction and cardiovascular disease similar to that seen in normal aging. Impairment in eNOS activity and blunted production of the vasodilator nitric oxide (NO) has been demonstrated to be the underlying cause of vascular dysfunction with exercise in the elderly with evidence in both the arms and legs [26, 19, 20]). Other characteristics and risk factors that arise from being overweight are analogous to those seen in an aging population, and specifically in conduit artery sheer stress postmenopausal women [24]. Increased oxidative stress and reduced antioxidant capacity, factors seen in both overweight and elderly individuals, impair endothelial function through oxidation of NO and uncoupling of eNOS [15], Hashimoto et al. 1995, Kawano et al. 1995 [3, 30]. Estrogen may have antioxidant effects at the cellular level, protecting young healthy women from dysfunction [16]. However, young overweight women with potentially impaired effects of estrogen could be at a similarly elevated risk as post-menopausal women. [25, 24]. Further investigation is necessary to decipher the potential disruption of normal vascular physiology during exercise in overweight young women statistically associated with lower levels of cardiovascular fitness [13, 14].

The aim of this study is to determine if elevated endogenous estrogen has an altered effect on working muscle vascular conductance in overweight women as compared to healthy control individuals. Limberg et al. [12] concluded there is a facilitating effect of estrogen on increases in vascular conductance with reduced alpha2-aderenergic vasoconstriction during isometric handgrip exercise. However, there was no significant difference between the male group and early follicular phase. The lack of change between the Early Follicular Menses phase and the luteal phase could have been from vascular antagonist effects of the rise in progesterone during this phase, although Limberg et al. unfortunately lacked the plasma measurement of estrogen and progesterone in the study. We hypothesize that the peak concentration of estrogen during the proliferative portion of the follicular phase of the menstrual cycle in female humans will increase vascular conductance during exercise when the effects of progesterone are negligible. In addition, we hypothesize that overweight abdominal obese females will have an attenuated conductance response to graded isotonic handgrip exercise during the same menstrual phase.

2 Methods

2.1 Participants

20 women (11 controls and 9 abdominal obese) aged 25 ± 1.29 years were examined in this study. All participants were volunteers and were non-smokers, sedentary (30 min of moderate exercise less than 4 times a week), and not on any form of birth control as determined by a physical examination form and a personal questionnaire. Participants were screened and separated into two groups based on physical characteristics: healthy sedentary control and sedentary abdominal obese (AO) (having at least two but not ≥ 3 criteria of the NCEP III categorization of the Metabolic Syndrome including a waist circumference > 96 cm) [9]. On study days, participants refrained from caffeine, aspirin, or ibuprofen ingestion 12 h prior to arrival. Participation took place over the course of three separate visits. All participants were recruited locally and gave written informed consent prior to any action taken in the laboratory. A schematic of the study design is presented in Fig. 1. All experimental procedures and protocols conformed to the Declaration of Helsinki and the study was approved by the Human Subjects Committee Institutional Review Board at Eastern New Mexico University.

2.2 Visit 1

Classification of participants to study groups was based on physical characteristics (BMI, body fat percentage, waist/hip ratio, resting blood pressure, fasted blood glucose, triglycerides, total cholesterol, and HDL). Group characteristics are reported in Table 1. All participants were asked to refrain from alcohol, exercise, caffeine, aspirin, and ibuprofen, for at least 12 h prior to all testing. We recorded anthropometric measurements at this physical screening visit with participants arriving fasted 10–12 h prior to arrival. BMI and percent body fat were measured through bioimpedance using an Omron Fat Loss Monitor (HBF-306C). Triglycerides, HDL, total cholesterol, and fasted blood glucose were measured from capillary blood sample obtained through finger prick (CardioChek Portable Blood Test System, PTS Panels, for cholesterol; OneTouch Ultra 2 Blood Glucose Monitoring System for glucose measurement). Two 6 mL venous blood samples were collected in 10.8 mg EDTA-treated 6 mL vacutainers (BD, Franklin Lakes, NJ) and

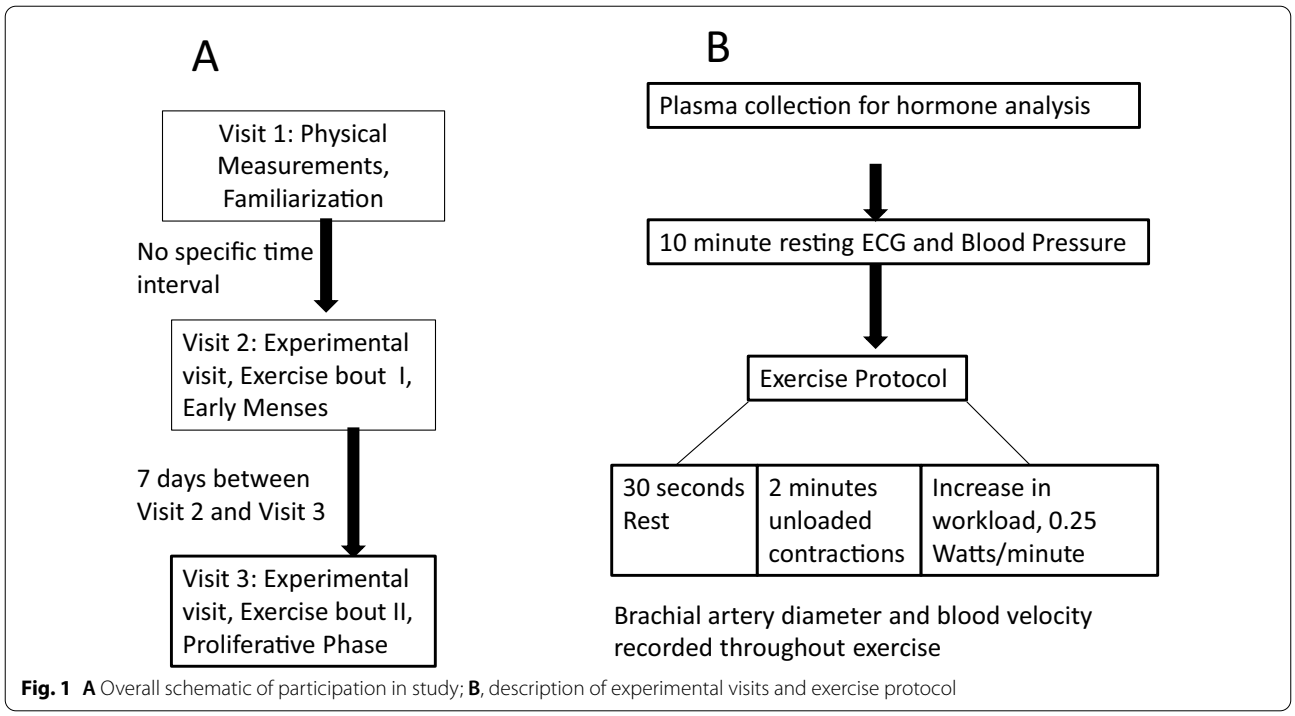

Fig. 1 **A** Overall schematic of participation in study; **B**, description of experimental visits and exercise protocol

Table 1 Physical characteristics of overall experimental groups displayed as averages ± SD; categories statistically different between groups are marked by * (*W:H* waist-to-hip ratio, *BMI* Body Mass Index, *BF%* percent Body Fat)

	Control/healthy (*n* = 11)	**Abdominal obese (*n* = 10)**	***p* value**
Height (cm)	163.98 ± 1.65	169.29 ± 2.71	0.168
Weight (kg) *	*56.53 ± 5.02*	*97.14 ± 5.57*	*< 0.001*
Waist size (cm)	*81.86 ± 6.9*	*113 ± 19.3*	*< 0.001*
W:H*	*0.81 ± 0.01*	*0.96 ± 0.03*	*0.002*
BMI*	*24.1 ± 0.59*	*33.82 ± 1.94*	*< 0.001*
BF%*	*24.47 ± 1.1*	*35.42 ± 1.86*	*0.001*
Fasted glucose (mg/dl)	*91.57 ± 1.71*	*99.9 ± 2.78*	*0.007*
SBP (mmHg)	115 ± 3.98	119.7 ± 4.79	0.586
DBP (mmHg)	73.7 ± 2.23	77.2 ± 3.53	0.493
Total Chol mg/dL	140.0 ± 8.6	148.4 ± 11.2	0.544
HDL Chol (mg/dL)	45.83 ± 3.29	40.9 ± 2.27	0.137
Trig (mg/dL)	97.0 ± 8.4	101.8 ± 19.2	0.841
Menses E2 (pg/mL)	75.55 ± 10.02	51.86 ± 6.37	0.186
Prolif E2 (pg/mL)	301.25 ± 34.58	346.03 ± 40.4	0.609
Menses Prog (pg/mL)	106.65 ± 63.15	155.2 ± 97.17	0.332
Prolif Prog (pg/mL)	141.57 ± 78.32	141.56 ± 97.74	0.915
Menses Prog: E2	1.93 ± 1.68	4.28 ± 4.82	0.39
Prolif Prog: E2	0.74 ± 0.55	0.51 ± 0.53	0.516
Testosterone (pg/mL)	45.6 ± 5.84	42.45 ± 8.55	0.39
oxLDL (pg/mL)	*98.1 + 40.6*	*146.06 ± 62.25*	*0.047*
Forearm volume (ml)	*791.1 ± 13.61*	*1021.43 ± 73.01*	*0.050*

centrifuged, and plasma was aliquoted stored in 1 mL microtubes and stored at − 80 °C for future analysis of 11-keto-testosterone, 17-β-estradiol and Progesterone and Oxidative LDL.

2.3 Visit 2

Upon arrival, participants were asked to provide venous blood samples for measurement of 17-β-Estradiol and Progesterone. This visit was scheduled during self-reported early menses when estrogen and progesterone levels can be expected to be at their lowest menstrual cycle concentrations. Forearm volume was measured using water displacement with participants placing the non-dominant arm into a column of water, the resulting volume of overflow being measured, and volume of the hand being subtracted for pure forearm volume. Participants were placed in a supine position, while three lead ECG was recorded for 10 min on a Biopac MP150 data acquisition unit using AcqKnowledge 4.4 data acquisition software (Biopac Systems Inc., Goleta, CA). Resting blood pressure was recorded after ECG using an Omron Blood Pressure Monitor. Participants remained in the supine position during the entire course of exercise; all participants exercised the non-dominant left forearm. Exercise was completed using a custom-built handgrip dynamic ergometer with participants exercising at a cadence of 30 handgrip contractions per minute. Participants began graded exercise with their non-dominant hand with 2 min of unloaded contraction (0 Watts) followed by a ramp increase in workload of 0.25 Watts (0.5 kg resistance) every minute [6, 9]. Participants were asked to continue exercise until task failure was reached. Task failure was defined as the inability to maintain the proper cadence of 30 contractions per minute. During exercise, blood velocity and vessel diameter were measured in the brachial artery of the exercising left arm using a linear array ultrasound probe operating at 4–7 MHz (Phillips HDI 5000 Ultrasound System, ATL Ultrasound, Bothell, WA). Doppler ultrasound was used for the measurement of blood flow in during in the graded handgrip exercise as prior research groups have determined that the strain-gauge plethysmography methods give an estimate of limb blood flow at rest during the end of exercise, but not for continuous exercise in a graded dynamic exercise setting, because the participant is required to stop contracting during the exercise to measure the limb flow changes. Additionally, as stated by Byström et al., the occlusion pressure required for obtaining the measurement of blood flow using occlusion plethysmography of the forearm causes a 28% reduction in muscle blood flow during graded handgrip exercise and significantly by underestimating the change in flow [2]. Doppler ultrasound with the linear flow probe allows for detection in diameter changes of conduit arteries continuously as well as changes in velocity without halting the graded workloads until task failure. Diameter was defined as the distance between intima layers of the vessel walls in a longitudinal section of the brachial artery. Diameter measurements were assessed during the final 15 s of each set for analysis. Velocity was continuously recorded through Doppler B-mode and a custom-built interface audio transducer unit which processed the high-resolution, angle corrected, intensity weighted Doppler audio information from the ultrasound system into a lower frequency velocity signal (0–20 Hz) (Herr et al. 2010) that could be sampled in real time by AcqKnowledge 4.4 data acquisition software. Post-processing analysis using AcqKnowledge acquisition yielded mean blood velocities calculated as mean of the final 30 s for ever stage [20].

2.4 Visit 3

This visit was scheduled 1 week after visit 2 during the proliferative phase of the menstrual cycle. Similar to visit 2, venous plasma samples were collected again to measure an expected rise in estrogen concentrations and to determine if progesterone had changed significantly. The handgrip exercise protocol of this visit repeated from that executed on the second visit and continuous resting and exercise ECG and blood pressure were also recorded on this visit.

2.5 Artery Diameter and Blood Velocity Analysis

Diameter recordings of the brachial artery collected during the handgrip exercise bouts of visits 2 and 3 were recorded digitally and analyzed post-test using edge detection software (Brachial Analyzer 5.10.11, Medical Imaging Applications, Iowa City, Iowa). Average diameter at each stage of exercise was recorded. Blood velocity recordings from each visit were analyzed by determining the average velocities seen at each stage of exercise. Arterial blood pressure and heart rate were also recorded at each stage of exercise via beat-to-beat finger plethysmography and ECG respectively. Diameter and mean velocity data were used to calculate blood flow to the forearm through the brachial artery at each stage using the formula $Q = \pi\ r^{2*}v$ (ml/min). Recorded beat-to-beat blood pressures were used to calculate mean arterial pressure (1/3 Pulse Pressure + Diastolic Pressure) which was used to calculate forearm vascular conductance ($C = Q/P$; ml/min/mmHg). Forearm volumes were used to correct blood flow and vascular conductance values per 100 mL of forearm volume for each participant. The intra-rater reliability for reproducibility of the Doppler ultrasound measurement was

measured by calculating the coefficient of variation on a control group ($n=10$) of participants. Measurements were taken of the brachial artery diameter and mean arterial velocity at rest on two separate visits by the same imaging technician.

2.6 Biomarker Analysis

Plasma samples from each participant were used to measure oxidative stress in an oxidized LDL (oxLDL) assay (Cell Biolabs, Inc., San Diego, CA). Additionally, 11-keto-testosterone, 17-β-estradiol, and Progesterone was also measured from the plasma samples collected from each participant using associated EIA kits (Caymen Chemical Company, Ann Arbor, MI).; absorbance was read at 412, 420, and 450 nm, respectively.

2.7 Statistical Analysis

Within group statistical variance between time points was assessed with a repeated-measure ANOVA with a Tukey's post hoc analysis comparing percent dilation from rest to 1.5 Watts (3 kg resistance), which was a common end point across participants and groups. Similar analysis assessed differences in flow and conductance corrected for forearm volume, along with mean arterial pressure. An unpaired *T* test was used to compare differences between groups including estrogen, progesterone, and testosterone concentrations. The potential rise in estrogen between these two menstrual phases was calculated as the percent difference; the change from early menses to the proliferative phase over the average of the two concentrations. Variation in the percent difference was assessed by an unpaired *T* test. $P<0.05$ was considered significant. To determine if the change in estrogen between the menses and proliferative time period had an altered effect on working muscle vascular conductance in the AO women as compared to the healthy control a 2×2 mixed ANOVA was compared. Data are resented as means ± SD.

3 Results

3.1 Physical Measurements and Functional Analysis

Comparison of the anthropometric data gathered from each group yielded significant difference between control participants and AO participants. Physical measurements revealed the AO participants had significantly ($P<0.05$) waist circumference, higher body fat percentages (BF%), and body mass indices (BMI) as well as significantly higher fasted blood glucose, weights and waist-to-hip ratios, oxLDL, and Forearm Volume; see Table 1. Measurements also revealed differences in triglycerides, total cholesterol, systolic and diastolic blood pressures, HDL cholesterol, between overweight and control participants. However, these comparisons were not statistically significant. All ECG recordings collected at visit 2 underwent heart rate variability analysis; statistical review of the resulting data yielded no significant results between groups.

3.2 Vascular Responses to Exercise

Percent dilation was calculated as the change from resting diameter to end of the 1.5 Watt stage of exercise divided by resting diameter × 100. The 1.5 Watt stage was determined as the common end point among participants. The overall change in diameter of the brachial artery from rest to 1.5 Watts was not significantly different in either of the experimental groups from early menses to the proliferative phase. This change in dilation was slightly, though not significantly, different between groups (Fig. 2). Percent dilation in the control group was not different during the proliferative phase (12.951% ± 7.4) as compared to that seen in early menses (13.5 ± 6.34). This relationship was not seen in the abdominal obese group. In addition, percent dilation in the AO group during the proliferative phase was greater (12.37 ± 3.52) than that seen in the AO group during early menses phase (10.4 ± 7.86) although not statistically different. (Fig. 2). However, these differences yielded no statistical significance ($p=0.868$ and 0.74, respectively). There was no statistical difference in resting diameter between groups or between time points within the two groups.

Systolic and diastolic blood pressure was assessed throughout the handgrip exercise and used to calculate

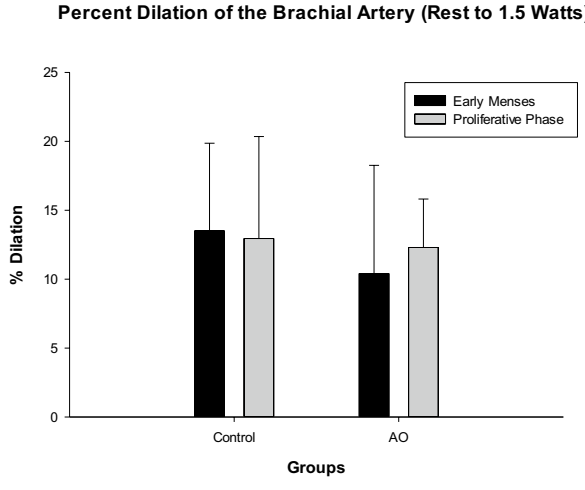

Fig. 2 Percent dilation during exercise within each group, comparing dilatory responses seen during early menses (low estrogen) and during the proliferative phase (high estrogen). Control percent dilation $p=0.868$. Between group comparison at proliferative phase $p=0.433$

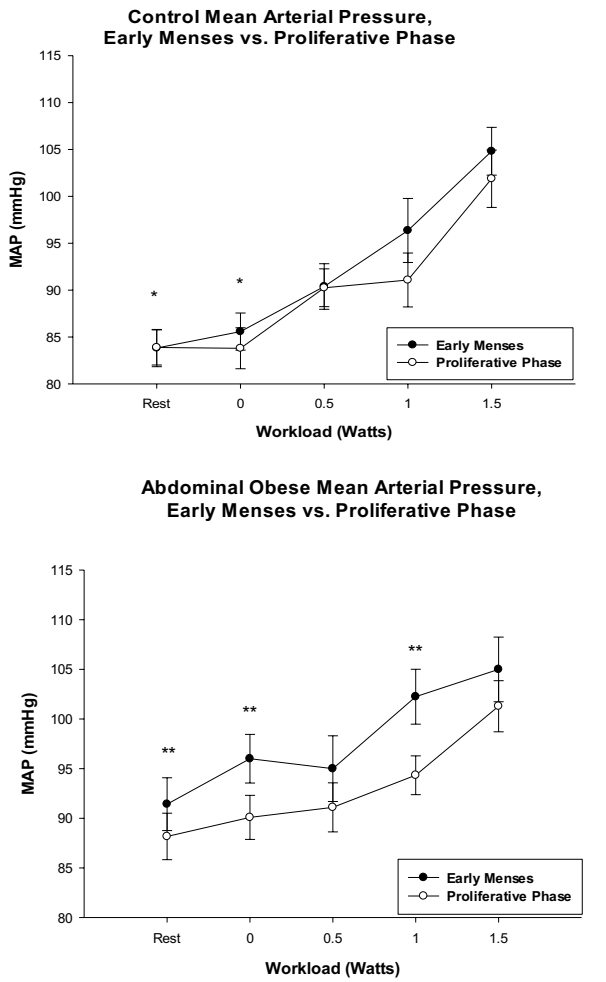

Fig. 3 Mean arterial pressure in control and overweight participants during dynamic handgrip exercise, rest to 1.5 Watts, *indicates a significant difference between the groups during the Early Menses phase ($p < 0.05$). **indicates a significant difference in the AO group between Early Menses and the Proliferative Phase

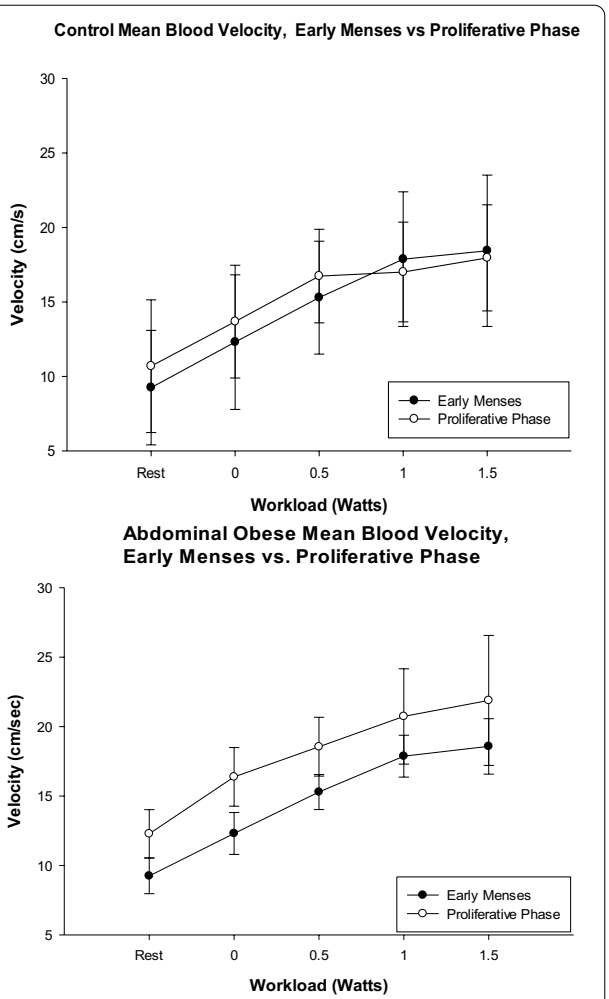

Fig. 4 Mean blood velocity in the brachial artery during isotonic graded handgrip exercise in control and overweight participants, rest to 1.5 watts

mean arterial pressure (MAP). During the Early Menses period, there was a significantly higher MAP in the AO group during rest, unloaded, and 0.5- and 1.0-Watts workloads compared to the Proliferative Phase (Fig. 3). MAP in Control participants from rest to the 1.5 Watts workload stage was not seen to be significantly different between experimental stages of the menstrual cycle, from early menses to the proliferative phase (Fig. 3). When comparing the control to the AO groups, the MAP was significantly lower in the Early Menses period (83.1 ± 7.1 vs 91.4 ± 7.7 mmHg, $p = 0.045$) at rest and the unloaded movement but was no longer significant; during the graded exercise, the MAP differences when estrogen was elevated; during the Proliferative Phase, there was no pressure differences between the groups at any point of the exercise. During the early menses exercise period in the AO group, the mean blood velocity measurements were lower compared with the proliferative phase in the AO participants when although no statistical difference was found at any workload. No difference was seen in blood velocity during exercise in control participants and no statistical differences were present between the groups (Fig. 4).

Vascular conductance of the brachial artery was calculated at each workload during exercise. No significant differences were observed within either group when conductance was compared between experimental menstrual cycle phases. In a comparison of conductance responses during early menses when estrogen concentrations were lowest, AO participants presented with lower vascular

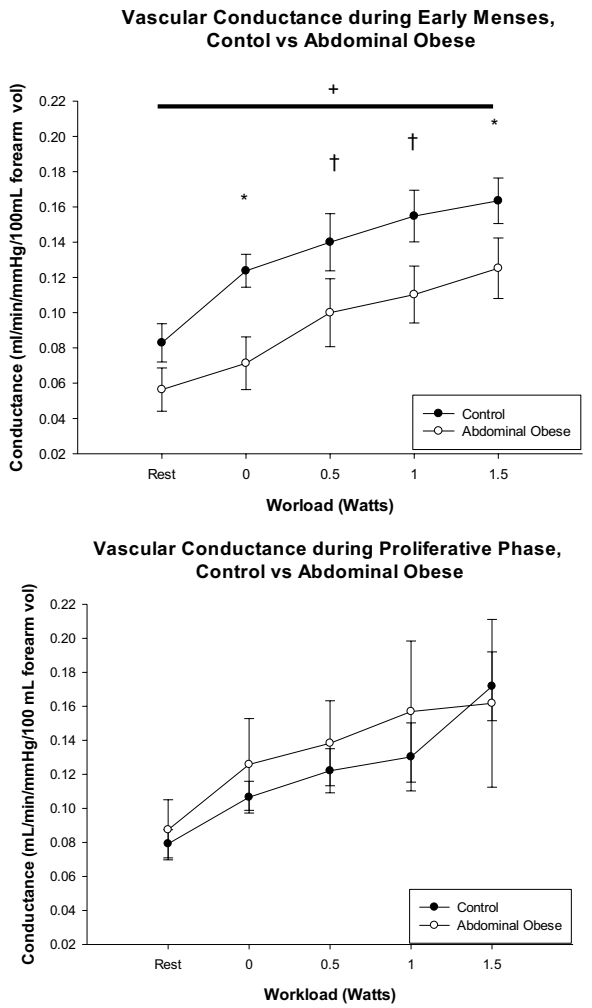

Fig. 5 Vascular conductance of the brachial artery during handgrip exercise compared between groups during early menses, *$p < 0.05$, †$p < 0.1$. During the Early Menses period, the comparison between the groups were significantly different (+, $p = 0.016$) for the delta between rest and peak conductance at 1.5-Watt workload

conductance responses compared to healthy controls all workloads (Fig. 5). The group difference in the high estrogen period of the proliferative phase was attenuated at the AO group presented with an increase in conductance response although not significantly difference from Early Menses and the control group at the same period. When comparison of the delta from rest to the 1.5-Watt workload using a 2×2 ANOVA, the difference in the mean values among the different levels of weight group is greater than would be expected by chance after allowing for effects of differences in ovarian phase. There is a statistically significant difference (+ in Fig. 5, $P = 0.016$). However, there is not a statistically significant interaction between Ovarian Phase and Weight group ($P = 0.778$).

Conductance responses were calculated per 100 mL of forearm volume. Comparison of forearm volumes revealed that AO participants had significantly higher forearm volumes compared to controls (Table 1). The coefficient of variation for participants during a time control for the measures of resting brachial diameter and mean blood velocity using the Doppler ultrasound method were 1.9% and 3.4% respectively.

Plasma samples were assayed, and estrogen concentrations were measured for both early menses and the proliferative phase in both control and AO groups. In both experimental groups, a rise in estrogen was measured in pg/mL from early menses to the proliferative phase. Estrogen concentrations were slightly higher in the control group as compared to the AO group at early menses (control = 67.15 pg/mL ± 13.4; overweight = 51.86 ± 9.005; $p = 0.556$); this relationship was reversed at the proliferative phase as the AO presented higher estrogen concentrations (control = 319.12 ± 40.6; AO = 346.03 ± 70.21; $p = 0.664$), although these concentrations were not significantly different. Progesterone plasma concentrations were also measured and calculated into pg/mL. The concentration from early menses to the proliferative phase in both groups determined no statistically significant change and remained well within the same range (control $p = 0.455$; AO $p = 0.65$). Testosterone concentrations were measured in plasma and compared between groups; no significant difference was seen. There was a significant difference between the measurements of oxLDL (pg/mL) with the AO group having a larger plasma concentration (146.1 ± 62.25 pg/mL) compared to the control group (98.1 ± 40.6 pg/mL) as measured during the early menses stage (Table 1).

4 Discussion

The major finding of the present study is that the overweight abdominal obese pre-menopausal women exhibit a lowered brachial conductance response to dynamic graded small muscle exercise during the early menses of the menstrual cycle follicular phase. This attenuated vascular response was also resolved equal to the age-matched health controls upon increases in Estrogen during the menstrual proliferative phase prior to ovulation. Previous studies have described a potential facilitating effect of estrogen on increases in vascular conductance during exercise [4], 5, 12]. Overall, the data in this study indicate that in young women, normally considered vascularly healthy can present with the characteristics of vascular deficiencies not presumed to be detrimental.

We found the MAP when compared between phases of the menstrual cycle within the control group exhibited no differences in MAP between early menses and

the proliferative phase; however, MAP in the AO women was higher during early menses than that seen during the proliferative phase (Fig. 3). MAP within the AO women during the proliferative phase was returned to levels consistent with those seen in the healthy control group (Fig. 3). This difference in arterial pressure during exercise at the two menstrual cycle time points could potentially serve as the cause for the difference in conductance during early menses in AO women when compared to controls. Vascular function assessed in the forearm of women across the menopausal transition in sedentary women suggests functional lost with the loss ovarian function [16, 17]. However, the ovarian functional changes to the vascular blood flow in sedentary overweight women at various stages of the menstrual cycle have not been demonstrated, although obesity is highly linked to hypertension and sympathetic reflex abnormalities [8, 11]. Additionally, abdominal obesity is highly correlated to higher Pulse Wave Velocity in women which could also indicate stiffer arteries causing elevated blood pressure and diminished flow (van den [27]. The relationship between abdominal obesity and PWV during the menstrual cycle phases was not accessed during this study. Although blood pressures were higher in the early menses phase for the overweight group during the exercise, an alternative approach is needed for the role of central and peripheral arterial stiffness during these different phases of the menstrual cycle in both groups.

The significance difference in vascular conductance seems to be mostly weighted in the changes in MAP and resulting changes in vascular conductance in the AO women. Our results indicate that AO pre-menopausal women have reduced vascular conductance during early menses when estrogen concentrations are lowest, only to return to within a normal healthy range during the proliferative phase when endogenous estrogen is increased. This significant rise in vascular conductance between early menses and the proliferative was not seen in the healthy control group; MAP and vascular conductance remained relatively similar between menstrual phases within this group (Figs. 3, 4, 5). Taking these results into consideration, estrogen could potentially play a more drastic cardiovascular protective role in abdominal obese pre-menopausal women compared to healthy weight pre-menopausal women. However, as seen in Table 1, there was not a significant difference in the overall Estradiol (E2) content between the groups during the Early Menses and Proliferative Phase. Additionally, in the study, analysis using the 2×2 ANOVA comparing the groups by weight, and menstrual cycle phase on the outcomes of vascular conductance indicated these was changes within the groups but not between the groups when comparing the menstrual cycle phase. Although there is an initial increase in Progesterone in the Early Menses Phase of the AO group, there was not a significant difference either between the control groups and overweight at either time point; neither was there a progesterone to estrogen ratio significance difference (Table 1). However, as indicated by Limberg et al., the increase in Progesterone during the follicular phase of the menstrual cycle in healthy controls and overweight abdominal obese women could potentially attenuate the benefits that estrogen may provide for vascular function [12]. The AO group exhibit a reduced conductance during exercise similar to that seen in post-menopausal women, but only during the Early Menses phase of the cycle. This reduced response is only reversed with increases in endogenous estrogen, similar to that seen in post-menopausal women after exogenous estrogen treatment [5]. Removal of estrogen through menopause in overweight women would leave no means of maintaining relative cardiovascular health. Reduced function is expected with aging (Parker et al. 2008),however, this reduced function is already present in young pre-menopausal women. Further endothelial dysfunction could potentially increase with the advancement of associated insulin resistance and diabetes, and worsen beyond even normal aging. As previously demonstrated, sedentary practices also have deleterious effects on vascular function [1], [22, 23, 18] Though exercise-induced shear stress can restore normal function in response to an acute sedentary period and even prevent it [22, 18], such benefits may not be fully realized in young overweight females. The present results indicate an important link between menstrual hormone concentrations and exercise-stimulated blood flow, and the attenuation of this dynamic in the presence of poor metabolic health. A sedentary lifestyle can lead to excess weight gain and metabolic dysfunction, both impairing vascular functions systemically. Though physical activity has the capability of maintaining vascular health, it is evident that the presence of estrogen in females is of equal importance.

This experiment faced some potential limitations such as assessment of neural activity during exercise and comparison of the activity of other hormones. Neural control of the vasculature from the sympathetic nervous system during exercise was not measured in participants. Increased sympathetic activity would increase heart rate and decrease artery diameter during exercise, a response that is abolished by the presence of estrogen [4, 5]. Future investigations should measure concentrations of norepinephrine spillover or Muscle Sympathetic Nerve Activity (MSNA) during exercise to determine if overweight women have higher levels of sympathetic activity compared to healthy controls. This increased sympathetic activity could potentially drive down vascular conductance. Concentrations of 11-keto-testosterone were measured to determine potential hormone

imbalances within the overweight women,however, no imbalance was seen. Future investigations should consider the role of insulin as a growth factor or the activity of other hormones such as leptin on the vasculature. Increased leptin has been associated with hyperinsulinemia, insulin resistance, and obesity [29],these heightened leptin concentrations have led to coronary endothelial dysfunction in rat and dog models [10]. Further investigation should consider metabolic regulation in overweight abdominal obese premenopausal women and the consequences that these conditions have on the vasculature.

The significance of this experiment lays in the observed vascular conductance responses in AO women when compared to controls. Overall results from these measurements were lower in AO women compared to controls. Similar results have been seen in previous studies between premenopausal and post-menopausal women during single leg knee kick [20]. Under these conditions, the premenopausal AO women exhibit vascular conductance responses similar to that seen in post-menopausal older women. Vascular function can only deteriorate further and require pharmacological intervention if no lifestyle changes are made. Our results have uncovered this condition at an early time point where simple changes in lifestyle may be all that is required to improve vascular function. This experiment provides a potential mechanistic target in vascular dysfunction in young overweight women at a time when complications can be rectified.

Abbreviations
AO: Abdominal obese; eNOS: Endothelial nitric oxide synthase; NO: Nitric oxide; HDL: High-density lipoprotein; BMI: Body mass index; BF%: Body fat percentage; ECG: Electrocardiogram; oxLDL: Oxidative low-density lipoprotein; MAP: Mean arterial pressure.

Author contributions
RMR and MAB conceived and designed research; RMR and MAB performed experiments; RMR analyzed data; MAB interpreted results of experiments; RMR and MAB prepared figures; RMR and MAB drafted manuscript; edited and revised manuscript; RMR, KC, and MAB approved final version of manuscript.

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Data availability
The data that support the findings of this study are available from the corresponding author, [M.A.B.], upon reasonable request.

Declarations

Conflict of interests
The authors declare that they have no competing interests.

Consent for publication
Not Applicable.

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References
1. Boyle LJ, Credeur DP, Jenkins NT, Padilla J, Leidy HJ, Thyfault JP, Fadel PJ. Impact of reduced daily physical activity on conduit artery flow-mediated dilation and circulating endothelial microparticles. J Appl Physiol. 2013;115(10):1519–25. https://doi.org/10.1152/japplphysiol.00837.
2. Byström S, Jensen B, Jensen-Urstad M, Lindblad LE, Kilbom A. Ultrasound-Doppler technique for monitoring blood flow in the brachial artery compared with occlusion plethysmography of the forearm. Scand J Clin Lab Invest. 1998;58(7):569–76. https://doi.org/10.1080/00365519850186193.
3. Dinenno FA, Jones PP, Seals DR, Tanaka H. Limb blood flow and vascular conductance are reduced with age in healthy humans: relation to elevations in sympathetic nerve activity and decliSnes in oxygen demand. Circulation. 1999;100(2):164–70. https://doi.org/10.1161/01.cir.100.2.164.
4. Fadel PJ, Zhao W, Thomas GD. Impaired vasomodulation is associated with reduced neuronal nitric oxide synthase in skeletal muscle of ovariectomized rats. J Physiol. 2003;549(Pt 1):243–53. https://doi.org/10.1113/jphysiol.2003.038828.
5. Fadel PJ, Wang Z, Watanabe H, Arbique D, Vongpatanasin W, Thomas GD. Augmented sympathetic vasoconstriction in exercising forearms of postmenopausal women is reversed by oestrogen therapy. J Physiol. 2004;561(Pt 3):893–901. https://doi.org/10.1113/jphysiol.2004.073619.
6. Gonzales JU, Thompson BC, Thistlethwaite JR, Harper AJ, Scheuermann BW. Forearm blood flow follows work rate during submaximal dynamic forearm exercise independent of sex. J Appl Physiol. 2007;103(6):1950–7. https://doi.org/10.1152/japplphysiol.00452.2007.
7. Gonzales JU, Thistlethwaite JR, Thompson BC, Scheuermann BW. Exercise-induced shear stress is associated with changes in plasma von Willebrand factor in older humans. Eur J Appl Physiol. 2009;106(5):779–84. https://doi.org/10.1007/s00421-009-1074-x.
8. Grassi G, Seravalle G, Dell'Oro R, Turri C, Bolla GB, Mancia G. Adrenergic and reflex abnormalities in obesity-related hypertension. Hypertension. 2000;36(4):538–42. https://doi.org/10.1161/01.hyp.36.4.538.
9. Gupta A, Gupta V. Metabolic syndrome: what are the risks for humans? Biosci Trends. 2010;4(5):204–12.
10. Knudson JD, Dincer UD, Zhang C, Swafford AN Jr, Koshida R, Picchi A, Focardi M, Dick GM, Tune JD. Leptin receptors are expressed in coronary arteries, and hyperleptinemia causes significant coronary endothelial dysfunction. Am J Physiol Heart Circ Physiol. 2005;289(1):H48–56. https://doi.org/10.1152/ajpheart.01159.2004.
11. Lambert E, Straznicky N, Eikelis N, Esler M, Dawood T, Masuo K, Schlaich M, Lambert G. Gender differences in sympathetic nervous activity: influence of body mass and blood pressure. J Hypertens. 2007;25(7):1411–9. https://doi.org/10.1097/HJH.0b013e3281053af4.
12. Limberg JK, Eldridge MW, Proctor LT, Sebranek JJ, Schrage WG. Alpha-adrenergic control of blood flow during exercise: effect of sex and menstrual phase. J Appl Physiol. 2010;109(5):1360–8. https://doi.org/10.1152/japplphysiol.00518.2010.
13. Lyerly GW, Sui X, Lavie CJ, Church TS, Hand GA, Blair SN. The association between cardiorespiratory fitness and risk of all-cause mortality among women with impaired fasting glucose or undiagnosed diabetes mellitus. Mayo Clin Proc. 2009;84(9):780–6. https://doi.org/10.1016/S0025-6196(11)60487-4.
14. McGuire KA, Janssen I, Ross R. Ability of physical activity to predict cardiovascular disease beyond commonly evaluated cardiometabolic

risk factors. Am J Cardiol. 2009;104(11):1522–6. https://doi.org/10.1016/j.amjcard.2009.07.023.
15. Moore DJ, Barlow MA, Gonzales JU, McGowan CL, Pawelczyk JA, Proctor DN. Evidence for the emergence of leg sympathetic vasoconstrictor tone with age in healthy women. Physiol Rep. 2015;3(1):e12275. https://doi.org/10.14814/phy2.12275.
16. Moreau KL, Donato AJ, Tanaka H, Jones PP, Gates PE, Seals DR. Basal leg blood flow in healthy women is related to age and hormone replacement therapy status. J Physiol. 2003;547(Pt 1):309–16. https://doi.org/10.1113/jphysiol.2002.032524.
17. Moreau KL, Hildreth KL, Meditz AL, Deane KD, Kohrt WM. Endothelial function is impaired across the stages of the menopause transition in healthy women. J Clin Endocrinol Metab. 2012;97(12):4692–700. https://doi.org/10.1210/jc.2012-2244.
18. Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ. Padilla J Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting. Am J Physiol Heart Circ Physiol. 2016;311(1):H177–82. https://doi.org/10.1152/ajpheart.00297.2016.
19. Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Herr MD, Proctor DN. Sex differences in leg vasodilation during graded knee extensor exercise in young adults. J Appl Physiol. 2007;103(5):1583–91. https://doi.org/10.1152/japplphysiol.00662.2007.
20. Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD. Proctor DN Sex-specific influence of aging on exercising leg blood flow. J Appl Physiol. 2008;104(3):655–64. https://doi.org/10.1152/japplphysiol.01150.2007.
21. Pinola P, Lashen H, Bloigu A, Puukka K, Ulmanen M, Ruokonen A, Martikainen H, Pouta A, Franks S, Hartikainen AL, Järvelin MR, Morin-Papunen L. Menstrual disorders in adolescence: a marker for hyperandrogenaemia and increased metabolic risks in later life? Finnish general population-based birth cohort study. Hum Reprod. 2012;27(11):3279–86. https://doi.org/10.1093/humrep/des309.
22. Restaino RM, Holwerda SW, Credeur DP, Fadel PJ, Padilla J. Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function. Exp Physiol. 2015;100(7):829–38. https://doi.org/10.1113/EP085238.
23. Restaino RM, Walsh LK, Morishima T, Vranish JR, Martinez-Lemus LA, Fadel PJ, Padilla J. Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress. Am J Physiol Heart Circ Physiol. 2016;310(5):H648–53. https://doi.org/10.1152/ajpheart.00943.2015.
24. Somani YB, Moore DJ, Kim DJ, Gonzales JU, Barlow MA, Elavsky S. Proctor DN Retrograde and oscillatory shear increase across the menopause transition. Physiol. Rep. 2019;7(1):e13965. https://doi.org/10.14814/phy2.13965.
25. Tirosh A, Potashnik R, Bashan N, Rudich Oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. J Biol Chem. 1999; 274(15): 10595–10602. https://doi.org/10.1074/jbc.274.15.10595
26. Trinity JD, Wray DW, Witman MA, Layec G, Barrett-O'Keefe Z, Ives SJ, Conklin JD, Reese V, Richardson RS Contribution of nitric oxide to brachial artery vasodilation during progressive handgrip exercise in the elderly. American journal of physiology. Regulatory, integrative, and comparative physiology. 2013; 305(8): R893–R899. https://doi.org/10.1152/ajpregu.00311.2013
27. van den Munckhof I, Holewijn S, de Graaf J, Rutten J. Sex differences in fat distribution influence the association between BMI and arterial stiffness. J Hypertens. 2017;35(6):1219–25. https://doi.org/10.1097/HJH.0000000000001297.
28. Virdis A, Ghiadoni L, Pinto S, Lombardo M, Petraglia F, Gennazzani A, Buralli S, Taddei S, Salvetti A. Mechanisms responsible for endothelial dysfunction associated with acute estrogen deprivation in normotensive women. Circulation. 2000;101(19):2258–63. https://doi.org/10.1161/01.cir.101.19.2258.
29. Widjaja A, Stratton IM, Horn R, Holman RR, Turner R, Brabant G. UKPDS 20: plasma leptin, obesity, and plasma insulin in type 2 diabetic subjects. J Clin Endocrinol Metab. 1997;82(2):654–7. https://doi.org/10.1210/jcem.82.2.3744.
30. Yang YM, Huang A, Kaley G, Sun D. eNOS uncoupling and endothelial dysfunction in aged vessels. Am J Physiol Heart Circ Physiol. 2009;297(5):H1829–36. https://doi.org/10.1152/ajpheart.00230.2009.