Alpha-Adrenergic Control of Blood Flow during Exercise: Effect of Sex and Menstrual Phase

By

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Running Head: Functional Sympatholysis in Men and Women

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Sex differences exist in autonomic control of the cardiovascular system. This study was designed to directly test sex or female menstrual phase-related differences in α-adrenergic control of blood flow during exercise. We hypothesized that women would exhibit reduced α-adrenergic vasoconstriction when compared to men during exercise; in addition, women would constrict less during the early luteal than the early follicular phase of the female menses. Young men (n=10) were studied once and women (n=9) were studied twice; once during the early follicular phase and once during the early luteal phase of female menses. We measured forearm blood flow (Doppler Ultrasound of the brachial artery) during rest and steady-state dynamic exercise (15% and 30% of maximal voluntary contraction; 20 contractions/min). A brachial artery catheter was inserted for the local administration of α-adrenergic agonists [phenylephrine (PE, α₁) or clonidine (CL, α₂)]. Blood flow responses to exercise (forearm vascular conductance, FVC) were similar between all groups. At rest, infusion of PE or CL decreased FVC in all groups (40-60% reduction). Vasoconstriction to PE was abolished in all groups at 15% and 30% exercise intensity. Vasoconstriction to CL was reduced at 15% and abolished at 30% intensity in all groups; women had less CL-induced constriction during the early luteal than early follicular phase (p<0.017, 15% intensity). These results indicate vasodilator responses to forearm exercise are comparable between men and women and are achieved through similar paths of α-adrenergic vascular control at moderate intensities; this control may differ at low intensities, specific to the female menstrual phase.

Key Words: blood flow, exercise vasodilation, functional sympatholysis, sex
Introduction

The understanding of vascular control mechanisms responsible for skeletal muscle blood flow during exercise stems from predominantly male participants. Given the potential for sex-specific differences in physiological control during exercise, these results are limited in application. Evidence from both animal and human studies indicate women demonstrate greater blood flow to exercising muscles when compared to men (19); (25);(28). The prominent role of the sympathetic nervous system in the integrated exercise response (29);(37) provides support that sex-differences in sympathetic control have the potential to influence muscle blood flow responses.

At rest, women exhibit reduced vasoconstrictor responses to sympathetic stimulation in both the forearm and calf when compared to men (18);(16). One potential mechanism may be related to the vasodilatory effect of estrogen and its ability to inhibit α-adrenoceptor binding (32); (33). After acute exposure to increased levels of estrogen, perimenopausal women exhibit blunted norepinephrine (NE)-responsiveness (33). Possible sex differences in sympathetic control during exercise remain unknown. Despite sympathetic activation during exercise, blood flow and oxygen delivery increase proportionally to meet the metabolic demand of the contracting muscle. Muscle contractions and subsequent metabolites reduce responsiveness to NE in the vascular beds of contracting skeletal muscle and blunted sympathetic vasoconstriction is observed – termed functional sympatholysis (26). As such, at higher exercise intensities, NE-mediated vasoconstriction is further limited (26);(1).

Female hormones appear to modulate functional sympatholysis. Specifically, impaired functional sympatholysis (enhanced adrenergic vasoconstriction) has been observed in the contracting muscles of ovariectomized rats (9) and postmenopausal women (8). After treatment with 17β-oestradiol (9) or oestrogen-replacement therapy (8), this impairment was attenuated; that is, adrenergic vasoconstriction was reduced during exercise with the presence of exogenous estrogen. The role of progesterone is less clear; currently the vascular effects of progesterone alone and in combination with estrogen are inconclusive (40)(10)(12, 24).
Given recent reports of blunted α-adrenergic responses in young women compared to men at rest, and greater muscle blood flow during exercise, we hypothesized that functional sympatholysis would be greater in women than men; that is, women would exhibit less α-adrenergic-mediated vasoconstriction than men during exercise.
Additionally, female hormones are associated with reduced sympathetic-mediated vasoconstriction, thus we hypothesized that women would exhibit enhanced sympatholysis during the early luteal phase compared to the early follicular phase of the menstrual cycle, when hormone levels are high. Important to study design, all women studied were naturally cycling and their hormone levels were not artificially controlled by supplements or contraceptives.
Materials and Methods

Subjects
Young healthy men (n=10) and women (n=9) participated in the study. All subjects completed a screening process in which physical activity and personal health history were assessed. All subjects were recreationally active, non-smokers, free from overt cardiovascular disease, and were not taking any cardiovascular medications. Female subjects were excluded if pregnant or using hormonal birth control. Women had a regular menstrual cycle and were studied once during the early follicular (EF) phase and once during the early luteal (EL) phase of the menstrual cycle. For logistical and safety purposes, female subjects completed the EF visit first and at least six weeks separated the two visits. Men visited the lab a single time.

Subjects were instructed to refrain from exercise, aspirin, NSAIDS, alcohol, and caffeine for 24-hours prior to the study day. Written informed consent was obtained from all subjects. All procedures were approved by the Institutional Review Board at the University of Wisconsin – Madison and conformed to the standards set by the Declaration of Helsinki.

Measurements
Weight and height measurements were performed, and BMI was calculated as weight in kilograms divided by height in squared meters (kg.m\(^{-2}\)). Heart rate was monitored continuously by a three-lead ECG. Blood pressure was measured continuously from a pressure transducer connected to the indwelling arterial catheter. Forearm volume was determined using water displacement.

Brachial Artery Catheterization: Subjects laid supine for insertion of the catheter. Under aseptic conditions and after local anesthesia (2% lidocaine), a 20 gauge, 5 cm catheter was placed in the brachial artery of the non-dominant forearm in the antecubital fossa (one male subject was studied in the dominant arm due to an anatomical anomaly in the non-dominant arm). The catheter was used for local administration of vasoactive drugs and for blood sampling (30); (41). The catheter was continuously flushed at 3 mL.hour\(^{-1}\) with heparinized saline.
Blood Sampling: Thirty minutes after catheter placement and prior to exercise or drug infusions, whole blood was drawn from the arterial catheter. Blood samples were centrifuged and plasma was frozen at -20°C. Samples were later analyzed for hormone levels (estradiol, progesterone) using Radioimmunoassay (RIA) and Enzyme Immunoassay (EIA), respectively.

Forearm Blood Flow: Blood flow (artery diameter, blood velocity) was measured with Doppler Ultrasound (Vivid 7, General Electric, USA). A 12 MHz linear array probe was placed approximately midway between the antecubital and axillary regions, medial to the biceps brachii muscle. The ultrasound probe operator continuously adjusted the probe position to maintain a fixed insonation angle of 60 degrees with the sample volume adjusted to cover the width of the brachial artery, compensating for small movements during exercise (21); (30); (31).

Dynamic Forearm Exercise: Each subject laid supine with non-dominant arm extended to the side at approximately 90°. Dynamic and rhythmic, forearm exercise required that participants squeeze and release two handles together 4-5 cm to raise and lower a weight over a pulley at a rate of 20 times per minute (at a duty cycle of 1 second contraction: 2 second relaxation) (21); (41);(30). Functional sympatholysis is graded with the level of exercise intensity; therefore forearm exercise was completed at two workloads. Forearm MVC (maximal voluntary contraction) of the non-dominant arm was determined as the average of the highest two measurements from 5 consistent trials using a hand dynamometer; forearm exercise was completed at 15% and 30% of MVC. This forearm exercise model is identical to that used in several laboratories (31);(41);(17).

Intra-arterial Drug Infusions: All drugs (Phenylephrine, Baxter Healthcare Corporation, Deerfield IL; Clonidine, Xanodyne Pharmaceuticals, Newport KY; Sodium Nitroprusside, Hospira Inc, Lake Forest IL) were infused via the brachial artery catheter and were mixed specifically for each study visit to standard concentrations. Phenylephrine (PE, 0.03125 ug/dL forearm volume/min normalized to blood flow) is a selective α₁-adrenergic agonist. Clonidine (CL, 0.15 ug/dL forearm volume/min normalized to blood flow) is a selective α₂-adrenergic agonist that acts primarily post-junctionally (3) (4). Both were infused to determine post-junctional α-adrenergic vasoconstrictor.
By administering all drugs locally (dose/L forearm volume) we were able to minimize systemic blood pressure changes and activation of other counter-regulatory systems. All drug infusions were also adjusted for the blood flow conditions, on the basis of steady-state forearm blood flow and forearm volume, in an effort to normalize the concentrations of each drug in the blood perfusing the forearm across conditions where blood flow might differ within and between groups (7); (41);(29). As a separate control condition, we assessed the effect of passive vasodilation on α-adrenergic vasoconstrictor responsiveness. To elevate resting forearm blood flow to similar levels observed during exercise, we infused sodium nitroprusside (NTP, 2 ug/dL forearm volume/minute normalized to blood flow) (37). Nitroprusside releases nitric oxide to directly relax vascular smooth muscle.

**Study Protocols**

All testing was performed in the supine position. A total of seven (7) study conditions were randomized and counterbalanced between subjects with a 10-minute rest period after each trial (Figure 1A). NTP was infused as a high-flow control and was followed by PE infusion. For each additional drug infusion (PE, CL), we assessed three (3) levels of exertion: 1) Rest, 2) forearm exercise at 15% of MVC, 3) forearm exercise at 30% of MVC. Each trial was 7 minutes in length, with PE or CL infused during the final 3 minutes (Figure 1B). Beat-to-beat heart rate, blood pressure, and brachial artery blood velocity measurements were obtained throughout each trial. This model is similar to those published previously (7); (17); (29); (37); (41).

**Data acquisition**

Data were collected using similar methods published previously (21). Heart rate (HR) was derived from the electrocardiogram and values were confirmed with blood pressure tracings. Blood flow (FBF) was determined as the product of mean blood velocity (MBV, cm.s⁻¹) and vessel cross sectional area (CSA, radius in cm²) and was reported in mL.min⁻¹ [FBF= (MBV)(CSA)(60 s.min⁻¹)]. Arterial blood velocity was continuously assessed throughout each study condition (except during intermittent artery diameter measurements). Reported pulse-wave velocities were measured beat-to-beat at the last 30 seconds of rest, steady-state exercise, and drug infusion to reduce contraction-to-contraction-induced variability in blood flow (Figure 1B). Diameter measurements
typically resulted in loss of pulse wave signal for 15 seconds. To determine vessel
cross sectional area, artery diameters were taken as an average of five measurements
in late diastole. Arterial diameter was measured on B-mode images in the part of the
artery running perpendicular to the ultrasound beam and was identified by strong wall
signals in the longitudinal section of the artery in each image. All measurements were
obtained from video images taken at rest, after 3 minutes of each condition and after 3
minutes of vasoconstrictor infusion (PE, CL) and were assessed off-line by a well-
trained operator.

A commercial interface unit (Multigon Industries) processed the angle-corrected,
intensity-weighted Doppler audio information from the GE Vivid ultrasound system into a
flow velocity signal via fast Fourier transform (FFT); this method has been validated
previously in our lab by measuring volumetric flow through a tube of known diameter. In
addition, the transfer of Doppler audio signal to PowerLab has been validated (2). This
signal was sampled in real time with signal-processing software (PowerLab,
ADinstruments). All hemodynamic data were digitized, stored on a computer at 400 Hz
and analyzed off-line using PowerLab; post-processing using PowerLab's Chart 5
application package yielded mean blood velocities, blood pressures, and heart rates.

Data Analysis
The primary analysis was to test whether vasoconstriction resulting from infusion of $\alpha$-
adrenergic agonists was different between groups (Men vs EF women vs EL women).
The main dependent variables were vascular conductance (FVC) normalized to forearm
limb volume and the percent changes in FVC after infusion of $\alpha$-adrenergic agonists. To
determine FVC at relative workloads, forearm blood flow (FBF) measurements (mL.min$^{-1}$
1) were normalized for blood pressure and forearm volume under each specific
condition; FVC was reported as mL.min$^{-1}$.100 mmHg$^{-1}$.100mL$^{-1}$. FVC measures are
most appropriate for sex comparisons because women exhibit lower blood pressure and
forearm volume when compared to men (Tables 1 and 2). A change in FVC from rest
was calculated as: [FVC during exercise] – [FVC at rest]. We used percentage
reduction in FVC as our standard index to compare vasoconstrictor responses to
agonists across conditions; this method has emerged as the most appropriate way to
compare vasoconstrictor responsiveness under conditions where marked differences
exist in steady-state blood flow (34);(37);(3). Percent reduction in FVC (% change FVC) after vasoconstrictor administration was calculated as:

\[
\frac{(FVC_{\text{post-vasoconstrictor}}) - (FVC_{\text{pre-vasoconstrictor}})}{FVC_{\text{pre-vasoconstrictor}}} \times 100
\]

Statistics: All statistics were done with the assistance of a biostatistician. Difference between rest/exercise as well as steady-state/drug infusion were assessed for normality (Shapiro-Wilk) and were compared using a student’s t-test approach to determine if the parameter of interest was different from zero within a group (sex/menstrual phase) at each workload. In case of non-normal data the equivalent non parametric version, Wilcoxon signed rank, was used. Groups were compared using ANOVA at each workload to determine the significance of sex/menstrual phase on various parameters of interest. All data are presented as mean±standard error. Significant main effects (p<0.05) were followed by a Bonferroni adjustment for multiple comparisons; thus p-values less than 0.017 were considered significant when making 3 comparisons (Men vs EF Women, Men vs EL Women, EF vs EL Women). All p-values reported were 2-sided; analyses were performed using SAS statistical software version 9.2 (SAS institute Inc. Cary, NC).
Results

Subject Characteristics: Ten men and nine women completed the study. Subject characteristics are summarized in Table 1. There were no significant differences between groups in regard to age and body mass index (p=NS). As expected, men had greater height, weight, MVC, and forearm volume than women (p<0.017). Each female participant was followed for a minimum of 3 months to confirm a regular menstrual cycle before participation (cycle length 28±1 days); early follicular (EF) visits were on day 3±0.3 and early luteal (EL) visits were on day 15±0.8 of the female menstrual cycle.

Blood plasma was collected from each study participant, however the majority of samples were lost in a freezer malfunction. The viable samples were from a subset of women studied (n=3). Each subject exhibited higher plasma [estradiol] and [progesterone] during the EL visit (105±34, 3738±3015 pg/mL, respectively) when compared to the EF visit (36±4, 590±85 pg/mL), although increases were not statistically significant (p=0.06, p=0.18).

Systemic Responses to Exercise: Heart rate was not statistically different between groups at rest; heart rate significantly increased 4-9 beats/min with exercise within groups (see Tables 2 and 3). Brachial artery diameter was greater in men at each condition (rest and exercise) when compared to women during either phase (p<0.017); diameter increased ~0.02 cm in each group with exercise, although changes were not significant for all trials (Tables 2 and 3). Due to differences in blood pressure between men and women (Table 2), all blood flow measures were normalized for perfusion pressure and are reported as FVC. In addition, small but significant changes in arterial blood pressure (3-7 mmHg) occurred between rest and exercise within some trials (Tables 2 and 3).

Systemic Responses to Drug Infusions: Brachial artery diameter, heart rate, and arterial pressure with drug infusion are summarized in Tables 2 and 3. Small but significant within group changes in brachial artery diameter, heart rate, and arterial blood pressure (~5 mmHg) occurred with PE infusion (p<0.05). In addition, small but significant within group changes in heart rate and blood pressure were seen with CL infusion (p<0.05).
Vasodilatory Responses to Exercise: Steady-state FVC (prior to drug infusion) were similar between PE and CL trials (p=NS) and are therefore presented as an average of the two trials (Figure 2). Resting FVC was greater in men than women during the EL phase (p<0.017). Both exercise intensities (15 and 30% MVC) increased blood flow in all groups from rest in an intensity-specific manner. FVC responses to exercise were similar between groups (Figure 2A). When FVC was normalized for differences in resting measures, the change in FVC from resting values was similar between groups (p=NS, Figure 2B).

Alpha₁-Adrenergic Vasoconstriction During Exercise: Percent reductions in FVC after PE infusion are summarized in Figure 3. The vasoconstrictor responses to PE during exercise were significantly blunted when compared to responses during rest (p<0.017). At both intensities, exercise abolished any reduction in FVC due to vasoconstriction with exogenous PE. This response was similar between groups (p=NS).

Alpha₂-Adrenergic Vasoconstriction During Exercise: Percent reductions in FVC after CL infusion are summarized in Figure 4. Infusion of CL decreased FVC by ~60% in all groups at rest. The vasoconstrictor responses to CL during 15% exercise intensity in men and EF women were significantly blunted when compared to responses during rest (p<0.017); this vasoconstrictor response was abolished in women during the EL phase (p<0.017). Exercise abolished any change in FVC during 30% intensity similarly between groups (p=NS).

Vasodilatory Responses to Nitroprusside: NTP infusion increased forearm vascular conductance (Men 58±9, EF 47±5, EL 55±7 mL.min⁻¹.100 mmHg⁻¹.100mL⁻¹) and steady-state levels of FVC were comparable to those seen during exercise at 30% MVC; these values were similar between groups (data not shown, p=NS). Infusion of PE decreased FVC similarly between groups (p=NS; Men -37±7, EF -44±8, EL -36±3%) to a level comparable to those seen with infusion of PE at rest (Men -36±9, EF -45±10, EL -32±9%).
Discussion

Current understanding of mechanisms behind the control of exercise blood flow stems from predominantly male participants. Given the potential for sex-specific differences in physiological control, it is important to determine whether adrenergic control mechanisms are dependent upon sex or menstrual phase. This study directly examined sex and menstrual phase-related differences in adrenergic-mediated vasoconstriction both at rest and during exercise in normally cycling women. The novel findings of this study include: 1) Exogenous $\alpha_1$-adrenergic vasoconstriction is abolished in both men and women at 15% and 30% exercise intensity, regardless of menstrual phase (Figure 3, Table 2); 2) During the early luteal phase of the female menses, women respond to an exogenous $\alpha_2$-adrenergic agonist with less vasoconstriction during exercise at 15% effort (Figure 4, Table 3); and 3) Exogenous $\alpha_2$-adrenergic vasoconstriction is abolished similarly in men and women at 30% exercise intensity, regardless of menstrual phase (Figure 4, Table 3). Findings from this study suggest blood flow responses to forearm exercise are not different between men and women and are achieved through a similar degree of functional sympatholysis at moderate intensities; however, $\alpha_2$-adrenergic vasoconstriction may differ at lower intensities relative to female menstrual phase.

Sex-Specific Differences in Exercise Blood Flow: Recent evidence both supports (19); (25) and refutes (14) the notion that women respond to exercise with greater blood flow to exercising muscle when compared to men. When conductance was expressed in relative units (mL.min$^{-1}$.100 mmHg$^{-1}$.100mL$^{-1}$) at relative workloads (%MVC), no differences in the steady-state exercise responses were observed between men and women (Figure 2B). Similarly, when vascular conductance was expressed in absolute units (mL.min$^{-1}$.100 mmHg$^{-1}$) on a continuum of absolute workloads (kg), no sex-specific differences were observed (data not shown). Results from the current study support the theory that forearm vasodilator response to forearm exercise are similar between men and women, regardless of menstrual phase. Discrepancies with other study results may be due to limb- (36); (27); (19), exercise intensity- (20);(27), or exercise modality-specific differences in vascular control of blood flow (14). Gonzales et al. report similar forearm blood flows between women and men in response to dynamic handgrip
exercises, however exercise was performed at gradually increasing intensities (ramping) to exhaustion (14), making it difficult to compare directly to our moderate steady-state exercise. During single-leg knee extension exercise, Parker et al. found the hyperemic response to exercise was greater in young women compared to men at workloads >40% of maximal effort (25). Subjects in the current study performed forearm exercise at workloads ≤30% of maximal effort; due to the research design, workloads >40% of maximal effort in the arm may be confounded by the development of muscle fatigue, increases in muscle sympathetic nerve activity, or other systemic responses (37). Similarly, performing this complex design in the leg can increase systemic responses in addition to increasing risk to human participants. Taken together, our data clearly exhibit similar exercise vasodilatory responses between men and women at intensities ≤ 30% of MVC. Future invasive studies of this nature will need to address this research question at higher intensities in both limbs.

**Functional Sympatholysis:** The current study locally infused NTP to increase forearm blood flow to levels similar to those observed during exercise (29). We observed no differences in endothelium-independent vasodilation between groups (data not shown). In addition, combined infusion of NTP with an α<sub>1</sub>-adrenergic agonist exhibited ~40% reduction in vascular hemodynamics similarly between groups (data not shown). Using NTP as a measure of high blood flow, these data indicate passive vasodilation does not impact α-adrenergic vasoconstrictor responsiveness in resting muscle. Thus any differences observed in vascular responses to adrenergic infusions during exercise are specific to the working muscle (functional sympatholysis).

In human forearms, the degree of sympatholysis in men has been shown to be similar between α<sub>1</sub>- and α<sub>2</sub>-adrenergic receptor subtypes during low-intensity (10-15% MVC) exercise (29). The current data confirm this finding and extend results both to women and to moderate-intensity exercise; our findings suggest at low- to moderate-intensities exogenous α<sub>1</sub>- (15% and 30% MVC) and α<sub>2</sub>- (30% MVC) adrenergic vasoconstriction in all groups is not only blunted, but abolished (Figures 3 and 4).

Complete elimination of exogenous α-adrenergic vasoconstriction has been previously observed during leg extension exercise in humans (42). However, results in the exercising leg suggest α<sub>2</sub>-adrenoceptors are more sensitive to metabolic inhibition than
α₁-adrenergic receptors (42). Similar results have been observed in animal models; functional sympatholysis at lower workloads may be primarily due to blunting of post-junctional α₂-adrenergic receptor-mediated vasoconstriction and α₁-mediated responses are preserved until heavy exercise (34); (3). Taken together, these observations suggest the functional distribution of α-adrenoceptors is not the same in the arm when compared to leg circulation ((29); (17); current study). Species- and limb-differences in adrenergic receptor density, distribution, and responsiveness may exist. In addition, relative exercise intensities and differences in pharmacological agonists may explain conflicting results.

Sex-Differences in Alpha-Adrenergic Control: At rest, women have been shown to exhibit blunted vasoconstrictor responses to sympathetic stimulation in both the forearm and calf when compared to male subjects (18); (16). This was not observed in the current study; vasoconstrictor responses to adrenergic agonists were similar between groups at rest (p=NS). Discrepancies may be due differences in experimental approach; a strength of our study was the direct assessment of alterations in α-adrenergic vasoconstrictor responses by local infusion of receptor-specific pharmacologic agonists directed at α₁- and α₂-adrenergic receptors. Previous studies have used non-specific pharmacological agonists; (18) or cold pressor test (16) to elicit sympathetic responses. Infusing exogenous NE or tyramine into the brachial artery (29); (18) limits the ability to discriminate between specific α-adrenergic receptors. In addition, NE may also bind β-adrenergic receptors. It has been shown that stimulation of β₂-adrenergic receptors causes greater forearm vasodilation in women at midmenstrual cycle than it does men (18); (15). Therefore, any group differences observed with NE infusion could be due to differences in α-adrenergic vasoconstriction, β₂-adrenergic vasodilation, or both. By directly testing vasoconstrictor responses to specific adrenergic agonists, we clearly present that α-adrenergic vasoconstriction is largely similar in the forearm circulation of men and women. Considering adrenergic responsiveness may not be consistent between rest and exercise (5), it was important to systematically address sex-specific differences that may exist specifically during exercise. Contrary to our hypothesis, responsiveness to α₁-adrenergic agonist, phenylephrine (PE), was similar between men and women both
at rest and during exercise (Figure 3). In addition, our results suggest responsiveness to $\alpha_2$-adrenergic agonist, clonidine (CL), to be similar between men and women (Figure 4).

**Menstrual-Phase Differences in Functional Sympatholysis:** To our knowledge, this is the first study to directly assess differences in vascular responsiveness to $\alpha$-adrenergic stimulation during natural hormone fluxes in the female menstrual cycle (no hormonal therapy or hormone contraceptives were used in these subjects). Impaired functional sympatholysis has been observed in both human (postmenopausal) and animal (ovariectomized) models exhibiting low levels of female hormones; this impairment was attenuated with estrogen therapy (8);(9). In the current study, we observed exogenous $\alpha_1$-adrenergic responses to be similar between groups when studying young women with regular menstrual cycles. In addition, $\alpha_2$-adrenoceptor sympathetic vasoconstrictor responses were blunted during exercise in women during the EL phase when compared to the EF phase, at low exercise intensity (15% MVC). Whereas this altered sympatholysis will not likely affect systemic blood pressure regulation at these low exercise intensities, our data suggest there is a difference in vascular control with changes in the menstrual cycle.

Acute changes in circulating estrogen levels may play a role in the observed difference; potential mechanisms include decreased NE-binding to receptors (32), suppressed receptor expression (44), altered sympathetic innervation (45), and altered gene transcription of vasoactive metabolites (22) with increases in plasma estrogen concentration. Increased estrogen levels have been shown to upregulate nitric oxide that normally opposes sympathetic vasoconstriction. However this idea may be limited given that nitric oxide appears to mediate sympatholysis in rats (35); (9) but is not obligatory in humans (29); (6).

Additionally, it is important to consider women during the early luteal phase of the menstrual cycle will exhibit increased levels of estrogen in addition to rises in progesterone; currently the vascular effects of estrogen and progesterone alone and in combination are inconclusive (40). Previous research suggests the favorable vascular effects of estrogen are attenuated (10) or maintained (12);(24) in the presence of progesterone.
Taken together, our results indicate that menstrual-phase related differences in adrenergic responsiveness are not observed with exogenous $\alpha_1$-adrenergic stimulation and are specific to $\alpha_2$-stimulation. Along these lines, Freedman et al. suggests, at rest, women are less responsive to exogenous $\alpha_2$-stimulation during the luteal phase than the follicular phase of female menses (11). Research in rabbits suggests estrogen will depress $\alpha_2$- but not $\alpha_1$-adrenergic responsiveness, possibly due to a reduced density of $\alpha_2$-receptors (13); this has yet to be shown in humans. In contrast to our results, Freedman et al. also observed greater responsiveness to exogenous $\alpha_1$-stimulation at rest during the luteal phase than the follicular phase (11). The relationship between $\alpha_1$- and $\alpha_2$-adrenergic vasoconstriction appears to be complex and may differ under a variety of hormonal and exercise conditions.

Our findings also suggest women achieve similar vasodilatory responses to exercise (Figure 2B) with lower $\alpha_2$-adrenergic vasoconstriction (Figure 4) during the EL phase when compared to women during the EF phase. However, resting sympathetic nerve activity has been shown to be higher during the EL phase (23), which might offset any reduction observed in $\alpha$-adrenergic vasoconstriction. Thus, acute changes in estrogen in normally cycling women may lead to reduced $\alpha$-adrenergic responsiveness during conditions of higher circulating NE. The combined effect may result in similar FVC measures between menstrual phases. Future research is needed to better understand the effect of estrogen with or without progesterone on adrenergic responsiveness, the role this plays during different phases of a normal female menses, and its influence at higher exercise intensities.

**Experimental Considerations:** Recent evidence in aging men suggests the level of sympatholysis is dependent upon whether responses are compared at relative or absolute workloads (43). In the current study, women exhibited lower MVC than male participants (Table 1), thus at a given relative workload women completed lower absolute work. In a subset of participants we were able to compare a similar absolute workload (~8.5 kg) at different relative intensities (15% for men and 30% for women). Results indicate the level of sympatholysis to be similar between sexes (Figure 5); the lack of sex differences in forearm adrenergic vasoconstriction does not appear to be dependent on whether responsiveness is assessed at relative or absolute intensities in
this population. However, it is important to consider these data are from only a subset of participants; future studies should include multiple exercise intensities in order to assess differences based on intensity, as the appropriateness of measuring blood flow levels at absolute and relative intensities is debatable (43).

A potential limitation of the present study is the lack of plasma catecholamine and hormone measures at rest and during exercise. However, catecholamine measures have been collected previously using a similar study design (41) and responses were similar between men and women during dynamic forearm exercise at 20% MVC. Given the small muscle mass and mild to moderate exercise intensities used in this study have been shown previously to maintain basal sympathetic nerve activity (39); (38), we likely avoided changes in muscle sympathetic nerve activity and activation of other counter-regulatory systems.

We also lacked plasma hormone measure in several subjects, due to sample loss in a freezer malfunction. Thus we cannot explicitly state each female subject exhibited elevated estrogen and/or progesterone. However, we recorded the menstrual cycle length (28±1 days) of each female participant for approximately 3 months before enrollment in the study, we studied women on day 3±0.3 and day 15±0.8 to correspond to EF and EL phases of the female menses, and obtained plasma hormone levels in a subset of women studied. In women studied (n=3), each exhibited higher plasma [estradiol] during EL than EF phase (p=0.06). Taken together, we are confident women were studied during two distinct phases of the menstrual cycle and differences observed in adrenergic responsiveness were likely due to increases in female hormone levels.

Our design does not, however, allow us to differentiate between effects due to estradiol and/or progesterone, as both were elevated. This elevation is similar to that seen during the majority of female menses, and thus holds physiological relevance.

Lastly, we collected blood velocity data throughout exercise+drug infusion, however only the last 30 seconds of data were presented. This decision was made based on assessment of steady-state data collected in our lab and results from previous studies (7); (41); (29). However, if this analysis missed an initial nadir response to an adrenergic agonist, our results may overestimate sympatholysis. Whereas post hoc analysis in a subset of participants does not change the interpretation of our results (data not shown),
researchers should consider a study design that would allow for this type of analysis in the future.

**Conclusion:** We assessed whether sex or menstrual cycle phase alters $\alpha$-adrenergic vasoconstrictor responses in human skeletal muscle during exercise. Our results indicate that sex differences do not exist in forearm blood flow during mild to moderate exercise; however, women exhibit reduced $\alpha_2$-adrenergic vasoconstriction during exercise at 15% effort during the early luteal when compared to the early follicular phase of the female menses. Further, exogenous $\alpha_1$- and $\alpha_2$-adrenergic constriction is abolished during exercise at 30% effort similarly between men and women, regardless of menstrual phase. This suggests blood flow responses to forearm exercise are similar between men and women and are achieved through similar adrenergic vascular control mechanisms at moderate intensities; however, these mechanisms in the forearm may differ at lower intensities, specific to menstrual phase.
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Table and Figure Legends

**TABLE 1:** Subject demographics. (Mean±SEM) *p<0.05

**TABLE 2:** Forearm hemodynamics with phenylephrine (PE) infusion. (Mean±SEM)
Steady-state values before drug infusion (SS), Steady-state values after drug infusion (PE, CL). EF = early follicular, EL = early luteal. * Difference between Men vs Women EF (p<0.017); † Difference between Men vs Women EL (p<0.017); a A significant change from rest to exercise within groups (p<0.05); b A significant change from rest to PE infusion within groups (p<0.05)

**TABLE 3:** Forearm hemodynamics with clonidine (CL) infusion. (Mean±SEM)
Steady-state values before drug infusion (SS), Steady-state values after drug infusion (PE, CL). EF = early follicular, EL = early luteal. * Difference between Men vs Women EF (p<0.017); † Difference between Men vs Women EL (p<0.017); a A significant change from rest to exercise within groups (p<0.05); b A significant change from rest to CL infusion within groups (p<0.05)

**FIGURE 1:** Experimental design.
A) Study protocol: This study required 7 study conditions involving rest, forearm exercise, or NTP infusion. Drug orders were randomized and counterbalanced. Each condition was followed by a 10-minute rest period (R).

B) Breakdown by condition: The NTP trial required NTP infusion for 7 minutes, with PE infused during the final 3 minutes (left). Exertion trials were 7 minutes in length, with PE or CL infused during the final 3 minutes (right). Black boxes (██) signify steady-state measures of blood velocity (followed by vessel diameter, ↓) used in data analysis.

**FIGURE 2:** Forearm vascular hemodynamics at rest and steady-state exercise.
(Mean±SEM) (A) Forearm Vascular Conductance (FVC) in men, EF women, and EL women at 3 levels of exertion (rest, 15% MVC, 30% MVC). FVC was greater in men than women during the early luteal phase at rest. ‡ p<0.017 Men vs Women EL
(B) A Change in FVC from rest in men, EF women, and EL women during forearm exercise at 15% and 30% MVC. Values were similar between groups.

**FIGURE 3: Alpha$_1$–adrenergic vasoconstriction to phenylephrine (PE).** (Mean±SEM) Constriction was calculated as a percent change (%) in FVC from steady-state to post-infusion of PE; the reduction in FVC observed at rest was abolished with exercise at 15 and 30% intensity.

**FIGURE 4: Alpha$_2$–adrenergic vasoconstriction to clonidine (CL).** (Mean±SEM) Constriction was calculated as a percent change (%) in FVC from steady-state to post-infusion of CL; the reduction in FVC observed at rest was abolished with exercise at 30% intensity. Values were different between women during the early follicular and early luteal phases during 15% exercise. † p<0.017 Women-EF vs Women-EL

**FIGURE 5: Adrenergic vasoconstriction at an average absolute workload.** (Mean±SEM) Data are reported at an average absolute workload (Men 8.5±0.5 kg; EF Women 8.5±0.4) in a subset of participants (Men n=4; EF Women n=5). Constriction was calculated as a percent change (%) in Forearm Vascular Conductance (FVC) from steady-state to post-drug infusion. **(A)** %FVC in men and EF women at rest and exercise at ~8.5 kg with infusion of PE. The reduction in FVC was blunted with exercise. **(B)** %FVC in men and EF women at rest and exercise at ~8.5 kg with infusion of CL. The reduction in FVC was blunted with exercise. Values were similar between groups.
Literature Cited


**FIGURE 1: Experimental design.**

**A) Study protocol:** This study required 7 study conditions involving rest, forearm exercise, or NTP infusion. Drug orders were randomized and counterbalanced. Each condition was followed by a 10-minute rest period (R).

**B) Breakdown by condition:** The NTP trial required NTP infusion for 7 minutes, with PE infused during the final 3 minutes (left). Exertion trials were 7 minutes in length, with PE or CL infused during the final 3 minutes (right). Black boxes (□) signify steady-state measures of blood velocity (followed by vessel diameter, ↓) used in data analysis.
FIGURE 2: Forearm vascular hemodynamics at rest and steady-state exercise. (Mean±SEM) (A) Forearm Vascular Conductance (FVC) in men, EF women, and EL women at 3 levels of exertion (rest, 15% MVC, 30% MVC). FVC was greater in men than women during the early luteal phase at rest. ‡ p<0.017 Men vs Women EL (B) A Change in FVC from rest in men, EF women, and EL women during forearm exercise at 15% and 30% MVC. Values were similar between groups.
FIGURE 3: Alpha₁–adrenergic vasoconstriction to phenylephrine (PE). (Mean±SEM) Constriction was calculated as a percent change (%) in FVC from steady-state to post-infusion of PE; the reduction in FVC observed at rest was abolished with exercise at 15 and 30% intensity.
FIGURE 4: Alpha2–adrenergic vasoconstriction to clonidine (CL). (Mean±SEM) Constriction was calculated as a percent change (%) in FVC from steady-state to post-infusion of CL; the reduction in FVC observed at rest was abolished with exercise at 30% intensity. Values were different between women during the early follicular and early luteal phases during 15% exercise. † p<0.017 Women-EF vs Women-EL
FIGURE 5: Adrenergic vasoconstriction at an average absolute workload. (Mean±SEM)
Data are reported at an average absolute workload (Men 8.5±0.5 kg; EF Women 8.5±0.4) in a subset of participants (Men n=4; EF Women n=5). Constriction was calculated as a percent change (%) in Forearm Vascular Conductance (FVC) from steady-state to post-drug infusion. (A) %FVC in men and EF women at rest and exercise at ~8.5 kg with infusion of PE. The reduction in FVC was blunted with exercise. (B) %FVC in men and EF women at rest and exercise at ~8.5 kg with infusion of CL. The reduction in FVC was blunted with exercise. Values were similar between groups.
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**TABLE 1: Subject Demographics.** (Mean±SEM) *p<0.05
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**TABLE 2: Forearm Hemodynamics with Phenylephrine (PE) Infusion.** (Mean±SEM) Steady-state values before drug infusion (SS), steady-state values after drug infusion (PE, CL). EF = early follicular, EL = early luteal. * Difference between Men vs Women EF (p<0.017); † Difference between Men vs Women EL (p<0.017); A significant change from rest to exercise within groups (p<0.05); b A significant change from rest to PE infusion within groups (p<0.05)
<table>
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<td>54±3</td>
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</table>

**TABLE 3: Forearm Hemodynamics with Clonidine (CL) Infusion.** (Mean±SEM) Steady-state values before drug infusion (SS), Steady-state values after drug infusion (PE, CL). EF = early follicular, EL = early luteal. * Difference between Men vs Women EF (p<0.017); † Difference between Men vs Women EL (p<0.017); a A significant change from rest to exercise within groups (p<0.05); b A significant change from rest to CL infusion within groups (p<0.05)