THE INFLUENCE OF ORAL CONTRACEPTIVES ON THE AUTONOMIC NERVOUS SYSTEM

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Abstract

This thesis aims to determine the effects of oral contraceptives (OC) on resting autonomic function, autonomic reflex activation, and cerebral blood flow (CBF). The first study systematically reviewed the effects of OC on any aspect of autonomic function. A search strategy was applied to several databases, and 6,148 citations were retrieved. Forty studies were included and grouped by measurement of autonomic function investigated. Physiological responses to isolated reflex activation (i.e., the chemoreflex, mechanoreflex and metaboreflex) were influenced by OC; however, the effect of OC on resting autonomic indices and response to autonomic stressors was less consistent. These inconsistencies may be caused by hormone dosage within OC formulations or stressor intensity. The second study investigated the influence of OC on the cardiorespiratory response to metabo- and mechanoreflex activation in the arm and leg. Two minutes of isometric handgrip or plantarflexion exercise-induced similar increases in blood pressure (BP) and ventilation (V_E) in both OC and non-OC users (NOC). While both exercise modalities increased V_E, neither OC nor NOC exhibited a sustained increase in V_E during 3 minutes of arm or leg post-exercise circulatory occlusion. All women increased BP and V_E during 3 minutes of arm or leg passive movement. Considering that all women had a cardiorespiratory response to mechanoreflex but not metaboreflex activation, we suggest that the mechanoreflex may drive V_E during exercise in women. The final study investigated the influence of OC and the menstrual cycle on CBF and cerebral autoregulation (CA) during 5 minutes of hypercapnia (5%). Regardless of menstrual or pill phase, all women improved high frequency and very low frequency dynamic CA (dCA) during hypercapnia, although low frequency dCA decreased in the high hormone phase of NOC and the low hormone phase of the pill cycle. During hypercapnia, the presence of endogenous hormones attenuated dCA, while the chronic use of exogenous hormones (i.e., OC) chronically attenuated dCA (i.e., when hormones were *not* present). The findings of this thesis shed light on the complex influence of OC on autonomic function, CBF and different physiological stressors, emphasizing the need for further research to fully elucidate its impact. There is a further need to control for menstrual cycle, OC formulation, dose, progestin generation and/or type when studying factors affecting autonomic physiology.

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Cheers to the last five years,

T Pereira

Tania Pereira.

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Abbreviations

ANS – Autonomic Nervous System

BMI – Body Mass Index

BP – Blood Pressure

BSA – Body Surface Area

CBF – Cerebral Blood Flow

cBRS – Cardiovagal Baroreflex Sensitivity

CO_2 – Carbon Dioxide

CPT – Cold Pressor Test

CrCP – Critical Closing Pressure

CVRi – Cerebrovascular Resistance Index

DAT – Doppler Audio Translator

DBP – Diastolic Blood Pressure

dCA – Dynamic Cerebral Autoregulation

$ETCO_2$ – End-tidal Carbon Dioxide

ETO_2 – End-tidal Oxygen

HF – High Frequency

HH – High Hormone

HR – Heart Rate

HRV – Heart Rate Variability

JBI – Joanna Briggs Institute

LF – Low Frequency

LH – Low Hormone

MAP – Mean Arterial Pressure

MAP_{MCAv} – Cerebral Perfusion Pressure

MCA_V – Middle Cerebral Artery Velocity

MCA_{Vmax} – Systolic Middle Cerebral Artery Velocity

MCA_{Vmean} – Mean Middle Cerebral Artery Velocity

MCA_{Vmin} – Diastolic Middle Cerebral Artery Velocity

MVC – Maximum Voluntary Contraction

MSNA – Muscle Sympathetic Nerve Activity

nGain – normalized Gain
NO – Nitric Oxide
NOC – non-OC user
OC – Oral Contraceptives
PECO – Post-exercise Circulatory Occlusion
PI – Pulsatility Index
PICO – Population (P), Intervention (I), Comparator (C) and Outcome (O)
PM – Passive Movement
PNS – Parasympathetic Nervous System
PRISMA – Preferred Reporting Items Systematic Reviews and Meta-Analyses
PROSPERO – International Prospective Register of Systematic Reviews
PWV – Pulse Wave Velocity
Q – Cardiac Output
Qi – Cardiac Output Index
RAP – Resistance Area Product
RI – Resistance Index
RMSSD – Root Mean Squared of Successive normal-normal or RR intervals
SA node – Sinoatrial node
SBP – Systolic Blood Pressure
SDNN – Standard Deviation of Normal-Normal intervals
SDRR – Standard Deviation of RR intervals
SNS – Sympathetic Nervous System
SV – Stroke Volume
SVi – Stroke Volume index
SVT – Sympathetic Vascular Transduction
TCD – Transcranial Doppler
TFA – Transfer Function Analysis
TPR – Total Peripheral Resistance
TPRi – Total Peripheral Resistance index
TSST – Trier's Social Stress Test
VLF – Very Low Frequency
VO_2 max – Maximal Oxygen Consumption

Chapter 1: **Introduction**

Globally, 16% of all reproductive-aged women currently use an oral contraceptive (OC) as their primary contraceptive method[1]. OCs function to prevent pregnancy through a combination of progestin and estradiol, which inhibits follicular development[2,3]; however, there are also pill formulations that contain only progesterone. Both endogenous and exogenous sources of estrogen and progesterone fluctuate in cyclical patterns throughout the menstrual cycle or OC pill regimen. During the natural menstrual cycle, estrogen peaks during the late follicular phase and progesterone peaks in the luteal phase alongside a concurrent high level of estrogen[4]. For OC, pill regimens contain an active dose of combined hormones and often include a placebo "dose" (i.e., sugar pill), mimicking the cyclical fluctuations of the menstrual cycle. This cyclical fluctuation allows researchers to investigate the effects of the presence of circulating synthetic hormones (active dose pill), and the long-term adaptation from hormonal contraceptive use in the absence of synthetic hormones (placebo pill). Additionally, OC can be further stratified into "generations" based on the source that the hormone was synthesized from and the progestin type/family (e.g., gonanes are derived from testosterone, levonorgestrel or norgestrel)[5].

Progestins are synthesized to bind to progesterone receptors and mimic the effects of their endogenous counterparts; however, structural differences in progestins can lead to interactions with other steroid receptors (i.e., estrogen, androgen, mineralocorticoid and glucocorticoid receptors) with varying affinities[6,7]. While all progestins are able to bind to androgen receptors, medroxyprogesterone acetate (1st generation)[8], norethisterone acetate (1st generation)[8], levonorgestrel (2nd generation)[9] and gestodene (3rd generation)[9] are equally potent androgen receptor agonists as dihydrotestosterone (i.e., a natural androgen) for transcriptional activation. Androgenic effects, such as acne, hirsutism and weight gain, were unwanted symptoms of early generations of OC and newly progestins were developed to prevent these symptoms[10]. It is important to note that testosterone is the most abundant sex hormone in women and that it has critical physiological roles as a precursor to estrogen and maintaining cardiometabolic, muscular and sexual health (reviewed in Davis and Wahlin-Jacobsen, 2015)[11]. Interestingly, norethisterone acetate, levonorgestrel and gestodene are also the only progestins that can act as estrogen agonists[9]. Comparatively, drospirenone and nomogestrol acetate (i.e., both 4th generation) are as potent as natural progesterone as androgen receptor antagonists[9] –

highlighting the fact that the newer progestins were specifically formulated to minimize the androgenic effects of the previous generations and to have more specific progesterone-like activity. Taken together, these findings suggest that the varying progestins may exert differential effects based on binding affinity to specific receptors, and the tissue-specific density of those receptors.

Considerations for both the estrogen and progestin components of OC may be equally important when assessing potential physiological impacts of OC, as the risk of venous thromboembolism is influenced by the estrogen dose of combined OC[12] and the progestin type[13]. While many OC formulations have reduced the ethinyl estradiol (i.e., the most common estrogen component) dose, other more natural estrogens have been investigated for their efficacy as a contraceptive – such as 17 beta-estradiol[14], estradiol valerate[15], and more recently, estetrol[16]. OC have progressively evolved over the last 60 years since their inception and there are many intricacies to their pill regimens and formulations that contribute to higher variability than their endogenous counterparts.

In a review by Coupal et al.[17], the surge of pubertal sex hormones can coincide with adolescent-onset conditions associated with perturbed autonomic function, such as vasovagal syncope, orthostatic intolerance, and postural orthostatic tachycardia syndrome. Further, a considerable body of knowledge demonstrates that both fluctuating endogenous hormones[18-29], and the age-related menopausal decline in endogenous sex hormones[29-35] can influence autonomic function. If endogenous female sex steroids can influence autonomic function, then it is likely that exogenous sources of female sex steroids may also affect the physiological responses controlled by the autonomic nervous system (ANS).

The introductory chapter of this thesis will outline autonomic function, the control of cerebral blood flow (CBF) and reflex control of physiological responses to complex and singular stressors (i.e., exercise, orthostasis, hypercapnia, etc.). In particular, this thesis will focus on the influence of combined OC on resting autonomic function and the physiological responses controlled through autonomic reflex pathways. Moreover, the isolated autonomic reflexes of interest are related to the control of the physiological response to exercise. By exploring the current state of literature, this thesis will first outline current gaps in our knowledge of the influence of OC on autonomic function and CBF, then address those gaps in the chapters that follow.

1.1 **Literature Review**

1.1.1 **Autonomic Nervous System**

The ANS controls all unconscious physiological processes and the physiological response to various stressors, through altering the balance of parasympathetic and sympathetic activity. The parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) work antagonistically and synergistically to maintain homeostasis under resting conditions and in response to stress[36], respectively. These opposing branches of the ANS are commonly known as the “rest and digest” or the “fight or flight” responses; therefore, the PNS induces bradycardia, muscle relaxation and digestion, whereas the SNS induces an excitatory state through tachycardia, increased blood pressure (BP), vasoconstriction and thus, diversion of blood flow to active muscle in order to respond to an immediate threat[36].

Both the PNS and SNS directly innervate the sinoatrial (SA) node of the heart – meaning that sympathovagal balance can directly affect the firing frequency of the SA node and thus, heart rate (HR). The balance of parasympathetic and sympathetic HR can be estimated using HR variability (HRV), which assesses the magnitude of variation in timing of successive RR intervals[37]. Higher HRV suggests that the body is better able to respond and react to external stressors as a result of effective and dynamic neural feedback[38,39]. HRV can be assessed in the time domain (i.e., variation of successive RR intervals) and in the frequency domains, representing vagal tone[40] and sympathovagal contributions on frequency oscillations between successive RR intervals[41], respectively.

Changes in HR can also be mediated through the baroreflex, which are stretch mechanoreceptors located in the carotid sinus and aortic arch that influence cardiac contractility, total peripheral resistance (TPR) and RR interval in response to arterial BP[42]. These stretch-sensitive mechanoreceptors sense the deformation of the arterial wall and relay this afferent feedback to the cardiovascular centre of the brain for sympathovagal balance adjustment[43]. In response to a rise in BP, the arterial walls stretch, and this causes an increase in baroreceptor firing rate, which reduces sympathetic output to lower HR, TPR and BP[44,45]. When BP levels drop, this reduction in baroreflex activity (i.e., reduced baroreceptor firing rate due to less wall strain) induces an increase in sympathetic control of HR and sympathetic vasoconstriction to drive HR, TPR and cardiac output (Q) higher to increase BP[46,47]. The magnitude of alteration in arterial BP is influenced by baroreflex sensitivity (BRS), which can be further divided into cardiovagal BRS (cBRS) and sympathetic BRS[48-50]. The cBRS relies on the fast acting PNS to exert influence on arterial BP via HR and represents the relationship between the RR interval and magnitude of change in arterial BP[51]. Conversely, the sympathetic BRS is slower acting and represents the magnitude of change in sympathetic output (i.e., muscle sympathetic nerve activity (MSNA)) in response to changes in arterial BP[52].

1.1.2 **Autonomic Function During Exercise**

During exercise, the ANS functions to maintain systemic BP and adequate CBF in the face of extensive vasodilation occurring in the skeletal muscle due to metabolite build up and therefore an increase in blood flow directed to exercising limbs[53]. The magnitude of the autonomic response to exercise reflects the combined feedforward input of central command with the feedback of the exercise pressor reflex and the baroreflex (amongst others)[54,55].

Exercise Pressor Reflex

The exercise pressor reflex consists of two interconnected reflexes, the mechanoreflex and the metaboreflex. During mechanoreflex activation, group III afferent nerve fibres are responsible for responding to static contraction and/or tendon stretch[56]. The mechanoreflex initiates an increase in cardiovascular drive at the onset of exercise, prior to sufficient metabolite build-up[57]. With increased exercise duration and metabolite accumulation, group IV muscle afferent nerves relay this metabolic environment feedback from the active muscle to the brainstem[57]. Metaboreflex activation elicits greater sympathetic activity and increases BP through increasing systemic vascular resistance, thus shifting blood from inactive regions of non-exercising muscle and other vascular beds to contracting muscle[58].

Chemoreflex

Ventilation (V_E) is stimulated by the exercise pressor reflex with simultaneous chemoreflex activation[59], suggesting that the chemoreflex may provide an additional reflex feedback mechanism during exercise[60]. The chemoreflex is activated centrally and peripherally through chemoreceptors in the brainstem or carotid and aortic bodies, respectively[61]. Central (medullary) chemoreceptors are carbon dioxide (CO_2) -sensitive and respond to local tissue changes in [H+] (i.e., acidosis), while peripheral arterial chemoreceptors are primarily oxygen-sensitive with some sensitivity to CO_2[61]. In response to rising arterial CO_2 during exercise, the chemoreflexes are primarily responsible for augmenting the ventilatory response[62]. Indeed, both increased acidity of arterial blood pH and CO_2 production significantly contribute to the increase in V_E throughout exercise[63].

1.1.3 **Exercise & CBF**

During exercise, CO_2 levels and BP rise exponentially, and blood volume is directed to the periphery to support the increased oxygen demand of the skeletal muscles - presenting a significant challenge to the maintenance of CBF. Despite only representing 2% of the average human's total body weight, the brain is highly metabolically active requiring 20% of total body oxygen[64], and roughly 12% of Q at rest[65]. Maintaining cerebral oxygen delivery is regulated through cerebral autoregulation (CA) and other mechanisms, such as CO_2-mediated cerebral artery vasodilation, baroreflex-mediated alterations in systemic cardiovascular responses and finally, plasma volume shifts[66].

CA is the process by which CBF is maintained, despite changes in perfusion pressure, to meet the high metabolic rate and waste production of the brain[67]. CA can either be assessed statically or dynamically. Static CA involves longer term adaptations of blood flow to changes in BP, compared to dynamic CA (dCA) that measures spontaneous changes of blood flow in response to pressure, while considering changes over time[68]. Cerebral blood vessels are very sensitive to changes in CO_2, such that high CO_2 increases CBF by changing vasomotor tone[69]. CO_2 stimulates the cerebral arterioles to dilate and significantly increases CBF[70]. Greater cerebrovascular reactivity is marked by greater increases of CBF in response to CO_2[71].

1.1.4 **The Effects of OC on Resting Autonomic Function**

At rest, numerous studies have observed no influence of OC use on resting HR or HRV[72-75], although other studies have observed either an increase[76] or decrease in HRV[77]. Interestingly, OC users have greater resting sympathetic activity while taking the active dose pill[78]; thus, some controversies within the literature could stem from when in the pill cycle that these observations were made. Additionally, Cardoso et al.[79] observed that long-term OC use had been shown to increase BP in normotensive women. However, decreased peripheral vascular resistance, increased pulse pressure and increased aortic pulse wave velocity (PWV) have also been observed in OC users, suggesting greater peripheral vasodilation yet increased central aortic stiffness, respectively[80]. To support this, Limberg et al.[81] observed greater β-adrenergic receptor-mediated vasodilation in the forearm of OC users when compared to naturally cycling women (i.e., NOC). The conflicting evidence within OC literature suggests that greater clarification is necessary on the physiological effects of OC use and implies that more in-depth analysis of the context (i.e., OC type and dosing cycle) of these studies is needed.

1.1.5 **The Effects of OC on the Autonomic Reflexes**

Standing or exercise is associated with lower arterial CO_2, increased passive muscular stretch and increased metabolite build-up in active contracting or postural muscles; these markers inhibit the chemoreflex, or activate the mechanoreflex and metaboreflex, respectively. Abidi et al.[76] found very few interaction effects of posture with OC and/or menstrual cycle during upright standing; however, this group did not investigate the autonomic response to isolated reflex stimulation. Indeed, OC users have a smaller chemoreflex activated sympathetic response to apnea (i.e., concurrent hypoxia and hypercapnia) during the active pill dose versus placebo[78]. Further, Assadpour et al.[72] reported no effect of menstrual or pill cycle on the mechano- or metaboreflex, yet OC users exhibited significantly different ventilatory and cardiovascular responses compared to non-OC (NOC) users. For example, OC users displayed attenuated pressor responses to activation of the mechano- and metaboreflex, with an increased ventilatory response during the metaboreflex only[72]. It is important to note that this study only investigated activation of the *arm* metaboreflex and *leg* mechanoreflex.

Conversely, Minahan et al.[82] found that while women have significantly reduced pressor responses during isometric handgrip compared to men, OC use completely abolishes this sex difference implying an enhanced response with OC use. Both studies utilized handgrip to activate the metaboreflex and both observed responses to metaboreflex activation during the early follicular phase of the menstrual cycle (~days 2-6)[72,82]. However, Minahan et al.[82] observed the pressor responses of monophasic pill users (i.e., same dose of estrogen and progesterone in every pill) and Assadpour et al.[72] had a mixed group with multiple OC types (i.e., both monophasic and multiphase OC with an increasing dose of progestin throughout the month) with a higher proportion of monophasic 2nd generation OC. Minahan et al.[82] did not report specific

OC brand or dosages, which underlines the importance of controlling for and/or reporting dosage, pill type, and formulations used when interpreting/investigating OC autonomic responses. Moreover, the above suggests that synthetic female hormones may influence individual reflex responses but may not affect autonomic responses to complex stimuli (e.g., exercise). Indeed, a systematic review by D'Souza and colleagues[83] found few effects of OC on varying aspects of exercise performance.

1.1.6 **The Effects of OC & the Menstrual Cycle on CBF**

In the low hormone (LH) phase of the menstrual cycle (i.e., low estrogen and low progesterone), women experience more light-headedness during orthostatic stress compared to other phases[84] - implying that cycling female hormones may attenuate the hemodynamic and autonomic changes associated with presyncope. Indeed, estrogen augments CBF by decreasing resistance in cerebral microvasculature, particularly in the late follicular phase of the menstrual cycle (i.e., highest level of estrogen and no progesterone)[85], yet Abidi et al.[76] observed a strong trend for greater pulsatility index (PI) and lower middle cerebral artery (MCA) velocity in the high hormone (HH) phase of the menstrual cycle (i.e., high estrogen and high progesterone) – potentially indicating an opposing effect of progesterone. However, Favre & Serrador[86] found that while women had greater dCA than men in a young healthy population, there was no effect of menstrual cycle on dCA. Taken together, this suggests that while indices of CBF and resistance may fluctuate during the menstrual cycle, dCA may be robust enough to be unimpeded by cycling female sex steroids.

Kastrup et al.[87] found that women had greater cerebrovascular CO_2 reactivity compared to men, yet more recent studies have found no sex differences[88,89]. In fact, Hazlett and Edgell[88] further observed no menstrual phase effects on the cerebrovascular response to CO_2 when comparing the LH and HH phases, suggesting that high levels of endogenous hormones may not influence the CO_2 response. Importantly, neither Peltonen et al.[89] nor Hazlett and Edgell[88] included women taking OC, whereas Kastrup et al.[87] did. Interestingly, Abidi et al.[76] also found that OC users had lower $ETCO_2$ compared to non-OC users; despite that difference, there was no difference in cerebrovascular resistance in response to the observed hypocapnia between OC and NOC. These results imply that OC users may have attenuated cerebrovascular responsiveness to CO_2; however, more studies with clearly defined OC use parameters are needed.

1.1.7 **OC, Autonomic Function & CBF**

Aside from the potential influence of OC on dCA and CO_2 cerebrovascular reactivity, OC may influence CBF through modifications in the baroreflex or alterations in the patterns of peripheral blood flow. The sympathetic and cardiovagal baroreflex are less sensitive in OC users during the active dose of the pill regimen[18], suggesting an attenuation in autonomic control of HR and BP in the HH phase of the pill cycle. OC use is also associated with lower mean arterial pressure (MAP) and lower cerebral perfusion pressure during the active dose of the pill[76], suggesting that use of exogenous cycling hormones may promote chronic peripheral vasodilation leading to lower BP. Indeed, the active dose of OC has also been associated with increased lower limb blood flow[18], and attenuated chemoreflex function[78] – all of which may influence the maintenance of CBF. Considering the implications of the above, it is logical to assume that exogenous changes in hormonal concentrations could affect cerebrovascular responses to hypercapnia.

1.2 **Dissertation Aims**

The overarching aim of this thesis is to examine the effects of OC on the ANS and CBF. These investigations will examine the autonomic, cardiovascular, ventilatory, and cerebrovascular responses to challenges that stimulate the autonomic reflexes (similar to those engaged while exercising), while accounting for fluctuating sex hormones, OC use and/or differences in muscle size or strength when appropriate.

1.2.1 **Specific Study Aims & Hypotheses**

Study 1:

The purpose of the systematic review was to summarize the current state of autonomic and OC literature and determine research questions that have yet to be explored. In addition to providing more detailed autonomic reflex summaries, and depending on the citations retrieved, we primarily examined the effects of OC use on autonomic function with secondary commentary on the effects of the exogenous OC pill cycle, pill generation (i.e., comparing altered hormonal formulations), and other hormonal contraceptives where applicable. We hypothesized that OC would have significant impacts on autonomic function and the effects would likely differ between pill dosages, concentrations, and formulations.

Study 2:

Traditionally, the isolated metaboreflex has been measured in the upper body, and the few studies which investigated the lower limbs involve dynamic leg cycling which includes input from central command and concurrently activates mechanoreceptors[90-92]. Further, mechanoreflex activation is traditionally induced through passive movement of the lower leg[72,93,94]. The aim of this study was to confirm previous cardiovascular and ventilatory responses to the activation of the leg mechanoreflex and arm metaboreflex in OC users while uniquely investigating the response of the arm mechanoreflex and the leg metaboreflex. With this comparison, we were able to consider the role of muscle size and/or limb specific responses during reflex activation in OC users. We hypothesized that OC users would have attenuated pressor responses to both reflexes in both limbs and an increase of V_E in response to metaboreflex activation.

Study 3:

This study was a secondary analysis of Assadpour et al.[72], focusing on the cerebrovascular responses to hypercapnia in OC and NOC users which were not included in the original study. The objective of this study was to investigate the influence of OC use and menstrual cycle on cerebrovascular reactivity to hypercapnia and CA. We hypothesized that women in the HH menstrual phase would have greater cerebrovascular resistance compared to women in the LH menstrual phase, due to the previously observed trend of increased cerebrovascular vasoconstriction with similar MAP in the HH phase of the menstrual cycle[76]. Premenopausal women are more likely than men to experience syncope, and syncope becomes more prevalent during puberty[95], which suggests that the presence of endogenous hormones may be associated with attenuated dCA. Therefore, we hypothesized dCA would be reduced in the presence of sex hormones in the HH menstrual phase. We also hypothesized that OC users would have enhanced CO_2 reactivity due to previous observations of lower $ETCO_2$, yet no differences in cerebrovascular resistance due reduced cerebral perfusion pressure and enhanced MCA flow velocity[76]. Pregnant women have greater dCA compared to non-pregnant women[96,97], which suggests that greater concentrations of sex hormones may be protective. Considering that OC users have similar total concentrations of sex hormones to pregnant women[98], higher concentrations of sex hormones than typically seen across the natural menstrual cycle may lead to improved dCA.

Chapter 2: **The Effects of Oral Contraceptives on Resting Autonomic Function and the Autonomic Response to Physiological Stressors: A Systematic Review**

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2.1 **Abstract**

Purpose: This systematic review aimed to summarize how OC affect resting autonomic function and the autonomic response to a variety of physiological stressors. **Methods:** A search strategy was created to retrieve citations investigating physiological responses comparing OC users to NOC in response to autonomic reflex activation. **Results:** 6,148 citations were identified across databases from inception to June 2nd, 2022, and 3,838 citations were screened at the abstract level after deduplication. Then, 134 texts were assessed at full-text, and only 40 studies met eligibility requirements. Included citations were grouped by the aspect of autonomic function assessed, including autonomic reflex (i.e., baroreflex, chemoreflex, mechanoreflex, metaboreflex, and veno-arterial reflex), or indicators (i.e., HRV, PWV, and sympathetic electrodermal activity), and physiological stressors that may alter autonomic function (i.e., auditory, cold, exercise, mental or orthostatic stress, altitude, cold pressor test, sweat test and vasodilatory infusions). **Conclusion:** OC influence the physiological responses to chemoreflex, mechanoreflex and metaboreflex activation. In terms of autonomic indices and physiological stressors, there are more inconsistencies within the OC literature, which may be due to estrogen dosage within the OC formulation (i.e., HRV) or the intensity of the stressor (exercise intensity/duration or orthostatic stress). Further research is required to elucidate the effects of OC on these aspects of autonomic function due to the relatively small amount of available research. Furthermore, researchers should more clearly define or stratify OC use by duration, dose and/or hormone cycling to further elucidate the effects of OC.

Key words: Autonomic, Oral Contraceptives, Reflex, Pharmaceutical Sex Hormone

2.2 **Introduction**

OC are the 2nd most popular short-acting contraceptive method, with current users accounting for 16% of the 1 billion reproductive-aged women worldwide[1]. OC prevent pregnancy through a combination of synthetic estradiol and progestin (i.e., combined OC) or progestin alone, which prevents ovulation, implantation, and/or inhibits follicular development[99]. One of the more commonly used OC types contains 28 pills, including 21 active pills that contain synthetic hormones followed by 7 inactive placebo pills[100]. This duration of pill regimen mimics the average menstrual cycle length of NOC, with a median or average cycle of roughly 28 days[101-103]. Some formulations may not follow this 28-day guideline or may have varying active/placebo pill ratios[100]. Additionally, OC pill regimens may fluctuate across the active phase in hormonal dosing, such that hormone levels incrementally increase throughout each pill week. To account for phasic hormonal dosages, pills are referred to as being monophasic, biphasic or triphasic to describe whether the hormonal dosage is maintained or increased and in what increment. For example, the hormonal dose of a triphasic OC increases every week of the 3 weeks of the active phase. This cyclical fluctuation allows researchers to investigate the effects of circulating synthetic hormones (i.e., active phase) and the long-term adaptation from hormonal contraceptive use in the absence of synthetic hormones (i.e., placebo phase). Furthermore, OC active and placebo phases are often compared within the literature to the corresponding HH and LH phases of the menstrual cycle, the luteal and follicular phases, respectively.

Recent work from our lab has demonstrated that OC affect various aspects of resting autonomic balance (i.e., HRV) and isolated autonomic reflex activation responses (i.e., baroreflex, central chemoreflex, metaboreflex, and mechanoreflex) compared to NOC[72]. Yet, we also found no influence of OC on the cardiovascular and respiratory responses to paced deep breathing, Valsalva, or posture changes[76,104], which are common clinical tests used to assess for dysautonomia[105]. Thus, the purpose of this systematic review was to determine if there is a consensus on the influence of OC by summarizing the current literature on the effects of OC on resting autonomic function and the autonomic response to any physiological stressor. Additionally, this review aimed to determine research questions that have not been explored or require further clarification. We primarily examined the effects of OC use on autonomic function with a secondary discussion of pill generation (i.e., comparing varying hormonal formulations/

progestin derivatives) or hormone cycling (i.e., incremental hormonal dosages throughout the active pill phase) where available. The results of this systematic review provide future directions and considerations for investigators, and provide clinicians with a better understanding of the physiological effects of OC.

2.3 **Methods**

This systematic review was conducted according to the Preferred Reporting Items Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 1)[106]. This review was registered in the International Prospective Register of Systematic Reviews (PROSPERO; CRD42023267733).

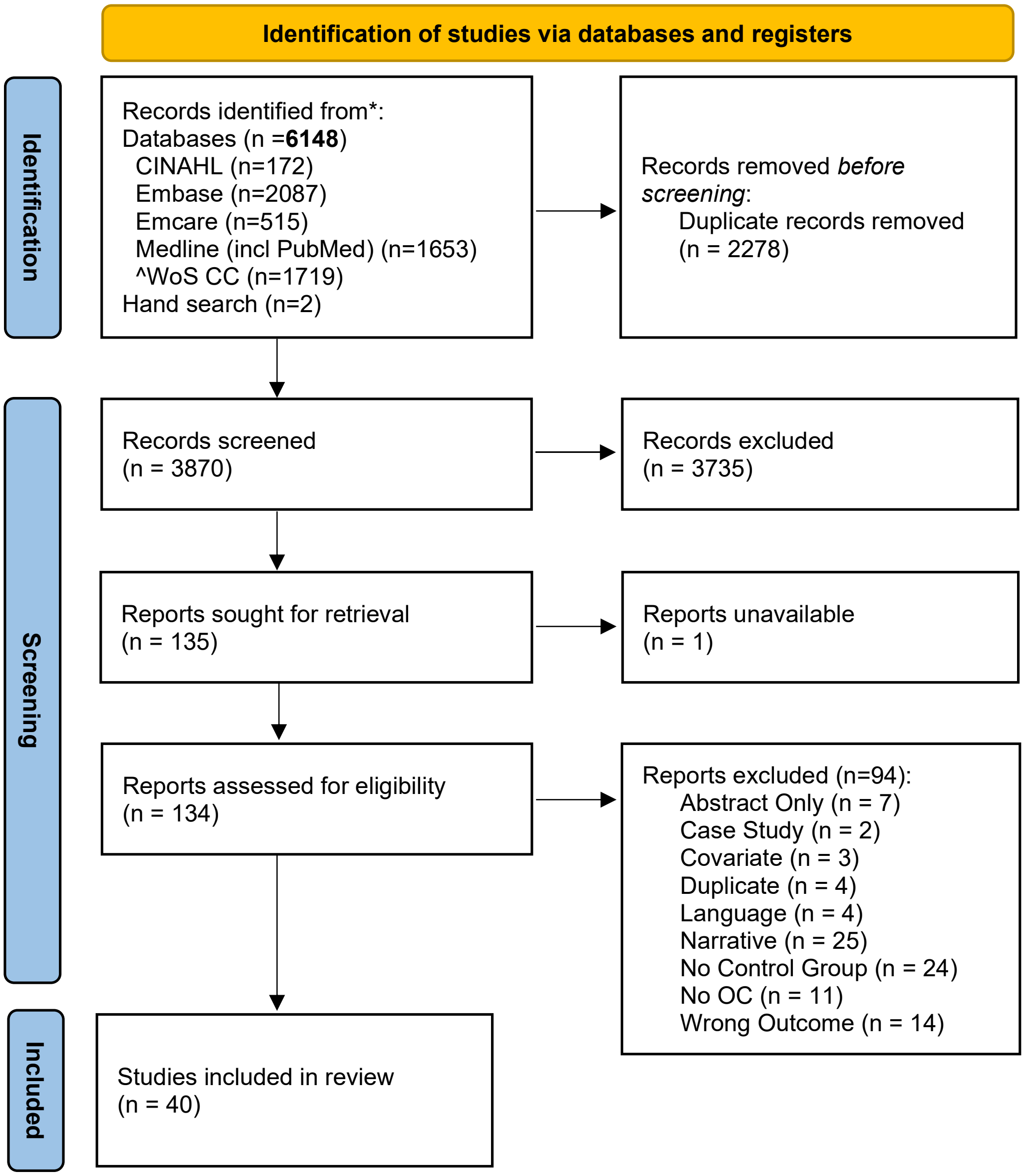

Figure 1. PRISMA flow diagram that demonstrates the total retrieved citations, removed duplicates, and the remaining citations throughout each screening level.

Search Strategy

In collaboration with an Information Specialist (MP), a search strategy (see *Appendix A*) was developed and organized with the PICO(s) framework, which categorizes search terms into Problem or Population (P), Intervention(I), Comparisons (C), Outcome (O) and Study Design. The autonomic function of healthy menstruating females (P) was assessed for an effect of OC use (I), as compared to non-users (C), on resting autonomic function or the autonomic response to any physiological stressor (O). The Outcome concept was not specified to purposefully broaden the citations captured, as a relatively small citation pool was expected. The following study designs were included: case-control, cohort, cross-sectional and randomized or non-randomized control studies. The results were limited to human females in applicable databases, and no language or date limits were applied during the search. The full Medline search strategy can be found in *Appendix A*. Citations were retrieved from six different databases: CINAHL Complete (EBSCOhost), Embase (Ovid), Emcare (Ovid), Medline (Ovid), PubMed (non-Medline), and Web of Science Core Collection. Databases were searched from inception to April 27th, 2021, and updated on June 2nd, 2022.

Study Selection

Citations were included if the study investigated the effects of OC use on any aspect of autonomic function (i.e., sympathetic or parasympathetic activity at rest or during stimulation, autonomic reflex activation, etc.) in healthy human females. Original research studies in French or English were included. Citations were excluded if the study design did not: include human participants, or directly compare OC to healthy controls, non-OC users, or themselves prior to using OC. Studies that only investigated hormone replacement therapy (i.e., for menopause) or alternative contraceptives (male contraceptives, intrauterine devices, transdermal patches, injections, etc.) were excluded.

Abstract Screening & Full-Text Review

All citations were uploaded and managed using the Covidence online software, which automatically removes duplicates. A screening tool was created for both screening levels to remove irrelevant citations. To ensure inter-reliability between review team members, a pilot screening using these tools was conducted on a small representative sample of citations[107]. Two review team members assessed each abstract to minimize bias (TP, JB, HJ, MP, JD, and HE), and a third reviewer's independent decision resolved any inclusion conflicts (TP and HE). After the initial abstract screening, the remaining citations were evaluated at the full-text level. Articles were retrieved using York University Library and other catalogues and article request systems (RACER, ResearchGate, OMNI catalogues, etc.).

Quality Assessment & Data Extraction

The quality and risk of bias of each study was appraised by the adapted Joanna Briggs Institute (JBI) Appraisal Checklist for Analytical Cross-Sectional Studies tool[108], as the majority of studies were cross-sectional. The quality appraisal tool assessed each study on clearly defined inclusion/exclusion criteria, appropriate participant or setting descriptions, identification of and mitigation strategies for confounding factors, reliability of outcome measurement, and appropriateness of statistical modelling. Two items on the checklist were amended to be specific to this systematic review; confirmation of OC use or type and if objective measurements were used to assess autonomic function. Studies were included if they met a majority of these 8 criteria, based on a consensus of two independent reviewers (TP and HE). Data extraction was conducted by a single reviewer (TP), and the extraction was independently reviewed by another team member (HE). Any conflicts were resolved with discussion.

Synthesis of Evidence

This systematic review was evaluated for publication using the PRISMA Item Checklist (see *Appendix B*)[106]. A meta-analysis was not performed due to the heterogeneity of all outcomes; therefore, a thematic synthesis was used to synthesize the retrieved citations. This type of synthesis involves coding included citations according to major themes or questions explored (i.e., autonomic indices, autonomic reflexes, specific stressors, etc.)[109]. Based on these groupings and descriptions of included study characteristics (methods, participants, protocols, etc.), a consensus was determined on the effects of OC on that specific aspect of autonomic function.

2.4 **Results**

After deduplication, 3,870 citations were screened at the title and abstract level, which resulted in 135 potential citations for full-text screening (Figure 1). Unfortunately, 1 text was unavailable for retrieval and could not be included in the full-text screening. A total of 40 studies progressed through all screening levels and were included in the subsequent data extraction. Information regarding participant characteristics and OC descriptions, sample size, aspects of autonomic function assessed, experimental protocol, physiological assessments, considerations for the menstrual cycle, statistically significant responses, and study conclusions were extracted.

All included studies were cross-sectional and met a threshold of $\geq 5/8$ for the quality appraisal; 28 studies were appraised at 8/8[73,77,81,82,110-133], 5 studies at 7/8[72,76,134-136], 5 studies at 6/8[104,137-140], and 2 studies were appraised at 5/8[141,142]. Most studies investigated healthy women; however, two studies specifically investigated the influence of OC in hypertensive women[113] or smokers[137]. Only seven studies did not specify the OC formulation[134-137,139,140,142], while 30 studies reported whether the OC was cyclic (i.e., biphasic or triphasic – incremental hormonal doses in 2 or 3 increments, respectively) or non-cyclic (i.e., monophasic – equivalent hormonal doses throughout active pill). More specifically, 17 studies investigated non-cyclic OC formulations[73,82,110-114,116,117,119,123-125,131,133,138,141], and 12 investigated combined cyclic and non-cyclic OC groups[72,76,81,104,115,121,122,128-130,132]. Five studies did not completely report the OC formulation or information about hormonal cycling (i.e., only the brand or the estrogen or progestin component was specified)[77,118,120,126,127]. Only 23 studies reported or controlled for the length of previous OC use, ranging from 2-18 months[72,73,77,82,110,111,114,116,117,119,120,124-126,128-131,133,135,137,139,141].

In the included studies, 39 studies included statistical modelling that varied from ANOVAs between groups and time and/or over the pill cycle (including ANCOVAs, general estimated equations or linear models), student t-tests (paired and unpaired) over time and between/within groups, and others, such as Mann-Whitney's U-test, Kruskal-Wallis test, and Wilcoxon Signed-Rank test. A single study did not report any information about the statistical methodologies used[141]. Two studies reported potential conflicts of interest, including; that two of the researchers were employed by the proprietary company of the multi-sensor wrist-worn device used in the study[139] and that one of the authors was on the editorial board of the publishing journal[131].

Studies were grouped by the aspect of autonomic function that was assessed; autonomic indicators such as HRV, autonomic reflexes, and physiological stressors that could influence autonomic function. Studies that evaluated multiple aspects of autonomic function were divided into the appropriate groupings while separately reporting each aspect assessed. Sixteen studies examined the effects of OC on resting autonomic function using various indices (Table 1), such as BP variability[123], HRV[72,73,76,77,113,123,126,127,133-135,139], and resting MSNA[115,120,129,130]. Eleven studies evaluated various autonomic reflexes, such as spontaneous baroreflex[72,76,113,123,129,138], central chemoreflex[72], mechanoreflex[72], metaboreflex (post-exercise circulatory occlusion (PECO) response)[72,82,125,129], sudomotor axon reflex[117,132], and veno-arterial reflex[112] (Table 2). Finally, the remaining texts evaluated a physiological stimulus that influenced autonomic function, including: altitude or altitude-related stressors (i.e., hypoxia)[136,142], auditory stress[77,141], cold pressor test (CPT)[114,116,118-121,128,137], exercise stress[82,122,125,129,140], mental stress[110,111,114,119,121,131,137], orthostatic stress[76,124,127,130], breathing maneuvers (i.e., paced deep breathing, Valsalva maneuver)[76,104], and vascular infusions[81,120,128](Table 3).

Table 1. Included studies that investigated an aspect of resting autonomic function (i.e., BP variability, HRV, and resting MSNA). Studies are grouped by the aspect of autonomic function assessed and alphabetically ordered.

Study ID	N	OC description	Methods	Results	Does the autonomic response of OC users differ from NOC?
Blood Pressure (BP) Variability					
Nisenbaum et al.[123]	NOC, n=33; OC, n=36	Combined (20 mcg EE + 3 mg drospirenone) OC (with 24 active days/ 4 placebo).	Using 5 minutes of supine rest, BP variability was calculated. OC users were tested prior to OC use in the LH phase and compared to 6months post OC use in the HH phase.	No change in systolic arterial BP variability in women after 6 months of OC use.	N
Heart Rate Variability (HRV)					
Abidi et al.[76]	NOC, n=12; OC, n=14;	Combined, cyclic and non-cyclic OC. • Tri-Cyclen (n=6) • Tri-Cyclen Lo (n=1) • Alesse (n=5) • Marvelon (n=1) • Novo-Cyprotenone/EE (n=1)	HRV (time domain and spectral analysis) was analyzed using an ECG recording. 5 minutes of data in the supine position was used. OC and NOC were compared across the LH and HH phases.	• Higher SDRR in OC compared to NOC. • No difference in LF power, HF power, and LF/HF ratio between OC and NOC.	Y
Armbruster & Stroebel[134]	NOC, n=47; OC, n=39	Unspecified.	HRV (time domain and spectral analysis) was analyzed using an ECG recording during seated rest. OC in the HH phase were compared to NOC (both phases combined).	• OC users had slightly higher %HF and log %HF power than NOC. • No difference in RMSSD and log RMSSD between OC and NOC. • Non-compared data within article: %LF power was 42.7±21.8 in OC and 54.6±21.6 in NOC. LF/HF ratio was 1.31±1.84 in OC and 2.11±2.47 in NOC.	Y

Assadpour et al.[72]	NOC, n= 12; OC, n=14	Combined, cyclic and non-cyclic OC for at least 3 months. • Tricyclen 28 (n=2, triphasic) • Cyclen 28 (n=1, monophasic) • Yaz (n=2, monophasic) • Alesse (n=7, monophasic) • Marvelon (n=2, monophasic)	HRV (time domain and spectral analysis) was analyzed using an ECG recording. Analyses used 3–5 minutes of data depending on the trial (i.e., 3 minutes for metaboreflex and mechanoreflex trials; 5 minutes for chemoreflex trial). OC and NOC were compared across LH and HH phases.	No difference in SDRR, RMSSD, pRR50, LF power, HF power, LF/HF ratio, and total power between OC and NOC at rest or in response to reflex activation.	N
Danel et al.[135]	NOC, n=113; OC, n=64	Unspecified OC for at least 3 months.	HRV (time domain) was analyzed using supine resting ECG. All women were tested 4-8 days after menstrual bleeding.	• Unlike the NOC group, OC users did not experience an association between pre-menstrual symptoms and SDNN or RMSSD. • No difference in SDNN and RMSSD between OC and NOC users.	Y
de Morais et al.[113]	NOC, n=26; OC, n=30	Combined (20 mcg EE + 3 mg drospirenone) OC (with 24 active days/ 4 placebo).	HRV (time and frequency domains) was analyzed during supine rest using ECG. Participants were tested prior to OC initiation in the LH phase and compared to 6months post OC use in the HH phase.	6 months of OC use did not influence %LF power, %HF power, LF/HF ratio in hypertensive OC users.	N

Milan et al.[77]	NOC, n=12; OC, n=10	Provided with combined (20-50 mg EE + norethindrone, drospirenone or desogestrel) OC for at least 6 months.	At least 500 RR intervals during rest or auditory stimulation were used for HRV (time and frequency domain analysis) using a Polar RS800CX HR monitor. Series required more than a 95% sinus rhythm to be included in the analysis. Menstrual phase was not specified.	OC had lower pNN50 and lower HF power at baseline compared to NOC.	Y
Nisenbaum et al.[123]	NOC, n=33; OC, n=36	Combined (20 mcg EE + 3 mg drospirenone) OC (with 24 active days/ 4 placebo).	HRV (time and frequency domains) was determined using a beat-to-beat finger BP waveform (Finometer) during supine rest. Participants were tested prior to OC initiation in the LH phase and compared to 6months post OC use in the HH phase.	No change in pNN50, RMSSD, VAR RR, %LF power, %HF power, and LF/HF ratio in women after 6 months of OC use.	N
Rebelo et al.[73]	NOC, n=80; OC, n=75	Combined, monophasic (20 µg EE and 150 µg gestodene) OC for at least 18 months.	HRV (non-linear methods symbolic analysis and conditional and Shannon's entropy) was determined using an ECG recording during supine rest with spontaneous breathing. ECG data were visually inspected to avoid artifacts, and 300 heart beats were used in the analysis. OC in the HH phase were compared to NOC in the LH phase.	No difference in Shannon's entropy, complexity index, normalized complexity index, percentage occurrence of patterns with no variation, one variation or patterns with two like or unlike variations between OC and NOC.	N

Rebelo et al.[126]	NOC, n=121; OC, n=111	Combined OC (20 mcg EE+ desogestrel, gestodene or levonorgestrel) for at least 18 months.	HRV (time and frequency domain) was analyzed from an ECG recording in both the supine and sitting positions while breathing spontaneously. The most stable ≥256 consecutive beats were evaluated. OC in the HH phase were compared to NOC in the LH phase.	No difference in RMSSD, SDNN, LF power, HF power, or LF/HF ratio during standing or sitting between OC and NOC.	N
Santos et al.[133]	NOC, n=10; OC, n=10	Combined, monophasic low dose OC for at least 6 months. • Fermiane (n=3, 0.02 mg EE + 0.075 mg Gestoden) • Harmonet (n=1, 0.02 mg EE + 0.075 mg Gestoden) • Tamisa 20 (n=2, 0.02 mg EE + 0.075 mg Gestoden) • Selena (n=1, 0.035 mg EE + 2.0 mg Cyproterone acetate) • Diane 35 (n=2, 0.035 mg EE + 2.0 mg Cyproterone acetate) • Yasmin (n=1, 0.03 mg EE + 3.0 mg Drospirenone)	HRV (time and frequency domain) was analyzed from an ECG recording in both the supine and sitting positions while breathing spontaneously. The region with greatest stability was used for these analyses if it included at least 5 minutes or 256 consecutive beats. OC in the LH and HH phases were compared to NOC in the late follicular phase.	No difference in RMSSD, RMSM, pNN50, LF power, HF power, LF/HF ratio in supine or sitting position between OC and NOC.	N

Schueller et al.[127]	NOC, n=27; OC, n=31	Combined (EE + progestin) OC. • levonorgestrel (n =8) • desogestrel/gestoden (n =9) • chlormadinon-acetate (n =6) • dienogest (n =5) • norgestimate (n =2) • cyproterone acetate (n =1)	HRV (time and frequency domain) was analyzed from 5 minutes of ECG while supine and with controlled breathing (15 cycles/min). OC in the HH phase were compared to NOC in the HH phase.	No difference in SDNN, pNN50, RMSSD, Total power, LF power, HF power, and LF/HF ratio between OC and NOC.	N

Sims et al.[139]	NOC, n= 3870; OC, n=455; Other, n=269	Combined and progestin-only OC for at least 9 months.	Resting HR was captured using a wrist-worn multi-sensor device (wrist photoplethysmography) during the last slow-wave cycle of sleep. RR-interval was used to determine HRV (domain undefined). "Recovery" is a proprietary metric of the WHOOP, Inc. platform that combines nighttime measures of HR, HRV, respiratory rate and sleep duration into a once-daily metric used to guide recommended training load. Higher recovery values (ranges from 0-100%) indicate readiness for a subsequent exercise training load. OC were compared to NOC throughout the pill/menstrual cycle.	• Compared to the menstrual cycle of NOC, OC have a different pattern of HRV change over the pill cycle. • OC users have lower HRV and Recovery during the early follicular phase than NOC. • OC start their cycle with similar HRV and Recovery to the late luteal phase, whereas NOC start their cycle with higher HRV and Recovery than in the late luteal phase. • Both OC and NOC experience a decrease in HRV in the late follicular phase, yet NOC still have higher HRV. Recovery is not different between OC and NOC in this phase. • In the latter half of the cycle (early and late luteal), OC increase HRV and Recovery over time and NOC decreases HRV and Recovery over time.	Y

Resting Muscle Sympathetic Nerve Activity (MSNA)					
Harvey et al.[115]	NOC, n=74; OC, n=53	Combined, cyclic and non-cyclic OC. • Monophasic (n=31) • Biphasic (n=3) • Triphasic (n=18) • Unknown (n=1)	Resting MSNA of the peroneal nerve was recorded while participants were supine. Baseline measurements were recorded for 5-10 minutes after 30 minutes supine rest. OC in the LH phase were compared to NOC in the LH phase.	No difference in MSNA (bursts/min or bursts/100 heartbeats) at rest between OC and NOC across the menstrual and pill cycle.	N
Middlekauff et al.[120]	NOC, n=10; OC, n=13	Combined OC for at least 12 months. • Orthotricyclen (n=6) • Orthotricyclen-Lo (n=3) • Levora (n=1) • Seasonique (n=1) • Kelnor (n=1) • Trinessa (n=1)	Resting MSNA of the peroneal nerve was recorded while participants were supine for a baseline period (10 minutes) after a 10min rest period. OC and NOC were compared across the LH and HH phases.	No difference in MSNA (bursts/min and bursts/100 heartbeats) between OC and NOC across the menstrual and pill cycle.	N
Takeda et al.[129]	NOC, n=14; OC, n=8	Combined, cyclic and non-cyclic OC for at least 6 months. • Yasmin (n=2, monophasic) • Ocella (n=1, monophasic) • Loryna (n=1, monophasic) • Natazia (n=1, triphasic) • Ortho Tri-Cylen Lo (n=1, triphasic) • Unknown (n=2)	Resting MSNA was recorded at the peroneal nerve for 6 minutes of steady-state supine rest after 30 minutes of quiet rest. OC in the HH phase were compared to NOC in the HH phase.	No difference in MSNA (bursts/min and bursts/100 heartbeats) between OC and NOC in the HH phase.	N

Usselman et al.[130]	NOC, n=9; OC, n=8	Combined, cyclic and non-cyclic (20-30 ug EE + progestin) OC for at least 6 months. • norelgestromin patch (n=1) • norgestimate (n=1, triphasic) • drospirenone (n=2, monophasic) • desogestrel (n=1, monophasic) • levonorgestrel (n = 3, monophasic)	Resting MSNA was recorded at the peroneal nerve while supine. OC in the LH and HH phases were compared to NOC in the LH and HH phases.	No difference in MSNA (bursts/min or bursts/100 heartbeats) at rest between OC and NOC across the menstrual and pill cycle.	N

BP, blood pressure; ECG, electrocardiogram; EE, estrogen estradiol; HF, high frequency; HH, high hormone; HR, heart rate; HRV, heart rate variability; LF, low frequency; LH, low hormone; MSNA, muscle sympathetic nerve activity; NOC, naturally cycling or non-oral contraceptive user; OC, oral contraceptive user; pNN50, proportion of normal successive RR intervals that differ by 50ms divided by total number of normal RR intervals; pRR50, proportion of successive RR intervals that differ by 50ms divided by total number of RR intervals; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal RR intervals; SDRR, standard deviation of RR intervals; VAR RR, RR interval variability.

Table 2. Included studies investigating the physiological response to the activation of an autonomic reflex. Studies are grouped in alphabetical order of the specific reflex activation investigated and separated into individual rows if multiple reflexes were assessed.

Study ID	N	OC description	Methods	Results	Does the autonomic response of OC users differ from NOC?
Baroreflex					
Abidi et al.[76]	NOC, n=12; OC, n=14;	Combined, cyclic and non-cyclic OC. • Tri-Cyclen (n=6) • Tri-Cyclen Lo (n=1) • Alesse (n=5) • Marvelon (n=1) • Novo-Cyprotenone/EE (n=1)	Spontaneous cBRS was determined with 5 minutes continuous beat-to-beat BP and ECG during supine rest (sequence method). OC and NOC were compared across the LH and HH phases.	No difference in cBRS slope between OC and NOC.	N
Assadpour et al.[72]	NOC, n=12; OC, n=14	Combined, cyclic and non-cyclic OC for at least 3 months. • Tricyclen 28 (n=2, triphasic) • Cyclen 28 (n=1, monophasic) • Yaz (n=2, monophasic) • Alesse (n=7, monophasic) • Marvelon (n=2, monophasic)	Spontaneous cBRS was assessed using 5 minutes continuous beat-to-beat BP and ECG during supine baseline (sequence method). OC and NOC were compared across the LH and HH phases.	No difference in cBRS slope at rest between OC and NOC.	N

de Morais et al.[113]	NOC, n=26; OC, n=30	Combined OC (20 mcg EE + 3 mg drospirenone with 24 active days/ 4 placebo).	Spontaneous cBRS was determined with 5 minutes continuous beat-to-beat BP and ECG during supine rest (α index). OC was tested at baseline (prior to use) in the LH and compared to 6months post OC use in the HH phase.	No difference in cBRS in hypertensive women after 6 months of OC use.	N
Nisenbaum et al.[123]	NOC, n=33; OC, n=36	Combined (20 mcg EE + 3 mg drospirenone) OC (with 24 active days/ 4 placebo).	Using 5 minutes of supine rest, BRS was analyzed using alpha index in the LF. Participants were tested prior to OC initiation in the LH phase and compared to 6months post OC use in the HH phase.	No change difference in Alpha LF in women after 6 months of OC use.	N
O'Brien et al.[138]	NOC, n=8; OC, n=9;	Combined, monophasic OC.	Spontaneous cBRS was determined using 5 minutes of SBP and ECG at rest while seated (Up, down, or total sequences and BEI). OC and NOC were compared across the LH and HH phases.	No difference in cBRS at rest between OC and NOC across the cycle.	N

Takeda et al.[129]	NOC, n=14; OC, n=8	Combined, cyclic and non-cyclic OC for at least 6 months. • Yasmin (n=2, monophasic) • Ocella (n=1, monophasic) • Loryna (n=1, monophasic) • Natazia (n=1, triphasic) • Ortho Tri-Cylen Lo (n=1, triphasic) • Unknown (n=2)	Spontaneous cBRS was assessed using the sequence method (down sequence only) for 6 minutes rest. sBRS was determined as the slope of the relationship between MSNA BI and DBP during 6min rest. OC in the HH phase were compared to NOC in the HH phase.	No difference in cBRS or sBRS between OC and NOC during rest.	N
Central Chemoreflex					
Assadpour et al.[72]	NOC, n=12; OC, n=14	Combined, cyclic and non-cyclic OC for at least 3 months. • Tricyclen 28 (n=2, triphasic) • Cyclen 28 (n=1, monophasic) • Yaz (n=2, monophasic) • Alesse (n=7, monophasic) • Marvelon (n=2, monophasic)	5 minutes of hypercapnic gas while supine (5% CO_2). Each participant was tested across the menstrual phase and pill cycle. OC and NOC were compared across LH and HH phases.	• OC in the LH phase had a greater increase in $ETCO_2$ compared to the OC in the HH phase and NOC in both phases. • PWV was lower in OC during the LH phase compared to NOC in the LH phase during CO_2. • No difference in the responses of HR, MAP, Qi, RR, Vt, V_E, SDRR, RMSSD, pRR50, LF power, HF power, LF/HF ratio, or total power to CO_2 between OC and NOC.	Y

Mechanoreflex					
Assadpour et al.[72]	NOC, n=12; OC, n=14	Combined, cyclic and non-cyclic OC for at least 3 months. • Tricyclen 28 (n=2, triphasic) • Cyclen 28 (n=1, monophasic) • Yaz (n=2, monophasic) • Alesse (n=7, monophasic) • Marvelon (n=2, monophasic)	BP cuff inflated +40 mmHg above SBP prior to passive leg movement (180° extension to 90° flexion at 1 Hz for 3 minutes while supine). OC and NOC were compared across LH and HH phases.	• OC users had an attenuated increase in MAP during PM compared to NOC, regardless of phase. • No difference in the responses of HR, Qi, RR, Vt, V_E or $ETCO_2$ to mechanoreflex activation between OC and NOC. • No difference in SDRR, RMSSD, pRR50, LF power, HF power, LF/HF ratio, and total power between OC and NOC in response to mechanoreflex activation.	Y
Metaboreflex					
Assadpour et al.[72]	NOC, n=12; OC, n=14	Combined, cyclic and non-cyclic OC for at least 3 months. • Tricyclen 28 (n=2, triphasic) • Cyclen 28 (n=1, monophasic) • Yaz (n=2, monophasic) • Alesse (n=7, monophasic) • Marvelon (n=2, monophasic)	2 minutes of isometric handgrip exercise at 40% MVC, followed by 3 minutes PECO at +40 mmHg above SBP (in supine position) in the supine position. OC and NOC were compared across LH and HH phases.	• OC users had smaller increases in MAP and Qi with greater increases in Vt and V_E during PECO compared to NOC. • No difference in the responses of HR, RR, $ETCO_2$, PWV, SDRR, RMSSD, pRR50, LF power, HF power, LF/HF ratio, or total power to PECO between OC and NOC.	Y

Minahan et al.[82]	NOC, n=15; OC, n=15	Combined, monophasic OC for at least 12 months.	3 minutes of isometric handgrip exercise at 30% MVC followed by 3 minutes with/ or without 230 mmHg PECO in a seated position. OC in the LH phase were compared to NOC in the LH phase.	• OC had higher SBP and DBP during PECO compared to NOC. • No difference in the HR response to PECO between OC and NOC.	Y
Parmar et al.[125]	NOC, n=9; OC, n=10	Combined, monophasic OC (EE 20-30 µg and progestin 100-150 µg) for at least 3 months.	2.5 minutes isometric handgrip exercise at 30% MVC followed by 2 minutes PECO at 250 mmHg in a semi-recumbent position. OC in the first week of the HH phase were compared to NOC in the LH phase.	• OC had greater increases of SBP, DBP and MAP peak responses during PECO compared to NOC. • No difference in HR peak response during PECO between OC and NOC.	Y
Takeda et al.[129]	NOC, n=14; OC, n=8	Combined, cyclic and non-cyclic OC for at least 6 months. • Yasmin (n=2, monophasic) • Ocella (n=1, monophasic) • Loryna (n=1, monophasic) • Natazia (n=1, triphasic) • Ortho Tri-Cylin Lo (n=1, triphasic) • Unknown (n=2)	Isometric handgrip was performed at 40% of MVC until fatigue (i.e., <80% of the desired force for ≥2sec) in the supine position followed by 2 minutes PECO at 250 mmHg. OC in the HH phase were compared to NOC in the HH phase.	• OC users had a larger increase in DBP, MAP, TPR and SVT during PECO compared to NOC. • No difference in responses of cBRS, sBRS, HR, Q, SV and MSNA BI during PECO between OC and NOC.	Y

Sudomotor Axon Reflex					
Grucza et al.[132]	NOC, n=10; OC, n=10	Combined, cyclic and non-cyclic OC. • Trikvilar (n=8, 30, 40, 30µg EE + 50, 75, 125µg levonorgestrel) • Neo-Gentrol (n=2, 30µg EE + 150µg levonorgestrel)	Exercise was performed at %50 VO$_{2max}$ on a cycle ergometer in a climactic chamber (24.0°C±0.5; 50% relative humidity) while wearing minimal clothing. Participants rested in the chamber for 20 minutes to reach temperature equilibrium and were in the chamber for a total time of 45 minutes. Sweating gain is defined as the slope difference between rectal temperatures at the onset of sweating and 63.1% steady sweating for unsteady-state, and the slope difference between rectal temperatures at the onset of sweating and sweating at 45 minutes of heat stress. OC was compared to NOC in the LH and HH phases.	• OC had a higher average sweating gain than NOC in the LH phase, and OC had a lower unsteady-state gain than NOC in the HH phase. • No difference in rectal temperature at sweating threshold, or rectal temperature at steady-state sweating during exercise between OC and NOC. • No difference in time to onset of sweating, time required for a 63.1% increase in sweating, or the sum of time delay + time required for a 63.1% increase in sweat rate during exercise between OC and NOC.	Y

Kenny et al.[117]	NOC, n=8; OC, n=8	Combined, monophasic OC for at least 18-24 months. • Minovral (n=2, 0.030 mg EE + 0.150 mg levonorgestrel) • Evra (n=1, 0.020 mg EE + 0.200 mg norelgestromin) • Alesse (n=2, 0.020-0.030 mg EE + 0.100 mg levonorgestrel) • Cyclen (n=1, 0.020 mg EE + 0.300 mg levonorgestrel) • Marvelon (n=2, 0.030 mg EE + 0.150 mg desogestrel)	Core and skin temperature were clamped at 32.5°C for 15 minutes while wearing a liquid-conditioned suit, which maintained temperature via a water-perfused circulation bath. Participants were pre-warmed at a rate of ~4.1°C per hour in skin temperature until a sustained increase in FBF and sweating was achieved (pre-exercise warming). After removing the suit and a small break (~7.5 minutes), participants cycled for 30 minutes at 75% VO_2 peak, followed by 15 minutes upright seated recovery period (post-exercise recovery). Then, participants donned the suit again and were warmed to 43°C until there was an elevated and sustained plateau in skin blood flow (post-exercise warming). OC and NOC were compared across LH and HH phases.	• No difference in MAP, HR, skin or esophageal temperature at rest, or at the start of pre- and post-exercise warming between OC and NOC. • No difference in MAP, esophageal temperature, sweat rate, and % cutaneous vascular conductance at the end of exercise or throughout post-exercise recovery between OC and NOC. • No difference in esophageal temperature at onset of vasodilation or sweating during pre- and post-exercising warming between OC and NOC.	N

Veno-arterial Reflex					
Bishop & Brown[112]	NOC, n=6; OC, n=8	Combined, monophasic (Microgynon 30; 30 µg EE + 150 µg levonorgestrel) OC.	During graded leg dependency, one foot was lowered below heart level for 5 minutes at each level (15, 25 and 35 cm). In the second trial, the participant's foot was lowered to 50cm below heart level for 5 minutes (i.e., single-level dependency). The final trial consisted of venous occlusion (+50 mmHg) of the thigh maintained for 5 minutes. All trials were conducted in the supine position. OC in the HH phase were compared to NOC in the LH and HH phases.	• OC had a smaller reduction in skin perfusion of the foot during graded leg dependency, single-level leg dependency and venous occlusion compared to NOC. • No difference in skin perfusion at the shin during graded leg dependency between OC and NOC.	Y

BEI, baroreflex effectiveness index; BMI, body mass index; BP, blood pressure; cBRS, cardiovagal baroreflex sensitivity; CO_2, carbon dioxide; DBP, diastolic blood pressure; ECG, electrocardiogram; EE, estrogen estradiol; $ETCO_2$, end-tidal carbon dioxide; FBF, forearm blood flow; HF, high frequency; HH, high hormone; HR, heart rate; LF, low frequency; LH, low hormone; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; MSNA BI, muscle sympathetic nerve activity burst incidence; maximum voluntary contraction; NOC, naturally cycling or non-oral contraceptive user; OC, oral contraceptive user; PECO, post-exercise circulatory occlusion; PM, passive movement; pRR50, proportion of successive RR intervals that differ by 50ms divided by total number of RR intervals; PWV, pulse wave velocity; Q, cardiac output; Qi, cardiac output index; RMSSD, root mean square of successive differences; RR, respiratory rate; SBP, systolic blood pressure; sBRS, sympathetic baroreflex sensitivity; SDRR, standard deviation of RR intervals; SV, stroke volume; SVT, sympathetic vascular transduction; TPR, total peripheral resistance; V_E, ventilation; VO_2, oxygen consumption; Vt, tidal volume.

Table 3. Study description of all included studies that assessed a physiological stressor that could influence autonomic function. Studies are grouped in alphabetical order by autonomic stressor investigated and separated into individual rows if multiple stressors were assessed.

Study ID	N	OC description	Methods	Results	Does the autonomic response of OC users differ from NOC?
Altitude/Hypoxia					
Harrison et al.[142]	NOC, n=37; OC, n=13	Unspecified.	Participants travelled to altitude (~3200 m) in less than 4 hours by airplane. Menstrual phase was not reported.	• OC had lower SBP, BP and MAP than NOC at sea level, yet greater DBP and MAP at altitude compared to NOC. • Compared to sea level, OC increased BP during altitude compared to NOC, who decreased BP. • No difference in seated HR, norepinephrine, epinephrine and dopamine between OC and NOC at altitude.	Y

Richalet et al.[136]	NOC, n=169; OC, n=336	Unspecified.	Participants were exposed to hypoxic gas during exercise for *simulated* altitude, which was compared to normoxia. All exercise was performed at an intensity of 30% maximal aerobic power and relatively 40-50% of HR max. The hypoxic cardiovascular response to exercise was calculated as the ΔHR divided by ΔSaO_2 between normoxia and hypoxia. The hypoxic ventilatory response was calculated similarly: ΔV_E divided by (ΔSaO_2/ body mass x 100). Menstrual phase was only investigated in NOC.	Neither the hypoxic cardiovascular nor hypoxic ventilatory responses during exercise were different between OC and NOC.	N

Auditory Stress					
Friedman & Meares[141]	NOC, n=21; OC, n=7;	Combined, monophasic (20 mcg EE + 0.25 mgd norgestrel) OC for at least 6 months.	21 pre-recorded tones of 1000 Hz frequency and 100 dB intensity were sounded at irregular intervals of 15-80secs with a tone duration of 1sec for the first 20, and the last tone was 5secs. A response in log skin conductance (i.e., indicator of skin sympathetic nerve activity) was considered greater than 0.003. The first stimulus point where no response was obtained for at least three successive stimuli was determined to be the habituation point. OC and NOC were compared over a pill or menstrual cycle at 6 intervals (3 prior to ovulation and 3 post-ovulation).	• OC users had lower arousal compared to NOC. • OC had a stable habituation point throughout the pill cycle compared to NOC, who had increased habituation prior to ovulation compared to after.	Y
Milan et al.[77]	NOC, n=12; OC, n=10	Provided with combined (20-50 mg EE + norethindrone, drospirenone or desogestrel) OC for at least 6 months.	At least 500 RR intervals during rest or auditory stimulation were used for HRV (time and frequency domain analysis) using a Polar RS800CX HR monitor. Series required more than a 95% sinus rhythm to be included in the analysis. Menstrual phases were not specified.	Unlike NOC, OC did not have a reduction in SDNN, RMSSD, pNN50, and LF power in response to music.	Y

Cold Pressor Test (CPT)					
Emmons & Weidner[137]	NOC, n=31; OC, n=38	Unspecified OC for a minimum of 2 months.	1min right hand submersion to the wrist in ice water (5°C). Smoking or non-smoking OC were compared to smoking or non-smoking OC. Menstrual phases were not specified.	No difference in SBP, DBP, and HR responses to CPT between OC and NOC.	N
Gamsakhurdashvili et al.[114]	NOC, n=24; OC, n=24	Combined, monophasic OC for at least 3 months.	3 minutes dominant hand immersion in ice water (at start: 2.2–3.8°C). OC in the HH phase were compared to NOC at mid and late points of the menstrual cycle.	• OC users had higher SBP 24 hours post-CPT compared to NOC. • No difference in HR, SBP, DBP, or cortisol response to CPT between OC and NOC. • No difference in HR, DBP, or cortisol 24hours post-CPT between OC and NOC.	Y

Jacob et al.[116]	NOC, n=11; OC, n=10	Combined, monophasic OC for at least 6 months. • Aviane/Falmina (n=1, 0.020 mg EE + 0.10 mg Levonorgestrel) • Estarylla (n=2, 0.035 mg EE + 0.25 mg Norgestimate) • Sprintec (n=2, 0.035 mg EE + 0.25 mg Norgestimate) • Femynor (n=1, 0.035 mg EE + 0.25 mg Norgestimate) • Enskyce (n=2, 0.030 mg EE + 0.15 mg Desogestrel) • Mircette (n=1, 0.020 mg EE + 0.15 Desogestrel) • Loryna/Gianvi (n=1, 0.020 mg EE + 3.00 mg Drospirenone)	2 minutes left foot submersion to the ankle in ice water (0 to -4°C). OC and NOC were compared across LH and HH phases.	• OC had a larger increase of HR, Q, FBF, and FVC throughout CPT exposure compared to NOC. • No differences in MAP, SV, TPR and FVR responses to CPT between OC and NOC.	Y
Kowalczyk et al.[118]	NOC, n=21; OC, n=17	Combined OC. • LoOvral (n=5) • Ortho-Cyclen (n=3) • Alesse-28 (n=3), • Desogen (n=1) • Levelen-ED (n=1), • Low Ogestrel (n=1), • Ortho-Novum 1/35 (n=1) • Orcon (n=1) • Ortho-Cept 28 (n=1)	3 minutes warm water (37°C) forearm immersion followed by cold water (4°C) for as long as tolerated, up to 3 minutes maximum. OC was compared to NOC across 5 intervals of the menstrual and pill cycle (menstruation, LH, Ovulation, HH and late HH).	No difference in SBP, DBP, HR or skin temperature response to either cold or warm water immersion between OC and NOC.	N

Merz et al.[119]	NOC, n=60; OC, n= 30;	Combined, monophasic (0.2-0.035 mg EE + desogestrel, levonegestrel, chlormadinone acetate, cyproterone acetate, dienogest, or drospirenone) OC for at least 3 months.	3 minutes warm (36-37°C) or cold (0-3°C) water immersion of the dominant hand up to the elbow. OC in the HH phase were compared to separate groups of NOC in the LH and NOC in the HH phase.	• OC had higher SBP and DBP at baseline, during CPT and 5 minutes post-CPT compared to NOC. • No difference in post-stress cortisol responses (at 20 and 30 minutes) between OC and NOC.	N
Middlekauff et al.[120]	NOC, n=10; OC, n=13	Combined OC for at least 12 months. • Orthotricyclen (n=6) • Orthotricyclen-Lo (n=3) • Levora (n=1) • Seasonique (n=1) • Kelnor (n=1) • Trinessa (n=1)	2 minutes of hand immersion in ice water slurry. OC and NOC were compared across the LH and HH phases.	No difference in responses of HR, MAP, MSNA (bursts/min, bursts/100 heartbeats, and total activity/min) and %MSNA (bursts/100 heartbeats and total activity/min) to CPT between OC and NOC in either phase.	N
Nielsen et al.[121]	NOC, n=60; OC, n=49	Combined, cyclic and non-cyclic OC. • Monophasic (n=41) • Triphasic (n=8)	3 minutes right hand immersion in ice water (1-4°C). OC users, pill phase undescribed, were compared to NOC in the HH phase.	• OC had a smaller increase in cortisol during CPT than NOC. • No difference in the number of CPT-induced cortisol responders and no difference in baseline or post-CPT (15 minutes or 25 minutes post-CPT) cortisol levels between OC and NOC.	Y

Straznicky et al.[128]	NOC, n=16; OC, n=16	Combined, cyclic and non-cyclic OC for at least 8 months. • Monophasic (n=10, 0.03 mg EE + 0.15 mg levonorgestrel, or n=1, 0.05 mg EE + 125 mg levonorgestrel) • Biphasic (n=1, 0.05 mg EE + 0.05 mg, 0.125 mg levonorgestrel). • Triphasic (0.03, 0.04, 0.03 mg EE + 0.05, 0.075, 0.125 mg levonorgestrel)	2 minutes hand immersion in ice water. OC in the HH phase were compared to NOC in the HH phase.	No difference in the maximum BP or HR response to CPT between NOC and OC.	N
Exercise Stress					
Minahan et al.[82]	NOC, n=15; OC, n=15	Combined, monophasic OC for at least 12 months.	3 minutes of isometric handgrip exercise at 30% MVC in a seated position. OC in the LH phase were compared to NOC in the LH phase.	• OC had higher SBP in response to handgrip. • No difference in the HR response to handgrip between OC and NOC.	Y
Nielsen & Mather[122]	NOC, n=42; OC, n=20	Combined, cyclic and non-cyclic OC. • Triphasic (n=3) • Monophasic (n=16) • Unknown (n=1)	3 minutes isometric handgrip exercise at 30-40% of their perceived MVC or 3 minutes of rhythmic maximal handgrip for three 18secs squeeze/60 s rest cycles. Menstrual phases were not compared.	No difference in salivary alpha-amylase or pupil diameter responses to both isometric and rhythmic hand grip between OC and NOC.	N

Parmar et al.[125]	NOC, n=9; OC, n=10	Combined, monophasic OC (EE 20-30 µg and progestin 100-150 µg) for at least 3 months.	2.5 minutes isometric handgrip exercise at 30% MVC in a semi-recumbent position. OC in the first week of the HH phase were compared to NOC in the LH phase.	• OC had greater increases of SBP, DBP and MAP peak responses during handgrip compared to NOC. • No difference in HR peak during handgrip between OC and NOC.	Y
Takeda et al.[129]	NOC, n=14; OC, n=8	Combined, cyclic and non-cyclic OC for at least 6 months. • Yasmin (n=2, monophasic) • Ocella (n=1, monophasic) • Loryna (n=1, monophasic) • Natazia (n=1, triphasic) • Ortho Tri-Cylin Lo (n=1, triphasic) Unknown (n=2)	Isometric handgrip was performed at 40% of MVC until fatigue (i.e., <80% of the desired force for ≥2sec) in the supine position. OC in the HH phase were compared to NOC in the HH phase.	• OC users had a larger increase in HR, MAP and DBP, and a larger decrease in SV due to handgrip compared to NOC. • OC users had a larger increase in SBP, TPR and MSNA burst frequency at handgrip fatigue compared to NOC. • OC users had reduced time to handgrip fatigue compared to NOC. • OC had a larger increase in SVT at handgrip fatigue compared to NOC.	Y

Teixeira et al.[140]	NOC, n=13; OC, n=17	Unspecified.	Cardiovagal index was calculated as a ratio between the longest and shortest RR interval prior to and at the end of 4secs exercise. Participants performed maximal inspiration, then held a 12secs apnea. Throughout the apnea, after 4secs of rest, the participant started pedalling as quickly as possible for 4secs, then stopped pedalling for an additional 4sec. OC and NOC were compared across 3 intervals of the menstrual and pill cycle (LH, Ovulation, and HH).	No difference in cardiovagal index response to exercise during apnea between NOC and OC in any phase.	N
Mental Stress					
Aleknaviciute et al.[110]	NOC, n=25; OC, n=15	Combined, monophasic (0.03 mg EE + 0.15 mg levonorgestrel) OC for at least 4 months.	Participants underwent the TSST, which included a preparation period, a free speech (i.e., simulated job interview) and a verbal mental arithmetic task (continuously subtracting 13 from 1022) performed in front of a 2-person panel who maintained neutral faces and did not give any feedback. Each period or task was 5 minutes. OC in the HH phase were compared to NOC in the HH phase.	• OC had a smaller increase in cortisol than NOC at 15, 30, 50 and 70 minutes post-TSST. • No difference in HR response to TSST between OC and NOC.	Y

Barel et al. 2018[111]	NOC, n=17; OC, n=20;	Combined, monophasic (25 mg EE + 75 mg Gestodene) OC for at least 12 months.	Participants underwent the TSST, which included a preparation period, a free speech (i.e., simulated job interview) and a verbal mental arithmetic task (continuously subtracting 13 from 1022) performed in front of a 2-person panel who maintained neutral faces and did not give any feedback. Each period or task was 5 minutes. OC in the HH phase were compared to NOC in the HH phase.	No difference in cortisol response to TSST between OC and NOC.	N
Emmons & Weidner[137]	NOC, n=31; OC, n=38	Unspecified OC for a minimum of 2 months.	Participants continuously added 3 to 300 (e.g., 300, 303, 309) for 1.5 minutes. The responses were tape-recorded, and participants were told that they would be evaluated for accuracy and compared to other participants. There was no feedback about their performance, and if a participant made an error, the participant was allowed to continue from the last digit remembered. Smoking or non-smoking OC were compared to smoking or non-smoking NOC. Menstrual phases were not specified.	• OC smokers had a larger increase in SBP during mental stress compared to NOC smokers or non-smokers. • No difference in the responses of DBP or HR to the mental stress test between OC and NOC.	Y

Gamsakhurashvili et al.[114]	NOC, n=24; OC, n=24	Combined, monophasic OC for at least 3 months.	Participants were presented with 60 emotional images (positive, negative and neutral images), while skin conductance responses were recorded (i.e., a marker of skin sympathetic nerve activity). OC in the HH phase were compared to NOC at mid and late points of the menstrual cycle.	OC had a smaller skin conductance response magnitude compared to NOC in the late cycle menstrual phase only during negative image viewing.	Y

Merz et al.[119]	NOC, n=60; OC, n= 30;	Combined, monophasic (0.2-0.035 mg EE + desogestrel, levonegestrel, chlormadinone acetate, cyproterone acetate, dienogest, or drospirenone) OC for at least 3 months.	Participants engaged in a CPT (described in Table 3) then (~20 minutes later) were instructed to memorize words (equal number of neutral, positive, and negative) in 2 minutes. Participants were instructed to recall as many words as they could remember (i.e., immediate recall). Participants returned to the lab to repeat the recall 24 hours later (i.e., delayed recall). After delayed recall, participants were given the first two letters of all the words and asked to complete the word stems with the previously learned words (i.e., delayed cued recall). OC in the HH phase were compared to separate groups of NOC in the LH or HH phases.	No difference in cortisol response to post-stress immediate recall, delayed recall, or delayed cued recall between OC and NOC.	N

Nielsen et al.[121]	NOC, n=60; OC, n=49	Combined, cyclic and non-cyclic OC. • Monophasic (n=41) • Triphasic (n=8)	Participants viewed an emotionally arousing story that was narrated and composed of 11 image slides with emotionally arousing elements. Arousal was determined as the average change in pupil diameter during the emotional story components. Immediately after, a CPT (described in Table 3) was administered. OC users, pill phase undescribed, were compared to NOC in the HH phase.	No difference in pupil diameter change in response to the emotional component of the story between OC and NOC.	N
Villada et al.[131]	NOC=17; OC=17	Combined, monophasic OC for at least 6 months.	Participants underwent the TSST, which included a preparation period, a free speech (i.e., simulated job interview) and a verbal mental arithmetic task (continuously subtracting 13 from 1022) performed in front of a 2-person panel who maintained neutral faces and did not give any feedback. Each period or task was 5 minutes. OC users, pill phase undescribed, were compared to NOC in the LH phase.	No difference in cortisol and HR responses to the TSST between OC and NOC.	N

Orthostatic Stress					
Abidi et al.[76]	NOC, n=12; OC, n=14;	Combined, cyclic and non-cyclic OC. • Tri-Cyclen (n=6) • Tri-Cyclen Lo (n=1) • Alesse (n=5) • Marvelon (n=1) • Novo-Cyprotenone/EE (n=1)	Participants were transitioned from supine (5 minutes) to seated (5 minutes) to standing (10 minutes). OC and NOC were compared across LH and HH phases.	No difference in SVi, HR, Qi, TPRi, MAP, $ETCO_2$, ETO_2, respiratory rate, and cBRS responses to standing between OC and NOC.	N
Odutayo et al.[124]	NOC, n=15; OC, n=10	Provided with combined (30 ug EE + 150 ug levonorgestrel) OC daily for at least 3 months. No placebo pill was taken.	Lower body negative pressure (LBNP) was applied for 15 minutes at each level (-15, -25, and -40 mmHg). OC users in the HH phase were compared to NOC in the follicular phase.	No difference in the MAP responses to LBNP between OC and NOC.	N
Schueller et al.[127]	NOC, n=27; OC, n=31	Combined (EE + progestin) OC. • levonorgestrel (n =8) • desogestrel/gestoden (n =9) • chlormadinon-acetate (n =6) • dienogest (n =5) • norgestimate (n =2) • cyproterone acetate (n =1)	Sequential beat-to-beat intervals of HR were recorded during 60° head-up tilt. cBRS was calculated from the immediate changes of RR-intervals and SBP during the short period of the last 2 heartbeats before head-up tilt and the following 10 beats. A regression was calculated between the decreases in SBP and in RR-interval. Measurements were only accepted for further analysis if a correlation coefficient >0.70 was obtained. OC in the HH phase were compared to NOC in the HH phase.	No difference in cBRS response during tilt between OC and NOC.	N

Usselman et al.[130]	NOC, n=9; OC, n=8	Combined, cyclic and non-cyclic (20-30 ug EE + progestin) OC for at least 6 months. • Patch user (n=1, norelgestromin) • norgestimate (n=1, triphasic) • drospirenone (n=2, monophasic) • desogestrel (n=1, monophasic) • levonorgestrel (n = 3, monophasic)	LBNP was applied for 3 minutes at each of -30, -60, and -80 mmHg, with 5 minutes of recovery between levels. The order of LBNP testing was quasi-random. OC in the LH and HH phases were compared to NOC in the LH and HH phases.	• OC users had a larger decrease in MAP at -80 mmHg LBNP compared to NOC whose MAP did not change across LBNP, regardless of phase. • No difference in the responses of MSNA burst frequency, MSNA burst amplitude, total MSNA, Q, HR, SV and TPR to LBNP between OC and NOC across both phases.	Y
Breathing Maneuvers					
Abidi et al.[76]	NOC, n=12; OC, n=14;	Combined, cyclic and non-cyclic OC. • Tri-Cyclen (n=6) • Tri-Cyclen Lo (n=1) • Alesse (n=5) • Marvelon (n=1) • Novo-Cyprotenone/EE (n=1)	Participants forcefully exhaled while maintaining 40 mmHg pressure for 15secs. The maximum HR during Valsalva and lowest HR within 30secs after exhalation were used to determine the Valsalva HR ratio. OC and NOC were compared across LH and HH phases.	No difference in the responses of DBP, SBP, or MAP during late phase II of the Valsalva maneuver or the Valsalva HR ratio when comparing OC and NOC.	N
Nili et al.[104]	NOC, n=12; OC, n=14	Combined, cyclic and non-cyclic OC. • Tri-Cyclen (n=6) • Tri-Cyclen Lo (n=1) • Alesse (n=5) • Marvelon (n=1) • Novo-Cyprotenone/EE (n=1)	Participants breathed at a frequency of 6 breaths/min. The ratio of maximum to minimum HR within a single breath and was averaged over 6 breaths to determine Exhalation to Inspiration ratio (E:I ratio).	No difference in MAP, $ETCO_2$, HR or E:I ratio responses to paced deep breathing between OC and NOC.	N

Vascular Infusions					
Limberg et al.[81]	NOC, n=10; OC, n = 13	Combined, cyclic and non-cyclic OC. • Microgestin Fe or Junel Fe (n=2, 0.020 mg EE + 1.0 mg norethindrone) • Yaz (n=2, 0.020 mg EE + 3.0 mg drospirenone) • Reclipsen, Desogen, or Apri (n=3, 0.030 mg EE + 0.150 mg desogestrel) • Kariva (n=1, 0.010, 0.020 mg EE + 0.150 mg desogestrel) • Tri-Sprintec, Tri-Linyah or Trinessa 28 (n=3, 0.035 mg EE + 0.180, 0.215, 0.250 mg norgestimate) • Unknown (n=2)	Isoproterenol was infused at four 3 minutes dosage levels (1, 3, 6, and 12 ng/100 g lean tissue/min). OC in the LH phase were compared to NOC in the LH phase.	• OC had higher HR and MAP than NOC at baseline and across all infusion doses. • Throughout isoproterenol infusion, OC had higher FBF, and FVC responses to isoproterenol compared to NOC, although there was no difference in vessel diameter.	Y
Middlekauff et al.[120]	NOC, n=10; OC, n=13	Combined OC for at least 12 months. • Orthotricyclen (n=6) • Orthotricyclen-Lo (n=3) • Levora (n=1) • Seasonique (n=1) • Kelnor (n=1) • Trinessa (n=1)	Phenylephrine was infused incrementally at doses of 0.3, 0.6, and 0.9 $\mu g \cdot kg^{-1} min^{-1}$ to activate the sympathetic arterial baroreflex. Nitroprusside was infused incrementally at doses of 0.4, 0.8, and 1.2 $\mu g \cdot kg^{-1} min^{-1}$, 5 min/ infusion to deactivate the sympathetic arterial baroreflex. OC and NOC were compared across the LH and HH phases.	No difference in MAP, % sympathetic nervous activity mean slope or %HR mean slope responses during either infusion in OC or NOC in either phase.	N

Straznicky et al.[128]	NOC, n=16; OC, n=16	Combined, cyclic and non-cyclic for at least 8 months. • Monophasic (n=10, 0.03 mg EE + 0.15 mg levonorgestrel, or n=1, 0.05 mg EE + 125 mg levonorgestrel) • Biphasic (n=1, 0.05 mg EE + 0.05 mg, 0.125 mg levonorgestrel). • Triphasic (0.03, 0.04, 0.03 mg EE + 0.05, 0.075, 0.125 mg levonorgestrel)	Noradrenaline was infused at a rate of 0.02-0.4 ug/kg/min. Each dose level lasted 6 minutes, and infusions were stopped when a Δ30 mmHg in MAP was achieved. OC in the HH phase were compared to NOC in the HH phase.	OC had greater SBP responsiveness to the dose of noradrenaline required for Δ20 mmHg compared to NOC during a low-fat diet.	Y

BP, blood pressure; cBRS, cardiovagal baroreflex sensitivity; CPT, cold pressor test; DBP, diastolic blood pressure; EE, estrogen estradiol; ETCO$_2$, end-tidal carbon dioxide; ETO$_2$, end-tidal oxygen; E:I ratio, expiration to inspiration ratio; FBF, forearm blood flow; FVC, forearm vascular conductance; FVR, forearm vascular resistance; HH, high hormone; HR, heart rate; LBNP, lower body negative pressure; LF, low frequency; LH, low hormone; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; MVC, maximum voluntary contraction; NOC, naturally cycling or non-oral contraceptive user; OC, oral contraceptive user; pNN50, proportion of normal successive RR intervals that differ by 50ms divided by total number of normal RR intervals; Q, cardiac output; Qi, cardiac output index; RER, respiratory exchange ratio; RMSSD, root mean square of successive differences; SaO$_2$, oxygen saturation; SBP, systolic blood pressure; SDNN, standard deviation of normal RR intervals; SV, stroke volume; SVi, stroke volume index; SVT, sympathetic vascular transduction; TPR, total peripheral resistance; TPRi, total peripheral resistance index; TSST, Trier Social Stress Test; V$_E$, ventilation.

2.5 **Discussion**

This is the first systematic review to comprehensively describe and summarize the effects of OC on autonomic function, both at rest and in response to stimuli. While most of the studies were of good quality, there was substantial variability in how each study described OC (e.g., formulation, generation, cyclic vs. non-cyclic, brand), and less than half of the studies controlled for the length of previous use. This inconsistency in reporting of OC limited secondary discussion of pill generation and hormone cycling. The primary finding of this systematic review is that OC does influence many aspects of autonomic function.

Resting Autonomic Indices

Resting MSNA was evaluated in 4 studies[115,120,129,130], and all of these studies found that there was no influence of OC on resting cardiovascular variables (HR, MAP, systolic BP (SBP), diastolic BP (DBP), Q, stroke volume (SV) and TPR) and MSNA (burst incidence or frequency). Only a single study investigated the effects of OC on the beat-to-beat variation in systolic arterial BP and there was no difference in OC users in the HH phase after 6 months of OC use compared to their baseline prior to OC-use in the LH phase[123]. Similarly, a majority of HRV studies cited no differences between OC and NOC with regard to HRV indices[72,73,113,123,126,127,133], while 5 found an effect of OC as compared to NOC[76,77,134,135,139]. Of the studies that observed HRV differences, two studies found that OC users had greater parasympathetic control than NOC (i.e., higher standard deviation of RR intervals and high frequency (HF) power) at rest[76,134]. Comparatively, Milan et al.[77] observed lower parasympathetic control in OC at rest compared to NOC (i.e., lower percentage of adjacent RR intervals with a duration difference of >50 ms and HF power). This study investigated OC with a higher estrogenic component, and varying estrogen doses in the OC formulation may explain inconsistencies within the literature. Its notable that HRV was not different between OC and NOC in studies that reported the use of OC containing lower estrogen levels (i.e., 20 micrograms)[73,113,123,133]. In contrast, one study reported lower parasympathetic control in OC users taking a combined OC with 20-50 milligrams of estrogen as compared to NOC[77].

Aside from varying estrogen doses, the included studies also differed in the type or dose of progestin, which could have influenced HRV. Although there is no literature describing the effects of the different OC progestins on HRV, research from hormone replacement therapy suggests that attenuation of HRV was only present in post-menopausal women whose therapy regimen included estrogen and progestin compared to estrogen alone or those without hormonal therapy[143]; therefore, the varying progestins may also play a role in the variability of the effects of OC on HRV. Unfortunately, not all studies reported any information about OC formulations or brands. Furthermore, Sims and colleagues[139] observed that HRV is elevated at the beginning of the placebo pill phase, then quickly declines towards the end of the placebo pill phase and incrementally increases throughout the subsequent active pill phase compared to NOC whose HRV started higher than that of OC in the LH phase then continued to decline throughout the HH phase. Not only does this demonstrate a difference in the effects of hormonal cycle between OC and NOC, but this may also potentially indicate that discrepancies may be influenced by when in the pill cycle that the evaluation occurred. Furthermore, comparisons between OC and NOC may be influenced by the experience of pre-menstrual symptoms in NOC, given that NOC who experience more pre-menstrual symptoms had higher sympathetic activity and lower parasympathetic activity in the HH phase compared to the LH phase[144]. Additionally, standard deviation of all normal-normal intervals (SDNN) and root mean squared differences of successive normal-normal intervals (RMSSD) were not associated with pre-menstrual symptoms (e.g., altered appetite, fatigue, irritability, mastalgia, mood swings) in OC, but were associated in NOC[135]. Therefore, the experience of pre-menstrual symptoms in NOC could contribute to exaggerated differences in HRV comparisons between OC and NOC. Considering the implications that this may have on OC comparisons, researchers should consider collecting information regarding pre-menstrual symptoms to use in covariate analysis when comparing HRV across NOC and OC users.

Autonomic Reflexes

All studies indicated no effect of OC on spontaneous baroreflex activation at rest[72,76,113,123,129,138]; therefore, OC likely do not influence the cardiovagal arm of the baroreflex. OC have long been associated with elevated BP, and a recent meta-analysis has demonstrated a positive relationship between the duration of OC use and the risk of hypertension[145]. At rest, Harvey and colleagues[115] observed that OC users had higher BP with similar MSNA (burst incidence and frequency) during the placebo pill phase compared to NOC during the LH phase. Further, resting BP and MSNA were positively correlated in NOC but not OC users[115], suggesting that OC alter neurovascular control (i.e., the vascular response to sympathetic outflow). Taken together with the findings of this review, this suggests that OC induced chronic hypertension may act on alternate mechanisms of BP control rather than direct autonomic reflex control. For example, OC increase hepatic angiotensinogen production leading to increased renin production in the kidneys and increased BP[146]. Therefore, any potential influence of OC on BP could also be mediated by altered end organ function rather than autonomic BP control.

There was only 1 study that investigated the influence of OC on cardiovascular responses to central chemoreflex activation[72]. During 5% hypercapnia, OC users had lower PWV in the LH phase compared to NOC in the LH phase[72]. Resting PWV is a marker of arterial stiffness that describes the velocity of BP wave propagation across a known distance[147], yet an increase in PWV in response to a stimulus can represent sympathetically-mediated vasoconstriction. Therefore, these results indicate that OC users may have less sympathetic output during central chemoreflex activation compared to NOC. As this was observed in the LH phase, this response is potentially due to a chronic adaptation to OC use. There was also a greater increase of $ETCO_2$ in the LH phase of OC compared to NOC in both phases. Considering that minute V_E and $ETCO_2$ are negatively associated (i.e., lower minute V_E allows for greater CO_2 accumulation)[148], this may suggest a depressed ventilatory response to central chemoreflex activation. In summary, there were similar cardiovascular, HRV and respiratory responses, yet potentially lower sympathetic and ventilatory responsiveness to CO_2 in the OC users in the LH phase compared to NOC across the menstrual phases. Interestingly, increased MSNA burst frequency and amplitude have been observed in response to severe chemoreflex activation (combined hypoxia and hypercapnia) during the LH phase compared to the HH phase of OC users; however this study did not compare OC users to NOC[78]. Future studies should consider isolating the central from peripheral chemoreflex to better characterize the individual contributions of each arm of the chemoreflex. There is no current literature on the effects of OC on the isolated peripheral chemoreflex (i.e., hypoxia alone), so further investigation is warranted. The effects of OC on the cardiovascular and V_E response to hypoxic exercise is discussed under *Autonomic Stressors*.

Assadpour et al.[72] was the only study to investigate the mechanoreflex (via passive leg movement), and a lower pressor response was observed in OC users compared to NOC, regardless of phase. However, HRV responses to passive leg movement were not different between OC users and NOC suggesting that there were no differences in parasympathetic or sympathetic output[72]. Previous work has demonstrated in males that the hyperemic response to passive leg movement is nitric-oxide dependant[149]. Therefore, considering that OC users have greater nitric-oxide dependant vasodilation than NOC[150], this greater reliance on nitric-oxide mediated vasodilation may contribute to the observed dampening of the mechanoreflex-activated pressor response. No effect of OC was found on the HRV response to PECO, yet an attenuated pressor response was observed in OC users during PECO compared to NOC in both menstrual/pill phases[72]. However, this was only observed by Assadpour et al.[72], and a larger increase in MAP was observed in OC users compared to NOC during PECO in 3 other studies[82,125,129]. Of the studies that noted greater pressor responses in OC users compared to NOC, two had longer protocols (2.5-3 minutes at 30% maximum voluntary contraction)[82,125] and a single study used handgrip until fatigue[129]. These longer exercise bouts may lead to a greater accumulation of metabolites, which could augment the pressor response. Interestingly, OC users had greater sympathetic vascular transduction (SVT) at fatigue and the last minute of PECO compared to NOC in the HH phase; however, the change in SVT was not different between OC and NOC when controlling for body mass index (BMI)[129]. During PECO, Takeda et al.[129] also found similar associations between the change in SVT and the change in MAP in OC users and NOC, and similar associations between the change in MAP and change in MSNA. Taken together, these results may indicate that sympathetic output may not contribute to increased pressor responses in OC and other factors such as BMI may have had an influence on this cohort. However, there were no differences between OC and NOC in body mass[82] or BMI[125] in the other studies that observed a greater pressor response to PECO in OC compared to NOC. More research is required to determine the role of OC and BMI on sympathetic and pressor responses to metaboreflex activation. Contrary to the suppressed pressor responses observed by Assadpour et al.[72], OC had a greater increase in V_E and tidal volume (Vt) than NOC during PECO[72]. Progestin administration is known to increase V_E[151]; thus, this hyperventilatory response may by influenced by the consumption of synthetic progestin.

Two studies investigated the effects of OC on the sweating response (i.e., sudomotor axon reflex) to cycling exercise and whole-body warming[117,132]. No differences existed between OC users and NOC in sweating onset during pre- or post-exercise warming in either menstrual phase[117,132]. Additionally, Kenny et al.[117] observed no differences in MAP, esophageal temperature, sweat rate, and percentage cutaneous vascular conductance during post-exercise recovery between OC users and NOC across both menstrual phases. There was no difference in resting rectal temperatures, or the temperature required for sweating onset or to reach steady sweating between OC users and NOC during whole-body aerobic exercise[132]. Both OC users and NOC had increased rectal temperature, and higher temperatures to reach sweating threshold in the HH phase compared to the LH phase, while only OC users had higher temperature threshold for steady sweating in the HH phase compared to the LH phase[132]; however, all comparisons were analyzed using student t-tests between OC users and NOC or LH and HH within each group, thus it is difficult to compare these cyclical fluctuations. OC users and NOC had similar gains during unsteady-state sweating (i.e., the transient phase of sweating prior to a constant rate of sweating), although the average gain for sweating over the total experimental period was higher for OC users than NOC in the LH phase[132]. Unlike NOC, OC users did not have higher unsteady-state or average gain in the HH phase, suggesting that OC users experience more uniform thermoregulatory responses to exercise over the pill cycle[132]. Further work should investigate both the onset of sweating and more comprehensive measures of sweating volumes or patterns and utilize repeated-measure ANOVAs for comparison to more accurately describe the differences between OC users and NOC over the menstrual/pill cycle.

A single study investigated the influence of OC on the veno-arterial reflex, which contributes to maintaining peripheral blood flow via vasoconstriction during postural changes[112]. Bishop and Brown[112] observed that OC users had a smaller decrease of skin perfusion of the foot during graded and single-level leg dependency in the HH phase compared to NOC in both menstrual phases, suggesting that OC users exhibit decreased skin postural vasoconstriction. Given that OC users have greater nitric-oxide mediated vasodilation compared to NOC[81], this may dampen their vasoconstrictive responses during postural challenges. In women, blunted peripheral vasoconstriction may contribute to the experience of orthostatic intolerance[152]; thus, this decreased postural vasoconstriction in OC users may contribute to altered orthostatic responses (further explored in *Autonomic Stressors*).

Autonomic Stressors

In response to altitude, OC users had a greater pressor response (DBP and MAP) compared to NOC[142]. This may suggest that OC users may have an exaggerated sympathetic response to altitude, especially considering that OC users experienced an augmented MSNA response to severe chemoreflex activation during the LH phase compared to HH phase[78]. However, Richalet et al.[136] found no differences between OC users and NOC in the HR or ventilatory response to hypoxic exercise. Therefore, the influence of OC on the cardiorespiratory response to altitude may not only be due to hypoxia[136]. Considering that altitude is a combined stressor of hypoxia and hypobaria, further research is required to determine if changes in barometric pressure contribute to the increased pressor response to altitude in OC users.

Freidman and Meares[141] observed that habituation as measured by a lack of sympathetic skin activity (measured via skin conductance) in response to repeated pre-recorded 1000 Hz and 100 dB tones was stable across the OC pill cycle compared to NOC, who had a higher habituation point prior to ovulation[141]. High arousal (i.e., autonomic response to a stimuli) increases the habituation point indicating that OC users had decreased arousal compared to NOC prior to ovulation[141]. Similarly, Milan and colleagues[77] observed that HRV did not change in response to music in OC users, unlike NOC who exhibited a decrease in parasympathetic activity (i.e., standard deviation of normal-normal RR intervals and HF power) in response to musical auditory stimulation. The results of these studies may suggest that OC users have some higher theoretical threshold for autonomic responses or that OC may blunt the autonomic response to auditory stressors. Friedman and Meares[141] did not report their statistical methodologies and some time points had relatively few observations, so conclusions should be drawn with caution. Additionally, it is difficult to base a consensus on only two studies that use varying types of auditory stress (e.g., a pre-recorded tone versus music); thus, more studies with larger participant populations and comparisons across various auditory stimuli are required to concretely define the effects of OC on auditory stress responses.

Eight studies investigated the influence of OC on CPT (sympathetic stressor), where 5 studies did not observe any differences between OC users and NOC in the cardiovascular[118-120,128,137] or sympathetic[120] response to CPT, and 3 studies did observe an influence of OC use on the cortisol[121], cardiovascular[114,116], or sympathetic[116] responses to CPT compared to NOC. Interestingly, Jacob and colleagues[116] observed that the pressor response to CPT was not different between OC users and NOC across both menstrual phases; however, the contributing mechanisms to the pressor response differed. OC users had a greater increase in HR, forearm blood flow and vascular conductance during CPT compared to NOC, regardless of menstrual phase[116]. Considering that OC users paradoxically vasodilated during a vasoconstrictive stimulus, this response may be mediated by an enhanced capacity for peripheral vasodilation or reduced capacity for sympathetically-mediated vasoconstriction[116]. Of the studies that reported temperature, a wide variety of temperatures were used to evoke a cold pressor response, which makes comparison more difficult considering that stimulus intensity seems to influence OC responses as noted in previous discussion. Studies with higher temperatures (4°C[118] and 5°C[137]) found no difference in SBP, DBP, HR[118,137], or skin temperature[137] responses in OC compared to NOC, while studies with lower temperatures (2.2-3.8°C[114], 0 to -4°C[116], and 1-4°C[121]) found larger increases in HR, Q, forearm blood flow and forearm vascular conductance[116], and a smaller cortisol response during CPT[121] in OC compared to NOC. Interestingly, no differences were observed between OC and NOC in post-stress cortisol response (5 minutes post-CPT) or SBP and DBP responses during 0-3°C CPT[119]. Considering that these temperature ranges overlap, it is difficult to determine the effect of temperature on CPT response; although, there are a greater number of studies that suggest that lower temperatures evoke greater cardiovascular responses and smaller cortisol responses between OC and NOC during CPT. Overall, there may not exist any differences in the pressor response between OC and NOC, and while OC have a smaller cortisol response than NOC during CPT – this difference may disappear post-CPT. We suggest that investigators should consider using lower temperatures or potentially compare a range of warmer and colder temperatures to confirm the effects of OC on the sympathetic response to CPT. Additionally, investigators should consider more tightly regulated temperature ranges or should report the range and mean temperature to fully investigate the effect of varying temperatures on the influence of OC on the sympathetic response to CPT. Timing of comparison may also be a factor to consider as Gamsakhurdashvili et al.[114] did not observe differences between OC and NOC in the acute CPT response yet OC users had higher SBP 24 hours post-CPT compared to NOC, which suggests that OC may have a delayed influence on the cardiovascular response to cold.

Five studies investigated the effects of OC on the autonomic response to an exercise stressor, either during full-body dynamic exercise[140], or handgrip[82,122,125,129]. Teixeira et al.[140] examined the effects of OC on cardiovagal withdrawal during exercise, and no difference was observed between OC users and NOC across the menstrual and pill cycle. This suggests that hormones do not influence cardiovascular autonomic control during the immediate transition into dynamic exercise. Similarly, Neilsen and Mathers[122] did not observe differences in salivary alpha-amylase or pupil dilation response to isometric or rhythmic handgrip exercise. Comparatively, the 3 other studies that investigated isometric handgrip found greater pressor[82,125,129], and sympathetic responses[129] in OC users compared to NOC. It is possible that OC may influence the autonomic response to handgrip yet not the response to dynamic exercise initiation, although these results do not represent all modalities of exercise, nor do they consider all autonomic variables and reflexes influenced by exercise (i.e., ventilatory). Further work is required to fully characterize the effects of OC on the autonomic response to exercise.

Seven studies investigated the effects of OC on the autonomic responses to various mental stressors, such as the Trier Social Stress Test (TSST)[110,111,131], mental arithmetic[137], and recall of emotional images[114], an emotional story[121], or words[119] after physiological stress (e.g., CPT). Most studies reported that there was no effect of OC use on the responses of cortisol[110,111,119,131], HR[110,131], or pupil diameter[121] to various mental stressors as compared to NOC. Three studies investigated the influence of OC on the cardiovascular[110,131] and cortisol response[110,111,131] to the TSST, a social stress test consisting of public speaking and mental arithmetic. Both Aleknaviciute et al.[110] and Villada et al.[131] found no difference in the cardiovascular response to the TSST in OC users and NOC. Only one study observed a blunted cortisol response to TSST in OC users compared to NOC[110]. It is unclear what caused this inconsistent finding, as all studies used similar methodologies. Emmons and Weidner[137] was the only study to investigate mental stress by a single arithmetic test, and a significant interaction between OC use and smoking status was observed, such that the smoking OC group exhibited greater systolic responses than smoking NOC during arithmetic[137]. Two studies observed cortisol response to memory recall 24 hours after CPT, and neither study observed differences between OC and NOC in the cortisol response to recall[114,119]. Gamsakhurdashvili and colleagues[114] also observed that the skin conductance response (i.e., a marker of skin sympathetic nerve activity) magnitude was smaller in OC users during negative image viewing compared to NOC in the late phase of the menstrual cycle, indicating a reduced sympathetic response to negative stimuli. Together, these findings suggest that OC do not influence the cardiovascular nor cortisol response to mental stress (i.e., TSST), although OC may act synergistically with other health-related lifestyle factors (i.e., smoking).

Clinical autonomic testing often uses orthostatic stress testing and breathing maneuvers, such as Valsalva and paced deep breathing[105]. Four studies investigated the relationship between

OC and orthostatic stress[76,124,127,130], yet only a single study found that OC influenced the cardiovascular response to lower-body negative pressure (LBNP), such that OC users experienced a larger decrease in MAP at -80 mmHg of LBNP compared to NOC[130]. Usselman and colleagues[130] used a greater stimulus than other studies that found no difference between OC users and NOC (-15 to -40 mmHg[124] or postural transition[76]), suggesting that greater orthostatic stress may be necessary to evoke differences between OC users and NOC. There were no differences between OC and NOC in the cardiovascular (HR, SV, Q, or TPR[76]), autonomic (MSNA[130], or cBRS[76,127]) or respiratory responses ($ETCO_2$, end-tidal oxygen (ETO_2), or respiratory rate[76]) to orthostatic stress. Two studies investigated the effects of OC on the Valsalva maneuver[76] and paced deep breathing[104]; neither study found differences in the pressor or HR responses between OC users and NOC[76,104].

Three studies investigated the effects of OC on cardiovascular[81,120,128] or sympathetic[120] responses induced by vascular infusions. A single study evaluated the effects of OC on the pharmaceutically-induced response to activation (i.e., phenylephrine) or deactivation (i.e., nitroprusside) of the baroreflex[120]. No differences were observed between OC and NOC in the cardiovascular (HR and MAP) or sympathetic (MSNA frequency or total activity) responses to incremental doses of phenylephrine or nitroprusside[120]. This further emphasizes the previously stated conclusion that the baroreflex is not influenced by OC, neither at rest[72,76,113,123,129,138], or during an induced stressor response[120]. Two studies infused systemic vasoconstrictive[128] or local vasodilatory[81] pharmaceuticals to determine the influence of OC on pressor responses. Both found differences between OC and NOC[81,128], suggesting that OC acts on the vasculature. More specifically, Straznicky et al.[128] observed that OC users experienced less responsive MAP yet more responsive SBP to noradrenaline infusion (i.e., vasoconstrictor) compared to NOC. These results may indicate greater ß1 and β2-adrenergic receptor sensitivity in OC compared to NOC - considering that norepinephrine activates β1 receptors increasing cardiac contractility allowing for augmented systolic contraction[153] (i.e., greater SBP) and vascular β2 receptors to counteract the α1-mediated vasoconstriction (i.e. unchanged MAP). Indeed, Limberg et al.[81] investigated the influence of OC on the vasodilatory effects of isoproterenol and found greater ß-adrenergic receptor-mediated vasodilation in OC compared to NOC[81], which could influence neurovascular control via attenuation of sympathetically-mediated pressor responses[32]. Further, OC users may experience attenuated pressor responses during mechanoreflex and metaboreflex activation[72], and larger drops in MAP during progressive LBNP compared to NOC[130], which could all be influenced by higher ß-adrenergic receptor-mediated vasodilation.

Limitations

A limitation of the current review is the small number of available studies investigating the effects of OC on autonomic function compared to NOC. It is difficult to ascertain the exact impact of OC on a particular reflex with so few studies to draw conclusions from. Further, this systematic review could not be quantitatively pooled in a meta-analysis due to the small number of included citations and the diversity of included autonomic indices, reflexes, or physiological responses. Many aspects of autonomic function require further investigation to reach a consensus within the literature or to confirm previous observations.

Additionally, OC are far more complicated than the specifications found in many of these studies. Not all investigators specified the type of OC included, and varying formulations may have divergent effects on autonomic function. For example, Herrera and colleagues[154] observed that second-generation progestins exacerbate the cortisol stress response to cold water immersion compared to other generations. Similarly, many investigators did not consider or compare hormonal fluctuations over the pill cycle. Indeed, the active dose of OC has been associated with reduced sympathetic and cardiovagal sensitivity[155], increased sweating thresholds[132,156,157], and reduced sympathoexcitation during severe chemoreflex activation[78]. Comparisons between active and placebo phases allow researchers to determine if the adaptations are due to the acute presence of exogenous synthetic hormones or a chronic adaptation of use (observed during placebo). This is further complicated by changing doses within a pill cycle, where increasing dosages of synthetic hormones are used over time. Relatively few studies compare autonomic function across mono-, bi- or triphasic OC. Of the small pool of included studies, few investigations accounted for the potential intra- and intergroup differences between different OC formulations, phases, and cycling patterns; thus, future researchers should consider using covariate analysis, and mixed model or repeated-measures ANOVA to account for the varying components of OC pills to fully elucidate the influence of synthetic hormones.

2.6 **Conclusion**

OC had varying effects on autonomic function; thus, further research is required to confirm the influence of synthetic hormones. Interestingly, some inconsistencies within the literature may derive from OC users requiring a greater sympathetic stimulus than NOC to evoke a similar physiological response. For example, some studies observed that higher levels of LBNP or longer duration/higher intensity handgrip exercise were needed to evoke altered cardiovascular (i.e., pressor, blood flow, or HR) or sympathetic responses in OC compared to NOC[116,119,130]. We strongly recommend that investigators utilize a stronger sympathetic stimulus to confirm if OC users are different than NOC, as OC users may have some higher theoretical threshold required to observe altered responses. Additionally, OC formulation may also contribute to inconsistencies across autonomic research. While resting HRV was the most widely studied, there was great variability in the observed effects of OC use. Indeed, while endogenous estrogen has been positively associated with various components (low frequency (LF), HF, and total power) of resting HRV[158], those studies that reported no difference in resting HRV had relatively lower doses of estrogen in the OC formulations[73,113,123,133] and one study which used a higher estrogenic OC observed lower parasympathetic control of HRV compared to NOC[77]. Clinicians should be mindful of prescribing OC formulations with higher estrogenic dosages to individuals with chronic diseases since many of these individuals already experience sympathetic overactivation and/or reduced parasympathetic activity[159]. Further, since we have found that OC use can influence resting autonomic function and the autonomic responses to stressors, yet OC use is not often described in clinical studies, we recommend that future studies include OC classification (i.e. type, duration, formation, cyclic vs non-cyclic) and/or control for OC use by exclusion or co-variate analysis to increase the accuracy of the determination of autonomic dysfunction when, and if, present.

Chapter 3: **The Influence of Oral Contraceptives on the Exercise Pressor Reflex in the Upper and Lower Body**

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Data Availability

This is not applicable to the current manuscript.

Competing Interests

The authors have no competing interests to declare.

Author Contributions

The experimental protocols were preformed in the Women's Cardiovascular Health lab at York University. Both authors (TJP and HE) were responsible for the study design, ethic's application, participant recruitment, data collection, data analysis, manuscript preparation and revisions. All authors have approved the final version of this manuscript for publication.

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3.1 **Key Points Summary**

- No differences between OC and NOC users in the cardiorespiratory responses to exercise (i.e., handgrip or plantarflexion), PECO (arm or leg) or passive movement (PM) (arm or leg) were observed.
- Disparities in muscle strength may have masked some differences between OC and NOC in the pressor response during metaboreflex but not mechanoreflex activation.
- No women exhibited increased V_E during metaboreflex activation in the arm or leg despite increasing V_E during both handgrip and plantarflexion exercise.
- Both OC and NOC increased V_E during arm and leg PM compared to baseline.
- Considering that exercise increases V_E and that women did not display a ventilatory response to arm or leg metaboreflex activation yet did increase V_E in response to mechanoreflex activation, we suggest that the mechanoreflex may primarily drive V_E during exercise in women. This may be due to an unmet metabolite threshold necessary for ventilatory stimulation.

3.2 **Abstract**

The exercise pressor reflex controls the cardiorespiratory response to exercise, which can be separated into the metabo- and mechanoreflexes. Previous research has demonstrated that OC enhance the ventilatory and pressor responses to arm metaboreflex activation (i.e., PECO) compared to NOC. OC users also experience attenuated pressor responses to leg PM than NOC. We investigated the cardiorespiratory responses to arm or leg metabo- and mechanoreflex activation in OC and NOC. Thiry-two women (OC, n=16; NOC, n=16) performed 4 trials: 40% handgrip or 80% plantarflexion followed by PECO and arm or leg PM. OC and NOC increased MAP similarly during handgrip, plantarflexion and arm or leg PECO compared to baseline. Despite increased V_E during upper and lower limb exercise, none of the women exhibited a sustained increase in V_E during arm or leg PECO. Additionally, OC and NOC similarly increased MAP and V_E during arm or leg PM compared to baseline. Therefore, OC and NOC were similar across all pressor and ventilatory responses to arm or leg metabo- and mechanoreflex activation. However, some differences between OC and NOC may have been masked by disparities in muscle strength. Since women are known to increase V_E during systemic exercise, we suggest that while women do not display a ventilatory response to arm or leg metaboreflex activation (perhaps due to not reaching a theoretical metabolite threshold to stimulate V_E), the mechanoreflex may drive V_E during exercise in women.

Keywords: Metaboreflex, Mechanoreflex, Oral contraceptives, Ventilation, Hemodynamics

3.3 **Introduction**

The exercise pressor reflex controls the cardiorespiratory response to dynamic exercise by the joint feedback of the metaboreflex and mechanoreflex, which provide information about the metabolic environment, and muscular deformation or tendon stretch of the exercising muscle, respectively. Previous research has demonstrated that women experience a blunted metaboreflex, yet this is unaffected by the cyclical fluctuations of the menstrual cycle[72,160,161]. Assadpour et al.[72] also observed that women who take OC had an enhanced increase in V_E and Vt in response to forearm metaboreflex activation via PECO after handgrip. While this was the only study to investigate the ventilatory response, there is conflicting evidence showing either a smaller[72] or larger increase in MAP[82,125,129] in OC users during arm PECO compared to NOC. Additionally, OC users have been shown to have a blunted pressor response compared to NOC during leg PM (i.e., mechanoreflex activation)[72], yet there was no effect of the menstrual or OC pill cycle on the ventilatory responses to leg PM[72]. These studies suggest that the monthly fluctuations of hormones may not affect cardiorespiratory responses to exercise pressor reflex activation, yet the chronic use of synthetic hormones may have an influence.

It has been suggested that differences in the magnitude of the response to activation of the metaboreflex or mechanoreflex could be due to the size and strength of the muscles involved[162-164]. For example, Lee et al.[162] and Tharpe et al.[163] observed that the sex difference in the pressor response to arm metaboreflex activation was attenuated when accounting for strength and muscle mass differences. Previous research has demonstrated that women have less metabolite production and acidosis than men during arm PECO[165]. Larger or stronger muscles may evoke greater acidosis or metabolite production, which could contribute to the previously observed sex differences in arm metaboreflex activation. Additionally, Vianna and colleagues[164] observed in males that the drop in RR interval induced by passive cycling with 4 limbs was greater than with a single limb only, suggesting that the muscle mechanoreflex is also dependent on the size or number of the muscles engaged. However, Fouladi et al.[166] observed that only males experienced an increase in BP in response to arm PM but not leg PM, suggesting that limb-specific sex differences in mechanoreflex responses are present regardless of muscle size.

The purpose of the study was to investigate the influence of OC on the cardiorespiratory responses to metaboreflex or mechanoreflex activation in both the forearm and the lower leg to determine if a larger or stronger muscle mass (i.e., leg versus arm) would enhance the cardiorespiratory response in women. Since the majority of studies have observed a greater pressor response to arm PECO in OC users, and OC users exhibit exaggerated sympathetic outflow and vascular transduction to arm PECO[129,167], we hypothesized that OC users would have an augmented pressor response to arm or leg metaboreflex activation compared to NOC. Nitric oxide (NO)-dependant vasodilation drives the hyperemic response to mechanoreflex activation[149,168,169]. Given that chronic OC use is associated with increased beta-receptor sensitivity[128] and beta-mediated vasodilation[81], we expect that OC will exhibit a blunted pressor response to PM due to their enhanced ability to vasodilate, as observed in Assadpour et al.[72]. Additionally, OC use may also lead to a hyperventilatory response to PECO due to previous observations that progestin administration has been shown to increase V_E through an increase in bronchiole smooth muscle relaxation[151]. Indeed, OC users exhibit an enhanced V_E response to arm PECO than NOC[72]; thus, we hypothesized OC would have a hyperventilatory response to both arm and leg metaboreflex activation compared to NOC. Lastly, we hypothesized that the ventilatory response to either arm or leg PM would be similar in all women, due to a lack of previously observed differences in the V_E response to leg PM[72].

3.4 **Methods**

Ethical Approval

This study was conducted in accordance with the ethical and safety standards set by the Declaration of Helsinki, except for database registration. Participants were informed about all experimental protocols and potential risks prior to providing their written consent. The experimental protocols were approved by the Office of Research Ethics at York University (Certificate #: e2018-254).

Participant Characteristics

Healthy individuals were included in this study with no history of any cardiovascular, respiratory, autonomic, or hormonal conditions. Participants were excluded if using medications that may have influenced their cardiorespiratory response or the use of any hormonal contraceptives other than OC. NOC were excluded if their menstrual cycle duration was outside of the range 26-30 days. OC users were required to have been taking their current OC for at least 3 months before testing. All testing occurred during the early follicular or placebo pill phase of the menstrual or pill cycle, given the lack of influence of the menstrual or pill cycle on either mechano- or metaboreflex activation[72]. Participants were asked to refrain from consuming fatty foods, alcohol, or caffeine, engaging in heavy exercise, and smoking 12 hours before their scheduled visit.

Thirty-two women (OC, n=16 and NOC, n=16) were recruited to participate in the current study. Types of OC included in the current cohort were: Alesse (n=5; Monophasic, 0.1 mg of levonorgestrel, 0.02 mg of ethinyl estradiol (EE)), Alysena (n=3; Monophasic, 0.1 mg of levonorgestrel, 0.02 mg of EE), Yasmin (n=1; Monophasic, 3 mg of dropspirenone, 0.03 mg of EE), Marvelon (n=1; Monophasic, 0.15 mg of desogestrel, 0.03 mg of EE), Linessa (n=1; Triphasic, 0.1, 0.125 and 0.15 mg of desogestrel and 0.025 mg of EE), Ortho Tri-cyclen (n=1; Triphasic, 0.18, 0.215 and 0.25 mg of norgestimate and 0.035 mg of EE) Ortho Tri-cyclen Lo (n=1; Triphasic, 0.18, 0.215 and 0.25 mg of norgestimate and 0.025 mg of EE), LoLo (n=1; Biphasic, 0-1 mg norethindrone acetate and 0.01 mg of EE), Freya (n=1; monophasic, 0.15 mg of desogestrel and 0.03 mg of EE) and Aviane (n=1; Monophasic, 0.1 mg of levonorgestrel and 0.02 mg of EE).

Height and weight were measured using a stadiometer, which was used to calculate BMI ($height/weight^2$) and body surface area (BSA; $0.007184x(weight^{0.425})x(height^{0.725})$)[170]. Predicted VO_2 max was estimated using anthropometrics and self-reported physical activity levels, using the Ainsworth equation[171]. The thickest and thinnest circumferences of the forearm and calf were measured to estimate muscle volume, as well as the distance between these points. Forearm or calf muscle volume was estimated using a modified equation for the volume of a truncated cone[172].

Experimental Protocol

Participants were supine for the duration of data collection and all exercise and movement were conducted with the limbs of the left side of the body. Prior to participating in any trials, maximum voluntary contraction (MVC) of handgrip and plantarflexion were determined using a pressure transducer and fixed plantarflexion device (MLT004/ST Grip Force, AD Instruments, Colorado Springs, USA), respectively. There were 4 trials that each participant underwent in a randomized order: upper or lower metaboreflex activation, and upper or lower mechanoreflex activation.

Metaboreflex Activation

After a 5-minute baseline, participants performed 2 minutes of isometric exercise maintained at an intensity of 40% MVC for handgrip or 80% MVC for static plantarflexion. The plantarflexion intensity was established during pilot work in an attempt to achieve equivocal ventilatory response to arm metaboreflex activation. Exercise was followed by 3 minutes of PECO at an occlusion pressure of +50 mmHg above resting SBP on the exercising limb below the cubital fossa or above the patella, respectively. A 2-minute recovery/reperfusion period followed PECO.

Mechanoreflex Activation

Immediately after supine rest (4 minutes), occlusion was applied distal to the cubital fossa or proximate to the patella for 1 additional minute prior to PM. While occluded, a research assistant passively moved the occluded limb through 90° of flexion to 180° of extension for 3 minutes, set to the pace of a metronome at 1 Hz or 30 full cycles per minute.

Cardiovascular Measures

HR was measured using a single-lead electrocardiogram (ECG; BioAMP, ADInstruments, Colorado Springs, USA), and 5 minutes of continuous ECG was used to analyze resting HRV to determine autonomic balance using the HRV module in the LabChart Pro software (Version 8.1.9, ADinstruments, Colorado, USA). Additionally, HRV was determined using 3 minutes of continuous ECG during baseline, PECO, and PM for both limbs. BP was determined by continuous beat-to-beat finger plethysmography (BMEye Nexfin, Amsterdam, NL). SV index (SVi) was determined via automated pulse contour analysis (ModelFlow algorithm), which was normalized to body size. Cardiac index (Qi) and TPR index (TPRi) were calculated using MAP and SVi (TPRi = MAP / Qi where Qi=SVi x HR). Hemodynamic averages were calculated from the last 30s of each timepoint: baseline, exercise, PECO, and recovery for metaboreflex activation or baseline and PM for mechanoreflex activation. Blood flow (flow= $(\pi \times (\text{diameter}/2)^2) \times$ velocity) was calculated using brachial artery diameter (average of 3 measures) and blood velocity (1-minute average) captured using duplex ultrasound (Vivid i, GE Healthcare Systems, Canada). Blood velocity was exported to PowerLab, using the DAT module (Doppler Audio Translator system, Penn State College of Medicine, Hershey, Pennsylvania). Ultrasound imaging was conducted on the right arm for all trials (i.e., contralateral to the exercising or moving limb), and images were captured within the end of the final minute of each timepoint.

Ventilatory Measures

A heated linear pneumotachometer was used to obtain Vt and respiratory rate (Series 3813, Hans Rudolph Inc, Shawnee Mission, USA), which were used to calculate V_E as a product. Expired gases were measured using CO_2 and oxygen (O_2) gas analyzers (Model 17630, Vacumed, Ventura, USA).

Data & Statistical Analysis

All data was acquired at a rate of 1000 Hz through PowerLab (16/35, ADInstruments, Colorado Springs, USA) and LabChart Pro (Version 8.1.9, ADinstruments, Colorado, USA) software. Statistical analyses were performed using Systat SigmaPlot software (Version 15.0, Inpixon, California, USA). Significance was set *a priori* to $p \leq 0.05$ and post-hoc was conducted using a Bonferroni correction. A one-way ANOVA was used to determine statistical differences between OC and NOC, such as anthropometrics, estimated fitness, muscular strength, resting BP and HRV. Some subject characteristics were not normally distributed (i.e., weight, height and arm volume), therefore these variables were compared with Kruskal-Wallis ANOVA on Ranks. A 2-way repeated-measures ANOVA (OC use x Time) was used to determine the cardiorespiratory responses to arm or leg metaboreflex and mechanoreflex activation in both OC and NOC.

As a secondary aim, this study investigated if muscle volume and/or strength influenced the magnitude of the pressor or ventilatory response to arm or leg PECO and PM – considering that these factors are known to influences sex-related differences in arm metaboreflex activation[162,163]. To account for the potential influence of muscle volume and strength, a one-way ANCOVA was conducted on the change (Δ) in MAP and V_E from baseline to either arm or leg PECO or PM during reflex activation in all women, with a post-hoc Holm-Sidak adjustment. Most variables (ΔMAP for arm or leg PECO and PM; ΔV_E for leg PECO and arm or leg PM) passed Levene's equal variance test (all $p>0.05$) and the equal slopes assumption (all $p>0.05$).

During arm PECO, ΔV_E did not pass the equal slopes test due to an interaction between the factor (i.e., OC use) and both covariates (i.e., group and handgrip strength, p=0.009; group and forearm muscle volume, p=0.029). Therefore, an Equal Slopes Model of Analysis of Variance was reported for most variables and individual linear regressions were reported for the ventilatory response in each group during arm PECO. Partial eta^2 (ηp^2) was used to estimate effect size. All raw data is displayed as means±SD, and the adjusted means are displayed as means±SE.

3.5 **Results**

Subject Characteristics and resting heart rate variability (HRV)

Compared to NOC, OC users were older (p=0.008), had higher SBP (p=0.047), higher DBP (p=0.045), and higher MAP (p=0.036; Table 4). OC also had lower predicted aerobic fitness (p=0.029) and had weaker grip strength than NOC (p=0.048; Table 4). There were no significant differences between OC and NOC in weight (p=0.27), height (p=0.76), BMI (p=0.25), forearm muscle volume (p=0.29), calf muscle volume (p=0.17), BSA (p=0.27), and maximum plantarflexion force (p=0.89; Table 4). OC had lower standard deviation of RR intervals (SDRR; p=0.045) and RMSSD (p=0.034) than NOC but there was no difference in pRR50 (p=0.06), LF power (p=0.08), HF power (p=0.13), and LF/HF ratio (p=0.17; Table 4).

Table 4. Subject characteristics (i.e., anthropometrics, resting BP, predicted fitness, muscle strength and HRV) for OC users, compared to NOC.

	NOC	**OC**	**P-value**
Age (years)	21±2	23±3*	**0.008**
Height (cm)	162±7	163±7	0.763
Weight (kg)	60±12	66±13	0.274
Body Mass Index (kg/m^2)	23±4	25±4	0.245
Body Surface Area (m^2)	1.6±0.2	1.7±0.2	0.271
Systolic Blood Pressure (mmHg)	102±7	109±10*	**0.047**
Diastolic Blood Pressure (mmHg)	66±7	72±8*	**0.045**
Mean Arterial Pressure (mmHg)	78±6	84±8*	**0.036**
Predicted VO$_2$ max (mL/kg/min)	40.4±3.0	37.8±3.5*	**0.029**
Estimated Forearm Volume (cm^3)	0.5±0.1	0.5±0.2	0.290
Estimated Calf Volume (cm^3)	1.4±0.2	1.6±0.4	0.173
Forearm MVC (N)	288±104	218±75*	**0.048**
Calf MVC (N)	257±143	262±96	0.892
SDRR (ms)	102±41	74±33*	**0.045**
RMSSD (ms)	128±64	82±52*	**0.034**
pRR50 (%)	64±23	49±22	0.059
LF power (nu)	19±11	30±21	0.078
HF power (nu)	79±11	70±21	0.125
LF/HF ratio	0.27±0.23	0.79±1.44	0.167

All values are mean±SD. HF, high frequency; LF, low frequency; MVC, maximal voluntary contraction; NOC, no oral contraceptive; OC, oral contraceptive; pRR50, proportion of RR interval differences greater than 50 ms; RMSSD, root mean square standard deviation; SDRR, standard deviation of RR intervals; VO$_2$ max, maximum oxygen consumption. Significance is represented by bold text and * ($p<0.05$).

Arm Metaboreflex

In all women, HR was higher during handgrip compared to baseline, arm PECO and recovery (Figure 2A; all $p<0.001$). During handgrip and arm PECO, MAP was higher than baseline and recovery in both OC and NOC (Figure 2B; all $p<0.001$). V_E was higher during handgrip compared to baseline, arm PECO, and recovery in all women (Figure 2C; all $p<0.001$).

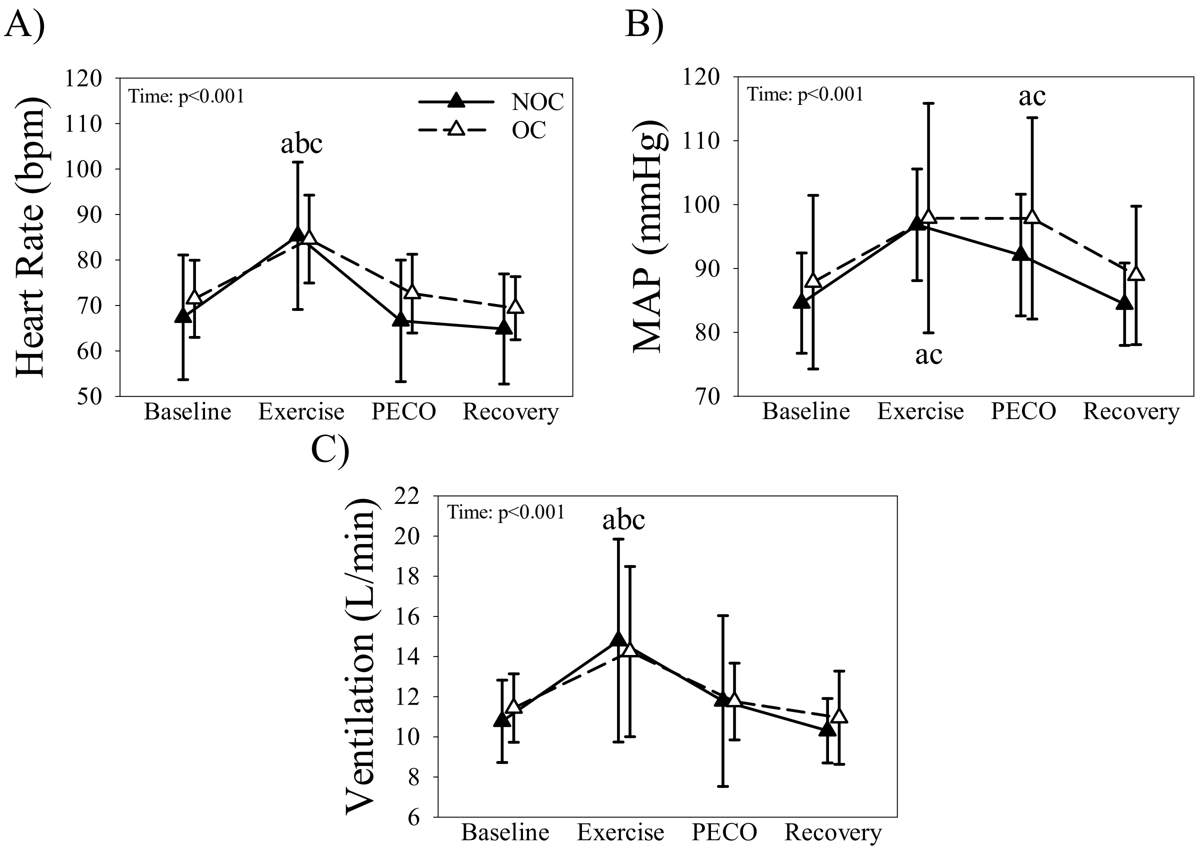

Figure 2. The heart rate (A), mean arterial pressure (MAP; B), and ventilation (C) responses to metaboreflex activation (i.e., post-exercise circulatory occlusion; PECO) in the **arm** of OC (dashed line & open triangles) and NOC females (solid line & closed triangles). Data presented as mean±SD. a indicates significantly different than Baseline in both groups. b indicates significantly different than PECO in both groups. c indicates significantly different than Recovery in both groups.

A main effect of time existed for average brachial artery diameter in the contralateral arm during arm metaboreflex activation ($p=0.049$; Table 5), yet post-hoc analysis revealed no statistical differences (all $p>0.10$). Brachial blood velocity and flow were higher in OC users than NOC, regardless of time ($p=0.026$ and $p=0.046$ respectively), and both variables were higher during handgrip compared to baseline, arm PECO and recovery (all $p<0.010$; Table 5). Qi and SVi were higher during handgrip than baseline, arm PECO and recovery in OC and NOC (all $p<0.001$; Table 5). During arm PECO, Qi and SVi were higher than baseline in all women (both $p<0.020$; Table 5). Additionally, SVi remained elevated during recovery compared to baseline in both groups ($p=0.010$; Table 5). In all women, TPRi was higher during arm PECO compared to handgrip ($p=0.004$; Table 5). DBP and SBP were higher during handgrip and arm PECO compared to baseline and recovery (all $p<0.001$; Table 5).

Breathing rate was higher during handgrip compared to baseline, arm PECO, and recovery in all women (all $p<0.001$; Table 5). $ETCO_2$ was lower during handgrip and arm PECO compared to baseline in all women (both $p<0.040$; Table 5). In OC and NOC, ETO_2 was higher during handgrip compared to baseline and recovery (both $p<0.005$; Table 5). There was no effect of time or OC on Vt during arm metaboreflex activation (all $p>0.05$; Table 5).

Table 5. The influence of OC on the cardiorespiratory response to metaboreflex activation in the **arm**.

	Arm Metaboreflex										
	OC				NOC				P-value		
	Baseline	Exercise	PECO	Recovery	Baseline	Exercise	PECO	Recovery	OC	Time	OC X Time
Brachial artery diameter (cm)	0.31±0.04	0.31±0.04	0.31±0.04	0.30±0.03	0.30±0.03	0.30±0.03	0.29±0.04	0.29±0.04	0.345	**0.049**	0.628
Brachial blood velocity (cm/s)	8.4±3.1 ^{a}	10.7±6.3 ^{a,b,c,d}	8.1±4.1 ^{a}	8.0±3.4 ^{a}	6.1±1.5	6.9±1.6 ^{b,c,d}	5.5±1.5	5.6±1.4	**0.026**	**<0.001**	0.310
Brachial blood flow (L/min/m^2)	0.61±0.30	0.79±0.49 ^{b,c,d}	0.60±0.37	0.58±0.29	0.44±0.16	0.50±0.15 ^{b,c,d}	0.40±0.19	0.41±0.15	**0.046**	**<0.001**	0.262
Qi (L/min/m^2)	6.5±0.9	7.5±1.3 ^{b,c,d}	7.0±1.0 ^{b}	6.5±0.9	6.0±1.3	7.0±1.1 ^{b,c,d}	6.2±1.1 ^{b}	6.2±1.2	0.159	**<0.001**	0.132
SVi (mL/m^2)	91±15	89±14 ^{b,c,d}	98±16 ^{b}	94±17 ^{b}	90±15	84±16 ^{b,c,d}	94±15 ^{b}	96±13 ^{b}	0.603	**<0.001**	0.053
TPRi (mmHg/L/min/m^2)	13.8±2.6	13.3±2.4 ^{c}	14.1±2.2	14.0±2.5	14.7±3.3	14.3±3.0 ^{c}	15.4±3.2	14.2±2.9	0.350	**0.006**	0.198
DBP (mmHg)	75±12	83±15 ^{b,d}	83±13 ^{b,d}	76±9	73±9	83±10 ^{b,d}	79±10 ^{b,d}	72±7	0.572	**<0.001**	0.809
SBP (mmHg)	116±20	124±24 ^{b,d}	127±22 ^{b,d}	118±17	111±8	123±10 ^{b,d}	121±13 ^{b,d}	113±8	0.445	**<0.001**	0.839
Breathing rate (breaths/min)	18±4	21±5 ^{b,c,d}	18±4	17±5	17±3	20±4 ^{b,c,d}	18±3	17±3	0.574	**<0.001**	0.186
ETCO$_2$ (mmHg)	41±4	38±5 ^{b}	40±4 ^{b}	41±4	40±3	38±3 ^{b}	38±4 ^{b}	39±2	0.149	**0.002**	0.186
ETO$_2$ (mmHg)	113±6	118±11 ^{b,d}	115±6	112±7	114±8	121±9 ^{b,d}	118±11	114±9	0.309	**<0.001**	0.789
Vt (L)	0.65±0.12	0.72±0.41	0.70±0.21	0.70±0.19	0.64±0.16	0.83±0.46	0.66±0.21	0.62±0.11	0.965	0.082	0.441

All values are mean±SD. DBP, diastolic blood pressure; ETCO$_2$, end-tidal carbon dioxide; ETO$_2$, end-tidal oxygen; NOC, no oral contraceptive; OC, oral contraceptive; PECO, post-exercise circulatory occlusion; Qi, cardiac index; SBP, systolic blood pressure; SVi, stroke volume index; TPRi, total peripheral resistance index; Vt, tidal volume. Significance is represented by bold text ($p<0.05$). a indicates significantly different than NOC. b indicates significantly different than Baseline. c indicates significantly different than PECO. d indicates significantly different than Recovery.

Leg Metaboreflex

HR was higher during plantarflexion compared to baseline, leg PECO and recovery in all women (all $p<0.001$) and was elevated during leg PECO compared to recovery ($p=0.003$; Figure 3A). In OC and NOC, MAP was higher during plantarflexion and leg PECO compared to baseline and recovery (all $p<0.001$; Figure 3B). V_E was higher during plantarflexion compared to baseline, leg PECO and recovery in all women (all $p<0.001$; Figure 3C).

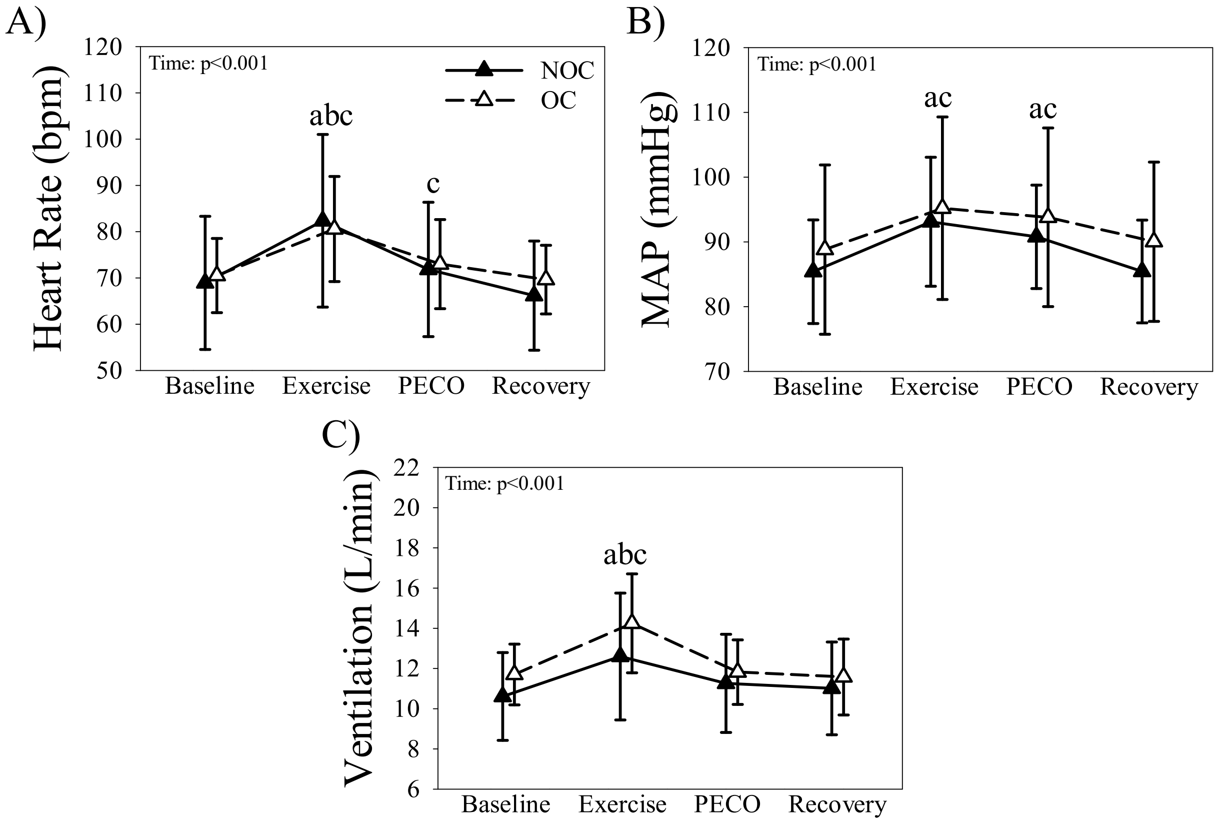

Figure 3. The heart rate (A), mean arterial pressure (MAP; B), and ventilation (C) responses to metaboreflex activation (i.e., post-exercise circulatory occlusion; PECO) in the **leg** of OC (dashed line & open triangles) and NOC females (solid line & closed triangles). Data presented as mean±SD. a indicates significantly different than Baseline in both groups. b indicates significantly different than PECO in both groups. c indicates significantly different than Recovery in both groups.

During leg metaboreflex activation, there was no effect of OC use or time on the average contralateral brachial artery diameter (all p>0.10). OC had higher brachial blood velocity and flow than NOC, regardless of time (p<0.030; Table 6). There was also a main effect of time for brachial blood velocity (p=0.024; Table 6); however, post-hoc analysis revealed that there were no differences between any timepoints (p>0.05). In all women, Qi was higher during plantarflexion compared to baseline, leg PECO and recovery (all p<0.001), and Qi was elevated during leg PECO compared to baseline and recovery in OC and NOC (both p<0.005; Table 6). In all women, SVi and TPRi were lower during plantarflexion compared to baseline, leg PECO and recovery (all p<0.035), and DBP and SBP were higher during plantarflexion and leg PECO compared to baseline and recovery (all p<0.001; Table 6). Additionally, DBP was also higher during plantarflexion compared to leg PECO (p=0.044; Table 6).

In all women, breathing rate was higher during plantarflexion compared to baseline, leg PECO, and recovery (all p<0.005; Table 6). $ETCO_2$ was lower during leg PECO compared to baseline (p=0.013), while ETO_2 was higher during plantarflexion and leg PECO compared to baseline in both OC and NOC (both p<0.005; Table 6). During leg metaboreflex, there was no main effect of time or OC use, nor any interaction for Vt in OC and NOC (all p>0.10; Table 6).

Table 6. The influence of OC on the cardiorespiratory response to metaboreflex activation in the **leg**.

	Leg Metaboreflex										
	OC				NOC				P-value		
	Baseline	Exercise	PECO	Recovery	Baseline	Exercise	PECO	Recovery	OC	Time	OC X Time
Brachial artery diameter (cm)	0.31±0.04	0.31±0.03	0.31±0.03	0.32±0.03	0.29±0.04	0.30±0.04	0.30±0.04	0.30±0.04	0.252	0.197	0.612
Brachial blood velocity (cm/s)	8.3±3.0 ^{a}	9.0±4.4 ^{a}	9.6±5.2 ^{a}	8.3±3.8 ^{a}	6.1±1.4	6.7±2.2	6.1±1.9	5.7±1.3	**0.020**	**0.024**	0.188
Brachial blood flow (L/min/m^2)	0.63±0.27 ^{a}	0.69±0.39 ^{a}	0.75±0.48 ^{a}	0.65±0.35 ^{a}	0.42±0.15	0.46±0.17	0.46±0.21	0.42±0.16	**0.027**	0.054	0.375
Qi (L/min/m^2)	6.6±0.7	7.3±0.7 ^{b,c,d}	6.9±0.7 ^{b,d}	6.7±0.7	6.3±1.1	7.2±1.1 ^{b,c,d}	6.6±1.1 ^{b,d}	6.2±0.9	0.295	**<0.001**	0.336
SVi (mL/m^2)	94±13	93±13 ^{c,d}	96±12	97±13	92±10	89±12 ^{c,d}	94±10	95±10	0.540	**<0.001**	0.664
TPRi (mmHg/L/min/m^2)	13.5±1.9	13.0±1.5 ^{b,c,d}	13.6±1.8	13.6±1.9	14.0±2.9	13.2±2.6 ^{b,c,d}	14.0±2.6	14.1±2.6	0.628	**<0.001**	0.867
DBP (mmHg)	76±11	81±12 ^{b,c,d}	80±11 ^{b,d}	77±10	73±9	80±10 ^{b,c,d}	77±8 ^{b,d}	73±8	0.430	**<0.001**	0.273
SBP (mmHg)	119±17	124±19 ^{b,d}	124±18 ^{b,d}	120±17	113±11	120±11 ^{b,d}	120±10 ^{b,d}	114±11	0.306	**<0.001**	0.447
Breathing rate (breaths/min)	18±4	21±6 ^{b,c,d}	18±4	18±4	19±4	22±8 ^{b,c,d}	19±4	19±3	0.671	**<0.001**	0.981
ETCO$_2$ (mmHg)	41±4	40±4	40±3 ^{b}	41±3	39±2	39±2	38±3 ^{b}	39±2	0.105	**0.015**	0.194
ETO$_2$ (mmHg)	113±6	117±5 ^{b}	115±6 ^{b}	114±6	115±7	117±7 ^{b}	118±7 ^{b}	117±6	0.346	**<0.001**	0.202
Vt (L)	0.68±0.21	0.73±0.27	0.68±0.18	0.67±0.13	0.59±0.19	0.62±0.22	0.63±0.19	0.60±0.16	0.191	0.369	0.733

All values are mean±SD. DBP, diastolic blood pressure; ETCO$_2$, end-tidal carbon dioxide; ETO$_2$, end-tidal oxygen; NOC, non-oral contraceptive; OC, oral contraceptive; PECO, post-exercise circulatory occlusion; Qi, cardiac index; SBP, systolic blood pressure; SVi, stroke volume index; TPRi, total peripheral resistance index; Vt, tidal volume**.** Significance is represented by bold text ($p<0.05$). a indicates significantly different than NOC. b indicates significantly different than Baseline. c indicates significantly different than PECO. d indicates significantly different than Recovery.

Arm Mechanoreflex

In both groups, HR, MAP and V_E increased during arm PM compared to baseline (all $p<0.005$; Figure 4A-C). OC users had higher brachial blood velocity and flow compared to NOC, regardless of time (both $p<0.030$; Table 7). In response to arm PM, both contralateral brachial artery diameter and TPRi decreased from baseline (both $p<0.040$), and Qi, DBP and SBP increased from baseline in all women (all $p<0.005$; Table 7). SVi was unaffected by time or OC use (all $p>0.10$; Table 7). In OC and NOC, breathing rate and ETO_2 increased (both $p<0.010$), while $ETCO_2$ decreased during arm PM compared to baseline ($p=0.004$; Table 7). There was no effect of time or OC use on Vt response to arm PM (all $p>0.55$; Table 7).

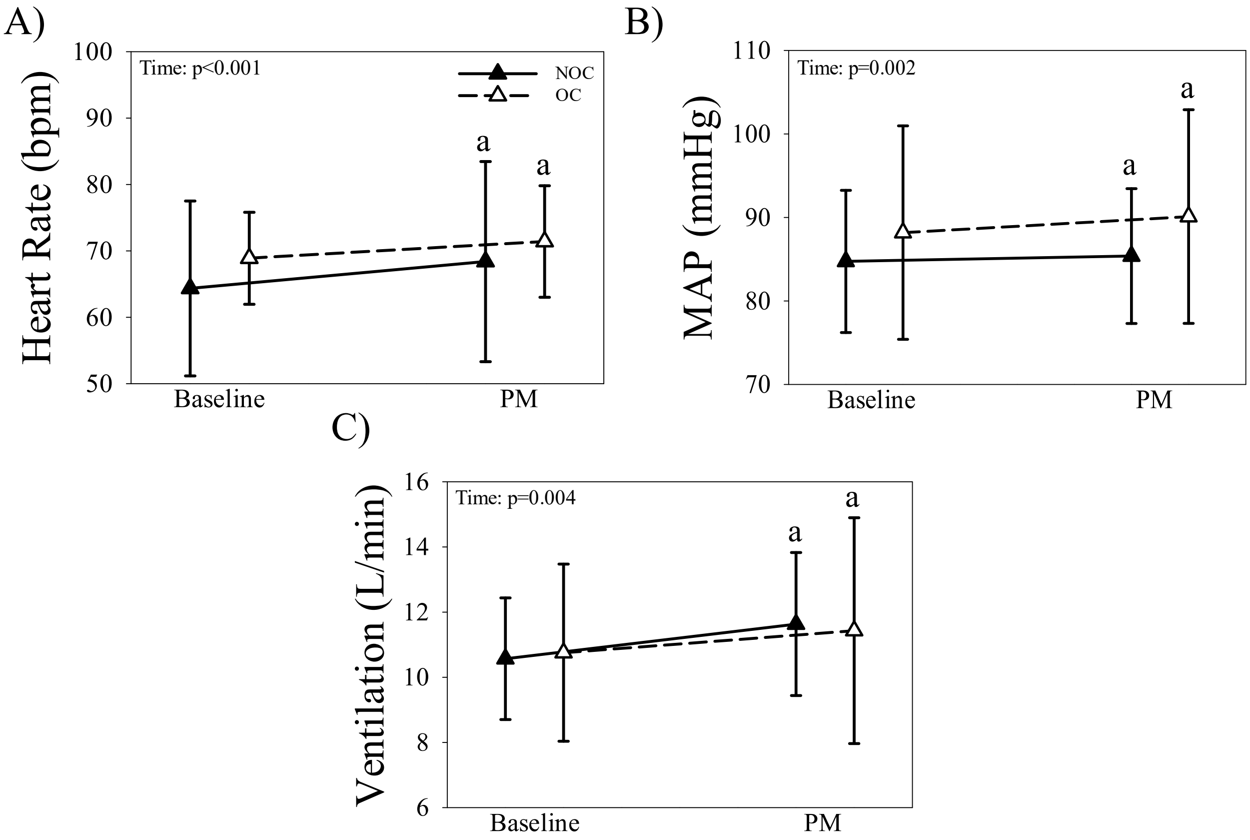

Figure 4. The heart rate (A), mean arterial pressure (MAP; B), and ventilation (C) responses to mechanoreflex activation (i.e., passive movement; PM) in the **arm** in OC (dashed line & open triangles) and NOC (solid line & closed triangles) females. Data presented as mean±SD. a indicates significantly different than Baseline within group.

Table 7. The influence of OC on the cardiorespiratory response to **arm** PM.

	Arm Mechanoreflex						
	OC		NOC		P-value		
	Baseline	PM	Baseline	PM	OC	Time	OC X Time
Brachial artery diameter (cm)	0.31±0.04	0.31±0.04 [b]	0.30±0.04	0.29±0.03 [b]	0.406	**0.016**	0.453
Brachial blood velocity (cm/s)	8.7±3.9 [a]	8.6±3.7 [a]	5.7±2.1	5.7±1.9	**0.012**	0.783	0.842
Brachial blood flow (L/min/m^2)	0.63±0.34 [a]	0.62±0.31 [a]	0.41±0.17	0.40±0.16	**0.024**	0.397	0.974
Qi (L/min/m^2)	6.5±0.6	6.7±0.7 [b]	5.9±1.5	6.2±1.5 [b]	0.243	**0.001**	0.755
SVi (mL/m^2)	95±14	95±14	92±15	91±14	0.524	0.135	0.235
TPRi (mmHg/L/min/m^2)	13.8±2.6	13.7±2.3 [b]	15.2±4.4	14.7±4.1 [b]	0.333	**0.038**	0.226
DBP (mmHg)	75±11	77±11 [b]	73±9	74±8 [b]	0.424	**<0.001**	0.133
SBP (mmHg)	117±16	120±17 [b]	112±10	113±11 [b]	0.212	**0.002**	0.101
Breathing rate (breaths/min)	19±3	21±4 [b]	19±2	21±4 [b]	0.892	**0.002**	0.533
ETCO_2 (mmHg)	41±4	40±4 [b]	39±3	39±2 [b]	0.127	**0.004**	0.751
ETO_2 (mmHg)	112±6	115±5 [b]	115±7	116±7 [b]	0.338	**0.009**	0.580
Vt (L)	0.59±0.17	0.58±0.19	0.57±0.10	0.57±0.16	0.826	0.562	0.665

All values are mean±SD. DBP, diastolic blood pressure; ETCO_2, end-tidal carbon dioxide; ETO_2, end-tidal oxygen; NOC, non-oral contraceptive; OC, oral contraceptive; PM, passive movement; Qi, cardiac index; SBP, systolic blood pressure; SVi, stroke volume index; TPRi, total peripheral resistance index; Vt, tidal volume. Significance is represented by bold text ($p<0.05$). a indicates significantly different than NOC. b indicates significantly different than Baseline.

Leg Mechanoreflex

HR (Figure 5A), MAP (Figure 5B) and V_E (Figure 5C) increased during leg PM in all women (all p<0.001). None of mean brachial artery diameter, blood velocity, blood flow, nor SVi were affected by OC use or time during leg mechanoreflex activation (all p>0.10; Table 8). Qi, DBP, and SBP increased from baseline during leg PM (all p<0.006), yet TPRi decreased in both OC and NOC (p=0.019; Table 8). In NOC only, breathing rate increased from baseline (p<0.001) and $ETCO_2$ decreased from baseline during leg PM in OC and NOC (p<0.001; Table 8). Vt and ETO_2 were unaffected by time or OC use (all p>0.10; Table 8).

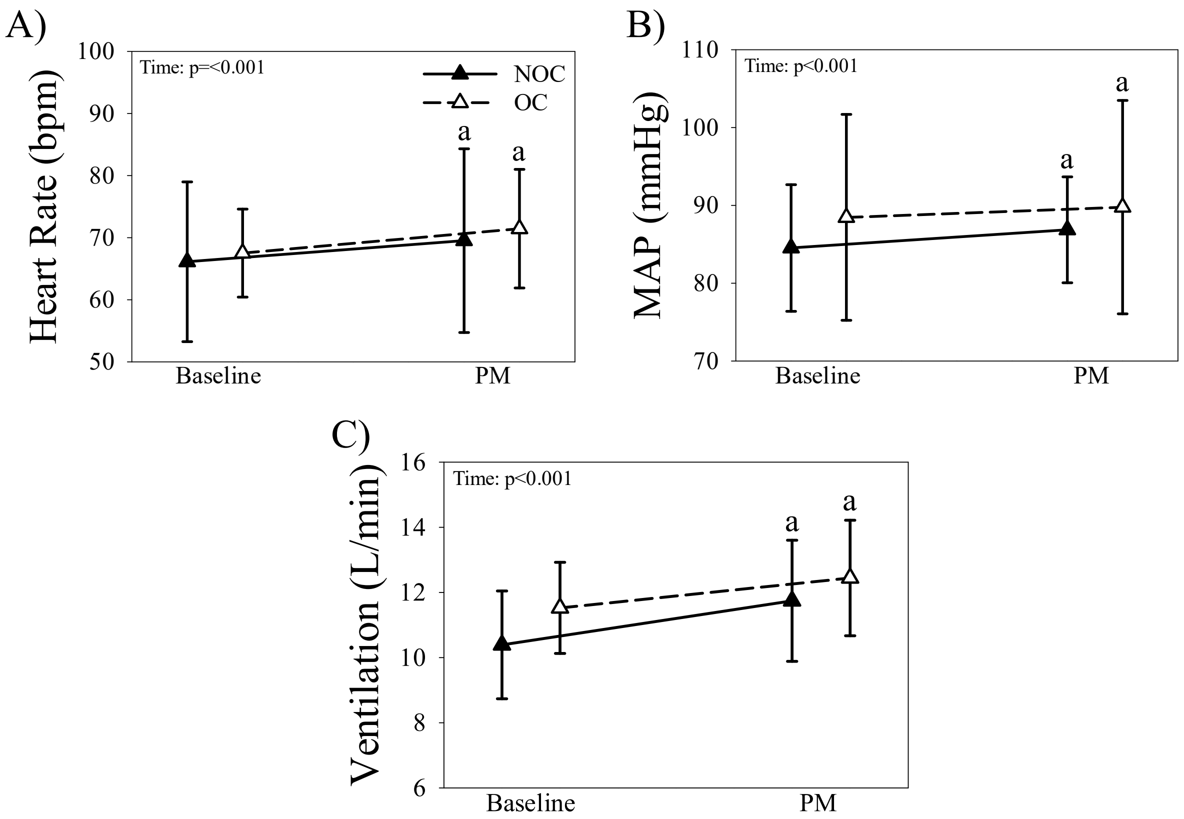

Figure 5. The heart rate (A), mean arterial pressure (MAP; B), and ventilation (C) responses to mechanoreflex activation (i.e., passive movement; PM) in the **leg** in OC (dashed line & open triangles) and NOC (solid line & closed triangles). Data presented as mean±SD. a indicates significantly different than Baseline within group.

Table 8. The influence of OC on the cardiorespiratory response to **leg** PM.

	Leg Mechanoreflex						
	OC		NOC		P-value		
	Baseline	PM	Baseline	PM	OC	Time	OC X Time
Brachial artery diameter (cm)	0.31±0.03	0.31±0.03	0.30±0.04	0.30±0.04	0.550	0.629	0.764
Brachial blood velocity (cm/s)	7.0±2.9	7.7±3.6	5.8±2.0	5.9±2.1	0.137	0.120	0.198
Brachial blood flow (L/min/m^2)	0.52±0.25	0.57±0.32	0.41±0.18	0.42±0.17	0.164	0.109	0.184
Qi (L/min/m^2)	6.3±0.7	6.7±0.8 [b]	6.0±1.0	6.2±1.1 [b]	0.209	**<0.001**	0.506
SVi (mL/m^2)	95±15	95±14	91±11	91±10	0.361	0.847	0.739
TPRi (mmHg/L/min/m^2)	14.1±2.3	13.5±2.4 [b]	14.6±3.5	14.4±3.4 [b]	0.500	**0.019**	0.277
DBP (mmHg)	75±11	76±11 [b]	72±8	75±7 [b]	0.497	**<0.001**	0.188
SBP (mmHg)	118±20	119±21 [b]	112±9	115±8 [b]	0.397	**0.005**	0.306
Breathing rate (breaths/min)	19±3	20±4	18±4	21±4 [b]	0.934	**<0.001**	**0.016**
ETCO$_2$ (mmHg)	41±3	40±3 [b]	39±2	38±2 [b]	0.111	**<0.001**	0.845
ETO$_2$ (mmHg)	116±12	116±5	115±6	118±6	0.851	0.235	0.229
Vt (L)	0.62±0.07	0.65±0.16	0.58±0.12	0.57±0.11	0.172	0.545	0.136

All values are mean±SD. DBP, diastolic blood pressure; ETCO$_2$, end-tidal carbon dioxide; ETO$_2$, end-tidal oxygen; NOC, non-oral contraceptive; OC, oral contraceptive; PM, passive movement; Qi, cardiac index; SBP, systolic blood pressure; SVi, stroke volume index; TPRi, total peripheral resistance index; Vt, tidal volume. Significance is represented by bold text ($p<0.05$). a indicates significantly different than Baseline.

Heart Rate Variability during PECO and PM

During arm metaboreflex activation, SDRR increased in response to arm PECO in both OC and NOC (p=0.021), yet there was no effect of time or group on RMSSD, pRR50, LF power, HF power and LF/HF ratio (all p>0.05; Table 9 - Supplemental). Compared to NOC, SDRR tended to be lower in OC during leg metaboreflex activation irrespective of time (p=0.052; Table 9 - Supplemental). In all women, during leg PECO, RMSSD and pRR50 decreased (p=0.024 and p=0.002, respectively), whereas HF power, LF power and LF/HF ratio were unaffected by OC or leg PECO (all p>0.10; Table 9 - Supplemental).

Table 9 - Supplemental. The influence of OC on the HRV response to arm or leg metaboreflex activation.

	PECO													
	Arm							Leg						
	OC		NOC		P-value			OC		NOC		P-value		
	Baseline	PECO	Baseline	PECO	OC	Time	OC X Time	Baseline	PECO	Baseline	PECO	OC	Time	OC X Time
SDRR (ms)	71±40	83±45[a]	99±42	113±53[a]	0.070	**0.021**	0.823	72±42	63±35	95±26	89±40	0.052	0.133	0.801
RMSSD (ms)	83±61	90±72	126±61	271±569	0.157	0.270	0.314	83±60	69±62[a]	119±46	104±66[a]	0.085	**0.024**	0.965
pRR50 (%)	49±26	44±30	61±22	59±23	0.136	0.199	0.462	49±20	37±30[a]	62±22	52±24[a]	0.088	**0.002**	0.665
LF (nu)	32±24	36±25	19±10	25±17	0.061	0.191	0.925	27±20	31±19	22±14	22±11	0.159	0.479	0.557
HF (nu)	67±22	62±24	77±9	73±16	0.088	0.180	0.942	72±19	68±18	75±13	75±11	0.251	0.466	0.568
LF/HF ratio	0.97±1.97	1.00±1.29	0.27±0.17	0.42±0.44	0.060	0.763	0.824	0.86±2.14	0.57±0.47	0.33±0.30	0.32±0.21	0.215	0.540	0.579

All values are mean±SD. HF, high frequency; HRV, heart rate variability; LF, low frequency; PECO, post-exercise circulatory occlusion; pRR50, proportion of RR interval differences greater than 50 ms; RMSSD, root mean square standard deviation; SDRR, standard deviation of RR intervals. a indicates significantly different than Baseline.

Regardless of time, SDRR was significantly lower ($p=0.040$) and RMSSD tended to be lower in OC compared to NOC during arm mechanoreflex activation ($p=0.052$; Table 10 - Supplemental). SDRR, RMSSD, and pRR50 all decreased during arm PM compared to baseline in OC and NOC (all $p<0.030$), yet neither OC use nor arm PM affected HF power, LF power, or LF/HF ratio (all $p>0.09$; Table 10 - Supplemental). During leg PM, SDRR, RMSSD, pRR50, and HF power decreased in both OC and NOC (all $p<0.005$; Table 10 - Supplemental). In contrast, LF power increased during leg mechanoreflex activation in all women ($p=0.003$; Table 10 - Supplemental). There was no effect of leg PM or OC use for LF/HF ratio (all $p>0.10$; Table 10 - Supplemental).

Table 10 – Supplemental. The influence of OC on HRV response to arm or leg passive movement (PM).

	Passive Movement													
	Arm							Leg						
	OC		NOC		P-value			OC		NOC		P-value		
	Baseline	PM	Baseline	PM	OC	Time	OC X Time	Baseline	PM	Baseline	PM	OC	Time	OC X Time
SDRR (ms)	70±34^{a}	63±30^{a,b}	96±38	87±38^{b}	**0.040**	**0.029**	0.766	77±43	70±35^{b}	92±36	78±28^{b}	0.349	**<0.001**	0.219
RMSSD (ms)	84±50	76±52^{b}	123±61	113±55^{b}	0.052	**0.022**	0.719	89±64	80±62^{b}	120±57	99±51^{b}	0.240	**<0.001**	0.076
pRR50 (%)	51±24	45±25^{b}	63±23	59±24^{b}	0.125	**0.029**	0.805	51±24	45±26^{b}	63±23	55±24^{b}	0.183	**0.002**	0.684
LF (nu)	24±18	27±17	19±12	21±11	0.228	0.345	0.807	23±19	34±20^{b}	18±12	23±13^{b}	0.134	**0.003**	0.254
HF (nu)	75±17	70±20	79±12	76±9	0.249	0.193	0.642	76±19	65±19^{b}	79±12	73±13^{b}	0.275	**0.004**	0.377
LF/HF ratio	0.44±0.64	0.52±0.50	0.27±0.22	0.30±0.17	0.091	0.611	0.785	0.60±1.36	0.69±0.62	0.26±0.26	0.35±0.24	0.132	0.598	0.988

All values are mean±SD. HF, high frequency; HRV, heart rate variability; LF, low frequency; PM, passive movement; pRR50, proportion of RR interval differences greater than 50 ms; RMSSD, root mean square standard deviation; SDRR, standard deviation of RR intervals. a indicates significantly different than NOC. b indicates significantly different than Baseline.

Influence of Muscle Strength and Size

During arm PECO, the ΔV_E failed the equal slopes assumption; thus, linear regressions were calculated in individual groups. In NOC, neither arm volume (R^2=0.243, F(1, 12)=3.849, p=0.07) nor handgrip strength (R^2=0.252, F(1, 13)=4.384, p=0.06) influenced ΔV_E during arm metaboreflex activation. During arm PECO in OC, neither arm volume (R^2=0.002, F(1, 13)=0.022, p=0.88) nor handgrip strength (R^2=0.008, F(1, 14)=0.117, p=0.734) influenced ΔV_E. The ANCOVA model which adjusted for muscle volume or strength was able to account for 12-41% of the variability (R^2) in the pressor responses to arm or leg metabo-and mechanoreflex activation; however, the model was less able to account for the variability (3-26%) for the ventilatory response to leg PECO and arm or leg PM (Table 11 - Supplemental). Handgrip strength significantly influenced ΔMAP arm PECO response (p<0.001; large effect ηp^2>0.14; Table 11 - Supplemental). After adjustment, OC had an enhanced pressor response to arm PECO than NOC (p=0.038; large effect ηp^2>0.14; Table 11 - Supplemental). During leg metaboreflex activation, plantarflexion strength significantly influenced ΔMAP to leg PECO (p=0.006; large effect ηp^2>0.14), yet there still were no differences between OC and NOC post-adjustment (p>0.1; Table 11 - Supplemental). During leg PM, plantarflexion strength significantly influenced ΔV_E (p=0.045; large effect ηp^2>0.14); however, this did not translate into any differences between either group after adjusting for the covariates (p>0.9; Table 11 - Supplemental). There was no effect of the main factor of OC use, nor an effect of the covariates muscle strength or volume on ΔMAP during arm or leg PM (all p>0.1) or ΔV_E during leg PECO or arm PM (all p>0.2; Table 11 - Supplemental).

Table 11 – Supplemental. Cardiorespiratory changes from baseline to arm or leg PECO and PM considering covariate analysis adjusting for the influence of muscle volume or strength.

				ANCOVA						
				Analysis of Variance for Equal Slopes Model						
		Unadjusted Means±SD		Model Fit		Adjusted Means ± Std. Err.		p-value (ηp^2)		
		OC	NOC	R^2	Adj. R^2	OC	NOC	OC use	Muscle Volume	Muscle Strength
Arm PECO	ΔMAP (mmHg)	10±8	8±8	0.406	0.334	12±2 *	6±2	**0.038** (0.16)	0.070 (0.13)	**<0.001** (0.37)
	ΔV_E (L/min)							*Assumptions for Equal Slopes Model not met*		
Leg PECO	ΔMAP (mmHg)	4±6	5±5	0.277	0.191	4±1	6±1	0.487 (0.02)	0.409 (0.03)	**0.006** (0.26)
	ΔV_E (L/min)	-0.009±0.7	0.6±1.3	0.161	0.0638	0.05±0.3	0.5±0.3	0.221 (0.06)	0.313 (0.04)	0.273 (0.05)
Arm PM	ΔMAP (mmHg)	2±2	1±2	0.171	0.0711	2±1	1±1	0.139 (0.09)	0.210 (0.06)	0.768 (0.004)
	ΔV_E (L/min)	0.6±1.7	1.0±1.6	0.0348	-0.0765	0.6±0.5	1.0±0.5	0.599 (0.01)	0.563 (0.01)	0.719 (0.005)
Leg PM	ΔMAP (mmHg)	1±2	2±3	0.116	0.0143	1±1	2±1	0.276 (0.05)	0.549 (0.01)	0.164 (0.07)
	ΔV_E (L/min)	1.1±0.9	1.2±1.1	0.262	0.170	1.2±0.3	1.1±0.2	0.949 (0.0002)	0.051 (0.15)	**0.045** (0.16)

ADJ, adjusted; ANCOVA, analysis of covariance; ANOVA, analysis of variance; MAP, mean arterial pressure; NOC, non-OC users; OC, oral contraceptives; V_E, ventilation; Raw data is presented as mean±SD and adjusted means are presented as mean±SE. Bolded text represents a significant difference ($p<0.05$). * indicates significantly different than NOC.

3.6 **Discussion**

The cardiorespiratory responses to exercise during metabo- or mechanoreflex activation were similar when comparing OC and NOC. Interestingly, neither group increased V_E during arm or leg PECO while exhibiting similar pressor responses. Considering that exercise and PM increases V_E, the above suggests that women may more heavily rely on afferent signaling from mechanoreflex activation during exercise to drive V_E, which may be due to some unmet theoretical metabolite threshold necessary to stimulate V_E or sexually dimorphic reflex control. Lastly, covariate analysis demonstrated that muscle strength may have masked some differences between OC and NOC in the pressor response during arm or leg PECO, but not arm or leg PM. Both muscle volume and strength were able to account for some of the variability in the current cohort.

Contrary to our hypothesis, we did not observe any differences between OC and NOC in the ventilatory or pressor responses to metaboreflex activation. Previously, Joshi & Edgell[173] observed that women do not increase V_E during arm PECO compared to men, which the authors postulated was potentially due to women having smaller muscles leading to reduced metabolite accumulation. Considering that the current study also investigated the ventilatory response to metaboreflex activation in the leg (i.e., a larger muscle mass) and we did not observe an increased V_E during leg PECO, this may suggest that all women may exhibit a blunted respiratory response to metaboreflex activation, regardless of OC use. Further, since it is well-established that women increase V_E during exercise, the mechanoreflex may be primarily responsible for providing sufficient input to stimulate V_E in women. Previous research observed that OC users had an increased ventilatory response to arm metaboreflex activation[72], yet we did not observe that in the current study. Unpublished data from Assadpour et al.[72] suggests that their cohort was evenly matched for forearm strength (OC: 216±59 N vs. NOC: 221±58 N; p=0.9) whereas our NOC group was stronger in the current study; therefore, any potential influences of OC on the ventilatory response to metaboreflex activation may have been confounded by disparities in muscle strength. In the present study, the covariate analysis demonstrated that there is indeed some influence of handgrip strength on the variation in the ventilatory response to metaboreflex activation.

Unexpectedly, OC did not display a greater pressor response to metaboreflex activation as observed in previous literature[82,125,129]. Cuff placement may have been responsible for this disparity, as the aforementioned studies used upper arm PECO rather than forearm PECO, which could have potentially captured a greater number of metabolites and thus, increased the pressor response to a greater degree. Since the NOC group were stronger in the current study, NOC could have potentially generated a greater concentration of metabolites confounding our results. Previous research comparing sexes suggests that the pressor response to arm metaboreflex activation is related to muscle strength[162,163] or muscle size[163]. Indeed, OC users had a greater pressor response to PECO in the arm after adjusting for muscular strength – yet a lack of difference between OC and NOC in V_E remained after controlling for these factors.

Our observation that women do not increase ventilation during metaboreflex activation is intriguing. There have been multiple studies showing interactions between the chemoreflexes and the metaboreflex[59,60,174-181]. Of those studies, most researchers investigated the reflex interactions in men only[174,176,177,179] or in mixed sex groups[59,60,178,180,181]. There was only a single study that investigated sex differences[175] which observed no sex differences in the ventilatory response to hypoxic PECO; however, neither menstrual cycle nor hormonal contraceptives were controlled for in the female group. It was previously observed that the CO_2 chemoreflex does not differ between OC and NOC[72], yet to our knowledge it is yet unknown if OC use influences the hypoxic chemoreflex. Van Klaveren and Demedts[182] suggest that individuals with similar lung size should have similar V_E response to hypoxia, therefore we hypothesize that OC use will not influence the peripheral chemorflex. Further, we suggest that there is no influence of OC use on the interactions between the metaboreflex and chemoreflexes since both groups in the current study had similar changes in $ETCO_2$ and ETO_2 with similar cardiovascular responses during PECO.

Our lab previously observed that OC users have reduced blood pressure responses to leg PM compared to NOC[72]. Considering that Limberg and colleagues[81] observed an enhanced NO-mediated vasodilatory capacity in OC compared to NOC and that the local hyperaemic response to leg PM is NO-dependant[149], this could contribute to the previously observed reduced pressor response to leg PM in OC compared to NOC. In the current study, there were no differences between OC and NOC in the absolute pressor responses during arm or leg PM. Covariate analysis demonstrated that neither leg volume nor strength influenced the pressor responses to arm or leg PM in the current study; however, differences in OC formulations could have contributed to the lack of observed differences. The current study included some participants who used lower hormonal dosage OC compared to Assadpour et al.[72], which may have exposed the current OC users to a smaller estrogenic dosage. Administering EE alone increases endothelium-dependant vasodilation in young healthy women[183]; although, specific progestin types, such as levonorgestrel[184] and desogestrel[183], in combined OC antagonizes these vasodilatory effects. Both studies had similar numbers of progestin types included, yet the current study included lower doses of estrogen and progesterone; therefore, the lack of observed differences in the pressor response to PM may be due to less exogenous hormone exposure.

As an indicator of sympathetic outflow, we measured brachial artery diameter and flow of the non-exercising arm throughout each protocol. Interestingly, we only noted a slight reduction of diameter during arm PM, potentially indicating increased sympathetic outflow. However, this did not translate to a change in flow. We observed increased brachial flow during arm exercise, likely a result of greater driving pressure, yet this was not observed during leg exercise or limb PM. Previous observations have shown that after infusion with a NO synthase inhibitor, the hyperemic response of the inactive upper limb was significantly diminished at low-moderate intensities (60 & 80 watts) of leg cycling; however, this non-active hyperemic response remained intact during contralateral handgrip exercise[185]. Additionally, Thijssen et al.[186] demonstrated that varying modalities of lower limb exercise (i.e., cycling, leg kicking, and walking) induced altered patterns of blood flow in the non-active upper limb. Taken together, this may suggest that lower limb plantarflexion may have not been a sufficient shear stress to increase contralateral non-active brachial flow.

At odds with our initial hypothesis, OC users did not display a greater pressor response nor did their brachial diameters reflect changes in SVT. There may be a balance between enhanced vasodilatory capacity[81] and the hypertensive effects of OC[187] that contribute to the lack of observed differences. However, OC users had higher brachial velocity and flow compared to NOC at all timepoints, potentially indicating a predominance of the vasodilatory effect of OC or anatomical differences. Alternatively, it is theoretically possible that the increased brachial blood flow observed in OC users could be due to a greater proportion of type I skeletal muscle fibres which can create more vasoactive metabolites and could be evidenced by the lower strength of the OC women.

The parasympathetic influence (i.e., SDRR) on cardiovascular control increased during arm PECO in both groups, yet the opposite was true for leg PECO, where parasympathetic control (i.e., RMSSD and pRR50) decreased during leg PECO in all women. A concurrent lack of change in sympathetic activity indices may indicate that the cardiac changes to isolated metaboreflex activation are mitigated by parasympathetic withdrawal in women. In support of this, previous research has also demonstrated that HRV increases during arm PECO even when controlling for tidal V_E and breathing rate[188]. We suggest that PECO of a smaller muscle mass (i.e., arm) may activate both vagal activity[189] and MSNA[190], but this vagal activity may be suppressed in the face of a larger muscle mass (i.e., leg)[90] and potentially greater MSNA[191]. In all women, parasympathetic control decreased in both limbs during PM (i.e., SDRR, RMSSD and pRR50); however, LF power (i.e. an index of combined sympathetic and parasympathetic influence on HR[192], or BRS[193]) only increased during leg PM in OC and NOC. Vianna et al.[164] observed a greater reduction in RR interval when more limbs or a larger muscle mass was involved in passive cycling. Larger muscle volume may mediate the observed increase in sympathetic control of HR during leg PM in both OC and NOC, while smaller muscles (i.e., arm) may rely more on parasympathetic withdrawal.

Limitations

A limitation of the present study is that the type (mono- vs. triphasic), hormonal dosage, or generation of OC (i.e., progestin type) were not controlled for; although at least half of OC users were on a similar monophasic OC with identical progestin and EE doses (i.e., Alesse and Alysena). There is limited data investigating the effects of varying dose over the pill cycle (mono- vs. bi- or triphasic OC) on autonomic, or respiratory function; however, Harvey and colleagues[115] did not observe any differences between mono-, bi- or triphasic OC in resting MAP or MSNA. Previous research has demonstrated that second-generation OC reduces endothelial function as observed via decreased flow-mediated dilation (FMD)[194-196], yet a fourth-generation OC had no influence on FMD[197]. Given that the FMD response is at least partly mediated by NO[198], this may suggest that varying OC generations may have different effects on NO-dependant vasodilation. This could influence the pressor response and may have contributed to some of the variation in the current cohort of OC users. Indeed, OC users had almost double the standard deviation in MAP at each timepoint during arm metaboreflex activation and both arm and leg mechanoreflex activation. Future research should consider comparing the pressor response to metabo- or mechanoreflex activation across pill generations or more tightly controlling for a single type of OC.

It is also important to note that other routes of synthetic hormone administration, such as patches, implants, or intrauterine devices, may have divergent physiological responses to exercise pressor reflex activation or effects on vascular function; thus, this study cannot be extended to other hormonal contraceptives. Furthermore, this study was conducted in relatively healthy young women and should not be extended to older, obese, or diseased populations, who may have additional influences exacerbating autonomic dysfunction.

3.7 **Conclusion**

In conclusion, there was no influence of OC use on the cardiovascular or ventilatory response to upper or lower body PECO or PM. The lack of observed differences between OC and NOC may be due to potential differences in strength, considering that OC users had weaker forearms and most previous research was conducted in cohorts that did not differ in strength. Neither OC nor NOC exhibited an increased ventilatory response during arm or leg metaboreflex activation – suggesting that metaboreflex activation may not stimulate breathing in women or that women may not meet a theoretical metabolite accumulation threshold necessary to stimulate V_E. All women had similar increased pressor and ventilatory responses to arm and leg mechanoreflex activation, suggesting that there are no limb-specific differences. Taken together, our results suggest that the mechanoreflex may be primarily responsible for initiating a ventilatory response in women during exercise or that concurrently activating the mechanoreflex provides additional input required to increase V_E during dynamic exercise.

Chapter 4: **Menstrual cycle and oral contraceptives influence cerebrovascular dynamics during hypercapnia**

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4.1 **Abstract**

Women experience fluctuating orthostatic intolerance during the menstrual cycle, suggesting sex hormones may influence CBF. Young (aged 18-30) healthy women, either taking OC (n=14) or not taking OC (NOC; n=12), were administered hypercapnic gas (5%) for 5 min in the LH (i.e., placebo pill) and HH (i.e., active pill) menstrual phases. Hemodynamic and cerebrovascular variables were continuously measured. Cerebral blood velocity changes were monitored using transcranial doppler ultrasound of the MCA to determine cerebrovascular reactivity. CA was assessed using steady-state analysis (static CA) and transfer function analysis (dCA). In response to hypercapnia, menstrual phase did not influence static cardiovascular or cerebrovascular responses (all $p>0.07$); however, OC users had a greater increase of mean MCA blood velocity compared to NOC (NOC– LH 12±6cm/s vs. HH 16±9cm/s; OC – LH 18±5cm/s vs. HH 17±11 cm/s; $p=0.048$). In all women, hypercapnia improved HF and very LF (VLF) CA (decreased normalized Gain (nGain); $p=0.002$ & $p<0.001$, respectively), whereas LF Phase decreased in the NOC HH phase ($p=0.001$) and the OC LH phase ($p<0.001$). Therefore, endogenous sex hormones impair LF dCA during hypercapnia in the HH phase. In contrast, pharmaceutical sex hormones (OC use) have no acute influence (HH phase) yet elicit a chronic attenuation of LF dCA (LH phase) during hypercapnia.

Keywords: brain blood velocity, cerebral autoregulation, hypercapnia, oral contraceptives, transcranial doppler

4.2 **New & Noteworthy**

We observed influences of both endogenous and exogenous hormones on the cerebrovascular response to hypercapnia. During hypercapnia, all women exhibit enhanced HF and VLF autoregulation, while naturally cycling women have reduced LF autoregulation in the HH phase of the menstrual cycle, yet this is observed in the LH phase of oral contraceptive use. This study highlights the influence of natural and synthetic hormones of CBF.

4.3 **Introduction**

CA is the process by which the cerebrovasculature responds to changes in perfusion pressure to ensure that CBF is maintained, in order to meet the high metabolic rate and waste production of the brain[67]. CA can be assessed statically or dynamically; static CA considers changes in pressure and flow over a longer time period, while dCA involves immediate changes[68,199]. There are four proposed mechanisms through which CBF is controlled; changes in vasomotor tone in response to pressure, activation of neural pathways to manipulate vasomotor tone, increased concentrations of metabolic by-products (H+, pCO_2, etc.), and the release of endothelial factors such as NO[200]. The cerebrovasculature is highly responsive to arterial CO_2 levels, which means that vasodilation will occur during hypercapnia and vasoconstriction will occur during hypocapnia to exponentially alter CBF[69,201-203].

In healthy women, estrogen has been associated with reductions in resting cerebrovascular resistance during the late follicular menstrual cycle phase (i.e., high level of estrogen, no progesterone) compared to the early follicular phase[85]. However, Hazlett and Edgell[88] observed no menstrual phase effects on the cerebrovascular response to CO_2 when comparing the early follicular and luteal (i.e. high estrogen and progesterone) phases, suggesting that progesterone may counteract the effect of estrogen on the CO_2 response. Notably, neither Peltonen et al.[89] nor Hazlett and Edgell[88] included women taking OC and did not concurrently investigate indices of dCA. OC users have been shown to have lower resting levels of $ETCO_2$[72,76], which could have an influence on CBF parameters. Additionally, Abidi et al.[76] observed that OC users did not concurrently increase cerebrovascular resistance in response to the observed hypocapnia. These results imply that OC users may have impaired cerebrovascular responsiveness to CO_2 in response to hypocapnia. Further, OC use is also associated with lower MAP and cerebral perfusion pressure during the active dose of the pill, suggesting that the use of exogenous cycling hormones may promote chronic peripheral vasodilation leading to lower BP[76]. Indeed, the active dose of OC has been associated with increased lower limb blood flow[204], reduced sympathetic BRS[204], and attenuated chemoreflex function[78]. Reduced sympathetic activity may impair dCA as evidenced by an increased magnitude of a change in CBF driven by BP (i.e., Gain) during sympathetic blockade[205].

This study aims to compare the effects of fluctuating endogenous and exogenous sex hormones on cerebrovascular CO_2 reactivity and dCA. Abidi et al.[76] observed a trend (p=0.065) for greater cerebrovascular resistance with similar MAP in the HH phase of the menstrual cycle. Therefore, we similarly hypothesized that women in the HH menstrual phase would have greater cerebrovascular resistance compared to women in the LH menstrual phase. In the presence of endogenous hormones, women are twice as likely to experience syncope than men, and syncope becomes more prevalent during puberty[95], suggesting that impaired dCA may be associated with the presence of sex hormones. Therefore, we hypothesize that in the presence of sex hormones in the HH menstrual phase, dCA will be impaired. Since OC users have lower $ETCO_2$[72,76] and no change in the cerebrovascular resistance response[76], we hypothesize that OC users would have enhanced CO_2 reactivity compared to non-users. Lastly, improved dCA has been observed during the second half of pregnancy, compared to non-pregnant individuals (i.e., sex hormone concentrations observed during pregnancy are greater than across a menstrual cycle)[96,97]. While pregnancy is also associated with severe hemodynamic changes, it represents a state of heightened levels of sex hormones and previous research has shown that OC use has similar levels of sex hormone-binding globulin which is reflective of total circulating sex hormone levels[98]. Taken together, this leads to our hypothesis that concentrations of sex hormones higher than typically seen through a natural cycle, such as those in OC active pills, may improve dCA.

4.4 **Methods**

Participants

All participants in this study concurrently participated in Assadpour et al.[72]. Written informed consent was obtained prior to participation, which was approved by the Office of Research Ethics at York University. Young, healthy women (n=26) between 18-30 years of age with a regular menstrual cycle (cycle length: 26-30 days) were recruited. Exclusion criteria included a history of hormonal or cardiovascular conditions and the inability to refrain from caffeine, fatty foods, or heavy exercise for a minimum of 12h prior to testing. We did not confirm chronic smoking status of the participants; however, individuals who could not abstain from smoking 12 hours prior to the testing were excluded from participation.

Women were separated into two groups, women not taking OC (NOC, n=12) and women taking OC (OC, n=14). OC participants reported using monophasic pills including Cyclen 28 (n=3), Yaz (n=2), Alesse (n=3), Alysena (n=4), and Marvelon (n=2). Transcranial doppler ultrasound (TCD) measurements were unsuccessful in two participants (NOC=1; OC=1, Cyclen

28); therefore, those participants were removed from the analysis. Additionally, not all participants met the coherence threshold (0.63) for suitable TFA and thus, fewer participants were included in the analysis of dCA (see Table 14 for n values). Testing occurred during days 2-5 and 18-24 of the menstrual cycle for NOC participants (LH and HH phases, respectively), while OC participants were tested during the placebo week (LH phase) and the final OC pill week (HH phase). Self-report was used to determine menstrual cycle phases. Progesterone production in the HH phase in NOC, and thus ovulation, was confirmed using a urine progesterone test (>5µg/mL; Progesterone (PDG) urine test, Easy@Home). Testing was randomized to reduce any ordering effects between LH or HH testing sessions, and researchers were not blinded to OC use, nor which phase was being tested.

Anthropometrics (height, body mass) were measured using a standard stadiometer and used to calculate BMI as body mass/ height^2. Information about anthropometrics and self-reported physical activity were used to obtain an index of maximal oxygen consumption (VO_2 max; mL·kg-1·min-1), calculated with the Ainsworth equation[171].

Hypercapnia Protocol

Participants laid supine for 5 min while breathing room air to establish a baseline. After baseline, participants started breathing a hypercapnic gas mixture (5% CO_2, 21% O_2, balance nitrogen) for 5 min, administered via a free-breathing Douglas bag system with no set flow rate.

Measurements

All measurements were conducted in the supine posture, and data was acquired at a rate of 1000Hz through PowerLab (16/35, ADInstruments, Colorado Springs, USA) and LabChart Pro (Version 8.1.9, ADinstruments, Colorado, USA) software. HR was continuously measured using a single-lead electrocardiogram (BioAMP, ADInstruments, Colorado Springs, USA). BP was determined by beat-to-beat finger plethysmography on the left middle finger (BMEye Nexfin, Amsterdam, NL) and was calibrated to an automated measurement using LabChart (BP Tru, BPM 200, Coquitlam, CA). $ETCO_2$ was measured using a CO_2 gas analyzer (Model 17630, Vacumed, Ventura, USA).

Right MCA blood velocity (MCA_V) was measured using a TCD system (Multigon Industries Inc., Yonkers, USA). A 2-MHz TCD probe was placed on the temporal window above the zygomatic arch and held by an adjustable headband (Marc600 Headframe, Spencer Industries, Redmond, USA). MCA_V is described as mean (MCA_{Vmean}), MCA_{Vmin} and systolic (MCA_{Vmax}). The ratio of the change in MCA_V to the change in $ETCO_2$ was calculated. Cerebrovascular resistance index (CVRi) was calculated as MAP divided by MCA_{Vmean}. Resistance index (RI) was calculated as the difference between MCA_{Vmax} and MCA_{Vmin}, divided by MCA_{Vmax}. PI was calculated as the difference between MCA_{Vmax} and MCA_{Vmin}, divided by the MCA_{Vmean}. Resistance area product (RAP) was calculated as the difference between MAP and DBP divided by the difference between MCA_{Vmean} and MCA_{Vmin}. Critical closing pressure (CrCP) was calculated as MAP minus the product of RAP and MCA_{Vmean}.

Transfer Function Analysis (TFA) was used to quantify dCA, according to the Claassen et al.[206] white paper. Briefly, MCA_V and BP were continuously obtained throughout each cardiac cycle, then were resampled at 4 Hz using Ensemble- R software (Ensemble-R, Elucimed Ltd., New Zealand). The data set was divided into 4 windows, which were passed through a Hann (cosine-bell) data window with an overlap of 50%. A fast Fourier transform was used to transform the signals into frequencies, which are binned into specific bands: HF (0.2-0.5 Hz), LF (0.07-0.2 Hz), and VLF (0.02-0.07 Hz). Spectral analysis provides power spectra for MCA_{Vmean} and cerebral perfusion pressure (MAP_{MCAv}), representing the magnitude of influence of similar frequency components on each variable. Cross-spectral analysis is applied to the power of MCA_{Vmean} and MAP_{MCAv} to create the transfer function, which describes how pressure fluctuations translate to changes in blood velocity via the amplitude (Gain) and timing (Phase) of the relationship. Gain can be normalized (nGain) by expressing velocity as a percentage of the mean signal to account for between-group differences. A large Gain implies changes in pressure lead to greater fluctuations in velocity, and a smaller Phase implies that pressure fluctuations are more quickly transmitted to velocity, indicating poor dCA[206]. Coherence (ranges from 0-1), an additional parameter calculated by TFA, describes the reliability of the relationship between Phase and Gain. Each variable of our dCA assessments (MCA_{Vmean} power, MAP_{MCAv} power, Gain, nGain, Phase and Coherence) are determined across each frequency band; HF, LF and VLF. Ensemble-R software quantifies parameters that meet a threshold of linearity based on a 95% confidence interval (default coherence threshold: 0.63). Phase wrap-around was present in the HF and LF bands, and those values were removed from analysis.

Data Analysis

For steady-state assessments, 1 min averages were determined to compare baseline to hypercapnia, and only the last minute of each was used for statistical analysis. Changes were calculated by subtracting the baseline values from the last minute of hypercapnia administration. Static participant characteristics (i.e., age and height) were compared between OC and NOC using independent t-tests, while other characteristics (i.e., body mass, BMI, exercise frequency and estimated VO_2 max) were compared using a 2-way repeated-measures ANOVA (OC use and menstrual phase as factors). Changes in cardiovascular (HR, MAP), static cerebrovascular (MCA_V, resistance indices) and ventilatory ($ETCO_2$) responses to hypercapnia administration were compared using a 2-way repeated-measures ANOVA (OC use and menstrual phase as factors). A 3-way repeated-measures ANOVA was used to compare TFA variables, with OC use as a between-participant factor and menstrual phase and CO_2 (baseline and hypercapnia) as within-participant factors.

Statistical analyses were performed using SPSS Statistics software (Version 25, IBM, Armonk, USA) and in consultation with a biostatistician. Significance was set a priori to $P< 0.05$, and all data in Tables are presented as mean ± SD while data in Figures are presented as median with 25th and 75th percentiles. Each variable was assessed for normality, using the Shapiro-Wilk test for small sample size and a visual analysis of boxplots. Variables that violated the assumption of normality and were skewed (outside the range of -1 to 1) had a gamma distribution applied and were log-transformed. Post-hoc analysis was conducted using estimated marginal means and pairwise comparisons, with a Sidak correction to account for multiple comparisons. Cohen's D (*d*) was used as a marker of effect size for all significant differences and was calculated as the mean difference between groups (grouped by hormone phase, time point and/or OC use depending on significant interactions) then divided by the pooled standard deviation.

4.5 **Results**

Participant characteristics, and cerebrovascular hemodynamics

Participant characteristics were not different between groups and/or menstrual phases compared by age, height, body mass, BMI, resting SBP, resting DBP, or predicted VO_2 max (all $p>0.10$; Table 12).

Table 12. Anthropometrics and predicted fitness for OC users, compared to NOC.

	NOC		OC		Comparisons (P-value)		
					Main Effects		Interactions
	LH	HH	LH	HH	OC	Phase	Phase*OC
Age (years)	22±4		22±4		0.78	-	-
Height (cm)	163±5		163±7		0.75	-	-
Body Mass (kg)	64±10	64±10	67±12	67±12	0.41	0.85	0.48
Body Mass Index (kg/m^2)	24±3	24±3	25±4	25±4	0.39	0.56	0.24
Systolic Blood Pressure (mmHg)	106±9	108±15	109±8	108±8	0.74	0.72	0.56
Diastolic Blood Pressure (mmHg)	68±8	68±11	72±7	73±6	0.10	0.87	0.87
Predicted VO_2 max (mL/kg/min)	37±4	37±3	36±2	36±2	0.50	0.79	0.78

All values are mean±SD. NOC – no oral contraceptive; OC – oral contraceptive; VO_2 max – maximum oxygen consumption; No significant differences between OC and non-OC users ($p>0.05$).

There was no significant difference in the HR response to hypercapnia between menstrual phases or OC use (all p>0.1; Table 13). Similarly, many steady-state cerebrovascular responses to hypercapnia were not different between menstrual phases or with OC use: MCA_{Vmin} (all p>0.06; Table 13), MCA_{Vmax} (all p>0.08; Table 13), RAP (all p>0.07; Table 13), CrCP (all p>0.2; Table 13), RI (all p>0.1; Table 13), and PI (all p>0.2; Table 13).

Table 13. Changes in cardiovascular and cerebrovascular variables in response to hypercapnia.

	NOC		OC		Comparisons (P-value)		
	LH	HH	LH	HH	Main Effects		Interactions
	Δ	Δ	Δ	Δ	OC	Phase	Phase*OC
HR (bpm)	2±3	5±4	2±4	3±4	0.57	0.29	0.19
MCAvmin (cm/s)	11±6	13±9	15±5	13±10	0.23	0.11	0.06
MCAvmax (cm/s)	11±5	15±9	17±7	18±13	0.08	0.25	0.65
CrCP (mmHg)	-2.19±2.38	-2.90±5.94	-2.61±2.5	-0.59±4.97	0.49	0.54	0.21
RAP (mmHg/cm/s)	-0.01±0.04	-0.02±0.06	-0.03+0.03	-0.06+0.06	0.07	0.15	0.42
RI	-0.04±0.03	-0.06±0.05	-0.06±0.02	-0.04±0.05	0.89	0.73	0.13
PI	-0.11±0.08	-0.16±0.12	-0.15±0.07	-0.13±0.15	0.82	0.72	0.24

All values are mean±SD. NOC – no oral contraceptive; OC – oral contraceptive; LH – low hormone; HH – high hormone; HR – heart rate; MCA_{Vmax} – maximum middle cerebral artery velocity; MCA_{Vmin} – minimum middle cerebral artery velocity; CrCP – critical closing pressure; RAP – resistance area product; RI – resistance index; PI – pulsatility index.

There was a significant interaction between menstrual phase and OC use on the MAP response to hypercapnia (p=0.036; Figure 6A); however, post hoc analysis revealed that there were no significant differences (all p>0.05). Regardless of menstrual phase (p=0.07), OC users had a significantly greater MCA_{Vmean} response to hypercapnia (p=0.048; *d* =0.43; Figure 6B). The $ETCO_2$ response to administration of hypercapnia was lower in NOC-LH compared to both NOC-HH (p=0.039; *d* =0.66) and OC-LH (p=0.03; *d* =0.87; Figure 6C). Regardless of menstrual phase, OC users had a higher slope between the change in MCA_{Vmean} over change in $ETCO_2$ (NOC-LH 1.85±0.68, NOC-HH 2.14±1.09, OC-LH 2.38±0.50, OC-HH 2.41±1.49; Main effect of OC p=0.03; OC vs. NOC *d* =0.41). There were no significant effects of the menstrual cycle (p=0.07) or OC use (p=0.08) on the change in CVRi during hypercapnia (Figure 6D).

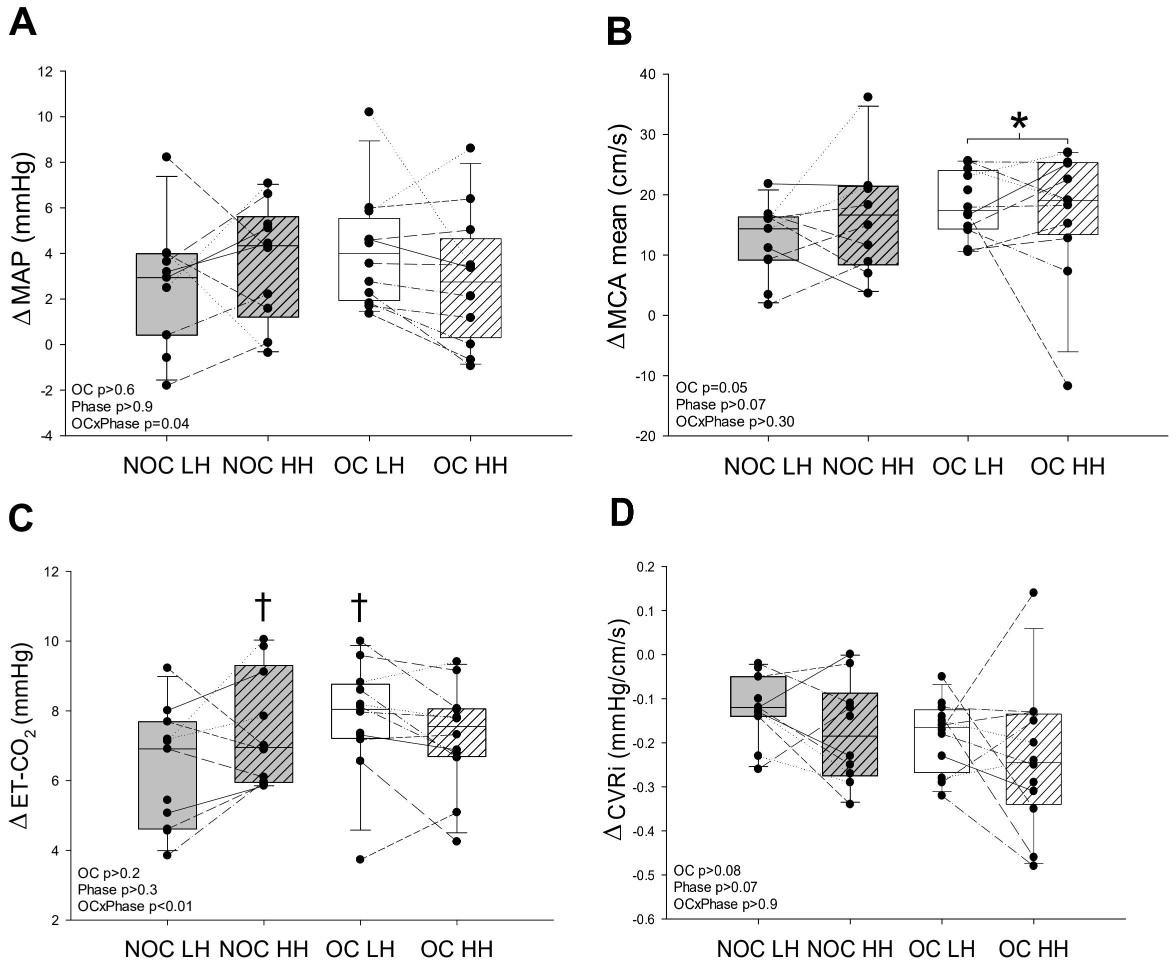

Figure 6. Change in cerebral mean arterial pressure (MAP; A), mean cerebral artery blood velocity (MCA$_{Vmean}$; B), end-tidal carbon dioxide ($ETCO_2$; C) and cerebrovascular resistance index (CVRi; D) in response to 5 minutes of hypercapnia in women taking oral contraceptives (OC; white bar) and not taking oral contraceptives (NOC; grey bar) during the low hormone (LH) and high hormone (HH) phases of their menstrual cycle. The centerline of each box represents the median response, while the upper and lower border of the box represents the 25th and 75th percentile. * indicates significantly different than NOC users; † indicates a significant difference from NOC-LH.

Dynamic Cerebral Autoregulation

Regardless of menstrual cycle or OC use, hypercapnia caused higher HF power of MAP_{MCAv} (p=0.003; *d* =0.26), HF Coherence (p=0.024; *d* =0.34), with lower HF nGain (p=0.002; *d* =0.21; Table 14). There was a significant interaction between the menstrual phase and hypercapnia for the HF power of MCA_{Vmean} (p=0.019), where the HF power of MCA_{Vmean} increased in response to hypercapnia in the HH menstrual phase only (p<0.001; HH baseline vs. HH hypercapnia *d* =0.02; Table 14). During hypercapnia, the HF power of MCA_{Vmean} was significantly higher in the HH than LH phase (p=0.036; LH hypercapnia vs. HH hypercapnia *d* = 0.55; Table 14). In the LF band, hypercapnia increased LF Gain (p=0.027; *d* =0.32) in all women, regardless of phase. LF Coherence increased above the threshold for linearity, only in the LH menstrual phase (p=0.001; LH baseline vs. LH hypercapnia *d* =0.51; Table 14). There was a significant interaction between OC, menstrual phase, and hypercapnia in LF Phase (p=0.025). In response to hypercapnia, LF Phase significantly decreased in the NOC group only during the HH menstrual phase (p=0.001; NOC HH baseline vs. NOC HH hypercapnia *d* =1.05; Table 14). In NOC, LF phase increased more during hypercapnia in the LH than HH phase (p=0.005; NOC LH hypercapnia vs, NOC HH hypercapnia *d* = 0.66; Table 14). In contrast, OC users significantly decreased LF Phase only during the LH menstrual phase (p<0.004; OC LH baseline vs. OC LH hypercapnia *d* =0.76; Table 14). In OC users, LF phase was larger in HH than the LH phase during hypercapnia (p=0.012, OC LH hypercapnia vs. OC HH hypercapnia *d* =0.85; Table 14). In the HH phase during hypercapnia, OC users had a larger LF phase than NOC (p<0.001, NOC HH hypercapnia vs. OC HH hypercapnia *d* =0.78; Table 14). In all women, hypercapnia decreased VLF power of MAP_{MCAv} (p=0.035; *d* =0.20), VLF Gain (p=0.001; *d* =0.53), and VLF nGain (p<0.001; *d* =0.72), regardless of menstrual phase (all Table 14). There was a significant interaction between menstrual phase and hypercapnia for VLF power of MCA_{Vmean} (p=0.050; Table 14), although there were no differences between menstrual phases (all p>0.25). In response to hypercapnia, the VLF power of MCA_{Vmean} decreased in the LH (p=0.002; LH baseline vs. LH hypercapnia *d* =0.76) and HH menstrual phase (p=0.025, HH baseline vs. HH hypercapnia *d* =0.49; Table 14). There was a significant interaction between menstrual phase and OC use for VLF Gain (p=0.040; Table 14), but post-hoc analysis revealed that there were no significant differences between groups (all p>0.12).

Table 14. Transfer function analysis of cerebrovascular response to hypercapnia.

		NOC				OC				Comparisons (P-value)						
		LH		HH		LH		HH		Main effects			Interactions			
Frequency Range		Baseline n=9	CO_2 n=9	Baseline n=10	CO_2 n=11	Baseline n=10	CO_2 n=10	Baseline n=12	CO_2 n=11	OC	Phase	CO_2	OC Phase	OC CO_2	Phase CO_2	OC Phase CO_2
HF	MCA_{Vmean} power, cm^2/s^2	5.71±4.04	6.30±4.84	6.95±3.76	10.73±8.04 *ǂ	6.99±6.58	7.61±4.83	6.88±6.41	10.33±7.71 *ǂ	0.731	-	-	0.487	0.921	**0.019**	0.959
	MAP_{MCAv} power, mmHg^2	2.11±3.09	2.47±4.01	2.77±1.87	3.50±2.34	1.35±0.97	1.53±1.21	1.72±1.54	2.79±2.52	0.225	0.170	**0.003**	0.834	0.516	0.094	0.282
	Coh, au	0.52±0.20	0.57±0.16	0.53±0.17	0.60±0.12	0.53±0.13	0.59±0.19	0.56±0.15	0.57±0.16	0.867	0.690	**0.024**	0.852	0.721	0.764	0.492
	Gain, cm/s/mmHg	1.89±0.74	1.83±0.82	1.53±0.40	1.66±0.70	2.06±0.93	2.40±1.40	2.02±0.89	2.23±1.41	0.141	0.325	0.055	0.601	0.190	0.689	0.285
	nGain, %/mmHg	2.30±1.04	1.97±0.93	2.13±0.79	1.95±0.91	2.52±1.01	2.46±1.21	2.65±0.78	2.34±1.05	0.201	0.842	**0.002**	0.834	0.593	0.753	0.232
	Phase, radians	0.42±0.56	0.32±0.22	0.34±0.33	0.17±0.13	0.27±0.17	0.11±0.15	0.25±0.22	0.16±0.13	0.122	0.662	0.060	0.356	0.778	0.990	0.506
LF	MCA_{Vmean} power, cm^2/s^2	7.27±4.97	6.89±3.05	7.67±4.99	8.43±5.93	7.48±4.06	6.99±4.53	6.50±3.24	9.46±8.53	0.996	0.446	0.355	0.866	0.479	0.136	0.457
	MAP_{MCAv} power, mmHg^2	5.97±9.59	4.57±5.13	3.65±2.21	4.55±4.39	2.95±2.71	4.06±5.34	2.68±2.41	3.51±3.38	0.296	0.506	0.219	0.814	0.151	0.365	0.267
	Coh, au	0.55±0.18	0.60±0.24 †	0.61±0.16	0.61±0.22	0.51±0.17	0.64±0.15 †	0.53±0.18	0.52±0.18	0.504	-	-	0.256	0.431	**0.004**	0.171
	Gain, cm/s/mmHg	1.36±0.61	1.45±0.58	1.37±0.43	1.50±0.54	1.38±0.43	1.63±0.69	1.42±0.48	1.78±0.92	0.344	0.737	**0.027**	0.869	0.322	0.666	0.906
	nGain, %/mmHg	1.70±0.99	1.58±0.79	1.85±0.73	1.72±0.66	1.87±0.62	1.70±0.59	1.84±0.52	1.87±0.66	0.542	0.602	0.256	0.857	0.769	0.670	0.618
	Phase, radians	0.52±0.20	0.44±0.24◊	0.60±0.40	0.28±0.18 *	0.58±0.33	0.34±0.23 †	0.66±0.38	0.68±0.33 ǂθ	-	-	-	-	-	-	**0.025**
VLF	MCA_{Vmean} power, cm^2/s^2	18.23±22.15	6.46±3.30 †	18.77±15.27	7.39±6.55*	25.47±29.13	7.99±6.17†	14.56±12.61	11.49±20.17*	0.554	-	-	0.677	0.440	**0.050**	0.120
	MAP_{MCAv} power, mmHg^2	9.52±15.71	6.04±7.18	5.72±6.50	3.51±3.12	4.01±2.46	3.08±2.00	3.41±2.81	4.23±4.26	0.129	0.427	**0.035**	0.284	0.057	0.302	0.239
	Coh, au	0.43±0.18	0.40±0.17	0.46±0.15	0.48±0.14	0.36±0.12	0.43±0.15	0.35±0.13	0.41±0.13	0.231	0.444	0.273	0.180	0.169	0.727	0.538
	Gain, cm/s/mmHg	2.29±1.28	1.56±0.78	1.64±0.69	1.26±0.55	1.44±0.77	1.27±0.63	2.18±1.50	1.69±0.82	-	-	**0.001**	**0.040**	0.375	0.952	0.337
	nGain, %/mmHg	2.90±1.92	1.67±0.93	2.21±1.19	1.48±0.74	1.72±1.03	1.43±0.67	2.66±1.95	1.80±0.70	0.670	0.673	**<0.001**	0.099	0.248	0.855	0.213
	Phase, radians	0.80±0.54	0.93±0.45	0.98±0.72	0.56±0.52	0.56±0.41	0.34±0.69	0.86±0.75	0.95±0.44	0.383	0.211	0.369	0.054	0.724	0.621	0.057

All values are mean±SD. NOC – no oral contraceptive; OC – oral contraceptive; HF – high frequency (0.2-0.4 Hz); LF – low frequency (0.07-0.2 Hz); VLF – very low frequency (0.02-0.07 Hz); MAP_{MCAv}, mean arterial pressure height-corrected to the middle cerebral artery; $MCAv_{mean}$, middle cerebral artery mean blood velocity; Coh – Coherence; nGain – normalized Gain; significance is represented by bold text for main effects and interactions ($p<0.05$). * indicates significantly different than baseline in HH phase; † indicates significantly different than baseline in LH phase; ǂ indicates higher than CO_2 in LH phase; ◊ indicates higher than CO_2 in HH phase; θ indicates different than NOC at this timepoint.

4.6 **Discussion**

The aim of this study was to describe the cerebrovascular and dCA response to hypercapnia during fluctuating phases of endogenous and exogenous sex hormones. In response to hypercapnia, neither the cardiovascular nor the cerebrovascular responses were influenced by menstrual phase, yet OC users had a greater increase in MCA_{Vmean}. Hypercapnia significantly decreases VLF and HF nGain in all women and menstrual phases. NOC users in the HH menstrual phase had decreased LF Phase in response to hypercapnia, whereas OC users experienced LF Phase decline in the LH menstrual phase in response to hypercapnia administration. Therefore, our key findings are that 1) there are minimal effects of menstrual phase or OC on hemodynamic or cerebrovascular resistance responses to hypercapnia, 2) all women exhibit enhanced HF and VLF dCA via dampening of BP fluctuations (nGain) in hypercapnia, and 3) while NOC have reduced timing (phase) of LF changes in BP translating to changes in CBF during hypercapnia in the HH menstrual phase, OC users exhibit the same reduction in the LH menstrual phase.

Cardiovascular, Ventilatory and Cerebrovascular response to Hypercapnia

In the parallel study, Assadpour et al.[72] found no influence of menstrual cycle nor OC use on resting cardiorespiratory variables. Similarly, the present study found no effects of menstrual phase or OC use on the hemodynamic responses to hypercapnia. Usselman et al.[78] also found that menstrual phase in OC users did not influence hemodynamic responses to hypoxic hypercapnia via apnea. $ETCO_2$ during 5% CO_2 administration was lower in NOC-LH compared to both NOC-HH and OC-LH. However, it is important to note that while minor differences in V_E and/or pulmonary perfusion could play a small role, the median values between groups differed by <1 mmHg. This small difference in $ETCO_2$ is unlikely to influence either cerebrovascular resistance or autoregulation. Indeed, a 7.5 mmHg increase in $ETCO_2$ during hypercapnia administration does not elicit a change in MCA diameter[207], suggesting that the present difference is not likely to influence diameter or perhaps dCA.

Contrary to our hypotheses, OC users had a similar MAP response to hypercapnia in the HH menstrual phase compared to the LH menstrual phase, yet the MCA_V response to hypercapnia was greater than NOC regardless of pill phase. Since there was a significantly greater MCA_V response but no change in MAP response in OC users, we expected to see a reduction in cerebrovascular resistance (i.e., CVRi, RAP, CrCP, RI, and PI), yet this was not statistically significant. A greater sample number and statistical power or controlling for OC pill generation type could have decreased variability and provided evidence for changes in resistance indices. However, OC use did not influence cerebrovascular resistance indices throughout a supine-sit-stand model, which includes both hypocapnia and baroreflex activation[76], nor does OC pill generation influence peripheral artery vasodilatory capacity[208]. Similarly, menstrual phase did not influence indices of cerebrovascular resistance in response to hypercapnia, corresponding to previous work in our lab, which found that there is no influence of the menstrual cycle on cerebrovascular resistance indices during upright posture[76,88].

Dynamic Cerebral Autoregulation

In all women during hypercapnia, the HF power of MAP control, HF Coherence, and the LF Gain (but not LF nGain) increased, while HF nGain, VLF power of MAP control, VLF Gain and VLF nGain decreased. These results suggest that the HF component of MAP control is elevated while the VLF component is suppressed during hypercapnia. Further, the HF and VLF components (nGain) of dCA are improved during hypercapnia (i.e., increased buffering of BP fluctuations). Interestingly, Ainslie et al.[209] observed no change in LF, HF, or VLF Gain, Coherence, MAP variability, or MCA_V variability during 4% hypercapnia in young recreationally active men. This may suggest that men are less likely to experience dCA dysregulation during hypercapnia compared to women. High levels of aerobic fitness are associated with both a reduced ability to dampen rapid or large changes in BP[210,211] and increased cerebrovascular CO_2 reactivity[212]. Groups in the current study did not differ by fitness; however, this highlights the importance of considering fitness in investigations of CBF and the cerebrovasculature.

The HF power of the MCA_V significantly increased during hypercapnia only in the HH menstrual phase, whereas LF Coherence increased during hypercapnia only in the LH menstrual phase, regardless of OC use. The exact physiological mechanisms for HF and LF control of CBF are unclear; however, the presence of estrogen and progesterone appear to augment the HF control while impairing the LF control in hypercapnia. Schmetterer et al.[213] observed that MCA_{Vmean} increases during hypercapnia, which was blunted by infusion of a NO synthase inhibitor, suggesting that NO plays a key role in the hypercapnic vasodilatory response. Indeed, since estrogen is known to upregulate endothelial NO synthase[214], an enhanced release of NO in the HH menstrual phase during hypercapnia could be responsible for the augmentation of HF control of CBF.

Coherence is based on the assumption of a linear relationship between MCA_V and BP. We observed that hypercapnia increases this relationship in the LF range when estrogen and progesterone levels are reduced; however, the relationship does not increase in the presence of high levels of these hormones. Since neurovascular transduction of sympathetic input is impaired in the presence of estrogen and progesterone[27], we hypothesize that the increased LF Coherence is due to enhanced sympathetic output during hypercapnia, but the lack of change in LF Coherence is due to impaired vascular transduction of this increased sympathetic activity. Korad et al.[215] observed a negative relationship between estradiol concentration and Rate of Regulation (i.e., the rate of change in CVRi related to the change in BP) during repeated squat maneuvers, suggesting that sex hormones are related to autoregulation. Since forced BP oscillations (e.g. repeated squats) have been shown to increase coherence compared to spontaneous oscillations[216], this may provide stronger evidence of diminished CA during high levels of sex hormones. Future research should investigate the effects of OC use on forced BP oscillations to improve the interpretation of the linear relationship between MCA_v and BP.

Importantly, NOC users had a reduction of LF dCA during hypercapnia in the presence of estrogen and progesterone, while OC users had a reduction of LF dCA during hypercapnia in the absence of estrogen and progesterone analogs. Therefore, the impairment of LF dCA during hypercapnia observed in the presence of natural estrogen/progesterone is not seen in the presence of pharmaceutical estrogen/progesterone, yet the chronic use of OC (as indicated by the placebo pill phase of OC) also causes a reduction of LF dCA during hypercapnia. Enhanced sympathetic BRS and an increased BP response to hypercapnia exist in the HH menstrual phase compared to the LH menstrual phase in NOC[18,217]. Further, sympathetic BRS is negatively associated with dCA, such that less effective dCA is associated with increased sympathetic BRS and vice versa[218-220]. Therefore, an enhanced sympathetic influence during hypercapnia in the HH menstrual phase of NOC may be responsible for the impaired LF dCA observed in the current study. Interestingly, it has also been observed that OC users have greater sympathetic BRS and increased sympathoexcitation in response to severe chemoreflex activation during the LH menstrual phase[78,204]. Along with our observations, these studies again suggest a relationship between increased sympathetic influence and impaired LF dCA in OC users during hypercapnia in the LH menstrual phase.

Perspectives

Although the human brain represents only 2% of the average human's total body mass[65], it requires roughly 20% of total body oxygen at rest[64]. This high demand for oxygenated blood means that the brain is susceptible to mismatches in perfusion and that decrements in the effectiveness of dCA can result in transient ischemia. While dCA was not different during baseline, the impact of hypercapnia might be particularly relevant to exercise, and a mismatch between perfusion pressure and flow could have detrimental effects for exercising women. Generally, dCA has been shown to be preserved in response to exercise of varying modalities and intensities[221-224]. However, more exhaustive aerobic or resistance exercise is associated with greater dCA dysregulation[225,226], suggesting that exercise can induce dCA dysregulation in an intensity-dependant manner. However, a majority of these studies are conducted solely on men[221,224,225], included insufficient female populations for statistical comparisons[223], or did not consider sex-related differences[222,226]. More exercise intervention studies that investigate women through the menstrual cycle while controlling for OC use are warranted.

Limitations

A limitation of the current study is that the analysis of OC was not separated by pill generation due to small sample sizes. It is possible that the type of progestin and formulation with estradiol could potentially affect physiological responses. Differences between OC generations have previously been observed by Shenouda et al.[208], where a negative relationship between the duration of OC use and flow-mediated dilation was observed in second-generation OC only. This suggests that different pill formulations may lead to different vascular responses. Future studies should enlarge sample sizes to separate OC generations for comparison. The current study did not quantify estrogen or progesterone levels; however, urine samples were tested for the presence of progesterone to confirm ovulation. If sufficient progesterone was not present (<5 μg/ml), participants were asked to return to the lab during their next cycle to ensure that an appropriate level of progesterone was present. Finally, the present observations are cross-sectional comparisons, which is a great first step to determining the effects of OC on autonomic functioning yet does not elucidate the longitudinal response within an individual. Longitudinal studies that observe women prior to starting their pill regimen are required to remove any baseline bias that could have led to the present observations. Using TCD ultrasound to estimate CBF is based on the assumption that the cerebrovascular diameter remains constant, in order to measure MCA_V. While cerebrovascular diameter stability has been demonstrated for changes in $ETCO_2$ smaller than 7.5 mmHg[207], the present study cannot confirm if cerebrovascular diameter was maintained. Furthermore, TFA was used to quantify autoregulation on spontaneous changes in BP, which is related to lower coherence (i.e., poorer signal-to-noise ratio)[216], and reproducibility (i.e., within-day)[227,228]. Methodologies regarding cerebrovascular CO_2 reactivity have also been highly debated. The duration of the CO_2 stimulus and steady state timepoints used for analysis can influence outcome variables[229]. Finally, these observations are limited to the present participant pool (i.e., healthy menstruating women) and cannot be generalized to other areas of cerebral circulation[230].

4.7 **Conclusions**

In response to hypercapnia, while OC users had a greater increase in MCA_{Vmean} compared to NOC, neither the hemodynamic nor the cerebrovascular resistance responses were influenced by OC use or menstrual phase. We also observed that MAP control becomes more reliant on HF and less reliant on VLF control during hypercapnia. Additionally, VLF control of MCA_V decreased significantly during hypercapnia in both phases, yet the response did not differ throughout the menstrual cycle. Comparatively, HF MCA_V control is menstrual phase dependent in hypercapnia, where hypercapnia increases HF power in the presence of estrogen and progesterone analogs. In women, exposure to CO_2 generally led to better autoregulation (HF and VLF nGain), although some aspects of dCA were dependent on menstrual phase and OC use (Coherence or Phase). Importantly, we found that LF dCA was impaired during hypercapnia in the HH menstrual phase of NOC yet the LH menstrual phase of OC users. This suggests that the response to hypercapnia in the presence of endogenous estrogen and progesterone is impaired LF dCA; however, this is not observed in the presence of pharmaceutical hormones. Further, the chronic use of pharmaceutical hormones (as evidenced by changes during the placebo pill) elicits impaired LF dCA during hypercapnia. The current study highlights the importance of considering the influence of an individual's hormonal milieu.

Chapter 5: **Conclusion**

In conclusion, this thesis has demonstrated the varying effects of OC on resting autonomic function, autonomic indices, CBF and the physiological responses to autonomic reflex activation. There is a paucity of available literature on the influence of OC on the ANS; however, the current thesis addresses some of the previous gaps in the literature. Previous literature has demonstrated that endogenous estrogen is positively associated with HRV[158], yet most available studies included OC with relatively low estrogen doses; thus, it is difficult to determine if synthetic estrogen has a similar association with HRV. Additionally, studies are further confounded by a lack of standardized intensity of stimuli utilized, as divergent cardiovascular and sympathetic responses between OC and NOC were only observed with more intense sympathetic stimuli (e.g., greater LBNP[130] or colder temperatures for CPT[116,119]).

We found that neither OC nor NOC increase V_E in response to PECO, yet both groups had a V_E response to arm or leg mechanoreflex activation, which suggests that the mechanoreflex may be primarily responsible for driving V_E during exercise in women. Secondly, the current study demonstrated that OC did not influence most cardiorespiratory responses to autonomic reflex activation - similar to a previously observed lack of effect of OC on the physiological responses during whole-body dynamic exercise[83]. However, disparities in strength between OC and NOC (e.g., OC had significantly lower handgrip strength than NOC) significantly attenuated the pressor response to arm metaboreflex activation in OC – indicating the importance of matching groups for strength in future studies.

Finally, we found nuanced interactions between the effects of OC and the menstrual cycle on CA. While most hemodynamic responses to hypercapnia were not influenced by OC or menstrual phase, OC users had larger increases in MCA_{Vmean} than NOC during hypercapnia - indicating greater cerebrovascular reactivity. Autoregulation generally improved in all women exposed to high CO_2; however, hypercapnic dCA became impaired during the HH menstrual phase of NOC and the LH menstrual phase of OC – suggesting that the presence of endogenous hormones acutely impairs dCA while the absence of synthetic hormones in OC users causes a similar impairment.

This thesis mainly focused on the effect of OC on the cardiorespiratory response to isolated reflex activation or the cerebrovascular response to hypercapnia. While we did not find many effects of OC, we did not investigate different types or doses of OC. Future studies should investigate specific OC groupings – given that second generation OC attenuate vascular responsiveness[194-196,208], while fourth generation OC do not[197]. Researchers should consider more strict OC stratification to determine the influence of different progestin generations or the effect of monophasic versus multiphasic OC. Furthermore, there are several other forms of hormonal contraceptives yet very little research investigates their effects on autonomic function and CBF. Comparing OC to other hormonal contraceptives (e.g., intrauterine devices, injections, post-coital emergency contraceptives, implants, rings, minipills, patches, etc.) would allow for investigation of the effect of administration route and the effects of altering the menstrual cycle with longer lasting contraceptives. Further investigation is required to elucidate whether there is an influence of all types of synthetic hormones and whether how those hormones are delivered affects their influence on autonomic function and CBF.

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APPENDIX

Appendix A: Study 1

Search Strategy

Autonomic Nervous System & Oral Contraceptives, Female
Search Strategy: Ovid MEDLINE(R) ALL <1946 to April 27, 2021>

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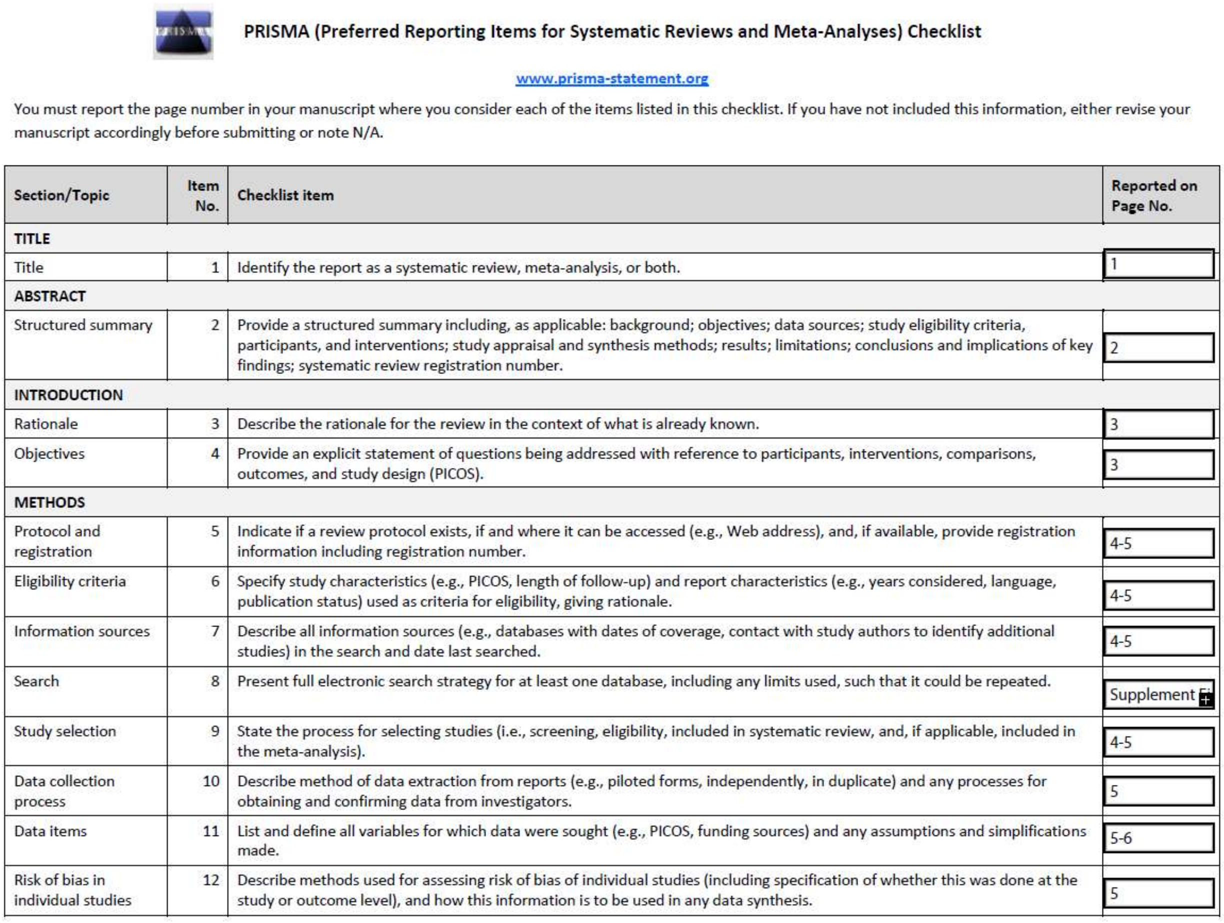
1 [Problem: Autonomic Functioning]
2 autonomic nervous system/ (27070)
3 exp parasympathetic nervous system/ (50510)
4 exp sympathetic nervous system/ (64688)
5 Baroreflex/ (6186)
6 Hypercapnia/ (8771)
7 Hypoxia/ (65860)
8 Valsalva Maneuver/ (4157)
9 exp Orthostatic Intolerance/ [includes Hypotension, Orthostatic/] (8422)
10 exp Syncope/ (13095)
11 (nervous system* adj3 (autonomic or sympathetic or parasympathetic)).tw,kw. (30747)
12 (autonomic adj3 (nervous or control or function* or reflex* or response* or tone or parameters or balance or activity or modulation)).tw,kw. (34712)
13 ((sympathetic or parasympathetic) adj3 (regulation* or control* or mechanism* or skin response* or activity or function*)).tw,kw. (26562)
14 ((heart rate or blood pressure) adj3 variability).tw,kw. (23044)
15 (baroreflex* or metaboreflex* or mechanoreflex* or chemoreflex* or hypercapnia or hypoxia or syncop* or presyncop* or pre-syncop*).tw,kw. (155682)
16 (valsalva* adj3 maneuver*).tw,kw. (3174)
17 (hypotension adj3 (orthostatic or postural)).tw,kw. (6894)
18 (orthostatic adj3 (test* or stress* or intolerance)).tw,kw. (2837)
19 (baroreceptor adj3 (reflex* or sensitivity)).tw,kw. (2797)
20 (pressor adj3 (test* or response*)).tw,kw. (11034)
21 (deep adj3 breathing).tw,kw. (2223)
22 (test adj3 (Sudomotor or thermoregulatory or sweat)).tw,kw. (1082)
23 (pulmonary adj3 (reflex* or stretch*)).tw,kw. (785)
24 or/2-23 (379122)
25 [Intervention: Oral Contraceptives]
26 exp Contraceptives, Oral/ (50306)
27 Contraception/ (20197)
28 exp Estrogens/ (163037)
29 exp Progestins/ (69149)
30 (birth control or contracepti*).tw,kw. (77386)
31 (contracepti* adj3 (hormon* or oral or phasic or triphasic or monophasic or pharma* or cyclic)).tw,kw. (32827)
32 (hormon* adj3 (synthetic or exogenous or pharma*)).tw,kw. (6768)
33 (oral adj3 contraceptives).nm. (27968)
34 (cyproterone* or chlormadinone* or desogestrel* or drospirenone* or epiestriol* or estradiol* or estrane* or estriol* or estrogen* or estrone* or ethinylestradiol* or gestodene* or gonane* or hydroxyestr* or ketoestradiol* or levonorgestrel* or mestranol* or norethindrone* or norethynodrel* or norgestimate* or oestrogen or pregnane* or progest*).tw,kw. (272943)
35 or/26-34 (404545)
36 [Limit to Human Females]
37 Female/ (8994956)
38 (female* or women).tw,kw. (1904341)
39 37 or 38 (9344838)
40 24 and 35 and 39 (2406)
41 limit 40 to "humans only (removes records about animals)" (1511)

Appendix B: Study 1

PRISMA Checklist



PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Checklist

www.prisma-statement.org

You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Section/Topic	Item No.	Checklist item	Reported on Page No.
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4-5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4-5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4-5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplement Fi
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4-5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5

Section/Topic	Item No.	Checklist item	Reported on Page No.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4-5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	22-42
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	22-42
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	6
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7-15
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			

Section/Topic	Item No.	Checklist item	Reported on Page No.
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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