The Influence of Menstrual Cycle and Oral Contraceptive Pill Phases on the Vascular Response to Hypoxia and Sympathetic Activation in Healthy Young Women

A Thesis

presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

_____

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

_____

by

Dain W Jacob

Dr. Jacqueline K Limberg, Thesis Supervisor

July 2021

The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

THE INFLUENCE OF MENSTRUAL CYCLE AND ORAL CONTRACEPTIVE PILL PHASES ON THE VASCULAR RESPONSE TO HYPOXIA AND SYMPATHETIC ACTIVATION IN HEALTHY YOUNG WOMEN

presented by Dain W Jacob,

a candidate for the degree of master of science,

and hereby certify that, in their opinion, it is worthy of acceptance.

_____

Dr. Jacqueline K. Limberg, PhD

_____

Dr. Victoria Vieria-Potter, PhD

_____

Dr. Jaume Padilla, PhD

_____

Dr. Camila Manrique, MD

_____

Dr. Brian Bostick, MD, PhD

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Abbreviation List:

CPT: Cold pressor test.

FBF: Forearm blood flow.

FVC: Forearm vascular conductance.

MSNA: Muscle sympathetic nerve activity.

NC: Naturally cycling.

OCP: Oral contraceptives.

TVC: Total vascular conductance.

ABSTRACT

INTRODUCTION: Hypoxia elicits peripheral vasodilation to preserve blood flow to metabolically active tissue and is accompanied by an increase in muscle sympathetic nervous system activity (MSNA). Prior data predominantly in male subjects, demonstrated the vasoconstrictive effects of MSNA are preserved in the setting of hypoxia. Because women are better able to attenuate or ignore the vasoconstrictive effects of MSNA at rest, we hypothesized women would exhibit greater hypoxic vasodilation (Aim 1). We further hypothesized women would attenuate the vasoconstrictive effects of sympathetic activation during both normoxia (Aim 2) and in the setting of hypoxia (Aim 3). The female sex hormone, estrogen, has been purported to be responsible for mediating the attenuated vasoconstriction to MSNA. As synthetic estrogen found in oral contraceptives is more biologically potent, we further hypothesized women taking oral contraceptives (OCPs) will better attenuate vasoconstriction relative to naturally cycling (NC) women during normoxia and hypoxia and this will be greatest during the high hormone phase of the pill or menstrual cycle. **METHODS:** Ten NC women, (25 ± 1 yrs, 23 ± 1 kg/m^2) and ten women on OCPs, (24 ± 1 yrs, 21 ± 1 kg/m^2) participated (N=20). Women were studied twice, once during the low hormone of the menstrual or pill cycle (menstrual cycle day, NC: 3.1 ± 0.4; pill cycle day: OCP: 5.2 ± 0.4) and once during the high hormone phase of the menstrual or pill cycle (menstrual cycle day, NC: 14.9 ± 0.9; pill cycle day OCP: 16.6 ± 0.6). Heart rate (ECG), blood pressure (finger photoplethysmography), forearm blood flow (venous occlusion plethysmography) and systemic blood flow (Modelflow) were continuously measured across three trials: 1) steady state hypoxia (80% S_pO_2); 2) sympathetic activation via two-minute cold pressor test (CPT); and 3) concomitant steady-state hypoxia and

sympathetic activation via two-minute CPT. Forearm and systemic blood flow were normalized for blood pressure and expressed as forearm vascular conductance (FVC) and total vascular conductance (TVC), respectively. Data are reported from the last one minute of each trial. **RESULTS:** Hypoxia elicited a relative increase in FVC (% change from baseline) that was greater during the high hormone phase of the menstrual or pill cycle compared to the low hormone phase in both NC and women taking OCPs ($p<0.01$). Sympathetic activation via CPT caused paradoxical local vasodilation in women taking OCPs, whereas NC women exhibited local vasoconstriction ($p<0.01$). Concomitant hypoxia and sympathetic activation via CPT resulted in less systemic vasoconstriction compared to normoxia that was not different between groups or hormone phases ($p=0.71$). **CONCLUSION**: The hypoxic vasodilatory response is augmented during the high hormone phase of the menstrual or pill cycle. Exogenous estrogen found in OCPs may cause paradoxical vasodilation to sympathetic activation, whereas sympathetic activation in NC women with endogenous hormones elicits vasoconstriction. We observed a hypoxia-mediated attenuation of the systemic vasoconstrictor response to sympathetic activation in both NC women and women taking OCPs across all hormone phases. These data demonstrate the vascular response to autonomic and environmental stressors are influenced by female sex hormones and menstrual/pill cycle phase.

CHAPTER 1: INTRODUCTION

Sex-related differences have been observed in the presentation and incidence of hypoxia-related disease states such as sleep apnea (63, 66). Furthermore, the incidence of hypertension and cardiovascular disease in such conditions differs by sex (12). Although mechanistic data are lacking, sex-related differences in cardiovascular disease risk may be attributed to the cardio-protective nature of the female sex hormones, estradiol and the loss of this effect following menopause (12). Data from healthy humans support the idea that neurovascular mechanisms integral to blood pressure control may differ between men and women (8, 11, 37); however, the majority of work has been conducted in the healthy, rested state and is not representative of physiological stressors observed in both everyday life and disease. The majority of work examining sex differences in vascular control are conducted in young, premenopausal women during the early follicular phase of the menstrual cycle – when endogenous sex hormones (e.g., estradiol, progesterone) are relatively low. This small hormonal window in which women are typically studied fundamentally limits physiologic understanding and practical translation to all women. Moreover, hormone phase and the role for endogenous (natural menstrual cycle) versus exogenous (oral hormonal contraceptive) hormones on mechanisms governing blood pressure and the vascular response to physiological stress remain unclear. Understanding the varied role of exogenous and endogenous hormones in modulating vascular responsiveness to stress is critical to enhance our understanding of mechanisms contributing to sex differences in disease incidence, and ultimately provide women with better sex-specific healthcare. Therefore, the goal of this thesis is to enhance our understanding of vascular control in the context of common physiological stressors, hypoxia and sympathetic nervous system

activation. We sought to determine how hormone source, as well as phase, influences the cardiovascular responses to physiological stimuli commonly experienced in disease states such as sleep apnea and hypertension. To do this, we examined local and systemic vascular response to hypoxia, sympathetic activation, and concomitant hypoxia and sympathetic activation in young healthy women. We studied women currently prescribed and taking oral contraceptives (OCPs) twice, during both the placebo and active pill phase. We further compared these results to data collected in women with natural menstrual cycles (NC) that were also studied twice, during both the early and late follicular phases. Therefore, we were able to examine both the effects of endogenous versus exogenous hormones, as well as high and low circulating hormone concentrations on vascular responsiveness to environmental stressors, respectively. The present investigation was framed in the context of the following specific aims and testable hypotheses:

AIM 1. Examine the effect of menstrual cycle phase and oral hormonal contraceptive pill phase on the vascular response to systemic hypoxia. *Hypotheses:* **Aim 1A.** Both NC women and women taking OCPs will exhibit greater local (forearm) and systemic hypoxic vasodilation during the high hormone phase when compared to the low hormone phase of the menstrual or pill cycle, respectively **Aim 1B.** Hypoxic vasodilation in the local and systemic circulations of healthy young women will be greater in women taking OCPs compared to NC women.

AIM 2. Examine the effect of menstrual cycle phase and oral hormonal contraceptive pill phase on the vascular response to sympathetic activation. *Hypotheses***: Aim 2A.** Both NC women and women taking OCPs will exhibit less

2

sympathetically-mediated local and systemic vasoconstriction in response to a cold pressor test during the high hormone phase compared to the low hormone phase of the menstrual or pill cycle, respectively. **Aim 2B.** Acute sympathetic activation (cold pressor test) will elicit local and systemic vasoconstriction that will be greater in NC women compared to women taking OCPs.

AIM 3. Examine the effect of a natural menstrual cycle and oral contraceptive pill on the vascular response to concomitant hypoxia and sympathetic activation. *Hypotheses***: Aim 3A.** Sympathetic activation will elicit local and systemic vasoconstriction during normoxia that will be attenuated during hypoxia. **Aim 3B.** The local and systemic vasoconstriction observed during normoxia will have more attenuation during hypoxia in women taking OCPs compared to NC women.

Results from the present investigation will advance our understanding of how circulating concentrations (hormone phase) and molecular formulation (endogenous, exogenous) of female sex hormones influence vascular control during environmental stress (i.e., hypoxia, sympathetic activation).

CHAPTER 2: LITERATURE REVIEW

Introduction

Cardiovascular disease is the most common cause of death worldwide, of which hypertension is a major risk factor (1, 2). Healthy premenopausal women exhibit lower blood pressure compared to age-matched healthy men, as well as lesser incidence and severity of hypertension (3, 12). Thus, it may not be surprising that mechanisms of blood pressure regulation have shown to differ between men and women (8, 12, 89). Interestingly, hypertension and cardiovascular disease incidence steeply increases following menopause (25), suggesting a critical role for the sex hormone estradiol as a cardioprotective modulator of blood pressure.

Systemic arterial blood pressure is the product of cardiac output and peripheral resistance to blood flow. The ability to increase or decrease total peripheral resistance plays a key role in blood pressure regulation when cardiac output is held constant (52). Resistance to flow is determined primarily by vascular tone or the diameter of a blood vessel at a given time. The tone of microvasculature or small blood vessels found in skeletal muscle are thus a main determinant of systemic blood pressure (52). As sex hormones play a role in the modulation of blood pressure, it is reasonable to suspect they influence the microvasculature as well.

A primary determinant of vascular tone, and thus blood pressure, is the autonomic nervous system. The autonomic nervous system is essential for beat-to-beat blood pressure regulation and ultimately maintenance of blood flow and oxygen delivery to the peripheral tissues. For example, a fall in arterial oxygen (i.e., hypoxia) is known to stimulate peripheral vasodilation in order to ensure oxygen delivery is preserved (44). Without a

reflex increase in the activity of the sympathetic (a branch of the autonomic) nervous system during systemic hypoxemia, blood pressure would fall and lead to hypotension and syncope (45). Thus, a balance between local vasodilation and sympathetically-mediated vasoconstriction is essential to maintain blood pressure and oxygen delivery (44). However, in women, this balance may be shifted to a greater propensity to vasodilate, as opposed to vasoconstrict in response to sympathetic activity. This is supported by literature demonstrating sympathetic support of blood pressure is lower in women from men (8, 30, 31). Animal data suggest female sex hormones are the primary mechanism by which blood pressure is differentially modulated in response to environmental stressors such as hypoxia (36); therefore, these data provide rationale to interrogate, in humans, the specific influence of female sex hormones on hypoxic and sympathetic regulation of the vasculature.

Blood flow control and hypoxia

Systemic hypoxia creates a mismatch between the peripheral supply of oxygen and demand of the muscle, and is observed in the context of exercise, altitude, or disease (e.g., sleep apnea, chronic obstructive pulmonary disease, peripheral artery disease, heart failure). Systemic hypoxia elicits local skeletal muscle vasodilation and increases in peripheral blood flow to match oxygen supply with demand (11). Mechanisms contributing to vasodilation in skeletal muscle during hypoxia (hypoxic vasodilation) is multifactorial, but has been primarily attributed to increases in nitric oxide and prostaglandins (16). In addition, adenosine, endothelium-derived hyperpolarizing factors and activation of β-adrenergic receptors have all been thought to contribute to skeletal muscle hypoxic vasodilation (54).

Interestingly, hypoxic vasodilation occurs despite a significant reflex increase in sympathetic nervous system activity directed toward the skeletal muscle vasculature (muscle sympathetic nerve activity, MSNA) (11). This rise in MSNA during systemic hypoxemia is in part mediated by the peripheral chemoreceptors located at the bifurcation of the common carotid artery (carotid body chemoreceptors) and aortic arch (60). These chemoreceptors are activated by a reduction in the partial pressure of oxygen which reflexively increases ventilation and sympathetic activity (60). An increase in MSNA results in release of norepinephrine from postganglionic sympathetic neurons which binds to α-adrenergic receptors on the vascular smooth muscle to cause vasoconstriction and an increase in vascular tone (17). These α-adrenergic receptors are G-coupled protein receptors and are important contraction mediators by activation of inositol trisphosphate and diacylglycerol (68). Also located within the carotid sinus are arterial baroreceptors, which are negative-feedback stretch receptors, such that a decrease in blood pressure increases sympathetic outflow in order to maintain blood pressure at homeostatic levels (71). It has been previously demonstrated that cardiac baroreflex sensitivity is attenuated during hypoxia, such that the lack of inhibition via the baroreceptors allows for more chemoreceptor-mediated sympathetic outflow during hypoxia (71). Consequently, the net interaction of local vasodilators combined with sympathetic outflow during hypoxemia will dictate vascular tone and ultimately blood pressure and oxygen delivery. It is important to note that these observations in the periphery occur in tandem with changes centrally, such as vascular reactivity of the gastrointestinal tract, kidneys, and heart that contribute to whole-body or total vascular conductance in response to acute hypoxia (69).

Hypoxic sympatholysis

"Hypoxic sympatholysis" is the ability of the vasculature to attenuate the vasoconstrictive effects of high sympathetic nervous system activity during hypoxia. The concept of "sympatholysis" is most often studied in the context of exercise, where despite an increase in MSNA, local vasodilators oppose or attenuate sympathetic activity in order to preserve blood flow and oxygen delivery to metabolically active tissue (16). Based on the information above, it would appear that in order to achieve compensatory hypoxic vasodilation in the face of high sympathetic nervous system activity, "hypoxic sympatholysis" is a likely explanation. However, there are data to suggest hypoxic sympatholysis does not occur in humans (17, 73), although controversy exists (35). Specifically, Dinenno and colleagues showed that sympathetically-mediated vasoconstriction is not attenuated by systemic hypoxia and rather α-adrenergic responsiveness is preserved in the setting of mild-to-moderate hypoxemia (i.e., lack of hypoxic sympatholysis) (17). In addition, Tan and colleagues found neurovascular transduction (defined as the transfer of sympathetic nerve activity into vascular tone) was increased (rather than decreased) during exposure to acute hypoxia in humans, suggesting sympathetic vasoconstriction is enhanced during hypoxia (73). However, an important limitation of these studies was the inclusion of relatively few women (n=2 out of 10) (17) and the failure to control for menstrual cycle phase (73), severely limiting the ability to directly examine the role of sex and/or sex hormones on vascular responsiveness to sympathetic activity and hypoxia. Given the presence of sex-related differences in the development of hypertension and other cardiovascular diseases in conditions associated with hypoxemia (i.e., sleep apnea) (12, 63), understanding sex differences in the

neurovascular control during hypoxemia, as well as underlying mechanisms (i.e., circulating sex hormones) is essential.

Sex differences in hypoxic vascular control

Under normoxic conditions, there are clear sex-related differences in neural control of the circulation. As noted above, release of norepinephrine from the sympathetic nerve terminals elicits smooth muscle contraction and vasoconstriction via binding to α-adrenergic receptors. Notably, vasoconstriction in response to sympathetic activity is greater in men compared to women (8), although controversy exists (65). Some of this controversy may be due to studies being conducted under β-adrenergic receptor blockade. Indeed, women exhibit greater β-adrenergic receptor sensitivity than men during normoxic conditions (46).   Consistent with this, any sex-related difference in sympathetic vasoconstriction between men and women are lost in the presence of β-adrenergic receptor blockade (8, 46). These data suggest women may exhibit preferential binding of norepinephrine to β-adrenergic receptors which promote smooth muscle relaxation and thus counteract the effects of α-adrenergic receptor mediated vasoconstriction. In further support of this, β-adrenergic receptor blockade uncovers a positive relationship between MSNA and blood pressure in young women that is otherwise absent when the β-adrenergic receptors are not blocked pharmacologically (32). In search of the mechanism by which women exhibit preferential β-adrenergic receptor activation, there are data which show estrogen enhances endothelial β-adrenergic receptor expression under normoxic conditions in humans (46). Furthermore, in rodents, estrogen treatment in ovariectomized female rats increases vascular endothelial β-adrenergic receptor expression, independent of other sex

hormones like testosterone or progesterone (43). Whether these findings directly translate to humans and whether these differences are important in vascular responsiveness to physiological stressors (i.e., hypoxia) is less clear.

In humans, the peak increase in sympathetic nervous system activity during systemic hypoxia has been shown to be no different between men and women (43). Despite similar increases in MSNA, there are data to suggest young women exhibit blunted vasoconstriction (62) or enhanced vasodilation during acute hypoxia (11) when compared to men – although controversy exists (57). Blunted vasoconstriction during systemic hypoxia could be due to decreased α-adrenergic receptor sensitivity to norepinephrine in healthy premenopausal women (54). Conversely, sex differences in β-adrenergic receptor activity may directly contribute to enhanced hypoxic vasodilation. Sympathetic activation of β-adrenergic receptors elicits smooth muscle relaxation via direct action on vascular smooth muscle cells as well as through the endothelial nitric oxide pathway. The β-adrenergic receptors contribute up to 50% of hypoxic vasodilation (81), with nitric oxide and prostaglandins thought to contribute to the other 50% (13, 16). Together, these data support sex differences in sympathetic modulation of the vasculature during the physiological stress of reduced arterial oxygen (i.e. hypoxia).

Estrogen appears to augment hypoxic vasodilation in a pre-clinical rat model (36). Specifically, Hinojosa-Laborde and colleagues found intermittent exposure to low oxygen (intermittent hypoxia) elicited an increase in activity of the sympathetic nervous system and blood pressure in male animals (36). In contrast, female rats were shown to be protected from an intermittent hypoxia mediated increase in blood pressure (36). Notably, any "protective" effects of female sex on the blood pressure response following

intermittent hypoxia was lost with ovariectomy (i.e., elimination of all ovarian hormones, including estradiol) thereby supporting a critical role for ovarian hormones in the systemic vascular response to hypoxia (36). Recent human data from our group support this (41), and showed the blood pressure response to acute intermittent hypoxia is greater in young men than young women. In a follow-up study, currently in review, we went on to show sympathetically-mediated vasoconstriction elicited via the cold pressor test is attenuated in young women during steady-state hypoxia compared to normoxia, whereas sympathetically-mediated vasoconstriction is preserved in young men during acute hypoxia (14). These preliminary results confirm previous findings that hypoxic sympatholysis is absent in young men (17), and provide new data to suggest it may be present in young women. Importantly, women had natural menstrual cycles and were studied during the early follicular phase of the menstrual cycle (days two to four, confirmed by self-report) when circulating estrogen is low. However, whether sex-related differences are inherent to female sex or specific to menstrual cycle phase, and the role for endogenous versus exogenous female sex hormones (i.e., estrogen, progesterone) remains unclear.

Sex hormones and neurovascular control: Naturally cycling vs. Oral contraceptives

Naturally cycling women

Women with natural menstrual cycles (i.e., naturally cycling, NC) experience a series of changes in steroid hormone (primarily estrogen and progesterone) production and release stimulated by the female reproductive system. This cyclical release of hormones occurs in an approximate 28 day cycle, with a range of 21-35 days. Because the amount of estrogen varies throughout the menstrual cycle and estrogen concentration may affect

vascular function (86), an argument has been proposed to not control for menstrual cycle phase when performing studies of vascular function in order to increase external validity (72). As discussed herein, a counter-point states we should not only control for-, but it is imperative to study women across the menstrual cycle to fully understand the impact of menstrual cycle phase and differing hormone concentrations on physiologic outcomes (83).

Although there are several forms of bioactive estrogen, for the purposes of this thesis, when referencing estrogen in context of women with natural menstrual cycles, it is intended to refer to 17-β estradiol unless otherwise specified. Circulating estrogen is lowest during the early follicular phase in naturally cycling women (approximate days 1-7 of the cycle), and peaks during the late follicular phase, right before ovulation (approximate days 10-14 of the cycle). Following ovulation, women enter the luteal phase in which both estrogen and progesterone remain high. Estrogen and progesterone have been shown to have divergent effects on the peripheral vasculature, with estrogen promoting an increase in the ability to vasodilate and progesterone antagonizing this vasodilatory response (55, 75). Due to these divergent effects, the majority of this thesis will focus on the follicular phase of the menstrual cycle when only estrogen is elevated. Thus, for congruity and in the context of estrogen, the early and late follicular phases will be referred to as the low and high hormone phases of the menstrual cycle, respectively.

Premenopausal women have a lower incidence of cardiovascular disease and hypertension compared to age matched men (82). Part of this lower incidence of hypertension may be due to less sympathetically-mediated vasoconstriction. In support of this, Hart and colleagues (31) have shown prior to the onset of menopause there is no relationship between MSNA and blood pressure in young women; this is in contrast to

11

relationships observed in post-menopausal women (31). Barnes and colleagues (2) went onto show sympathetic support of blood pressure is greater in postmenopausal compared to premenopausal women. Together these data suggest the presence or absence of estrogen may be important in the transduction of sympathetic activity to vascular tone (8). However, a limitation of this work is the inability to examine differences independent of age. Seals and colleagues have shown previously that age can independently increase sympathetic support of blood pressure in men (84). Thus, to examine the potential role for female sex hormones (i.e., estrogen) independent of the potential confounding effects of age, women may be studied across the natural menstrual cycle, when circulating estrogen levels are known to fluctuate.

As concentrations of estrogen fluctuate across the regular menstrual cycle, MSNA is also known to fluctuate ((59), **Table 1**). Specifically, MSNA and plasma norepinephrine levels are higher during the high estrogen phase of the menstrual cycle when compared to the low estrogen phase (59). Levels of MSNA observed during the high-hormone phase are inversely associated with the net change or "surge" in circulating estradiol relative to the low hormone phase across of the menstrual cycle (10). In other words, the greater the surge or increase in estrogen between phases from the low to high-hormone phase, the greater the change in resting, basal MSNA (10). Interestingly, despite changes in sympathetic outflow at different phases of the menstrual cycle, cyclical changes in vascular transduction are not observed (i.e., no change in vasoconstriction or blood pressure in response to a given amount of sympathetic activation) (59). The lack of change in neurovascular transduction across the menstrual cycle may be due to cycle-dependent fluctuations in β-adrenergic receptor or other local vascular dilators. For example, β-

adrenergic receptor responsiveness appears to be greater during the mid-luteal phase (46) than during the follicular phase (50). Although definitive studies have not been conducted in humans, these combined data suggest any changes in MSNA during the menstrual cycle are likely met with increases in local vasodilation, resulting in no net change in vascular tone.

In addition to differences in neural control of the circulation across the natural menstrual cycle, key local vascular control mechanisms (e.g., nitric oxide) are known to fluctuate across the menstrual cycle. Specifically, serum nitrate/nitrite (metabolites of nitric oxide) increase in the high hormone phase of the menstrual cycle (67). Consistent with this, flow mediated vasodilation (FMD, a measure of endothelium-dependent dilation) is greater during the high hormone phase when compared to the low hormone phase of a natural cycle (1, 22). This is also consistent with data showing endothelial independent (nitroglycerin) mediated vasodilation is augmented during the high hormone phase of the menstrual cycle compared to the low hormone phase (34).

In the context of hypoxia, the low hormone phase of the menstrual cycle is associated with greater increases in sympathetic activity during a hypoxic apnea when compared to the high hormone phase (mid-luteal) in naturally cycling women (76). In men, this rise in MSNA during hypoxic apnea results in vasoconstriction and an increase in blood pressure. Interestingly, young, NC women studied during the early follicular (low-hormone) phase do not exhibit vasoconstriction in response to apnea (62). Notably, any "beneficial" effect of female sex is lost following menopause, such that hypoxic apnea causes subsequent vasoconstriction and a reduction in blood flow of the brachial artery in postmenopausal women that is not observed in premenopausal women (62). Together,

these data suggest: 1) higher circulating estrogen dampens or attenuates sympathoexcitatory effects of hypoxia, and 2) an uncoupling of sympathetic activity and vascular tone occurs during hypoxia in young women. An estrogen-mediated disconnect between sympathetic activity and vascular tone during hypoxia could have important implications for the development of hypertension in hypoxia-related disease states, such as sleep apnea which is more prevalent in post- compared to pre-menopausal women (12). Lastly, this may have implications for the development of hypertension in conditions where estrogen is chronically high (i.e., polycystic ovarian syndrome) or in women lacking a natural menstrual cycle (i.e., oral hormonal contraceptive pills).

Oral contraceptives

Over 80% of American women will use OCPs in their lifetime (6, 53). OCPs modulate female sex hormone levels by supplementing a combined synthetic estrogen (ethinyl estradiol) and progesterone into a once-daily pill. There are several formulations of synthetic progesterone that accompany synthetic estrogen and are used to sub-classify generation of OCP (i.e., first, second, third and fourth generation). Monophasic OCP provide a fixed dose of synthetic estrogen and progesterone for 28 continuous days, as opposed to bi- or tri-phasic contraceptives in which the synthetic hormones have two or three increasing concentrations, respectively, to be more analogous to a natural menstrual cycle. In most cases, 28 days of active (synthetic estrogen hormone containing) pills are followed by approximately 7 days of placebo or non-hormone pills to mimic the low circulating hormones of the early follicular phase and to induce menstruation. The synthetic estradiol found in OCP has been shown to bind to uterine estrogen receptors from rodents

with 400 greater relative binding affinity compared to endogenous 17-β estradiol (5). Additionally, ethinyl estradiol's pharmacokinetics are such that blood concentrations peak every day, approximately four hours after ingestion of the active (hormone-containing) pill (84) – this is in contrast to the twice-a-month peak of 17-β estradiol that occurs in naturally cycling women.

Notably, MSNA and plasma norepinephrine levels do not differ between naturally cycling women and women taking OCPs when studied during the early follicular and placebo phase, respectively (33). Despite no difference in resting sympathetic activity compared to NC, OCP use has a relatively non-specific, positive impact on peripheral vascular function. For example, women taking OCPs exhibit greater β-adrenoreceptor mediated vasodilation when compared to naturally cycling women (51). In further support of this, OCP use has been shown to enhance basal nitric oxide production and release (42). Moreover, endothelial-dependent vasodilation was shown to be greater in women taking OCPs compared to naturally cycling women (51). Amongst women taking OCPs, researchers also observed greater endothelium-dependent vasodilation (FMD) during the active pill (high hormone) phase compared to the placebo (low hormone) phase (56). These changes in endothelial function with OCP use appear to occur independent of changes in endothelial-independent vasodilation (42, 80). Together these findings suggest endothelial-mediated vasodilation is greater in women taking OCPs compared to NC women, and this difference may be augmented during the active pill phase compared to the placebo pill phase.

Despite widespread use of OCPs and their potential impact on vascular control, very little is known about the effect of OCP use on the vascular response to physiological

stress. To our knowledge there is only one study which examined the MSNA response to hypoxic apnea and results show the rise in MSNA may be attenuated in the active pill phase compared to the placebo pill phase (77). It may be concluded that synthetic estradiol may limit sympathetic outflow or attenuate the sympathoexcitatory response to stress at the level of the vasculature (77), but further research is needed.

There are many gaps in our understanding of the effects of OCP use on neurovascular control, and whether these effects mirror those which occur in NC women, is limited. A summary of the current literature can be found in **Table 1**. Furthering this research is extremely important for all women, and especially those currently taking OCPs or supplementing estrogen, such as hormone replacement therapy during menopause. Additionally, these results may also have clinical implications for synthetic estrogen and the vasculature in hypoxic conditions like stroke or peripheral artery disease.

CHAPTER 3: METHODS

All proposed experiments were approved by the Institutional Review Board at the University of Missouri (IRB #2011312) and conform to the ethical principles of the Declaration of Helsinki, including registration with ClinicalTrials.gov (ID: NCT04436731).

SCREEN PROCEDURES

Participants

Women were recruited from Columbia, MO and surrounding areas using advertisements, word-of-mouth, and approved databases of prior research participants. We enrolled 16 participants taking OCP, of which 10 women completed both visits and compared the data to results from 10 naturally cycling women (see **Figure 4**). Inclusion criteria for all women consisted of: non-obese (BMI<30 kg/m^2), normotensive (<140/90 mmHg), non-nicotine users between the ages of 18-45 years taking no medications known to affect autonomic or cardiovascular function.

All women taking OCPs completed two study visits, once during the placebo pill phase (days 4-7) and once during the active pill phase (days 14-20). First generation pills were excluded as their high dosage ethinyl estradiol (>50mcg) has been known to cause adverse vascular effects (28). Therefore, inclusion criteria for women taking oral hormonal contraceptives included 2nd, 3rd, and 4th generation conventional (28-day length) monophasic oral contraceptive pills. Women were required to have been consistently taking their prescribed formulation for >6 months. The ethinyl estradiol dosage was required to be between 15-35μg, as high doses have been linked with adverse

cardiovascular events (28). Data were compared to results from naturally cycling female participants studied once during the low hormone phase of the menstrual cycle (menstrual bleeding, days 2 – 4, self report) and once during the high hormone phase (late follicular, days 14 – 20, determined using commercially available ovulation kit).

Screen Visit and Informed Consent

Women completed one 1-hour virtual screen visit. Due to the COVID-19 pandemic, screen visits were held virtually via Zoom. After completing a verbal waiver of informed consent, medical history was evaluated via prescreen questionnaire to confirm participants were free from chronic disease or medication affecting autonomic and/or cardiovascular function.

Participants refrained from caffeine, strenuous exercise, alcohol, and non-steroid anti-inflammatory agents for 24-hours prior to their scheduled study start. Participants arrived to the laboratory after 4-hour fast (no food or drink other than sips of water). Study visits were at 8:00am. If a participant was not able to complete the study visit at 8:00am (n=2), they were required to complete their second visit at the same chronological time as their first visit (11am and 1pm).

The morning of the study visit participants provided written informed consent, after which height, weight, and vital signs (blood pressure, heart rate) were assessed. All participants were required to have a negative pregnancy (urine) test. Individuals were then familiarized with the lab space and underwent a practice cold pressor test (CPT, described below). Following the test participants were asked to rate their perceived thermal and pain sensation of the CPT on a modified Borg scale (7). During the CPT, the participant's left

foot was immersed up to the ankle in an ice-water bath maintained at 0-4°C. CPT is a well-established sympathoexcitatory stimulus used to assess α-adrenergic receptor mediated vasoconstriction (18, 47). Notably, the level of sympathetic nervous system activation in response to the CPT is reliably repeatable within study visits (15).

Instrumentation

Participants were instrumented with a three-lead electrocardiogram to measure heart rate (Lead II, Bio Amp FE132, ADinstruments, Colorado Springs, CO, USA) and finger photoplethysmography for blood pressure (Human NIBP Controller ML282, ADinstruments, Colorado Springs, CO, USA) which was calibrated to upper arm blood pressure (automatic sphygmomanometer). Oxygen saturation was monitored by finger pulse oximetry (Oximeter Pod, Fingerclip ML320/F, ADinstruments, Colorado Springs, CO, USA) and respiration by use of a piezo respiratory belt transducer (MLT1132, ADinstruments, Colorado Springs, CO, USA). Participants wore a mask connected to a non-rebreathing valve (2600 Medium 2-way NRBV, Hans Rudolph, Shawnee, KS, USA), and equipped to an air-gas blender (Oxyen/air blender #81-1920, Tri-anim Health Services, Dublin, OH, USA). Inspired/expired gases were analyzed at the mouth using a gas analyzer (Gemini 14-10000 Respiratory Monitor, CWE Inc., Ardmore, PA, USA). Forearm blood flow was assessed via venous occlusion plethysmography on the participant's left arm (D.E. Hokanson Inc, Bellevue, Washington, USA). Briefly, a blood pressure cuff was placed around the wrist and inflated to 220 mmHg to exclude hand circulation from the measurement. A second blood pressure cuff was placed around the upper arm and

cyclically inflated to 50 mmHg for 7 seconds then deflated for 8 seconds (0 mmHg) to obtain one blood flow measurement every 15 s (85).

Blood Sampling

On each study visit, after a 15-min quiet rest period, individuals had a 40 mL blood sample taken via venipuncture. The samples were centrifuged, serum was pipetted off, and stored at 4°C prior to assessment of estradiol, progesterone, luteinizing hormone, and total testosterone (all immunoassay). For measures of norepinephrine and epinephrine, samples were centrifuged, plasma was pipetted off, and samples were stored at -80°C degrees prior to assessment (high performance liquid chromatography) (Quest Diagnostics Laboratories, Columbia, MO).

Protocol

Individuals wore a mask covering the nose and mouth connected to an air-gas blender for the entirety of the data collection period. All testing was done in the supine position. Following blood sampling, hemodynamic and breathing data were collected continuously during 5-minutes of quiet rest. After the resting period, participants had their left foot passively placed in a bucket of ice water (normoxic CPT) to acutely activate the sympathetic nervous system. The normoxic CPT was followed by a minimum 10-minute washout period to allow hemodynamic variables to return to baseline.

Following the washout period and once hemodynamic variables returned to baseline (**Supplemental Table**), participants underwent a 3-minute normoxic baseline. After a 3-minute baseline, participants oxygen levels were titrated via blending of room air

and 5% oxygen to a target saturation of 80% SpO2 (finger pulse oximeter). Once the desired oxygen saturation was achieved, this level of hypoxia was maintained for 3 minutes, at which time women completed a second CPT (2-min) during the steady-state hypoxia (**Figure 2**).

Data Analysis

Main outcome variables included forearm vascular conductance and total vascular conductance. Measures of forearm blood flow (FBF) are expressed as ml of blood per 100 ml of forearm volume per minute from an average of 3-5 total flows. FBF was normalized for mean arterial blood pressure to determine forearm vascular conductance (Forearm blood flow ÷ mean arterial blood pressure x 100 mmHg, FVC) and expressed as mL of blood /100 mL of forearm volume/100mmHg/min. FVC is preferential over resistance indices, as it linearly relates to flow and is indicative of downstream vascular tone; an increase in FVC indicative of peripheral vasodilation and a decrease in FVC signifying peripheral vasoconstriction (49).

Stroke volume was estimated from the finger blood pressure waveform using the Modelflow method (LabChart, ADinstruments), which incorporates age and sex. Cardiac output was calculated as the product of stroke volume and heart rate. Total vascular conductance (TVC) was calculated as the product of stroke volume and heart rate, divided by mean arterial blood pressure and expressed as mL/mmHg.min.

An absolute change in main outcome variables (FVC, TVC) in response to physiological stress (Aim 1: hypoxia; Aim 2: cold pressor test) was calculated (e.g., Δ = CPT – Baseline). To control for differences in baseline levels, relative changes (% = Δ ÷

Baseline x 100) were also assessed. To determine hypoxia-mediated attenuation of sympathetic vasoconstriction (Aim 3), differences in the vascular response between normoxia and hypoxia trials (ΔHypoxia – ΔNormoxia) were also calculated.

Statistical Analysis

Participant demographics were compared within and between groups using a two-way repeated measures analysis of variance (multiple comparison, Holm-Sidak). The effect of hypoxia (Aim 1) and cold pressor test (Aim 2) on main outcome variables was assessed within groups using a two-way repeated measures analysis of variance (multiple comparison, Holm-Sidak). Comparisons between groups (NC, OCP) and phase (low, high hormone) on the change (% change from baseline) in main outcome variables were conducted using a two-way repeated measures analysis of variance (multiple comparison, Holm-Sidak) test. Normality was assessed using the Shapiro-Wilk and equal variance assessed with the Brown-Forsynthe test. Two-tailed $p < 0.05$ was considered statistically significant. Data are reported as mean ± standard error of the mean (SEM).

CHAPTER 4: RESULTS

Subject demographics

Thirty-four women were enrolled in the present investigation, with complete data from ten NC women and ten women taking OCPs presented herein (N=20, **Figure 4**). Two visits for each participant were conducted. NC women were studied during the low hormone (early follicular) and high hormone (late follicular) phases of the menstrual cycle. Women taking OCPs were studied during low hormone (placebo pill) and high hormone phase (active pill) of the pill cycle. Visits were completed in random order.

All demographic data are reported in **Table 2**. NC women and women taking OCPs were matched for age (p=0.52) and body mass index (p=0.14). Estradiol was significantly higher during the high hormone phase compared to the low hormone phase of the menstrual cycle in NC women (p<0.01). There were no differences detected in epinephrine or norepinephrine across groups or phase (main effect of group, p=0.48; main effect of phase, p=0.59).

AIM 1. The effect of menstrual cycle phase and oral hormonal contraceptive pill phase on the vascular response to systemic hypoxia

Hypoxia was achieved in both groups of women, shown by a decrease in oxygen saturation (SpO_2; main effect of hypoxia, p<0.01, **Table 3**). Hypoxia elicited an increase in heart rate in both the NC group (main effect of hypoxia, p<0.01) and the OCP group (main effect of hypoxia, p<0.01). In both groups, mean blood pressure and cardiac output increased and stroke volume fell during hypoxia (main effect of hypoxia, all p<0.05).

In both groups, hypoxia elicited an absolute increase in TVC (Main effect of

hypoxia: NC p<0.01, OCP, p=0.05). See **Table 3**. This increase in TVC did not differ by phase in either group of women (Main effect of phase, p>0.05; **Table 3**). Additionally, when examined as a relative change (%) in TVC (**Figure 5A**), this increase from baseline during hypoxia did not differ between groups or phases (Main effect of group, p=0.59; phase p=0.56).

Systemic hypoxia resulted in an increase in FBF in NC women (Main effect of hypoxia, p=0.03), with a trend for an increase in FBF in women taking OCPs (Main effect of hypoxia, p=0.059). There was no observable increase in FVC in either group with hypoxia (Main effect of hypoxia, p>0.05). FVC did not differ by phase in women taking OCPs (Main effect of phase, p=0.11), but increased from the low to high hormone phase in NC women (main effect of phase, p=0.03) (**Table 3**). The relative rise from baseline in FVC with hypoxia (%) was found to be greater during the high hormone phase compared to the low hormone phase in both NC women and women taking OCPs (Main effect of phase, p<0.01) and this response did not differ between groups (Interaction of group and phase, p=0.30) (**Figure 5B**).

AIM 2. The effect of menstrual cycle phase and oral hormonal contraceptive pill phase on the peripheral vascular response to acute sympathetic activation

As expected, sympathetic activation via CPT produced a significant increase in mean blood pressure and heart rate in both groups (Main effect of CPT, p<0.01, **Table 4**). Heart rate was greater at rest and in response to the CPT in the OCP group during the high hormone phase compared to the low hormone phase of the pill cycle (OCP, main effect of phase, p<0.03). In the NC women, CPT elicited an increase in heart rate that was not

different between phases (NC, main effect of phase, p=0.58). There were no differences in perceived cold or pain scores in response to CPT both between groups and phases within groups (low hormone, high hormone) (p>0.05, **Supplemental Tables**).

In contrast to FVC, both NC women (Main effect of CPT, p<0.01) and women taking OCPs (Main effect of CPT, p=0.067) tended to decrease TVC during the CPT (See **Table 4**) and conclusions were maintained when assessed as a relative (%) change (Main effect of group, p=0.40; **Figure 6A**). Contrary to our hypothesis, there was no effect of menstrual cycle or pill phase on the local (FVC) or systemic (TVC) vascular response to acute sympathetic activation in either group (Both groups, main effect of phase, p>0.05). See **Table 4** and **Figure 6**.

Acute sympathetic activation resulted in a significant decrease in FVC in NC women (Main effect of CPT, p<0.01). In women taking OCPs, there was a trend for an increase in FVC during CPT that did not reach statistical significance (Main effect of CPT, p=0.07). When examined as a relative (%) change in FVC, conclusions were maintained. Specifically, NC women exhibited a reduction in FVC to CPT, whereas women taking OCPs exhibited a paradoxical increase in FVC (%) (Main effect of group, p<0.01; **Figure 6B**).

AIM 3: The effect of menstrual cycle phase and oral hormonal contraceptive pill phase on the peripheral vascular response to concomitant hypoxia and sympathetic activation

In both groups, concomitant hypoxia and sympathetic activation resulted in significant increases in mean blood pressure (both groups, interaction effect of gas and

CPT, p<0.01, **Tables 5 and 6**). Perceived cold scores following the hypoxic CPT were lower compared to the normoxia CPT trial in both groups (NC and OCP, main effect of hypoxia, p<0.05). Additionally, perceived pain scores were lower in the OCP group in response to the hypoxic CPT compared to the normoxic CPT (p<0.05), with no change in the NC group between hypoxia and normoxia CPT (p>0.05, **Supplemental Tables**).

Acute sympathetic activation via CPT elicited a decrease in TVC that did not differ between normoxia and hypoxia in NC women (Main effect of CPT, p<0.01; Interaction of hypoxia and CPT, p>0.05; **Table 5**). Similarly, when examined as a relative (%) change from steady-state, the fall in TVC with the CPT did not differ between normoxia and hypoxia in NC women (Main effect of hypoxia, p=0.44; **Figure 5A**). These responses were not specific to menstrual cycle phase in the NC women (Interaction of hypoxia and phase, p=0.88; **Figure 7A**).

In women taking OCPs, acute sympathetic activation via CPT elicited a decrease in TVC that did not differ between normoxia and hypoxia during the high hormone portion of the pill cycle phase (Main effect of CPT, p=0.04; Interaction of hypoxia and CPT, p=0.84; **Table 6**). When examined as a relative (%) change from steady-state, the fall in TVC with the CPT also did not differ between normoxia and hypoxia (Main effect of hypoxia, p=0.23) nor across pill cycle phase (Main effect of phase, p=0.67; Interaction of hypoxia and phase, p=0.60; **Figure 7B**).

Because the CPT elicited vasodilation in women taking OCPs, it cannot be used a model of sympathetic vasoconstriction and therefore, we cannot interpret the results in comparison to NC. However, for transparency, the results have been included herein. Concomitant hypoxia and CPT cause an absolute reduction in FVC in NC women during

the high hormone phase only and no change during the low hormone phase (Main effect of CPT, high hormone p = 0.02; low hormone p=0.24; **Supplemental Table 1**). Women on OCPs had a significant increase in FVC in response to CPT while hypoxic, that did not differ between low and high hormone phases (Main effect of CPT, all p<0.05; **Supplemental Table 1**). When expressed as a relative percent change from baseline the NC women had a reduction in FVC that did not differ from normoxic CPT or between hormone phases (Interaction of hypoxia and phase, p=0.18; **Supplemental Figure 1**). Conversely, women on OCPs had an increase in FVC in response to a hypoxic CPT that was no different compared their normoxic CPT or between pill phases (Interaction of phase and hypoxia, p=0.181; **Supplemental Figure 1**).

		MSNA	MSNA Transduction	β-adrenergic vasodilation	Nitric Oxide	Endothelial dependent vasodilation	Endothelial independent vasodilation	Hypoxia MSNA
Naturally Cycling	Low hormone	↓[60]	↔[60]	↓[51]		↓[1,23]	↓[35]	↑[63]
	High hormone	↑[60]	↔[60]	↑[48]	↑[68]	↑[1,23]	↑[35]	↓[63]
Oral Contraceptives	Low hormone	↔[34]		↑[52]	↑[44]	↑[52]	↔[34,65]	↔[77]
	High hormone					↑[57]	↔[34,65]	↓[77]

Table 1: Influence of Hormone and Concentration on the Vascular Response to Autonomic, Pharmacologic, and Environmental Stimuli. Muscle sympathetic nerve activity (MSNA), ↑ = an increase relative to the other phase of the menstrual or pill cycle; ↓ = a decrease relative to the other phase of the menstrual or pill cycle; ↔ = no change relative to the other phase of the menstrual or pill cycle; numbers correlate to reference number.

	Naturally Cycling (NC)		Oral Contraceptive (OCP)		P-value		
	Low	High	Low	High	Group	Phase	Interaction
Count	10	10	10	10	---	---	---
Age (years)	25±1		24±1		0.518	---	---
Height (cm)	166±1		164±2		0.429	---	---
Weight (kg)	62±2		56±2		0.087	---	---
Body mass index (kg/m^2)	23±1		21±1		0.143	---	---
Estradiol (pg/mL)	28±4	185±51*	35±5	17±1†	0.005	0.004	**<0.001**
Progesterone (ng/mL)	0.6±0.1	1.1±0.2*	0.5±0.0	0.5±0.0†	0.006	0.017	**0.017**
Luteinizing Hormone (pg/mL)	4±1	21±9*	4±1	2±1†	0.049	0.059	**0.027**
Testosterone (Total) (ng/dL)	28±3	42±4*	37±6	27±4*†	0.554	0.442	**<0.001**
Epinephrine (pg/mL)	30±6	25±2	25±2	24±4	0.483	0.302	0.481
Norepinephrine (pg/mL)	237±87	184±12	200±25	190±14	0.717	0.430	0.586
Testing day	3.1±0.4	14.9±0.9*	5.2±0.4	16.6±0.6*†	**0.003**	**<0.001**	0.774

Table 2: Participant Demographics. Data are reported as Mean±SEM from NC (n=10) or OCP (n=10) unless otherwise noted. NC (Progesterone/Estradiol/Testosterone/LH, n=8; Norepinephrine/Epinephrine, n=6); OCP (Progesterone/Estradiol, n=10; Testosterone/LH, n=8; Norepinephrine/Epinephrine, n=6). Demographics compared using a two-way repeated measures analysis of variance and multiple comparison with Holm-Sidak test. *p<0.05 Low Hormone; †p<0.05 vs NC. Testing day = number of days since start of self-report menstrual or pill cycle.

	Naturally Cycling		Oral Contraceptive			P-value	
	Low	High	Low	High	Phase	Hypoxia	Interaction
Oxygen saturation (% SpO2)							
Normoxia	98±0	98±0	99±0	99±0	NC: 0.416	**<0.001**	0.828
Hypoxia	81±1†	81±1†	81±1†	81±1†	OCP: 0.984	**<0.001**	0.647
Mean blood pressure (mmHg)							
Normoxia	82±3	82±2	84±1	82±1	NC: 0.962	**0.005**	0.850
Hypoxia	87±3†	88±3†	89±2†	88±2†	OCP: 0.206	**0.003**	0.414
Heart rate (beats/min)							
Normoxia	64±2	69±2*	69±3	74±2*	NC: **0.019**	**<0.001**	0.237
Hypoxia	82±4†	86±3†*	87±4†	93±5†*	OCP: **0.028**	**<0.001**	0.968
Stroke volume (mL/beat)							
Normoxia	78±3	71±4	83±4	84±3	NC: 0.124	**0.020**	0.311
Hypoxia	72±2†	69±4†	78±4†	79±4†	OCP: 0.903	**0.017**	0.891
Cardiac output (L/min)							
Normoxia	4.9±0.3	4.7±0.3	5.7±0.4	6.2±0.4	NC: 0.149	**<0.001**	0.607
Hypoxia	5.8±0.3†	5.5±0.3†	6.9±0.5†	7.2±0.6†	OCP: 0.277	**0.003**	0.566
Total vascular conductance (mL/mmHg.min)							
Normoxia	59±2	58±3	73±6	75±4	NC: 0.238	**0.008**	0.909
Hypoxia	66±2†	64±3†	81±8†	81±7†	OCP: 0.783	**0.050**	0.562
Forearm blood flow (mL/dL/min)							
Normoxia	2.1±0.2	2.4±0.2*	2.0±0.2	2.4±0.2	NC: **0.022**	**0.031**	0.066
Hypoxia	2.3±0.2†	3.0±0.3†*	2.3±0.3	2.9±0.4	OCP: 0.126	0.059	0.434
Forearm vascular conductance (mL/dL/100 mmHg/min)							
Normoxia	2.6±0.3	3.0±0.3*	2.4±0.3	2.9±0.3	NC: **0.029**	0.171	0.104
Hypoxia	2.7±0.3	3.5±0.3*	2.6±0.3	3.3±0.5	OCP: 0.109	0.214	0.547

Table 3: Hypoxic Vasodilation. Data are reported as Mean±SEM from NC (n=10) or OCP (n=10). Two-way repeated measures analysis of variance and multiple comparison with Holm-Sidak test within groups. *p<0.05 low hormone; †p<0.05 vs normoxia.

	Naturally Cycling		Oral Contraceptive		*P*-value		
	Low	High	Low	High	Phase	CPT	Interaction
Mean blood pressure (mmHg)							
Steady-state	83±3	80±2	81±1	93±1	NC: 0.699	**<0.001**	0.070
CPT	106±5†	107±4†	107±4†	106±3†	OCP: 0.923	**<0.001**	0.453
Heart rate (beats/min)							
Steady-state	66±3	68±2	69±3	75±2*	NC: 0.576	**0.005**	0.180
CPT	76±2†	76±3†	85±1†	90±3†*	OCP: **0.032**	**<0.001**	0.543
Stroke volume (mL/beat)							
Steady-state	79±3	71±4*	83±4	82±4	NC: 0.235	0.039	**0.038**
CPT	67±3†	67±4	74±6	75±5	OCP: 0.962	0.055	0.755
Cardiac output (L/min)							
Steady-state	5.0±0.3	4.7±0.3	5.7±0.4	6.2±0.4	NC: 0.523	0.354	0.129
CPT	5.1±0.3	5.0±0.3	6.3±0.5	6.8±0.6	OCP: 0.128	0.123	0.572
Total vascular conductance (mL/mmHg.min)							
Steady-state	61±2	60±3	74±6	74±4	NC: 0.962	**0.003**	0.754
CPT	48±3†	48±4†	63±7	66±7	OCP: 0.680	0.067	0.528
Forearm blood flow (mL/dL/min)							
Steady-state	2.4±0.3	2.6±0.3	2.0±0.2	2.6±0.2	NC: 0.278	0.308	0.646
CPT	2.1±0.2	2.5±0.3	3.8±0.4†	4.1±0.7†	OCP: 0.174	**0.007**	0.629
Forearm vascular conductance (mL/dL/100 mmHg/min)							
Steady-state	2.9±0.3	3.2±0.3	2.5±0.3	3.1±0.2	NC: 0.188	**0.008**	0.890
CPT	2.0±0.2†	2.4±0.3†	3.7±0.5	3.9±0.7	OCP: 0.150	0.072	0.384

Table 4: Sympathetic Vasoconstriction. Data are reported as mean±SEM from NC (n=10) or OCP (n=10) from the last minute of steady-state or CPT. Two-way repeated measures analysis of variance and multiple comparison with Holm-Sidak test within groups. *p<0.05 vs low hormone; †p<0.05 vs steady-state.

	Naturally Cycling				P-value		
	Low		High				
	Steady-state	CPT	Steady-state	CPT	Hypoxia	CPT	Interaction
Mean blood pressure (mmHg)							
Normoxia	83±3	106±5*	80±2	107±4*	Low: 0.557	<0.001	**0.040**
Hypoxia	87±3	104±4*	88±3†	106±4*	High: 0.076	<0.001	**0.004**
Heart rate (beats/min)							
Normoxia	66±3	76±2*	68±2	76±3*	Low: **<0.001**	**0.002**	0.190
Hypoxia	82±4†	89±3†*	86±3†	92±3†*	High: **<0.001**	**0.006**	0.547
Stroke volume (mL/beat)							
Normoxia	79±3	67±3*	71±4	67±3	Low: **0.043**	**0.014**	0.227
Hypoxia	72±2†	63±2†*	67±4	60±3	High: 0.100	0.083	0.354
Cardiac output (L/min)							
Normoxia	5.0±0.3	5.1±0.3	4.7±0.3	5.0±0.3	Low: **0.004**	0.985	0.562
Hypoxia	5.8±0.3†	5.7±0.4†	5.5±0.3†	5.5±0.3†	High: **0.009**	0.344	0.126
Total vascular conductance (mL/mmHg.min)							
Normoxia	61±2	48±3*	60±3	48±4*	Low: **0.032**	**0.002**	0.758
Hypoxia	66±2†	54±3†*	64±3	52±3*	High: 0.088	**0.003**	0.853

Table 5: Effect of Acute Hypoxia and CPT on Hemodynamic Variables in Naturally Cycling Women. Data are reported as Mean±SEM from n=10 from the last minute of steady-state and of the CPT. The effect of hypoxia and CPT within phase (low hormone, high hormone) was assessed using a two-way repeated measures ANOVA. Normality (Shapiro-Wilk), Variance (Brown-Forsynthe), Multiple comparison (Holm-Sidak). *p<0.05 vs steady-state, †p<0.05 vs normoxia.

	Oral Contraceptive				P-value		
	Low		High				
	Steady-state	CPT	Steady-state	CPT	Hypoxia	CPT	Interaction
Mean blood pressure (mmHg)							
Normoxia	81±1	107±4*	83±1	106±3*	Low: 0.305	**<0.001**	**0.002**
Hypoxia	89±2†	103±3*	89±2†	102±3*	High: 0.370	**<0.001**	**0.002**
Heart rate (beats/min)							
Normoxia	69±3	85±1*	75±2	90±3*	Low: **<0.001**	**<0.001**	0.148
Hypoxia	87±4†	100±3*†	93±5†	102±3*†	High: **<0.001**	**<0.001**	0.060
Stroke volume (mL/beat)							
Normoxia	83±4	74±6	82±4	75±5*	Low: 0.312	0.109	0.267
Hypoxia	78±4	74±5	79±4	75±5*	High: 0.318	**0.005**	0.221
Cardiac output (L/min)							
Normoxia	5.7±0.4	6.3±0.5	6.2±0.4	6.8±0.6	Low: **0.004**	0.154	0.976
Hypoxia	6.9±0.5†	7.4±0.6†	7.2±0.6†	7.5±0.5†	High: **<0.001**	0.078	0.457
Total vascular conductance (mL/mmHg.min)							
Normoxia	74±6	63±7	74±4	66±7*	Low: **0.024**	0.176	**0.019**
Hypoxia	81±8	78±9†	81±6†	74±5*†	High: **0.022**	**0.037**	0.836

Table 6: Effect of Acute Hypoxia and CPT on Hemodynamic Variables in Women Taking Oral Contraceptives. Data are reported as Mean±SEM from n=10 from steady-state normoxia/hypoxia and minute 2 of the cold pressor test (CPT). The effect of hypoxia and CPT within phase (low hormone, high hormone) was assessed using a two-way repeated measures ANOVA. Normality (Shapiro-Wilk), Variance (Brown-Forsynthe), Multiple comparison (Holm-Sidak). *p<0.05 vs steady-state, †p<0.05 vs normoxia.

| | Naturally Cycling | | | | P-value | | |
| | Low | | High | | | | |
	Steady-state	CPT	Steady-state	CPT	Hypoxia	CPT	Interaction
Forearm blood flow (mL/dL/min)							
Normoxia	2.4±0.3	2.1±0.2	2.6±0.3	2.5±0.3	Low: 0.172	0.702	0.080
Hypoxia	2.3±0.2	2.9±0.5	3.5±0.3	2.8±0.3	High: 0.079	0.569	0.820
Forearm vascular conductance (mL/dL/100 mmHg/min)							
Normoxia	2.9±0.3	2.0±0.2	3.2±0.3	2.4±0.3*	Low: 0.233	0.238	0.067
Hypoxia	2.7±0.3	2.8±0.4	3.5±0.3	2.8±0.3*	High: 0.176	**0.021**	0.802

| | Oral Contraceptive | | | | P-value | | |
| | Low | | High | | | | |
	Steady-state	CPT	Steady-state	CPT	Hypoxia	CPT	Interaction
Forearm blood flow (mL/dL/min)							
Normoxia	2.0±0.2	3.8±0.4*	2.6±0.2	4.1±0.7*	Low: 0.705	**0.001**	0.182
Hypoxia	2.3±0.3	3.6±0.4*	2.9±0.4	4.9±0.7*	High: 0.089	**0.005**	0.526
Forearm vascular conductance (mL/dL/100 mmHg/min)							
Normoxia	2.5±0.3	3.7±0.5*	3.1±0.2	3.9±0.7*	Low: 0.950	**0.013**	0.706
Hypoxia	2.6±0.3	3.6±0.4*	3.3±0.4	4.9±0.8*	High: 0.141	**0.027**	0.276

Supplemental Table 1: Effect of Acute Hypoxia and CPT on Forearm Blood Flow. Data are reported as Mean±SEM from n=10 from the last minute steady-state and of the CPT. The effect of hypoxia and CPT within phase (low hormone, high hormone) was assessed using a two-way repeated measures ANOVA. Normality (Shapiro-Wilk), Variance (Brown-Forsynthe), Multiple comparison (Holm-Sidak). *p<0.05 vs steady-state, †p<0.05 vs normoxia.

	Naturally Cycling		Oral Contraceptive		P-value		
	Low	High	Low	High	Phase	Hypoxia	Interaction
Thermal Scale (scale)							
Normoxia	7.5±0.5	7.7±0.4	7.6±0.6	7.8±0.4	NC: 0.775	**0.042**	0.820
Hypoxia	7.1±0.4†	7.1±0.7†	7.3±0.4†	7.1±0.4†	OCP: 1.000	**0.023**	0.373
Pain Scale (scale)							
Normoxia	9.4±0.8	10.4±0.7	11.7±0.7	11.4±0.7	NC: 0.787	0.909	0.067
Hypoxia	10.3±0.7	9.6±0.6	10.8±0.7†	10.3±0.3†	OCP : 0.558	**0.013**	0.876

Supplemental Table 2: Thermal and Pain Scales Following Normoxic and Hypoxic CPT. Data are reported as Mean±SEM from n=10 immediately following the CPT. The effect of phase (low/high hormone) and trial (normoxia/hypoxia) within group (naturally cycling, oral contraceptive) was assessed using a two-way repeated measures ANOVA.   Normality (Shapiro-Wilk), Variance (Brown-Forsynthe). *p<0.05 vs low-hormone, †p<0.05 vs normoxia.

	Naturally Cycling		Oral Contraceptive		*P*-value		
	Low	High	Low	High	Phase	Trial	Interaction
Mean Blood Pressure (mmHg)							
Trial 1 Baseline	83±3	80±2	81±1	83±1	NC: 0.393	0.964	0.424
Trial 2 Baseline	82±3	82±2	84±1	83±2	OCP: 0.833	0.074	0.073
Heart Rate (beats/min)							
Trial 1 Baseline	66±3	68±2	69±3	75±2	NC: 0.051	0.276	0.167
Trial 2 Baseline	64±2	69±2	69±3	74±2	OCP: **0.013**	0.336	0.529
Forearm Blood Flow (mL/dL/min)							
Trial 1 Baseline	2.4±0.3	2.6±0.3	2.0±0.2	2.6±0.2	NC: 0.357	0.188	0.736
Trial 2 Baseline	2.1±0.2	2.5±0.3	2.0±0.2	2.4±0.2	OCP: 0.064	0.230	0.264
Forearm Vascular Conductance (mL/dL/100 mmHg/min)							
Trial 1 Baseline	2.9±0.3	3.2±0.3	2.5±0.3	3.1±0.2	NC: 0.243	0.256	0.973
Trial 2 Baseline	2.6±0.2	3.0±0.3	2.4±0.3	2.9±0.3	OCP: 0.061	0.141	0.576
Total Vascular Conductance (mL/mmHg.min)							
Trial 1 Baseline	61±2	60±3	74±6	74±4	NC: 0.761	0.367	0.416
Trial 2 Baseline	59±2	58±3	73±6	75±4	OCP: 0.688	0.890	0.293

Supplemental Tables 3: Normoxic Baseline Before and Following Washout. Data are reported as Mean±SEM from n=10 from steady-state normoxic conditions before each trial. The effect of phase (low/high hormone) and trial (1/2) within group (naturally cycling, oral contraceptive) was assessed using a two-way repeated measures ANOVA. Normality (Shapiro-Wilk), Variance (Brown-Forsynthe).

Figure 1: Conceptual Framework. Sympathetic neuronal activity travels down the axon to the nerve terminal causing depolarization and a release of norepinephrine into the synaptic cleft. Norepinephrine then binds to α-adrenergic receptors to cause constriction or β-adrenergic receptors to cause dilation on the smooth muscle. Circulating molecules in the bloodstream may bind to receptors on the endothelium to also cause constriction or dilation. α: α-adrenergic receptor; β: β-adrenergic receptor; EPI: epinephrine; ER: estrogen receptor; NE: norepinephrine; NO: nitric oxide; 17-β: 17-β estradiol.
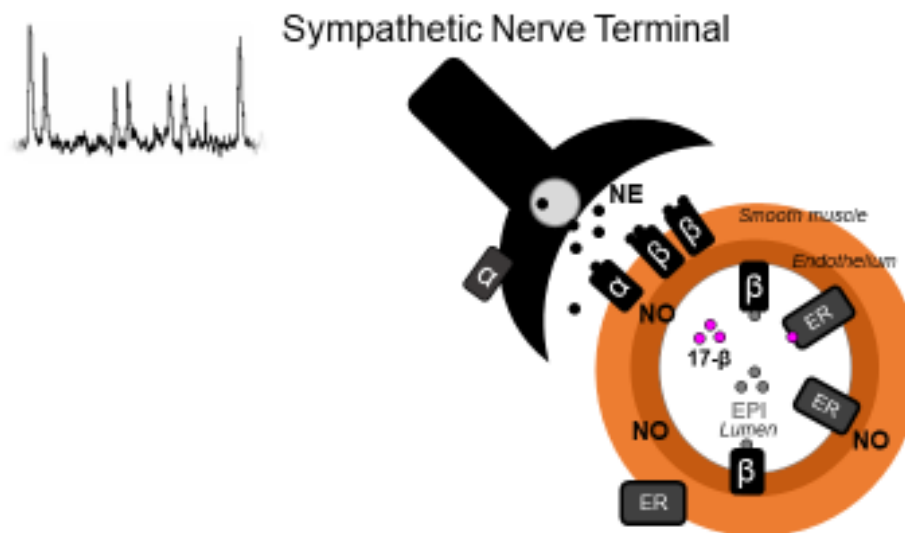
Figure 2: Study Timeline.

| 5 min baseline | 2 min CPT | 10-min washout | 3 min baseline | Hypoxia transition | Steady state hypoxia, 3 min | 2 min CPT |

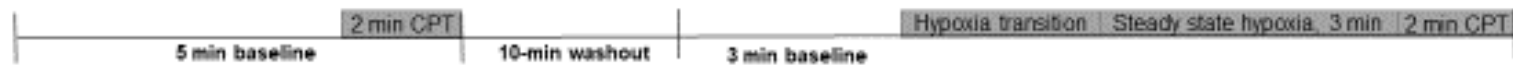The image spans most of the page. It's a scientific figure.

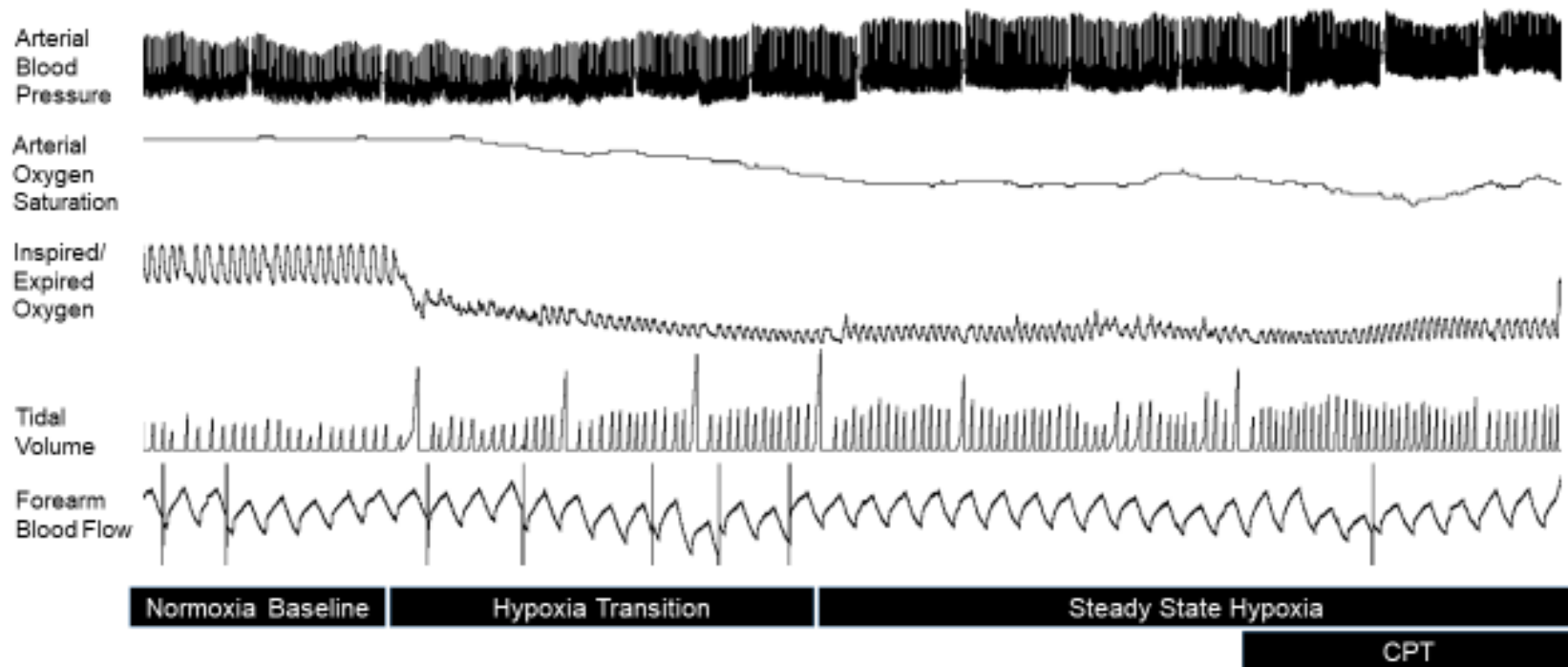Figure 3: Trial 2 Hypoxia Cold Pressor Protocol and Data Representation.
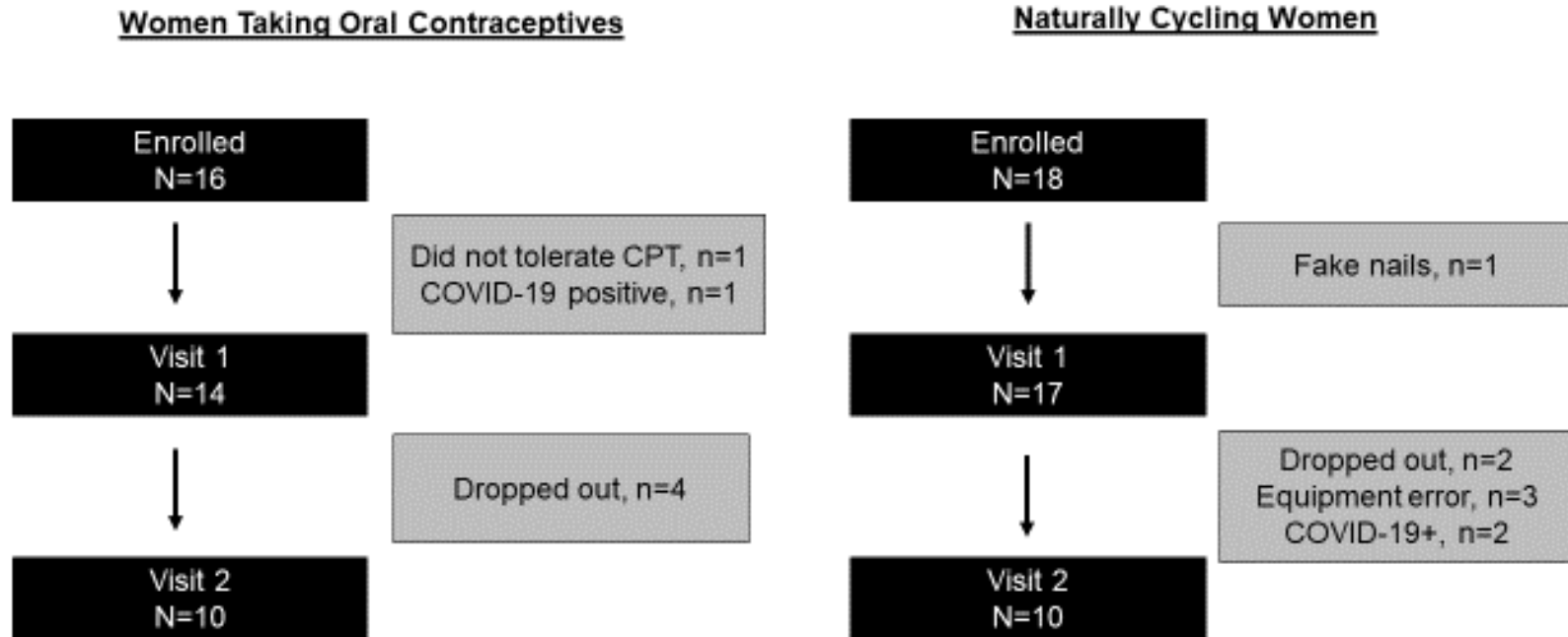
Figure 4: Participation Enrollment and Attrition.

Figure 5: Aim 1. Vascular Response to Hypoxia. Two-way Repeated Measures ANOVA (Normality: Shapiro-Wilk, Equal Variance: Brown-Forsynthe), Multiple comparisons (Holm-Sidak). Data are reported as mean ± SEM. *$p < 0.05$ Low hormone vs high hormone.
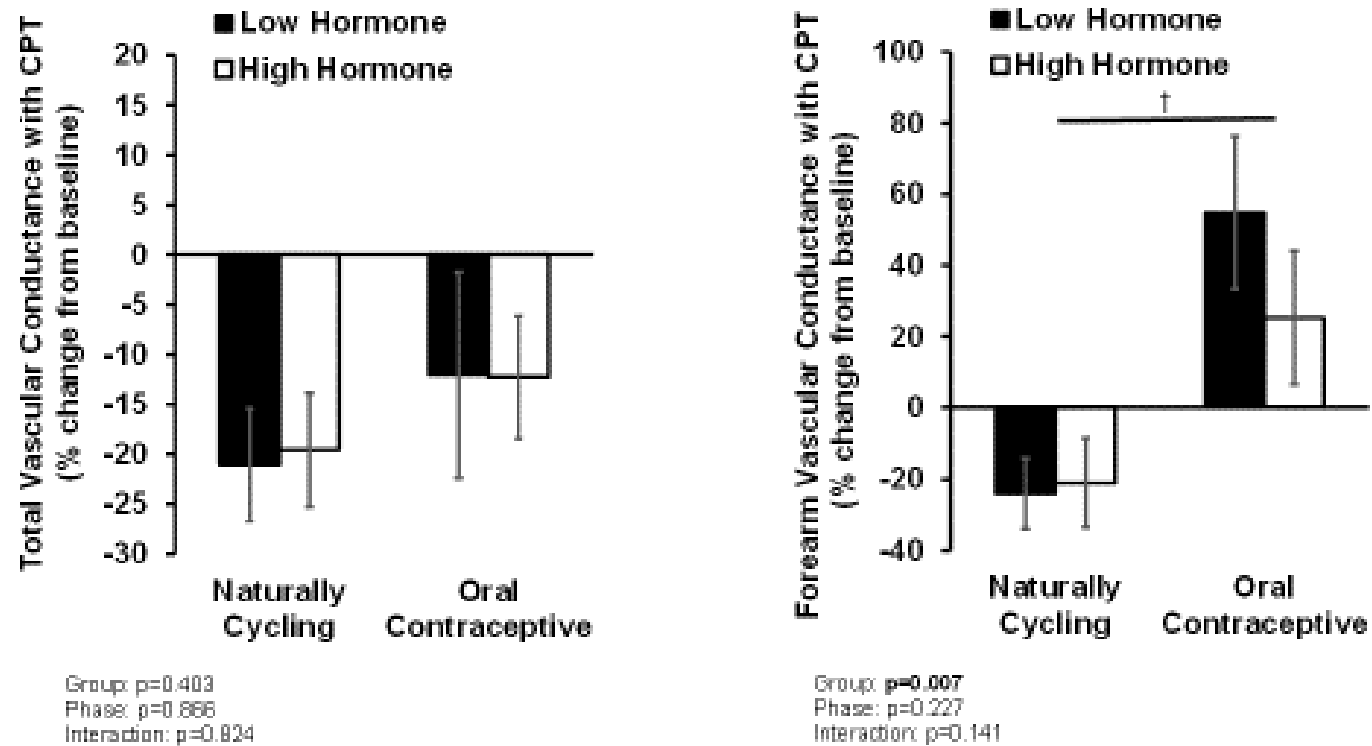
Figure 6: Aim 2. Vascular Response to Sympathetic Activation. Two-way Repeated Measures ANOVA (Normality: Shapiro-Wilk, Equal Variance: Brown-Forsynthe), Multiple comparisons (Holm-Sidak) within-group. Data are reported as mean ± SEM. †$p < 0.05$ NC vs OCP.
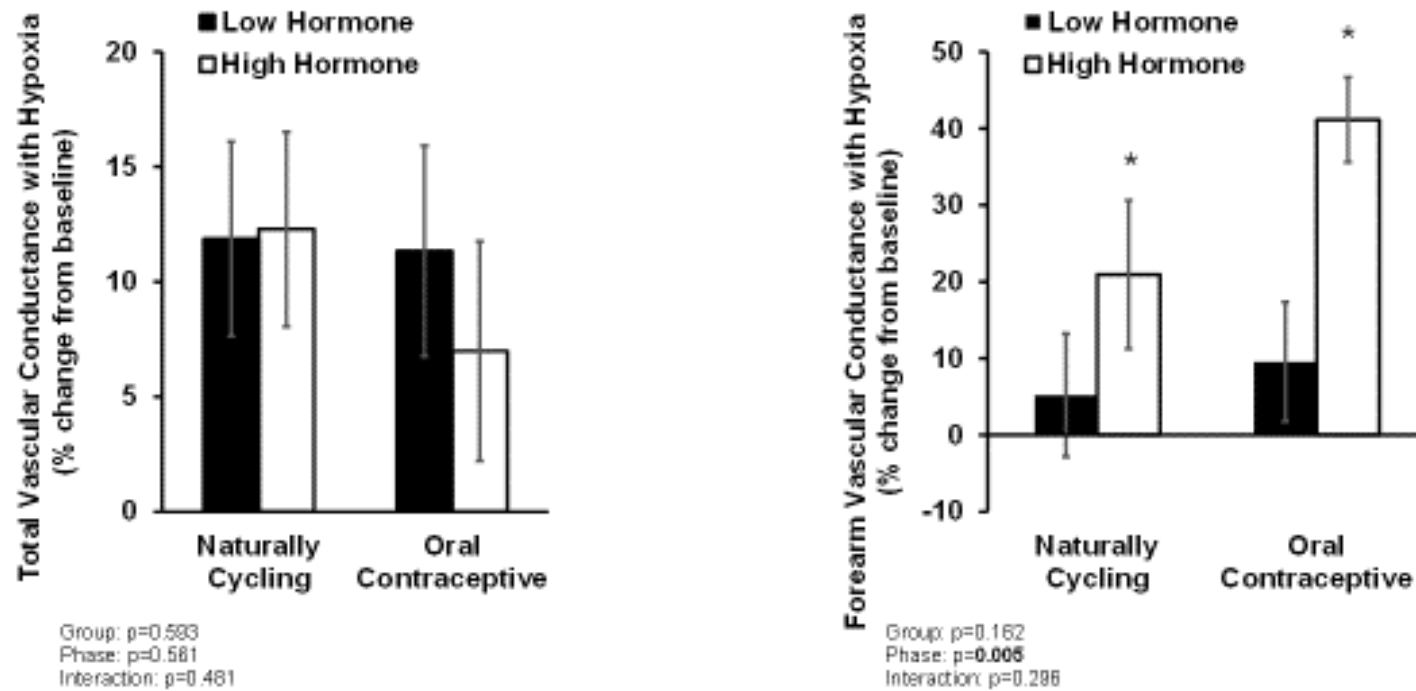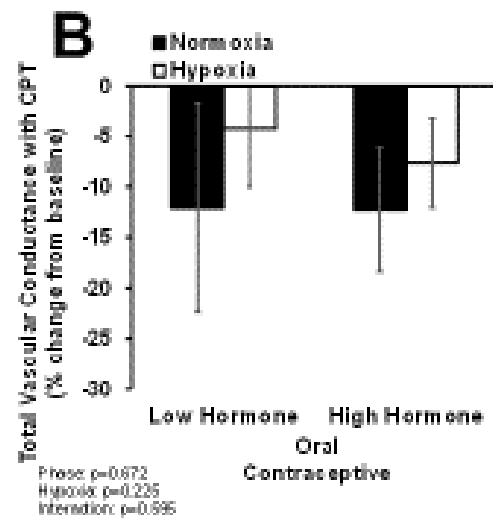
Figure 7: Aim 3. Change in TVC during Normoxia and Hypoxia CPT. Two-way Repeated Measures ANOVA (Normality: Shapiro-Wilk, Equal Variance: Brown-Forsynthe), Multiple comparisons (Holm-Sidak) within-group. Data are reported as mean ± SEM.

Figure 8: Aim 3. Difference in the systemic vascular response to CPT under normoxic versus hypoxic conditions (Hypoxia CPT - Normoxia CPT). Two-way Repeated Measures ANOVA (Normality: Shapiro-Wilk, Equal Variance: Brown-Forsynthe), Multiple comparisons (Holm-Sidak). Data are reported as mean ± SEM.

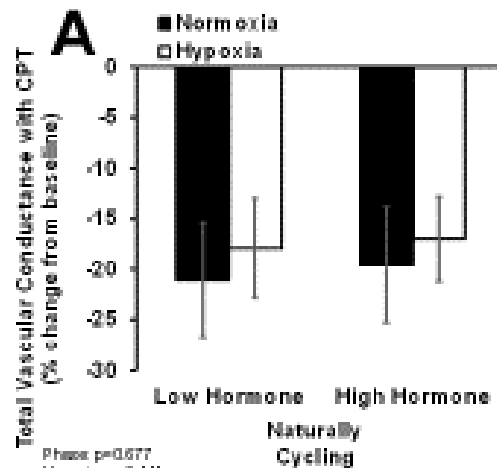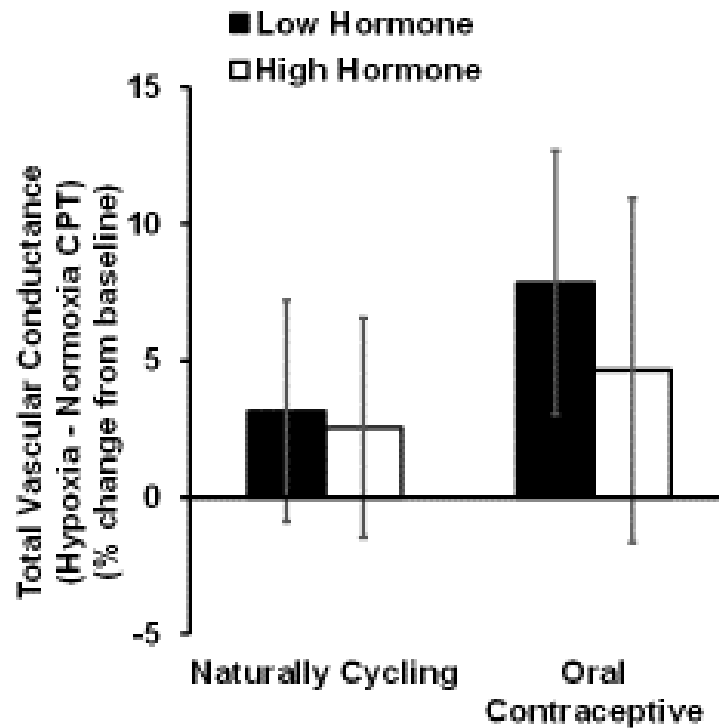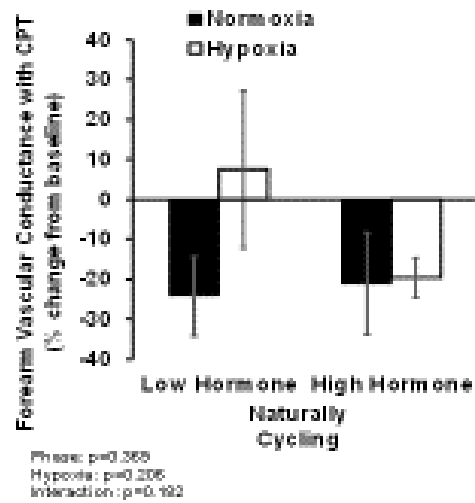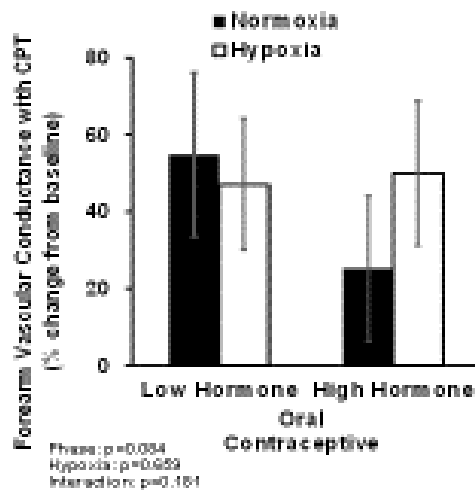Supplemental Figure 1: Difference in the local forearm vascular response to CPT under normoxic versus hypoxic conditions (Hypoxia CPT - Normoxia CPT).

CHAPTER 5: DISCUSSION

Overview

The main findings from the present study are three-fold. First, we show for the first time that hypoxic vasodilation in the forearm, but not in the systemic circulation, is greater during the high hormone phase compared to the low hormone phase of the menstrual cycle and/or oral contraceptive pill use (**Figure 5**). Second, we found acute sympathetic activation elicits forearm vasoconstriction in NC women, but paradoxical vasodilation in forearm circulation in women taking OCP (**Figure 6B**). Despite these observed differences in the forearm circulation, acute sympathetic activation elicits systemic vasoconstriction in both NC women and women taking OCP (**Figure 6A**). Last, we show sympathetic activation elicits similar levels of systemic vasoconstriction under normoxic and hypoxic conditions (**Figure 7**) and this effect is independent of group (NC, OCP) and cycle phase (low hormone, high hormone) (**Figure 8**); however women on OCP only exhibit peripheral dilation of the forearm to sympathetic activation during both normoxia and hypoxia CPT, whereas NC women constrict (**Supplemental Figure 1**). Together, these findings add to a growing body of evidence highlighting the modulatory effect of endogenous and exogenous hormones on the vascular response to physiological stress in healthy young women.

Aim 1. The effect of menstrual cycle phase and oral hormonal contraceptive pill phase on the vascular response to systemic hypoxia.

Systemic hypoxia creates a metabolic inequality of oxygen supply and oxygen demand, resulting in vascular adjustments to compensate and restore the system to

equilibrium. In response to hypoxia, a chemoreflex-driven increase in sympathetic nervous system activity and release of norepinephrine by postganglionic sympathetic nerve terminals occurs. During hypoxemia, despite this increase in sympathetic activity, vascular tone is maintained or decreased in order to preserve oxygen delivery (16). Given that norepinephrine mediated vasoconstriction in the periphery would limit blood supply during systemic hypoxia, opposition by local vasodilators within the skeletal muscle must occur to preserve blood flow.

There is precedence in the literature for sex-related differences in hypoxic vasodilation which have been attributed to differences in circulating estrogen. Specifically, Miller and colleagues examined hypoxic dilation in a cohort of young women and older postmenopausal women (57) where they showed hypoxic vasodilation measured in the femoral artery was greatest in young, premenopausal women compared to postmenopausal women. Of note, their cohort of young, premenopausal women was a mixture of naturally cycling women (n=4) and women taking oral contraceptives (n=8). Furthermore, the study did not control for pill or menstrual cycle phase. Although confounded by age differences between the groups and a varied group of pre-menopausal women, these data suggest female sex hormones contribute to greater hypoxic vasodilation and inversely, the lack thereof may be deleterious to the ability to dilate in response to hypoxia. Additionally, other studies have shown that while young women have a greater compensatory hypoxic dilation relative to age-matched men (11), these differences in hypoxic vasodilation are not observed in between older men and women, which may again be in part due to the loss of estrogen following menopause.

In the context of our data, we show that systemic vasodilation during hypoxia in

women and the relative magnitude of this vasodilation (% change from baseline) is no different between NC or OCP women or respective hormone phases. Second, when expressed as absolute values, we did not observe a significant hypoxia-mediated localized vasodilatory response of the forearm (FVC). Interestingly, when examining the relative increase in forearm blood flow (% change from baseline), we found a greater increase in hypoxic dilation during the high hormone phase of both NC and OCP groups compared to the low hormone phase. As such, these data demonstrate that hypoxic dilation occurs systemically in women irrespective of whether hormones are exogenous or endogenous, but the concentrations of these (low hormone vs high hormone) may be important when examining the magnitude of the local skeletal muscle vascular response to hypoxia.

Two plausible explanations for greater dilation of the skeletal muscle during the high hormone phase exist: 1) any hypoxia-mediated increase in sympathetic outflow is attenuated during the high hormone phase or 2) greater local vasodilation during the high hormone phase opposes sympathetic vasoconstriction during hypoxia. A pair of studies in NC (76) and women taking OCPs (77) observed greater increases in sympathetic nervous system activity in response to hypoxic apneas during low hormone phases compared to high hormone phases of the menstrual and/or pill cycle. These data suggest the lower local vasodilatory response to hypoxia during the low hormone phase may be due to a greater rise in MSNA and thus more sympathetically-mediated restraint of peripheral blood flow relative to the high hormone phase.

The mechanisms responsible for hypoxic vasodilation are primarily attributed to nitric oxide and β-adrenergic receptors within the vascular smooth muscle (11). Data suggest estrogen may enhance both nitric oxide bioavailability (26) and either β-receptor

expression, sensitivity or both (64). Based on this and in combination with our data, we speculate that the high hormone phase of the menstrual or pill cycle may enhance these pathways within the skeletal muscle vasculature. Furthermore, it is reasonable to speculate the β-receptor mechanism has a greater propensity to oppose sympathetic vasoconstriction as it proportionally causes norepinephrine or other adrenergic ligands to non-selectively bind to β-receptors, thereby offsetting α-mediated vasoconstriction. Conversely, an increase in nitric oxide during the high compared to low hormone phase (56) would augment the local dilatory capacity of the endothelium or vascular smooth muscle in response to hypoxia.

Aim 2. The effect of menstrual cycle phase and oral hormonal contraceptive pill phase on the vascular response to sympathetic activation

Use of CPT is well described as a noninvasive tool to increase activity of the sympathetic nervous system and assess α-adrenergic mediated vasoconstriction (18, 47) and has been shown to be reliably reproducible within human subjects (15).

If we start first with our measures of systemic vasoconstriction, we see results are contrary to our hypothesis. In both NC and OCP women, CPT elicited systemic vasoconstriction (TVC %) that did not differ between groups nor hormone phases. In contrast to the systemic circulation, when examining the local FVC response to acute sympathetic activation with CPT, we observed differing responses between women with circulating endogenous hormones (NC) and exogenous hormones (OCP).

First, as expected, we observed a reduction in FVC in NC women during the CPT and this reduction in FVC was to a level consistent with what has been published previously

(61). This sympathetically-mediated vasoconstriction did not differ between low and high hormone phases – suggesting circulating endogenous estrogen does not influence vascular responsiveness to CPT in naturally cycling women.

Quite surprisingly, and in contrast to results from NC women, women taking OCPs exhibited paradoxical vasodilation and increased FVC during CPT, irrespective of pill phase. There was no change in cardiac output in response to CPT during either pill phase therefore the increase in blood flow during CPT does not appear to be a result of changes in central hemodynamics (i.e., cardiac output). This lack of central hemodynamic effect is further supported by a reduction in TVC during CPT similar to NC (**Figure 6A**). With this, we speculate any sympathetically mediated increase in FVC in women taking OCPs is likely driven by local vasodilation in the forearm. However, the specific tissues (e.g., muscle, adipose, skin) in which this vasodilation is occurring is more difficult to discern.

Because the methodology of VOP requires occlusion on the hand, acral skin is not likely to contribute to vasodilation observed during CPT (85). Additionally, VOP has historically not been thought to detect changes in skin blood flow of the forearm when performed in a thermoneutral environment (85). All of our studies were completed in a temperature-controlled room and the acute, localized cold stress to the foot of the participant has not been shown to significantly alter core body temperature (88) and thus conclude it is not skin where vasodilation of the forearm occurs. Alternatively, there are some data to suggest subcutaneous adipose tissue does contribute a percentage to total blood flow of the forearm, and linearly increases with obesity (4); however, adrenergic influences do not appear to significantly modulate adipose tissue blood flow (24). Combined with the relatively low body mass index of women taking OCPs, it is unlikely

adipose tissue significantly contributed to the vasodilation that occurred in response to CPT.

Taken together, we speculate the vasodilatory response to sympathetic activation (CPT) observed only in the OCP group is likely due to local mechanisms within the skeletal muscle vasculature. For example, nitric oxide and/or β-receptors mediated vasodilation within the skeletal muscle vasculature are enhanced in women taking OCPs (42, 51), possibly due to the influence of estrogen on the β-receptors (64). This notion is supported by data demonstrating women taking OCPs having significantly greater β-receptor sensitivity compared to naturally cycling women (51). It is therefore reasonable to speculate the more biologically potent synthetic ethinyl estradiol (5) has a greater influence on β-receptors and would in theory, further augment nitric oxide-mediated vasodilation compared to endogenous estrogen. Thus, in the setting of sympathetic activation, the potential for a relative greater β-receptor mediated dilation and an increase in flow may occur. Given paradoxical vasodilation in response to sympathetic activation was observed during both phases of OCP use, our results indicate any contributing mechanisms are the result of a chronic, rather than acute effect of synthetic estrogen. In a short term supplementation model, postmenopausal women (whom experience chronically low endogenous estrogen) were treated for three days with transdermal exogenous estrogen (70). The transdermal estrogen did not attenuate norepinephrine spillover or sympathetically mediated hemodynamic responses to CPT following treatment. This suggests exogenous estrogen needs to be ingested orally and circulated systemically to potentiate any putative effects in response to sympathetic activity (70).

Aim 3. The effect of menstrual cycle phase and oral hormonal contraceptive pill phase on the peripheral vascular response to concomitant hypoxia and sympathetic activation

Hypoxic sympatholysis is the concept that during systemic hypoxia, local vasodilators within the peripheral skeletal muscle vasculature in order to oppose post-junctional α-adrenergic constriction in order to preserve or increase blood flow. In the context of exercise, sympatholysis is an important mechanism which helps to preserve or maintain oxygen delivery and blood flow during systemic sympathoexcitation. Data suggest that hypoxic sympatholysis does not exist, as vasoconstriction during hypoxia was preserved (17) or augmented (73) in response to norepinephrine or sympathetic activity, respectively. However, controversy exists (35). Specifically, intra-arterial forearm infusions of tyramine to evoke norepinephrine release elicited the same sympathetically-mediated vasoconstriction during normoxia as it did during moderate hypoxia (17). The authors concluded from this study that post-junctional α-adrenergic responsiveness is preserved in the setting of hypoxia (i.e. the absence of hypoxic sympatholysis) (17). Contrary to our hypothesis, but consistent with prior studies from primarily men, we show hypoxic sympatholysis is also not present in healthy young women irrespective of hormone or pill phase (**Figure 7**). Specifically, we show that the systemic vasoconstriction elicited by CPT is similar under normoxic and hypoxic conditions in all groups studied. These conclusions are maintained when total vascular conductance is represented as a relative change from baseline (**Figure 7A and 7B**) and when comparing the magnitude of difference between normoxic and hypoxic CPTs (**Figure 8**). Notably, although not directly tested statistically- all women have positive values (hypoxia CPT – normoxia CPT)

indicative of a hypoxia-mediated attenuation of sympathetic vasoconstriction across all groups and conditions (**Figure 8**), confirming our hypothesis for Aim 3. Due to methodological limitations, definitive conclusions in the forearm may not be made using current methods. However, as we observed the CPT elicited robust and paradoxical forearm vasodilation in the OCP group during both hypoxia and normoxia, it appeared hypoxic sympatholysis (**Aim 3**) was also appeared to be present in the forearm of women taking OCPs only. These data are presented within the **Appendix**. Further work is needed using gold-standard, targeted approaches such as pharmacological injection to either confirm or refute our data.

In regards to systemic circulations, we confirm the reduction in TVC does not differ between NC and women taking OCPs or their respective circulating hormone concentrations. A potential reason we did not observe sympatholysis may be the rate at which sympathetic activity is increased. For example, data show increasing local dilatory factors may inhibit norepinephrine release at low levels of sympathetic activity, but not proportionally to increases in sympathetic activity (78), such that with a rapid increase in sympathetic activity (such as with CPT) any previous inhibition is lost and a large amount of norepinephrine is released. Additionally, norepinephrine is more rapidly cleared from circulation during hypoxia (48). Hypoxia elicits a low level of sympathetic activation. When hypoxia is coupled with a CPT, a rapid and large increase in norepinephrine will occur. In this context, it may be that clearance of norepinephrine is not sufficient thus resulting in binding to adrenergic receptors and vasoconstriction.

It may be that as with functional sympatholysis attenuation of sympathetic vasoconstriction during exercise a blunted vasoconstrictor response is due to actively

contracting muscle creating localized tissue hypoxia. A study found that when sympathetic activation by use of lower body negative pressure was combined with hypoxia, vasoconstriction was preserved i.e. absence of hypoxic sympatholysis (29). However, when the researchers repeated the measurements with the addition of handgrip exercise, the vasoconstrictor response to hypoxia and sympathetic activation was abolished and attributed to local tissue hypoxia (29). It could be that, in our study we did not achieve a significant or not low enough localized P_{O2} to observe blunted vasoconstriction with hypoxia alone and/or muscle contraction is required to further reduce this local P_{O2}. Nonetheless, our data suggest the systemic vasoconstrictor response is indeed preserved during sympathetic activation and concomitant hypoxia in women.

Perspectives

Foremost, it should be noted that our findings were from acute conditions and whether these observations are maintained during either chronic conditions (hypoxia or sympathetic activation) is currently unknown. However, because we found during lower circulating estrogen concentrations, women had a lower relative vascular increase in hypoxia, these data may have practical and translational implications for women. For example, lower local blood flow in response to hypoxia (or an individual living at altitude) with chronically low estrogen (i.e. menopause) may potentiate a greater risk of conditions where impaired blood flow is observed, like peripheral artery disease.

Second, we observed a paradoxical dilation in women taking OCPs in response to acute sympathetic activation. This could be interpreted as an abnormal vascular response to autonomic stimulation, which inherently is the etiology of many syndromes and

conditions. Specifically, dilating instead of constricting peripheral vascular beds in the wrong context could lead to postural blood pooling, reduced perfusion to the brain, and ultimately syncope. This could potentially explain the higher prevalence of syncope and orthostatic hypotension in women relative to men (9), but further research is needed.

Conclusion

Main findings are 3-fold. First, both women who take OCPs and NC women exhibit similar levels of systemic vasodilation, but an augmented local peripheral blood flow response to hypoxia during the high hormone phase compared to the low hormone phase of the pill/menstrual cycle, respectively. Second, despite systemic vasoconstriction, women on OCPs have a paradoxical local peripheral vasodilatory response to acute sympathetic activation. The local vascular response to sympathetic activation in women taking OCPs is also contrary to vasoconstriction observed in NC women, regardless of menstrual/pill cycle phase. Lastly, there is no difference in systemic blood flow between OCP and NC in response to concomitant hypoxia and sympathetic activation; however, only women on OCPs exhibit sympathetically mediated dilation in the both settings of normoxia and hypoxia. Together these data highlight hormone-related differences in neurovascular control mechanisms and has potential implications for disease states in which hypoxia and sympathetic activation are observed.

CHAPTER 6: LIMITATIONS AND FUTURE DIRECTIONS

Although there are many strengths to our study, there are several limitations that should be acknowledged. First, CPT is widely accepted as a stimulus to activate the sympathetic nervous system (79). Furthermore, CPT shows good reliability and repeatability within human subjects (15). Despite its reproducible nature within participants and study days, the level of sympathetic activation achieved may vary between individuals (40). Because we did not use microneurography to directly quantify the level of sympathetic activation achieved, we cannot confirm that the sympathetic stimulus was the same between subjects and/or conditions (i.e., normoxia, hypoxia). Pain scores were lower during hypoxia CPT in women on OCPs, but no different in NC women and perceived thermal scores were lower during the hypoxia trial in all groups (**Supplemental Tables**). Although this suggests hypoxia may alter the perception of pain and/or cold, neither thermal nor pain scores have been associated with the level of sympathoexcitation achieved with a CPT (20, 21). Indeed, there are data to suggest hypoxia alters thermoperception (27, 74) but the implications on peripheral vascular control are currently unknown and warrants further investigation. Alternatively, because we did not randomize trial order, the lower thermal scores during hypoxia could have been an effect of repeated exposure to CPT. In the future, quantifying sympathetic activity with microneurography will allow for more specific analytics to determine the relationship between sympathetic activity and the influence on vascular tone.

Intra-arterial infusion of vasoactive drugs, such as phenylephrine or isoprenaline would allow for pharmacologic dissection to determine the relative contributions of α- and β- adrenergic receptors to vascular tone. In addition, the forearm has been shown to exhibit

lesser vasoconstriction in response to MSNA compared to the leg (23). Therefore, our results may be strengthened by examining blood flow in both the upper and lower extremities. This is especially important given differences observed between systemic and local (forearm) vascular responses to the environmental stressors applied herein.

Due to our inclusion of 2nd, 3rd and 4th generation oral contraceptives, the synthetic progesterone used may be an unknown confounder. Each generation of OCP uses a different formulation of synthetic progesterone. Direct effects of progesterone on the vasculature may be both dilatory or constrictive depending on location and concentration (19). Data also shows progesterone antagonizes the effects of exogenous estradiol on endothelial function (58). While several studies have examined the effect of differing doses of synthetic progesterone on vascular function (39, 55), to the best of our knowledge, none have compared the effects of formulation/generation of progesterone, thus leaving a gap in the literature. This notion of controlling for and examining the effects of formulations and dosages of synthetic progesterone used in oral contraceptives on vascular physiology studies has been recently proposed by other researchers as well (87). Also related to OCP use, we did not control for the time the active pill was taken in women taking OCPs and as plasma levels of synthetic estrogen peak ~4 hours following ingestion (84), this should be taken into consideration for future studies.

Last, although we put in great effort to quantify and control for hormones, we did not focus on the effects of testosterone on the vasculature. Testosterone is an independent negative predictor for developing arterial stiffness or hypertension in men (38), but whether this is the same in women is unknown. Our data show a reduction in circulating testosterone during their active pill phase compared to the placebo phase in women taking OCPs,

opposite of that in NC women. As the implications of testosterone's impact on endothelial function has shown both positive and negative effects (19), it is difficult to discern the specific role of testosterone on our data. More research is required to determine testosterones impact on the vasculature in women.

Although OCP use has a clinical role as a pregnancy preventative, the vascular effects of OCP remain incompletely understood. Whether OCP can be cardioprotective in some populations like postmenopausal women or how OCP use in healthy young individuals may increase risk of cardiovascular disease is currently unknown. Our data provide the cornerstone for this future investigation into the role female sex hormones play in modulating the vasculature's response to environmental stressors.

REFERENCES

1.   **Adkisson EJ**, **Casey DP**, **Beck DT**, **Gurovich AN**, **Martin JS**, **Braith RW**.
      Central, peripheral and resistance arterial reactivity: Fluctuates during the phases
      of the menstrual cycle. *Exp Biol Med* 235: 111–118, 2010. doi:
      10.1258/ebm.2009.009186.

2.   **Barnes JN**, **Hart EC**, **Curry TB**, **Nicholson WT**, **Eisenach JH**, **Wallin BG**,
      Charkoudian N, **Joyner MJ**. Aging enhances autonomic support of blood
      pressure in women. *Hypertension* 63: 303–308, 2014. doi:
      10.1161/HYPERTENSIONAHA.113.02393.

3.   **Benjamin EJ**, **Blaha MJ**, **Chiuve SE**, **Cushman M**, **Das SR**, **Deo R**, **De
      Ferranti SD**, **Floyd J**, **Fornage M**, **Gillespie C**, **Isasi CR**, **Jim'nez MC**, **Jordan
      LC**, **Judd SE**, **Lackland D**, **Lichtman JH**, **Lisabeth L**, **Liu S**, **Longenecker CT**,
      MacKey RH, **Matsushita K**, **Mozaffarian D**, **Mussolino ME**, **Nasir K**, **Neumar
      RW**, **Palaniappan L**, **Pandey DK**, **Thiagarajan RR**, **Reeves MJ**, **Ritchey M**,
      Rodriguez CJ, **Roth GA**, **Rosamond WD**, **Sasson C**, **Towfghi A**, **Tsao CW**,
      Turner MB, **Virani SS**, **Voeks JH**, **Willey JZ**, **Wilkins JT**, **Wu JHY**, **Alger
      HM**, **Wong SS**, **Muntner P**. Heart Disease and Stroke Statistics'2017 Update: A
      Report from the American Heart Association. *Circulation* 135 Lippincott Williams
      and Wilkins: e146–e603, 2017.

4.   **Blaak EE**, **Van Baak MA**, **Kemerink GJ**, **Pakbiers MTW**, **Heidendal GAK**,
      Saris WHM. Total forearm blood flow as an indicator of skeletal muscle blood
      flow: Effect of subcutaneous adipose tissue blood flow. *Clin Sci* 87: 559–566,
      1994. doi: 10.1042/cs0870559.

5. **Blair RM**. The Estrogen Receptor Relative Binding Affinities of 188 Natural and Xenochemicals: Structural Diversity of Ligands. *Toxicol Sci* 54: 138–153, 2000. doi: 10.1093/toxsci/54.1.138.

6. **Boldo A**, **White WB**. Blood pressure effects of the oral contraceptive and postmenopausal hormone therapies. *Endocrinol. Metab. Clin. North Am.* 40: 419–432, 2011.

7. **Borg G**. Psychophysical scaling with applications in physical work and the perception of exertion. In: *Scandinavian Journal of Work, Environment and Health*. 1990, p. 55–58.

8. **Briant LJBB**, **Burchell AE**, **Ratcliffe LEKK**, **Charkoudian N**, **Nightingale AK**, **Paton JFRR**, **Joyner MJ**, **Hart EC**. Quantifying sympathetic neuro-haemodynamic transduction at rest in humans: insights into sex, ageing and blood pressure control. *J Physiol* 594: 4753–4768, 2016. doi: 10.1113/JP272167.

9. **Bryarly M**, **Phillips LT**, **Fu Q**, **Vernino S**, **Levine BD**. Postural Orthostatic Tachycardia Syndrome: JACC Focus Seminar. *J. Am. Coll. Cardiol.* 73 Elsevier USA: 1207–1228, 2019.

10. **Carter JR**, **Fu Q**, **Minson CT**, **Joyner MJ**. Ovarian cycle and sympathoexcitation in premenopausal women. *Hypertension* 61: 395–399, 2013. doi: 10.1161/HYPERTENSIONAHA.112.202598.

11. **Casey DP**, **Shepherd JRA**, **Joyner MJ**. Sex and vasodilator responses to hypoxia at rest and during exercise. *J Appl Physiol* 116: 927–936, 2014. doi: 10.1152/japplphysiol.00409.2013.

12. **Colafella KMM**, **Denton KM**. Sex-specific differences in hypertension and

associated cardiovascular disease. *Nat. Rev. Nephrol.* 14 Nature Publishing Group: 185–201, 2018.

13. **Coney AM**, **Bishay M**, **Marshall JM**. Influence of endogenous nitric oxide on sympathetic vasoconstriction in normoxia, acute and chronic systemic hypoxia in the rat. *J Physiol* 555: 793–804, 2004. doi: 10.1113/jphysiol.2003.058156.

14. **Dain W. Jacob, Jennifer L. Harper, Elizabeth P. Ott, Clayton L. Ivie JKL**. Sex differences in response to acute sympathetic activation during steady state hypoxia. .

15. **Dillon GA**, **Lichter ZS**, **Alexander LM**, **Vianna LC**, **Wang J**, **Fadel PJ**, **Greaney JL**. Reproducibility of the neurocardiovascular responses to common laboratory-based sympathoexcitatory stimuli in young adults. *J Appl Physiol* 129: 1203–1213, 2020. doi: 10.1152/japplphysiol.00210.2020.

16. **Dinenno FA**. Skeletal muscle vasodilation during systemic hypoxia in humans. *J. Appl. Physiol.* 120 American Physiological Society: 216–225, 2016.

17. **Dinenno FA**, **Joyner MJ**, **Halliwill JR**. Failure of systemic hypoxia to blunt α-adrenergic vasoconstriction in the human forearm. *J Physiol* 549: 985–994, 2003. doi: 10.1113/jphysiol.2003.042507.

18. **Dinenno FA**, **Tanaka H**, **Stauffer BL**, **Seals DR**. Reductions in basal limb blood flow and vascular conductance with human ageing: Role for augmented α-adrenergic vasoconstriction. *J Physiol* 536: 977–983, 2001. doi: 10.1111/j.1469-7793.2001.00977.x.

19. **Dos Santos RL**, **Da Silva FB**, **Ribeiro RF**, **Stefanon I**. Sex hormones in the cardiovascular system. *Horm. Mol. Biol. Clin. Investig.* 18 Walter de Gruyter

GmbH: 89–103, 2014.

20. **El Sayed K**, **Macefield VG**, **Hissen SL**, **Joyner MJ**, **Taylor CE**. Rate of rise in diastolic blood pressure influences vascular sympathetic response to mental stress. *J Physiol* 594: 7465–7482, 2016. doi: 10.1113/JP272963.

21. **El Sayed K**, **Macefield VG**, **Hissen SL**, **Joyner MJ**, **Taylor CE**. Blood pressure reactivity at onset of mental stress determines sympathetic vascular response in young adults. *Physiol Rep* 6: e13944, 2018. doi: 10.14814/phy2.13944.

22. **English JAL**, **Jacobs LO**, **Green GG**, **Andrews TC**. Effect of the menstrual cycle on endothelium-dependent vasodilation of the brachial artery in normal young women. *Am J Cardiol* 82: 256–258, 1998. doi: 10.1016/S0002-9149(98)00322-1.

23. **Fairfax ST**, **Holwerda SW**, **Credeur DP**, **Zuidema MY**, **Medley JH**, **Dyke II PC**, **Wray DW**, **Davis MJ**, **Fadel PJ**. The role of α-adrenergic receptors in mediating beat-by-beat sympathetic vascular transduction in the forearm of resting man. *J Physiol* 591: 3637–3649, 2013. doi: 10.1113/jphysiol.2013.250894.

24. **Frayn KN**, **Karpe F**. Regulation of human subcutaneous adipose tissue blood flow. *Int. J. Obes.* 38 Nature Publishing Group: 1019–1026, 2014.

25. **Fuchs FD**, **Whelton PK**. High Blood Pressure and Cardiovascular Disease. *Hypertens (Dallas, Tex 1979)* 75: 285–292, 2020. doi: 10.1161/HYPERTENSIONAHA.119.14240.

26. **Gavin KM**, **Seals DR**, **Silver AE**, **Moreau KL**. Vascular endothelial estrogen receptor α is modulated by estrogen status and related to endothelial function and endothelial nitric oxide synthase in healthy women. *J Clin Endocrinol Metab* 94:

3513–3520, 2009. doi: 10.1210/jc.2009-0278.

27. **Golja P**, **Kacin A**, **Tipton MJ**, **Eiken O**, **Mekjavic IB**. Hypoxia increases the cutaneous threshold for the sensation of cold. *Eur J Appl Physiol* 92: 62–68, 2004. doi: 10.1007/s00421-004-1058-9.

28. **Hannaford PC**. Combined oral contraceptives: Do we know all of their effects? *Contraception* 51 Contraception: 325–327, 1995.

29. **Hansen J**, **Sander M**, **Hald CF**, **Victor RG**, **Thomas GD**. Metabolic modulation of sympathetic vasoconstriction in human skeletal muscle: Role of tissue hypoxia. *J Physiol* 527: 387–396, 2000. doi: 10.1111/j.1469-7793.2000.00387.x.

30. **Hart EC**, **Charkoudian N**, **Wallin BG**, **Curry TB**, **Eisenach J**, **Joyner MJ**. Sex and ageing differences in resting arterial pressure regulation: The role of the β-adrenergic receptors. *J Physiol* 589: 5285–5297, 2011. doi: 10.1113/jphysiol.2011.212753.

31. **Hart EC**, **Charkoudian N**, **Wallin BG**, **Curry TB**, **Eisenach JH**, **Joyner MJ**. Sex differences in sympathetic neural-hemodynamic balance implications for human blood pressure regulation. *Hypertension* 53: 571–576, 2009. doi: 10.1161/HYPERTENSIONAHA.108.126391.

32. **Hart EC**, **Joyner MJ**, **Wallin BG**, **Charkoudian N**. Sex, ageing and resting blood pressure: Gaining insights from the integrated balance of neural and haemodynamic factors. *J Physiol* 590: 2069–2079, 2012. doi: 10.1113/jphysiol.2011.224642.

33. **Harvey RE**, **Hart EC**, **Charkoudian N**, **Curry TB**, **Carter JR**, **Fu Q**, **Minson CT**, **Joyner MJ**, **Barnes JN**. Oral Contraceptive Use, Muscle Sympathetic Nerve

Activity, and Systemic Hemodynamics in Young Women. *Hypertension* 66: 590–597, 2015. doi: 10.1161/HYPERTENSIONAHA.115.05179.

34. **Hashimoto M**, **Akishita M**, **Eto M**, **Ishikawa M**, **Kozaki K**, **Toba K**, **Sagara Y**, **Taketani Y**, **Orimo H**, **Ouchi Y**. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation* 92: 3431–3435, 1995. doi: 10.1161/01.CIR.92.12.3431.

35. **Heistad DD**, **Wheeler RC**. Effect of acute hypoxia on vascular responsiveness in man. I. Responsiveness to lower body negative pressure and ice on the forehead. II. Responses to norepinephrine and angiotensin. 3. Effect of hypoxia and hypocapnia. *J Clin Invest* 49: 1252–1265, 1970. doi: 10.1172/JCI106338.

36. **Hinojosa-Laborde C**, **Mifflin SW**. Sex differences in blood pressure response to intermittent hypoxia in rats. *Hypertension* 46: 1016–1021, 2005. doi: 10.1161/01.HYP.0000175477.33816.f3.

37. **Hissen SL**, **Macefield VG**, **Brown R**, **Taylor CE**. Sympathetic baroreflex sensitivity is inversely related to vascular transduction in men but not women. *Am J Physiol - Hear Circ Physiol* 317: H1203–H1209, 2019. doi: 10.1152/ajpheart.00501.2019.

38. **Hougaku H**, **Fleg JL**, **Najjar SS**, **Lakatta EG**, **Harman SM**, **Blackman MR**, **Metter EJ**. Relationship between androgenic hormones and arterial stiffness, based on longitudinal hormone measurements. *Am J Physiol - Endocrinol Metab* 290: 234–242, 2006. doi: 10.1152/ajpendo.00059.2005.

39. **Houghton BL**, **Holowatz LA**, **Minson CT**. Influence of progestin bioactivity on cutaneous vascular responses to passive heating. *Med Sci Sports Exerc* 37: 45–51,

2005. doi: 10.1249/01.MSS.0000150075.81511.FE.

40. **Huang M**, **Yoo JK**, **Stickford ASL**, **Moore JP**, **Hendrix JM**, **Crandall CG**, **Fu Q**. Early sympathetic neural responses during a cold pressor test linked to pain perception. *Clin Auton Res* 31: 215–224, 2021. doi: 10.1007/s10286-019-00635-7.

41. **Jacob DW**, **Ott EP**, **Baker SE**, **Scruggs ZM**, **Ivie CL**, **Harper JL**, **Manrique-Acevedo CM**, **Limberg JK**. Sex differences in integrated neuro-cardiovascular control of blood pressure following acute intermittent hypercapnic-hypoxia. .

42. **John S**, **Jacobi J**, **Schlaich MP**, **Delles C**, **Schmieder RE**. Effects of oral contraceptives on vascular endothelium in premenopausal women. *Am J Obstet Gynecol* 183: 28–33, 2000. doi: 10.1067/mob.2000.105739.

43. **Jones PP**, **Davy KP**, **Seals DR**. Influence of gender on the sympathetic neural adjustments to alterations in systemic oxygen levels in humans. *Clin Physiol* 19: 153–160, 1999. doi: 10.1046/j.1365-2281.1999.00158.x.

44. **Joyner MJ**, **Casey DP**. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol Rev* 95: 549–601, 2015. doi: 10.1152/physrev.00035.2013.

45. **Joyner MJ**, **Wallin BG**, **Charkoudian N**. Sex differences and blood pressure regulation in humans. *Exp Physiol* 101: 349–355, 2016. doi: 10.1113/EP085146.

46. **Kneale BJ**, **Chowienczyk PJ**, **Brett SE**, **Coltart DJ**, **Ritter JM**. Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. *J Am Coll Cardiol* 36: 1233–1238, 2000. doi: 10.1016/S0735-1097(00)00849-4.

47. **Kooijman M**, **Rongen GA**, **Smits P**, **Van Kuppevelt HJM**, **Hopman MTE**. The role of the α-adrenergic receptor in the leg vasoconstrictor response to orthostatic

stress. *Acta Physiol* 195: 357–366, 2009. doi: 10.1111/j.1748-1716.2008.01904.x.

48. **Leuenberger U**, **Gleeson K**, **Wroblewski K**, **Prophet S**, **Zelis R**, **Zwillich C**, **Sinoway L**. Norepinephrine clearance is increased during acute hypoxemia in humans. *Am J Physiol - Hear Circ Physiol* 261, 1991. doi: 10.1152/ajpheart.1991.261.5.h1659.

49. **Limberg JK**, **Casey DP**, **Trinity JD**, **Nicholson WT**, **Wray DW**, **Tschakovsky ME**, **Green DJ**, **Hellsten Y**, **Fadel PJ**, **Joyner MJ**, **Padilla J**. Assessment of resistance vessel function in human skeletal muscle: guidelines for experimental design, Doppler ultrasound, and pharmacology. *Am. J. Physiol. Heart Circ. Physiol.* 318 NLM (Medline): H301–H325, 2020.

50. **Limberg JK**, **Johansson RE**, **Peltonen GL**, **Harrell JW**, **Kellawan JM**, **Eldridge MW**, **Sebranek JJ**, **Schrage WG**. β-Adrenergic-mediated vasodilation in young men and women: Cyclooxygenase restrains nitric oxide synthase. *Am J Physiol - Hear Circ Physiol* 310: H756–H764, 2016. doi: 10.1152/ajpheart.00886.2015.

51. **Limberg JK**, **Peltonen GL**, **Johansson RE**, **Harrell JW**, **Kellawan JM**, **Eldridge MW**, **Sebranek JJ**, **Walker BJ**, **Schrage WG**. Greater beta-adrenergic receptor mediated vasodilation in women using oral contraceptives. *Front Physiol* 7: 215, 2016. doi: 10.3389/fphys.2016.00215.

52. **Magder S**. The meaning of blood pressure 11 Medical and Health Sciences 1102 Cardiorespiratory Medicine and Haematology Luigi Forni. *Crit. Care* 22 BioMed Central Ltd.: 2018.

53. **Maguire K**, **Westhoff C**. The state of hormonal contraception today: Established

and emerging noncontraceptive health benefits. *Am. J. Obstet. Gynecol.* 205
Mosby Inc.: S4, 2011.

54. **Marshall JM**. Interactions between local dilator and sympathetic vasoconstrictor
influences in skeletal muscle in acute and chronic hypoxia. *Exp. Physiol.* 100
Blackwell Publishing Ltd: 1400–1411, 2015.

55. **Meendering JR**, **Torgrimson BN**, **Miller NP**, **Kaplan PF**, **Minson CT**. Ethinyl
estradiol-to-desogestrel ratio impacts endothelial function in young women.
Contraception 79: 41–49, 2009. doi: 10.1016/j.contraception.2008.07.025.

56. **Meendering JR**, **Torgrimson BN**, **Miller NP**, **Kaplan PF**, **Minson CT**. A
combined oral contraceptive containing 30 mcg ethinyl estradiol and 3.0 mg
drospirenone does not impair endothelium-dependent vasodilation. *Contraception*
82: 366–72, 2010. doi: 10.1016/j.contraception.2010.03.009.

57. **Miller AJ**, **Cui J**, **Luck JC**, **Sinoway LI**, **Muller MD**. Age and sex differences in
sympathetic and hemodynamic responses to hypoxia and cold pressor test. *Physiol
Rep* 7: e13988, 2019. doi: 10.14814/phy2.13988.

58. **Miner JA**, **Martini ER**, **Smith MM**, **Brunt VE**, **Kaplan PF**, **Halliwill JR**,
Minson CT. Short-term oral progesterone administration antagonizes the effect of
transdermal estradiol on endothelium-dependent vasodilation in young healthy
women. *Am J Physiol - Hear Circ Physiol* 301, 2011. doi:
10.1152/ajpheart.00405.2011.

59. **Minson CT**, **Halliwill JR**, **Young TM**, **Joyner MJ**. Influence of the menstrual
cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in
young women. *Circulation* 101: 862–868, 2000. doi: 10.1161/01.CIR.101.8.862.

60. **Mozer MT**, **Holbein WW**, **Joyner MJ**, **Curry TB**, **Limberg JK**. Reductions in carotid chemoreceptor activity with low-dose dopamine improves baroreflex control of heart rate during hypoxia in humans. *Physiol Rep* 4: e12859, 2016. doi: 10.14814/phy2.12859.

61. **Parker BA**, **Smithmyer SL**, **Jarvis SS**, **Ridout SJ**, **Pawelczyk JA**, **Proctor DN**. Evidence for reduced sympatholysis in leg resistance vasculature of healthy older women. *Am J Physiol Circ Physiol* 292: H1148–H1156, 2007. doi: 10.1152/ajpheart.00729.2006.

62. **Patel HM**, **Heffernan MJ**, **Ross AJ**, **Muller MD**. Sex differences in forearm vasoconstrictor response to voluntary apnea. *Am J Physiol - Hear Circ Physiol* 306, 2014. doi: 10.1152/ajpheart.00746.2013.

63. **Quintana-Gallego E**, **Carmona-Bernal C**, **Capote F**, **Sánchez-Armengol Á**, **Botebol-Benhamou G**, **Polo-Padillo J**, **Castillo-Gómez J**. Gender differences in obstructive sleep apnea syndrome: A clinical study of 1166 patients. *Respir Med* 98: 984–989, 2004. doi: 10.1016/j.rmed.2004.03.002.

64. **Riedel K**, **Deussen AJ**, **Tolkmitt J**, **Weber S**, **Schlinkert P**, **Zatschler B**, **Friebel C**, **Müller B**, **El-Armouche A**, **Morawietz H**, **Matschke K**, **Kopaliani I**. Estrogen determines sex differences in adrenergic vessel tone by regulation of endothelial β-adrenoceptor expression. *Am J Physiol Circ Physiol* 317: H243–H254, 2019. doi: 10.1152/ajpheart.00456.2018.

65. **Robinson AT**, **Babcock MC**, **Watso JC**, **Brian MS**, **Migdal KU**, **Wenner MM**, **Farquhar WB**. Relation between resting sympathetic outflow and vasoconstrictor responses to sympathetic nerve bursts: sex differences in healthy young adults. *Am*

J Physiol Integr Comp Physiol 316: R463–R471, 2019. doi: 10.1152/ajpregu.00305.2018.

66. **Roca GQ**, **Redline S**, **Claggett B**, **Bello N**, **Ballantyne CM**, **Solomon SD**, **Shah AM**. Sex-specific association of sleep apnea severity with subclinical myocardial injury, ventricular hypertrophy, and heart failure risk in a community-dwelling cohort. *Circulation* 132: 1329–1337, 2015. doi: 10.1161/CIRCULATIONAHA.115.016985.

67. **Rosselli M**, **Imthurm B**, **Macas E**, **Keller PJ**, **Dubey RK**. Circulating nitrite/nitrate levels increase with follicular development: Indirect evidence for estradiol mediated NO release. *Biochem Biophys Res Commun* 202: 1543–1552, 1994. doi: 10.1006/bbrc.1994.2107.

68. **Ruffolo RR**, **Hieble JP**. α-Adrenoceptors. *Pharmacol. Ther.* 61: 1–64, 1994.

69. **Sidi D**, **Kuipers JRG**, **Teitel D**, **Heymann MA**, **Rudolph AM**, **Developmental AMR**. *Developmental changes in oxygenation and circulatory responses to hypoxemia in lambs*. 1983.

70. **Sofowora GG**, **Singh I**, **He HB**, **Wood AJJ**, **Stein CM**. Effect of acute transdermal estrogen administration on basal, mental stress and cold pressor-induced sympathetic responses in postmenopausal women. *Clin Auton Res* 15: 193–199, 2005. doi: 10.1007/s10286-005-0261-z.

71. **Somers VK**, **Mark AL**, **Abboud FM**. Interaction of baroreceptor and chemoreceptor reflex control of sympathetic nerve activity in normal humans. *J Clin Invest* 87: 1953–1957, 1991. doi: 10.1172/JCI115221.

72. **Stanhewicz AE**, **Wong BJ**. Counterpoint: Investigators should not control for

menstrual cycle phase when performing studies of vascular control that include women. *J. Appl. Physiol.* 129 American Physiological Society: 1117–1119, 2020.

73. **Tan CO**, **Tzeng YC**, **Hamner JW**, **Tamisier R**, **Taylor JA**. Alterations in sympathetic neurovascular transduction during acute hypoxia in humans. *Am J Physiol - Regul Integr Comp Physiol* 304: R959-65, 2013. doi: 10.1152/ajpregu.00071.2013.

74. **Tattersall GJ**, **Milsom WK**. Hypoxia reduces the hypothalamic thermogenic threshold and thermosensitivity. *J Physiol* 587: 5259–5274, 2009. doi: 10.1113/jphysiol.2009.175828.

75. **Torgrimson BN**, **Meendering JR**, **Kaplan PF**, **Minson CT**. Endothelial function across an oral contraceptive cycle in women using levonorgestrel and ethinyl estradiol. *Am J Physiol - Hear Circ Physiol* 292, 2007. doi: 10.1152/ajpheart.00762.2006.

76. **Usselman CW**, **Gimon TI**, **Nielson CA**, **Luchyshyn TA**, **Coverdale NS**, **Van Uum SHM**, **Kevin Shoemaker J**. Menstrual cycle and sex effects on sympathetic responses to acute chemoreflex stress. *Am J Physiol - Hear Circ Physiol* 308: H664–H671, 2015. doi: 10.1152/ajpheart.00345.2014.

77. **Usselman CW**, **Luchyshyn TA**, **Gimon TI**, **Nielson CA**, **Van Uum SHM**, **Shoemaker JK**. Hormone phase dependency of neural responses to chemoreflex-driven sympathoexcitation in young women using hormonal contraceptives. *J Appl Physiol* 115: 1415–1422, 2013. doi: 10.1152/japplphysiol.00681.2013.

78. **Vanhoutte PM**, **Verbeuren TJ**, **Webb RC**. Local modulation of adrenergic neuroeffector interaction in the blood vessel well. *Physiol. Rev.* 61: 151–247,

1981.

79.   **Victor RG**, **Leimbach WN**, **Seals DR**, **Gunnar Walljn B**, **Mark AL**. Effects of
the cold pressor test on muscle sympathetic nerve activity in humans.
Hypertension 9: 429–436, 1987. doi: 10.1161/01.HYP.9.5.429.

80.   **Virdis A**, **Pinto S**, **Versari D**, **Salvetti G**, **Bernini G**, **Fruzzetti F**, **Genazzani
AR**, **Taddei S**, **Salvetti A**. Effect of oral contraceptives on endothelial function in
the peripheral microcirculation of healthy women. *J Hypertens* 21: 2275–2280,
2003. doi: 10.1097/00004872-200312000-00015.

81.   **Weisbrod CJ**, **Minson CT**, **Joyner MJ**, **Halliwill JR**. Effects of regional
phentolamine on hypoxic vasodilation in healthy humans. *J Physiol* 537: 613–621,
2001. doi: 10.1111/j.1469-7793.2001.00613.x.

82.   **Wenger NK**, **Arnold A**, **Bairey Merz CN**, **Cooper-DeHoff RM**, **Ferdinand KC**,
Fleg JL, **Gulati M**, **Isiadinso I**, **Itchhaporia D**, **Light-McGroary KA**, **Lindley
KJ**, **Mieres JH**, **Rosser ML**, **Saade GR**, **Walsh MN**, **Pepine CJ**. Hypertension
Across a Woman's Life Cycle. *J. Am. Coll. Cardiol.* 71 Elsevier USA: 1797–1813,
2018.

83.   **Wenner MM**, **Stachenfeld NS**. Point: Investigators should control for menstrual
cycle phase when performing studies of vascular control that include women. *J
Appl Physiol* 129: 1114–1116, 2020. doi: 10.1152/japplphysiol.00443.2020.

84.   **Westhoff CL**, **Torgal AH**, **Mayeda ER**, **Pike MC**, **Stanczyk FZ**.
Pharmacokinetics of a combined oral contraceptive in obese and normal-weight
women. *Contraception* 81: 474–480, 2010. doi:
10.1016/j.contraception.2010.01.016.

85.   **Wilkinson IB**, **Webb DJ**. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol* 52: 631, 2001. doi: 10.1046/J.1365-2125.2001.01495.X.

86.   **Williams JS**, **Dunford EC**, **MacDonald MJ**. Impact of the menstrual cycle on peripheral vascular function in premenopausal women: Systematic review and meta-analysis. *Am. J. Physiol. - Hear. Circ. Physiol.* 319 American Physiological Society: H1327–H1337, 2020.

87.   **Williams JS**, **MacDonald MJ**. Influence of hormonal contraceptives on peripheral vascular function and structure in premenopausal females: A review. *Am. J. Physiol. - Hear. Circ. Physiol.* 320 American Physiological Society: H77–H89, 2021.

88.   **Winchester WJ**, **Gore K**, **Glatt S**, **Petit W**, **Gardiner JC**, **Conlon K**, **Postlethwaite M**, **Saintot PP**, **Roberts S**, **Gosset JR**, **Matsuura T**, **Andrews MD**, **Glossop PA**, **Palmer MJ**, **Clear N**, **Collins S**, **Beaumont K**, **Reynolds DS**. Inhibition of TRPM8 channels reduces pain in the cold pressor test in humans. *J Pharmacol Exp Ther* 351: 259–269, 2014. doi: 10.1124/jpet.114.216010.

89.   **Yoon SSS**, **Carroll MD**, **Fryar CD**. Hypertension Prevalence and Control Among Adults: United States, 2011-2014. [Online]. *NCHS Data Brief* : 1–8, 2015. http://www.ncbi.nlm.nih.gov/pubmed/26633197 [13 Nov. 2019].