Sympathetic nervous system regulation of cardiovascular function:

effect of acute and chronic exercise on the normative adaptations in healthy pregnancy

by

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Abstract

Introduction: Pregnancy is associated with profound changes to the sympathetic nervous system control of blood pressure while maintaining blood pressure itself. This involves a coordinated increased muscle sympathetic nerve activity (MSNA) alongside reduced sympathetic baroreflex gain (BRG) and neurovascular transduction (NVT) that has been observed in the third trimester (TM3) of normotensive pregnancy. The mechanisms by which blood pressure is raised during stress in pregnant women are also altered. Exercise has been shown to have many health benefits in pregnancy including a 40% reduction in the risk of developing gestational hypertension. In fact, exercise is recommended for all pregnant women who do not have explicit absolute contraindications. These recommendations include a combination of aerobic and resistance exercise to achieve 150-minutes of moderate intensity activity per week. We know very little about the mechanisms behind which exercise is beneficial in reducing hypertension risk in pregnancy, and the studies here aim to address these questions from the perspective of the sympathetic nervous system.

Aims and hypotheses: The aim of our first study was to addresses whether there are differences between pregnant and non-pregnant women in the blood pressure and MSNA response during acute exercise (i.e. isometric handgrip; IHG). We hypothesized that the blood pressure response to IHG would not be different, but that pregnant women in TM3 would have a greater MSNA response to elicit this (i.e. blunted NVT). The aim of our second study was to identify whether a structured aerobic exercise program could alter the sympathetic regulation of blood pressure. We hypothesized that exercise would attenuate the rise in MSNA (and subsequent blunting of BRG and NVT) and would augment the decrease in arterial stiffness (pulse wave velocity; PWV) and other positive vascular changes associated with healthy pregnancy (i.e. changes in carotid

distensibility). We also hypothesized that an aerobic exercise program would reduce the blood pressure and MSNA response to cold pressor test (CPT).

Methodology: Two studies were used to test the hypotheses. First, a cross sectional study comparing the response to two minutes of isometric handgrip (IHG) and two minutes of post-exercise circulatory occlusion (PECO) between pregnant women in TM3 and non-pregnant women. In this study we measured heart rate, blood pressure, MSNA, and blood samples to measure catecholamine (norepinephrine and epinephrine) concentrations.

Second, we conducted a randomized controlled trail (prenatal exercise and cardiovascular health; PEACH study). We enrolled women into exercise (3-4x/week 25-40minutes, moderate intensity, aerobic exercise, for 14±1 weeks) or control (standard care) groups and we tested their neurovascular health before (16-20weeks) and after (34-36weeks) the intervention. The neurovascular assessment involved measuring resting heart rate, blood pressure, MSNA, PWV, and blood vessel diameter and flow in the carotid and superficial femoral arteries. We determined from these resting measures of sympathetic baroreflex gain (BRG) and neurovascular transduction (NVT), central and peripheral PWV, carotid artery distensibility measures, and femoral blood flow resistance (FVR) and conductance (FVC). In addition to resting measures, we also conducted a three-minute-long CPT (hand in ice water) where we measured the heart rate, blood pressure, MSNA, and the reactivity of the superficial femoral artery (FVR and FVC).

Results: Compared to non-pregnant women, here we show that MSNA reactivity during IHG and PECO is not different in TM3 of normotensive pregnancy. Next, we showed that an aerobic exercise intervention resulted in attenuation of the rise in MSNA (and blunting of NVT) in pregnancy. This occurred without differences in resting blood pressure, carotid artery

distensibility, FVR, FVC, PWV, concentrations of sex hormones, metabolic markers, or angiogenic factors. Aerobic exercise intervention also did not alter the blood pressure, MSNA or superficial femoral artery response to CPT during pregnancy.

Discussion: Pregnancy is associated with changes in blood pressure regulation independent of exercise. However, aerobic exercise between TM2 and TM3 can alter the trajectory of the rise in basal sympathetic activity within a group of normotensive pregnant women. More research is needed to elucidate if greater positive changes, including those of the vasculature (e.g. carotid distensibility and PWV) can be impacted with a longer duration intervention (i.e. starting before or earlier in pregnancy) or in women who are at higher risk for the development of gestational hypertension.

Preface

This thesis is original work by Rachel Skow. There are two projects, of which this thesis is a part, having received research ethics approval from the University of Alberta Research Ethics Board:

1) project name: *"The effects of pregnancy on blood pressure control during handgrip exercise"* Pro00058560, first approved April 28, 2017.

2) project name: "*Exercise and neurovascular function in pregnancy*" Pro00061045, first approved April 6, 2016.

All data collection and analysis are my original work. The custom software for conducting the neurovascular transduction analysis in Chapter 2 and Chapter 3 were written by Dr. Graham Fraser. Blood sample analysis was completed in part by Dr. Laura Reyes and Mr. Andrew Steele in combination with a local Dynalife blood processing center.

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Abbreviations List

A	Area (cross-sectional)
ANOVA	Analysis of variance
BF	Burst frequency
BI	Burst incidence
BMI	Body mass index (kg/m^2)
BRG	Baroreflex gain
CC	Compliance coefficient
CPT	Cold pressor test
DBP	Diastolic blood pressure
DC	Distensibility coefficient
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EPI	Epinephrine
FVC	Femoral vascular conductance
FVR	Femoral vascular resistance
GDM	Gestational diabetes mellitus
GWG	Gestational weight gain
HOMA-IR	Homeostatic model assessment of insulin resistance
HRR	Heart rate reserve
IHG	Isometric handgrip
IMT	Intima-media thickness (of the carotid artery)
MAP	Mean arterial blood pressure
METS	Metabolic equivalent; a measure of energy expenditure (1 MET ~ 3.5ml/min/kg)
MSNA	Muscle sympathetic nerve activity
MVC	Maximal voluntary contraction
MVPA	Moderate-to-vigorous physical activity

NE	Norepinephrine
NIRS	Near infrared sensor
NVT	Neurovascular transduction
P	Pressure
PCOS	Polycystic ovary syndrome
PEACH	Prenatal exercise and cardiovascular health; NCT 02948439
PECO	Post exercise circulatory occlusion
PlGF	Placental growth factor
PWV	Pulse wave velocity
Q	Cardiac output
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
sFlt-1	Soluble-fms like tyrosine kinase-1
SVR	Systemic vascular resistance
TM2	Trimester 2; 12+0 to 27+6 weeks gestation
TM3	Trimester 3; 28+0 to delivery of infant
VEGF	Vascular endothelial growth factor
VO_2	Volume of Oxygen; measure of Oxygen uptake (measured as L/min or ml/min/kg)

1 Introduction

Exercise during pregnancy is established to have significant health benefits for both mother and baby and is recommended for all women who do not have contraindications (Mottola *et al.*, 2018). Women who engage in regular physical activity both before and during pregnancy have a 40% reduction in the odds of developing hypertensive disorders during pregnancy (Kasawara *et al.*, 2012; Davenport *et al.*, 2018c). This is especially important because together, gestational hypertension (blood pressure greater than 140/90 after 20 weeks gestation; Watanabe *et al.*, 2013) and preeclampsia (gestational hypertension with de novo proteinuria; Roberts *et al.*, 2013) are the leading causes of maternal and fetal morbidity and mortality (Bellamy *et al.*, 2007; Mosca *et al.*, 2011b; Hollegaard *et al.*, 2017). The exercise-mediated reduction in risk for developing these disorders during pregnancy cannot be explained by improved glucose tolerance, decreased weight gain, or improved metabolic status alone (Mora *et al.*, 2007; Thijssen *et al.*, 2010). The direct effect of exercise is likely mediated by adaptations to the cardiovascular and nervous systems.

To support the demands of the growing fetus, the maternal cardiovascular system undergoes many adaptations during pregnancy. The longitudinal hemodynamic changes of pregnancy have been extensively reviewed elsewhere (Davenport *et al.*, 2016; Meah *et al.*, 2016; Fu, 2018). Specifically, these include a progressive increase in heart rate which reaches a peak approximately 15 beats per minute (26%) higher at term compared to pre-pregnancy levels (Meah *et al.,* 2016). This is accompanied by an increase in cardiac output of 30% that increases linearly in the first half of pregnancy and plateaus in the middle of the second trimester (Meah *et al.*, 2016). Further, pregnancy is associated with a progressive increase in blood volume that plateaus around 30-weeks and increases by approximately 1.1-1.2L (Longo, 1983; de Haas *et al.*, 2017) that results in vasodilation and outward remodeling of the arteries (Skow *et al.*, 2017). The rapid

adaptation of the maternal vasculature is mainly attributed to vasodilatory mechanisms which are stimulated by estrogen (Beldekas *et al.*, 1981) and mediated by vascular growth factors (angiogenic factors). In pregnancy, the relationship between two vascular growth factors, placental growth factor (PlGF) and soluble fms-like tyrosine kinase 1 (sFlt-1), is indicative of angiogenic stasis; PlGF is pro-angiogenic and sFlt-1 is anti-angiogenic and thus a higher PlGF:sFlt-1 ratio is associated with poorer vascular outcomes and pre-eclampsia risk (Zeisler *et al.,* 2016).

Together, these hemodynamic and hormonal changes result in minimal change in blood pressure throughout pregnancy (Green *et al.*, 2020) or a curvilinear decrease in blood pressure (~10%) which nadirs in the second trimester (TM2), returning to pre-pregnancy values at term (Meah *et al.*, 2016). The aforementioned observation is mirrored in the curvilinear decreases in systemic vascular resistance (SVR) up to 30% (Meah *et al.*, 2016) and a 6-12% decrease in vascular stiffness (measured as central pulse wave velocity; PWV) observed in mid-pregnancy (Edouard *et al.*, 1998; Choi *et al.*, 2002). Further, there is differential adaptation of the vascular beds as some reduce their stiffness (e.g., aorta) while others appear to become stiffer (e.g., carotid) (Mersich *et al.*, 2005). Together with increased vasodilation/ vasodilatory capacity (e.g., flow mediated dilation; FMD) and improved angiogenic status (i.e., increase in vascular growth factors), these are interpreted as improved cardiovascular health during pregnancy (reviewed in Skow *et al.*, 2017). However, little is known about how these vascular changes can be modified (i.e., further enhanced) during a normotensive pregnancy.

Pregnancy is established to be a state of "healthy" sympathetic hyperactivity. However, the increase in muscle sympathetic nerve activity (MSNA) observed in the third trimester (TM3) of a normotensive pregnancy (Reyes *et al.*, 2018a; Hissen & Fu, 2020) rivals that of cardiovascular morbidities characterized by poorer vascular outcomes (e.g., hypertension) in both non-pregnant

(Carthy, 2014) and pregnant (Reyes *et al.*, 2018a) populations. Elevated MSNA concurrent with decreased mean arterial blood pressure (MAP), SVR, and PWV during mid-pregnancy suggest that the effect of nervous system activity on maternal blood vessels (i.e., neurovascular transduction, NVT) is blunted. Indeed, we have recently shown that NVT is reduced in TM3 of normotensive pregnancy compared to the non-pregnant state. In other words, pregnant women in TM3 (who have increased MSNA but similar MAP) increase their blood pressure to a lesser extent following any given burst of MSNA. Similarly, sympathetic baroreflex gain (BRG) is blunted in TM3 compared to the non-pregnant state (Usselman *et al.*, 2015a). This means that for a given change in diastolic blood pressure (DBP) the reflexive change in MSNA is smaller during late pregnancy compared to the non-pregnant state. Although pregnancy is characterized by dynamic changes in cardiovascular function, neither NVT nor BRG have been investigated in TM2 and thus the patterning of these reflexes and their relationship to the cardiovascular changes is unknown.

Functional hemodynamic tests such as the cold pressor test (CPT) or isometric handgrip (IHG) have been utilized in both pregnant and non-pregnant populations to evaluate the responsiveness of the sympathetic nervous and cardiovascular systems to stress (Meah *et al.*, 2018b; Reyes *et al.*, 2018a). An exaggerated blood pressure response to CPT or IHG are predictive for the development of hypertension during pregnancy (Degani *et al.*, 1985b; Woisetschlager *et al.*, 2000; Meah *et al.*, 2018b). The MSNA response to CPT has been shown to be increased in TM3 compared to non-pregnant women (Usselman *et al.*, 2015b). Further, in response to CPT, NVT has been shown to be blunted in healthy pregnant women in TM3 compared to non-pregnant women (Usselman *et al.*, 2015b). To date, only one study has compared the MSNA response during IHG in pregnant women with and without hypertension. They demonstrated an augmented response in the women with hypertension (increase in MSNA burst frequency by 23.6 ± 11.1 and

37.8 ± 12.2 bursts per minute, in healthy pregnant and hypertensive women, respectively) (Greenwood *et al.*, 1998); however, a non-pregnant control group was not included. Together with the sole investigation of MSNA response to IHG in non-pregnant women (Shoemaker *et al.*, 2007), these data suggest that pregnant women in TM3 will have an augmented MSNA response to IHG. Moreover, the direct influence of the muscle metaboreflex (i.e., part of the exercise pressor reflex responsible for increasing MSNA) can be isolated by occluding blood flow to the exercising limb (forearm) during a period of ischemic relaxation (e.g. Mark *et al.*, 1985). The isolated effect of the muscle metaboreflex in pregnancy (i.e., MSNA response during post exercise circulatory occlusion [PECO]) has not been investigated.

Chronic aerobic exercise is associated with a reduction in resting MSNA and improve cardiovascular function in various non-pregnant populations, especially those with sympathetic hyperactivity (Carter & Ray, 2015). The improvements in cardiovascular function (e.g., lower resting blood pressure) are likely mediated through a reduced action of the sympathetic nervous system on the vasculature, resulting in reductions in arterial stiffness (PWV) and SVR (Nardone *et al.*, 2020). In pregnancy, exercise interventions are associated with improvements in cardiovascular health measured by lower resting heart rate and blood pressure, (Cai *et al.*, 2020a) and increases in FMD (Ramírez-Vélez *et al.*, 2011) and angiogenic status (Weissgerber *et al.*, 2010). However, we do not know the role of exercise training on MSNA in pregnancy. Although the finding is not universal (Svedenhag *et al.*, 1984; Sheldahl *et al.*, 1994; Ray, 1999; Laterza *et al.*, 2007; Ray & Carter, 2010), reductions in resting MSNA following aerobic training have been shown in young healthy subjects (Grassi *et al.*, 1994), and populations characterized by sympathetic hyperactivity with (Roveda *et al.*, 2003; de Mello Franco *et al.*, 2006; Fraga *et al.*, 2007; Laterza *et al.*, 2007; Ueno *et al.*, 2009; Antunes-Correa *et al.*, 2010; Antunes-Correa *et al.*,

2012) and without cardiovascular complications (e.g. obesity; Trombetta *et al.*, 2003; Tonacio *et al.*, 2006). Overall, these studies show that resting MSNA is more likely to be reduced in persons with initial hyperactivity. Therefore, pregnancy, which is characterized by sympathetic hyperactivity, may be a condition in which aerobic exercise modifies the sympathetic nervous system regulation of blood pressure.

Exercise training is also known to reduce the reactivity of the sympathetic nervous system and blood pressure during stress (Carter & Ray, 2015). However very little is known on the effects of aerobic exercise training on either CPT or IHG reactivity. There is only one study in healthy males which showed that aerobic exercise training did not alter the MSNA response to CPT (Sheldahl *et al.*, 1994); however, the reactivity of the sympathetic nervous system (i.e., MSNA response) during CPT has not been evaluated following aerobic exercise training in persons with sympathetic hyperactivity (e.g., heart failure) or in women. We know that the CPT response is altered in pregnancy (Usselman *et al.*, 2015b) and that altered blood pressure response to CPT is predictive of gestational hypertension risk (Woisetschlager *et al.*, 2000); therefore, knowing if exercise training can alter the reactivity to CPT in pregnancy will provide important information on how exercise helps reduce hypertension risk in pregnancy. Exercise training has also been shown to reduce the absolute MSNA response during IHG (30% MVC) by 10%, without altering the blood pressure response in women with obesity (Trombetta *et al.*, 2003) suggesting improved NVT with exercise training. In healthy men (i.e., a population with normal basal MSNA), there was no effect of aerobic exercise training on the MSNA response to IHG (Sheldahl *et al.*, 1994). However, we do not know if the reactivity to IHG is altered in pregnancy; as such, this would be a necessary first step in the research process.

Overall, normotensive pregnancy is deemed to be a state of heightened cardiovascular health; however, the mechanisms by which blood pressure is regulated by the sympathetic nervous system, and how they are altered during pregnancy are not well understood. Moreover, whether an intervention known to improve both cardiovascular health and nervous system activity (i.e., exercise) can further impact the presumably positive changes that occur in normotensive pregnancy is necessary. Understanding how the mechanisms of blood pressure regulation can be modified by prenatal aerobic exercise is of critical importance. It will not only provide vital information about the ways in which prenatal aerobic exercise helps reduce the risk of developing hypertension and preeclampsia and may also elucidate mechanisms by which these disorders may occur.

1.1 Purpose and Hypothesis

The overall purpose of my thesis was to determine the effects of acute and chronic exercise on the sympathetic nervous system control of blood pressure at rest and in response to physiological stress (CPT or IHG) during pregnancy. To determine this, I have investigated the responses of the sympathetic nervous system during acute exercise (IHG), and the changes in resting and reflex sympathetic activation (i.e., CPT) in response to an aerobic exercise intervention between TM2 and TM3. Sympathetic nervous system activity (MSNA) was assessed directly using microneurography, and indirectly using measures of blood pressure, blood flow (carotid and femoral artery), and arterial stiffness (PWV). The communication between the sympathetic nervous system and cardiovascular system (i.e., NVT) was assessed at rest and in response to the stressors (IHG and CPT).

The first aim of my thesis was to add to the literature regarding the influence of pregnancy on the sympathetic nervous system reactivity during stress, specifically the response to acute

exercise. Blood pressure and MSNA are increased during exercise through the actions of the exercise pressor reflex (including the muscle metaboreflex, mechanoreflex, and central command). Therefore, I sought to determine whether there were differences in the MSNA response during 2-minutes of IHG (30% maximal voluntary contraction; MVC) and 2-minutes of PECO between pregnant women in TM3 and non-pregnant women.

The second aim of my thesis was to determine whether a structured exercise intervention starting at TM2 (16-20weeks) alters MSNA at rest or in response to a sympathetic stressor (i.e., CPT; a response known to be altered in TM3; Usselman *et al.*, 2015b). To determine whether an exercise intervention influences other basal measures of blood pressure regulation by the sympathetic nervous system, measurements of sympathetic BRG and NVT were determined. This study also measured how the sympathetic nervous system control of blood pressure changes longitudinally between the TM2 and TM3 independent of exercise, thus adding a substantial amount of data on TM2 responses to the literature. Additionally, we determined secondary measures of vascular structure and function including carotid artery distensibility, arterial stiffness (PWV), and blood flow resistance in the superficial femoral artery. Vascular reactivity during CPT was used to identify if the communication between the sympathetic nervous system and the blood vessels (e.g., superficial femoral artery) is altered during stress with respect to increasing gestation or in response to exercise intervention.

1.1.1 Study 1: The effects of pregnancy on blood pressure control during handgrip exercise

The primary aim of this study was to investigate the effect of late pregnancy (TM3) on the muscle metaboreflex activation of the sympathetic nervous system (i.e., MSNA) and blood pressure response during IHG at 30% MVC and PECO.

In line with our previous research using CPT as a sympathetic stressor (Usselman *et al.*, 2015b), I hypothesized that the MSNA response during muscle metaboreflex activation will be increased in TM3 compared to non-pregnant women, but that the blood pressure response will not be different. That is to say, I hypothesized lower neurovascular transduction (NVT) at rest (Steinback *et al.*, 2019), but an unaltered NVT response (ΔMAP/ΔMSNA or ΔSVR/ΔMSNA) in pregnant compared to non-pregnant women.

Inclusion/ exclusion criteria:

Pregnant women in the third trimester (>28 weeks) were included. Non-pregnant women were recruited in their self-reported follicular phase of their menstrual cycle. If non-pregnant women were using contraceptives which eliminated their cycle (e.g. intrauterine device), they were tested at their convenience. All women were over the age of 18 years and free from cardiovascular, respiratory, and metabolic disorders. Moreover, pregnant women were not recruited if they had gestational hypertension, preeclampsia, or gestational diabetes mellitus. If a pregnant woman developed any of the exclusion criteria after her assessment, her data were excluded from the study.

1.1.2 Study 2: Exercise and neurovascular function in pregnancy

The primary aim of this study was to investigate the impact of exercise training during pregnancy on resting MSNA, BRG, NVT, and the sympathetic responsiveness during a CPT.

I hypothesized that aerobic exercise training would be associated with an attenuated rise resting MSNA and the blunting of BRG and NVT at rest. I also hypothesized that during a CPT, the blood pressure and MSNA response would be blunted in the exercise group at TM3 compared to women in the control group.

The secondary aim of this study is to longitudinally assess the impact of pregnancy on resting MSNA, BRG and sympathetic responsiveness during a CPT.

I hypothesized that advancing gestation (TM2 vs TM3) would be associated with an increase in resting MSNA and a decrease in BRG and NVT. Further, I hypothesized that advancing gestation would be associated with augmented MSNA and blood pressure responses during CPT.

Secondary measures for this study included measures of arterial stiffness (carotid distensibility and PWV), blood flow/resistance in the femoral artery, and NVT at rest. Femoral artery blood flow responsiveness was determined during the CPT.

I hypothesized that arterial stiffness would increase from TM2 to TM3, consistent with a recent review indicating a nadir decrease in PWV in the second trimester (Skow *et al.*, 2017). I also hypothesized that PWV would be decreased in women who participated in the structured exercise program compared to controls. I did not expect to see differences in femoral artery blood flow at rest, as I hypothesized an increase in MSNA and decreased NVT across gestation. Further, I hypothesized that women who participated in the exercise intervention would have an increase in NVT compared to women in the control group (i.e., less blunting across gestation).

Inclusion/ exclusion criteria:

Pregnant women less than 20 weeks gestation were recruited. Women were over the age of 18 years and free from cardiovascular, respiratory, and metabolic disorders at the time of inclusion. If a woman developed gestational hypertension or gestational diabetes mellitus, they remained in all aspects of the study (including exercise sessions) unless advised differently by their health care provider. If a woman developed preeclampsia or any other absolute contraindication to exercise (e.g., persistent TM2 and TM3 bleeding) they discontinued exercise but still completed the post-intervention neurovascular assessment.

1.2 Significance

Pregnancy is a time of profound physiological change. To support the demands of the growing fetus, the maternal cardiovascular and nervous systems undergo substantial adaptations. One such adaptation includes a two-fold increase in sympathetic nervous system activity, which is thought to help off-set the rapid blood volume expansion and vasodilatory response to changing hormone levels and maintain arterial blood pressure. During acute exercise (single bouts), blood pressure may increase similarly between pregnant and non-pregnant women (Davenport *et al.*, 2016), but the mechanisms facilitating this change are largely unknown. The muscle metaboreflex is one mechanism by which blood pressure is increased by the sympathetic nervous system during acute exercise and therefore warrants investigation to determine whether the mechanisms responsible for regulating blood pressure control during exercise are modified during pregnancy.

Chronic aerobic exercise during pregnancy has been shown to reduce the odds of developing gestational complications (e.g., preeclampsia) by up to 40%. However, we do not know the mechanisms by which this is occurring in women who have otherwise healthy pregnancies. Importantly, the reduction in odds cannot be explained by traditional risk factors (e.g., gestational weight gain) alone, and therefore the nervous and cardiovascular system have been suggested to play an important role in maintaining a healthy pregnancy. Specifically, exercise in non-pregnant populations has been shown to reduce cardiovascular disease risk through the actions of the sympathetic nervous system on the vasculature (e.g., reducing arterial stiffness and blood pressure). Therefore, understanding how sympathetic nervous system regulation of blood pressure (e.g., sympathetic BRG) and blood vessel mechanics (e.g., arterial stiffness or NVT) is altered in healthy pregnancy, and whether it can be influenced by aerobic exercise is important.

2 The sympathetic muscle metaboreflex is not different in the third trimester in normotensive pregnant women compared to non-pregnant women

2.1 Introduction

Isometric handgrip (IHG) is a safe non-invasive method for determining blood pressure and sympathetic nervous system reflex reactivity that has proven useful at predicting future cardiovascular risk in both pregnant (Meah *et al.*, 2018a) and non-pregnant (Garg *et al.*, 2013) populations. IHG coupled with a post-exercise circulatory occlusion (PECO) further delineates the specific role of the metaboreflex in exercise mediated sympathetic nervous system activation and associated blood pressure response. Specifically, in men, IHG at ≥25% maximal voluntary contraction (MVC) has been shown to elicit a two-fold increase in MSNA and subsequent 25-30% increase in blood pressure (Mark *et al.*, 1985). Importantly, these responses persist during PECO which isolates the specific influence of the metaboreflex.

Healthy pregnant women in their third trimester (TM3) have similar blood pressure responses during aerobic (e.g. Sady *et al.*, 1989; Purdy *et al.*, 2019) and IHG exercise (Greenwood *et al.*, 1998; Shoemaker *et al.*, 2007; Jarvis *et al.*, 2011). However, during healthy pregnancy (i.e., normotensive, euglycemic), blood pressure regulation by the sympathetic nervous system is altered at rest such that in TM3 muscle sympathetic nerve activity (MSNA) is increased (reviewed in Reyes *et al.*, 2018a) and neurovascular transduction (NVT; i.e., the increase in blood pressure resulting from increases in MSNA) is blunted (Steinback *et al.*, 2019). We have previously demonstrated that pregnant women in TM3 require a greater increase in MSNA to achieve the same increase in blood pressure during cold pressor stress (Usselman *et al.*, 2015b). Whether this relationship holds true during an exercise stress is yet to be determined; to date no study has compared the MSNA response during IHG and PECO between pregnant and non-pregnant women.

Understanding the mechanisms governing the blood pressure response to IHG in pregnancy will help to build on the current literature and provide a stepping-stone in the work that is being done addressing the role of the sympathetic nervous system in the exaggerated blood pressure responses observed in women who later develop gestational hypertension or preeclampsia. Therefore, the aim of this study was to determine if the increase in MSNA and blood pressure during IHG and PECO are altered in TM3 of normotensive pregnancy relative to non-pregnant controls. We hypothesize that the MSNA response to IHG and PECO will be increased but the blood pressure response will not be different in pregnant women in TM3.

2.2 Methodology

Thirty-six women (22 pregnant; 14 non-pregnant) were recruited for participation in a cross-sectional study investigating the effects of pregnancy on the sympathetic nervous system responses to IHG (muscle metaboreflex handgrip study). Recruitment occurred at the University of Alberta through convenience sampling of women who contacted the *Program for Pregnancy and Postpartum Health* to participate in research. Briefly, 25 non-pregnant women and 47 pregnant women contacted the *Program for Pregnancy and Postpartum Health* for interest in this study. 14 non-pregnant women consented to participate (no drop-out after consent) and 11 women declined to participate and did not give reasons for this (n= 9, no reply to our initial email). 22 pregnant women consented to participate in the study. Two pregnant women dropped out of the study prior to participation (due to injury and illness) and 20 women participated in the study, the other 25 pregnant women either declined to participate via email (n=8, no reason given) or did not reply (n=17) to our initial email. A sample of some of our screening questions can be found in ***Appendix A, Figure A1***.

Pregnant women were recruited if they were over 18 years of age, had a singleton pregnancy, and had no contraindication to exercise (Mottola *et al.*, 2018). Pregnant women were tested in the third trimester (>28weeks gestation) and were excluded if they developed gestational diabetes mellitus or gestational hypertension. Non-pregnant women were recruited if they were over 18 years of age, were currently not pregnant (at least one year postpartum) and had either 1) regular menstrual cycles not on birth control, or 2) intrauterine devices which ceased menstruation. Women were tested during their self-reported early follicular phase of their menstrual cycle (i.e., during menses) and women with intrauterine devices were tested at their convenience. All women were free from cardiovascular or metabolic conditions (e.g., diabetes, hypertension) and were not taking any medications. One woman was excluded following her assessment as she developed gestational hypertension. All women signed a written informed consent which was approved by the University of Alberta Research Ethics Board (Pro00058560) and conforms to the standards set by the *Declaration of Helsinki*.

2.2.1 Protocol

Women arrived after a 12 hour (overnight) fast where they avoided all food and drink except water. Women were instructed to refrain from having caffeine, over-the-counter medication (e.g. Tylenol), alcohol, or participating in strenuous exercise for at least 12 hours prior to the assessment. Upon arrival, an intravenous catheter was inserted into an antecubital vein on the non-dominant arm and a fasted blood sample was taken to evaluate sex hormones (estrogen, progesterone, and testosterone) and metabolic factors (i.e., fasted blood glucose and insulin). This was done in order to determine if these were related to any of our primary outcome measures (i.e., MSNA response to IHG) Following this, women were fed a standardized breakfast (multigrain

bagel & jam with juice). Following breakfast, the woman's height (cm) using a stadiometer and weight (kg) using a calibrated analog scale were recorded.

Women sat in a semi-reclined (45º) chair in a temperature-controlled room and were instrumented to measure heart rate (ECG; lead II; FE132, ADInstruments, Colorado Springs, USA), blood pressure on the non-dominant arm using finger photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, the Netherlands), oxygen saturation (pulse oximeter, Nellcor Oximax N-600X, Medtronic, USA), and breathing movements (respiratory belt transducer, MLT1132, ADInstruments). Blood pressure measures from the Finometer were calibrated offline using an average of three automated blood pressure measurements (BP 785; Omron Healthcare, Toronto, Canada). Each of systolic, diastolic, and mean arterial pressure (SBP, DBP, and MAP, respectively) were determined on a beat-by-beat basis from the calibrated blood pressure waveform. Cardiac output (derived model flow) was determined using the Finometer and used to determine systemic vascular resistance (Jansen *et al.*, 2001). MVC was be determined using a hand grip dynamometer (MLT004/ST, ADInstruments). Briefly, women were instructed to squeeze the dynamometer maximally with their dominant hand (without performing a Valsalva maneuver) three times. The peak contraction force was set to 100% and used to standardize submaximal hand-grip intensity between participants.

MSNA was recorded directly using microneurography (662C-3; Iowa University Bioengineering, Iowa). Briefly, a tungsten microelectrode (35 mm long, 200 µm in diameter; tapered to a 1-5µm un-insulated tip) was inserted into the Peroneal (Fibular) nerve. A reference needle was inserted just under the skin 1-3cm from the recording site to allow for background noise to be filtered from the signal. A useable signal was determined according to the following criteria: 1) signal-to-noise ratio of 3:1; 2) responsive to an end-expiratory breath hold; 3) not

responsive to loud noise or light touch; 4) pulse synchronicity (Delius *et al.*, 1972). The raw MSNA signal was amplified (100,000x), band-pass filtered (700-2000Hz), rectified and integrated (0.1-s time constant) to obtain a mean voltage neurogram according to standard practice. Raw MSNA data were sampled at 10,000Hz and stored for off-line analysis (Lab Chart; version 8.1.5; ADInstruments).

MSNA was quantified as frequency (BF; bursts per minute), incidence (BI; bursts/ 100 heart beats), amplitude (%), and total MSNA (frequency x amplitude; a.u.). MSNA BI was used to account for differences in resting heart rate between subjects and burst amplitude was used to determine total activity for comparisons to baseline during IHG and PECO. Total activity changes are useful in differentiating increases in amplitude versus frequency in terms of activation of the nervous system, which may be differentially regulated (Kienbaum *et al.*, 2001). Burst amplitude was normalized by setting the mean burst amplitude during the baseline period to 100%. All other burst amplitudes during the protocol were expressed as a percentage compared to this.

Sympathetic baroreflex gain (BRG) was measured to determine the influence of normative fluctuations in resting blood pressure (DBP) on sympathetic activation (i.e., baroreceptor sensitivity). Briefly, BRG was determined from the 15-minute resting period using spontaneous fluctuations in DBP and calculated based on weighted probability slope as we have done previously in pregnancy (Usselman *et al.*, 2015a; Steinback *et al.*, 2019). NVT was determined at rest to determine the relative contribution of the sympathetic nervous system to fluctuations in resting MAP. Briefly, tNVT slopes (gains) were determined by quantifying the peak MAP responses to associated sequences of MSNA bursts. Sequences of MSNA bursts were identified and grouped based on the number of consecutive bursts (i.e., singlets, doublets, triplets, quadruplet or more). Sequences were further binned into quartiles based on the sum of burst amplitudes. This

resulted in 16 bins (4 sequence types x 4 quartiles of total activity). The MAP response to each sequence type across quartiles of burst amplitude (i.e., by total MSNA) was plotted for each individual using a weighted linear regression based on the number of sequences and associated quartile using SPSS (IBM, USA). Linear slopes were compared so long as the weighted regression p-value was <0.05 and the correlation coefficient (R) was >0.3 (i.e. a moderate effect; Cohen, 1992). The decrease in MAP following non-burst sequences were also compared across burst sequence types (i.e., singlets, doublets, triplets, and quadruplets+) as done previously (Steinback *et al.*, 2019).

Following the initial baseline period, women were instrumented with a near-infrared sensor (NIRS) on the forearm of their exercising arm (dominant arm) over the flexor digitorum profundus muscle to determine the nadir deoxygenation (decrease in oxygen saturation) in the working tissue as an indication of exercise stimulus. The sensor was covered with a dark towel to avoid light contamination. Women were then positioned to be able to squeeze the handgrip device with their dominant arm at heart level, and we collected a new three-minute resting baseline. Subsequently, women squeezed the hand grip dynamometer at 30% of their MVC (i.e., an isometric contraction) for two minutes. The intensity and duration were selected to match the previous literature in women (Greenwood *et al.*, 1998; Shoemaker *et al.*, 2007) and the recommendations of the *International Working Group on Maternal Hemodynamics (Meah et al., 2018a)*. During the handgrip, women were aided by a visual representation of their relative handgrip force displayed on a screen in front of them to maintain 30%. Women were encouraged verbally to breathe throughout the IHG contraction and watched (both physically and via the respiratory belt signal in LabChart) to ensure they were not performing a Valsalva maneuver or holding their breath. Five to ten seconds before the end of the IHG contraction, a blood pressure cuff was inflated on their

upper arm to >220mmHg. The participants then relaxed their hand and the cuff remained inflated for two additional minutes (PECO). We obtained a capillary blood lactate sample (Lactate Pro, Cosmed, USA) both at rest and immediately before cuff deflation (last 30s of occlusion) to determine the net change in blood lactate during the exercise and occlusion stimulus. We obtained intravenous blood samples from the previously inserted catheter during the last minute of each baseline, handgrip, and occlusion to determine the catecholamine response (epinephrine and norepinephrine; EPI and NE, respectively). Blood samples were immediately spun down, and plasma was separated and aliquoted into 0.5ml samples and frozen for future analysis. Fasted blood samples were sent to a local commercial laboratory (DynaLIFE, Edmonton, AB, Canada) for the determination of glucose (hexokinase, Seimens Advia 1800), insulin (chemiluminescence microparticle immunoassay, Abbott Architect i2000), estradiol (electrochemiluniscence, Roche Cobas), progesterone (chemilunimescence competitive immunoassay, Siemens Centaur), and testosterone (two-site sandwich chemiluninescence, Siemens Centaur) concentrations. Catecholamines (NE and EPI) were analyzed on-site by a trained technician (ARS) using 2-CAT ELISA according to manufacturers directions (Fast track; Labor Diagnostika Nord, Germany).

Each minute of handgrip and PECO were analyzed individually to determine if any time dependent pattern exists between groups (Incognito *et al.*, 2018). Arterial blood pressure (SBP, DBP, and MAP) were analyzed in a similar fashion (i.e., as an average during each minute during handgrip and PECO). The three minutes of baseline recording immediately preceding the IHG were compared with the handgrip and PECO to determine absolute and relative changes. Blood catecholamine (EPI and NE) concentration responses were compared to baseline values during each IHG and PECO. MVC (N) was compared between groups to determine potential differences

in grip strength, and changes in capillary blood lactate, and deoxygenated blood (via NIRS) were compared to quantify the exercise stimulus.

Following the assessment, women were given an accelerometer (WGT3X-BT; Actigraph, Florida, USA) to objectively measure their level of physical activity for 7 consecutive days. Women were also given a *Pregnancy Physical Activity Questionnaire* (PPAQ) to determine their physical activity profile (including occupational, child-rearing, and sedentary activities; Chasan-Taber *et al.*, 2004). Accelerometry data were analyzed using Actilife analysis software (version 6.13.3). Briefly, accelerometers recorded accelerations over 60-second time intervals (epochs), to assess the intensity and duration of movement behavior (Melanson & Freedson, 1995). Freedson accelerometer count ranges (Freedson *et al.*, 1998a) were used to determine time spent engaging in moderate-to-vigorous physical activity (MVPA; ≥ 1952cpm) behaviors that occurred in bouts of ten-minutes or more. Data was included in the final analysis if total wear time was > 600 minutes per day on at least 4 of the 7 days.

2.2.2 Statistical Analysis

Subject characteristics (e.g., age, height, BMI), resting hemodynamics, resting MSNA (including BRG and NVT), grip strength (MVC; N), MVPA (minutes/week), and the change in blood lactate (mmol/L) and deoxygenated blood (%) were compared using an unpaired t-test. Data was assessed for normal distribution using the Shapiro-Wilk normality test; if data were not normally distributed, the Mann-Whitney test was used to compare results between groups. Repeated measures ANOVA was used to determine group and sequence type differences in the peak and nadir MAP response to burst and non-burst sequences, respectively (Steinback *et al.*, 2019). Two-way repeated measures ANOVA were used to compare the changes in MSNA (BF, BI, and total MSNA), and arterial blood pressure (SBP, DBP, and MAP) during each minute of

handgrip and occlusion between pregnant and non-pregnant women. $p<0.05$ was used to define significance. Post-hoc analysis to determine the time-points where the differences exist were complete using the Sidak's multiple comparisons method. Effect size was determined using https://www.psychometrica.de/effect_size.html (Lenhard & Lenhard, 2016). All statistical analysis was completed using GraphPad Prism (v8.4.3).

2.2.3 a-priori sample size calculation

Our primary hypothesis is that isometric handgrip and PECO will result in an augmented MSNA response in pregnant women. Data from nonpregnant females shows an increase in MSNA from 13 ± 6 to 28 ± 14 bursts per minute (approximately two-fold) during isometric handgrip that will remain increased during PECO (Shoemaker et al. 2007). A separate study investigating isometric handgrip only (no PECO) in the third trimester showed an increase of 23.6 ± 11.1 bursts per minute in normotensive pregnant women. Therefore, in this study burst frequency increased from 21 ± 2.1 to 44.6 bursts per minute. Using this data, I determined that six women will be needed in each group to see differences (see **Appendix F** for calculations). Further, in pregnant women blood pressure responses to both isometric handgrip and cold pressor test are similar (15% increase: Nissel et al 1985). Therefore, our power calculation should be sufficient to see differences in secondary outcomes. Using these data, we estimated six pregnant and six non-pregnant women would be required to observe a significant difference in the change in MSNA during IHG (80% power, $\alpha = 0.05$; G*Power v3.1.9; Faul *et al.*, 2009).

2.3 Results

Data from 19 pregnant and 14 non-pregnant women is included in the analysis here. All data is presented as mean ± SD unless otherwise indicated. **Table 2.1** shows the subject characteristics for all women included in the study. Pregnant women were tested at 32 ± 3 weeks gestation (range: 28-39). Due to the inherent difficulties in obtaining MSNA in pregnancy (e.g. high incidence of pre-syncope; Meah *et al.*, 2019b), we did not need to recruit as many non-pregnant women to obtain adequate measures of MSNA during IHG and PECO in both groups. There were no differences in BMI (pre/non-pregnant values) or resting hemodynamics (heart rate, blood pressure) between the women in whom we obtained MSNA and those whom we did not (see ***Appendix A, Table A1***). Maternal age was slightly higher in pregnant women without MSNA compared to those with MSNA (35 ± 3 and 33 ± 3 years, respectively; p=0.04). Non-pregnant women were tested during day 1-7 of their menstrual cycle (early follicular; n=8), immediately before menstruation (late luteal phase; n=2), or those who had intrauterine devices which ceased menstruation (n=4). Nonetheless, all non-pregnant women were not pregnant at the time of assessment. Briefly, women did not differ in age, non-pregnant BMI, physical activity levels, or parity.

Specifically, in terms of physical activity measured by accelerometry during the week of the assessment, non-pregnant women participated in 13-344 minutes of MVPA per week and pregnant women participated in 0-313 minutes per week. From the accelerometry data, 33% of non-pregnant and 22% of pregnant women met the recommended physical activity guidelines (≥150minutes of MVPA per week; Mottola *et al.*, 2018) the week they wore the accelerometer (see **Table 2.1**). Reported activities for both pregnant and non-pregnant women ranged in intensity from yoga to high intensity interval training (2.5-10.3 METS) and from 30 to >180minutes per week.

Table 2.1 ***Participant characteristics and physical activity measurements***

	Non-Pregnant	Pregnant	p-value
	n=14	n=19	
Participant characteristics			
Age (years)	32 ± 4	33 ± 3	0.28
Gestational age (weeks)	--	32 ± 3	--
Height (cm)	165 ± 6	167 ± 7	0.40
Pre/non-pregnant weight (kg)	68 ± 8	67 ± 15	0.78
Pre/non-pregnant BMI (kg/m^2)	25.2 ± 2.7	24.1 ± 4.8	0.48
Weight at assessment (kg)	68 ± 8	78 ± 15	**0.046**
BMI at assessment (kg/m^2)	25.2 ± 2.7	27.9 ± 5.0	0.07
Nulliparous; n (%)	9 (64)	12 (63)	>0.99
History of gestational hypertension; n (%)	0 (0)	1 (5)	
Physical activity measures (accelerometry)			
Number of assessments*	n=12	n=18	
Sedentary time (% of day)	67 ± 7	69 ± 7	0.55
Light activity (% of day)	29 ± 7	28 ± 7	0.69
MVPA (minutes per week)	126 ± 95	78 ± 107	0.22
Achieving ≥150min/week MVPA; n (%)	4 (33)	4 (22)	0.68
Ethnicity			
Caucasian; n (%)	11 (79)	16 (84)	
Asian; n (%)	2 (14)	2 (11)	0.918
Hispanic; n (%)	1 (7)	1 (5)	
Highest level of education completed**			
High School; n (%)	0 (0)	0 (0)	
College/ Certificate; n (%)	1 (8)	1 (6)	
Bachelors; n (%)	4 (33)	4 (22)	0.419
Masters; n (%)	3 (25)	10 (56)	
PhD or MD; n (%)	4 (33)	3 (17)	

Marital Status**			
Married; n (%)	7 (58)	16 (89)	
Common-Law; n (%)	3 (25)	2 (11)	0.073
Single; n (%)	2 (17)	0 (0)	

Values are mean ± standard deviation unless otherwise specified. BMI, body mass index; MVPA, moderate-to-vigorous physical activity. Physical activity was determined based on measures obtained from accelerometers worn for one week. MVPA was determined as activity occurring in bouts of 10-minutes or longer (Freedson *et al.*, 1998b). *Physical activity data is missing due to women not returning the device (n=2) or corrupt data (n=1). Parity, the number of women achieving ≥150min/week MVPA, and demographics were compared using a chi-squared (Fisher's exact) test. **Two non-pregnant women and one pregnant woman did not answer the questions about marital status or highest education level.

For pregnant women, the average gestation at delivery was 39 ± 1 weeks and the average birth weight was 3566 ± 637g; three women delivered infants >4000g. One woman subsequently developed gestational hypertension and delivered at 33wks. Her data were excluded and are not reported here.

2.3.1 Fasted blood sample analysis

We obtained fasted blood samples in 10 non-pregnant and 18 pregnant women. Details are presented in **Table 2.2.** Briefly, as expected, pregnant women had higher concentrations of estrogen, progesterone, and testosterone. Fasted blood glucose was lower, and insulin was higher in pregnant compared to non-pregnant women.

Table 2.2 ***Blood sample analysis.***

	Non-pregnant	Pregnant	p-value
Fasted blood sample analysis			
Number of women	10	18	
Estrogen (pmol/L)* #	232 ± 197	60,462 ± 21,605	**<0.001**
Progesterone (nmol/L) #	6.2 ± 11.8	433.8 ± 94.2	**<0.001**
Testosterone (pnmol/L)	1.0 ± 0.5	2.0 ± 0.9	**0.002**
Blood glucose (mmol/L)	4.8 ± 0.4	4.2 ± 0.4	**<0.001**
Insulin (pmol/L)	29.8 ± 10.8	50.9 ± 20.3	**0.001**
Resting catecholamine concentrations			
Number of women	n=8	n=13	
Norepinephrine (pg/ml) #	333 ± 203	271 ± 233	0.24
Epinephrine (pg/ml)	41.5 ± 16.3	33.4 ± 22.0	0.44

Fasted blood samples were analyzed commercially via DynaLife Medial Labs (Edmonton, AB). Catecholamine samples were analyzed on site by a trained technician (AS) using a 2-CAT ELISA (fast track; Labor Diagnostika Nord, Germany). Catecholamine samples were not obtained in all women. *Estrogen levels were out of range (too high) in two pregnant women and are not included (n=16); #Estrogen, progesterone, and norepinephrine concentrations were not normally distributed ($p<0.05$; Shapiro Wilk test) and were compared using Mann-Whitney test (on ranks).

2.3.2 Resting hemodynamics and sympathetic nerve activity

Resting hemodynamics and sympathetic nervous system activity for all women in the study are shown in **Table 2.3**. As expected, resting heart rate was increased in pregnant women compared to non-pregnant women (84 ± 12bpm vs 71 ± 7bpm, respectively, p=0.001). SBP, and DBP were not different between pregnant women (p= 0.16, 0.12, respectively), however, resting MAP was slightly lower in pregnant women compared to non-pregnant women (78 ± 7mmHg vs 83 ± 8mmHg, respectively; p=0.04). Similarly, cardiac output was higher in pregnant women (p=0.07, d=0.67) resulting in an expected decrease in SVR (p=0.003).

Table 2.3 *Resting hemodynamics, blood pressure variability, and sympathetic nerve activity in non-pregnant and pregnant women.*

	Non-pregnant	Pregnant	p-value
Resting hemodynamics			
Number of women	n=14	n=19	
Heart rate (bpm)	71 ± 7	84 ± 12	**0.001**
SBP (mmHg)	105 ± 8	100 ± 9	0.16
DBP (mmHg)	69 ± 9	64 ± 7	0.11
MAP (mmHg)	83 ± 8	78± 7	**0.04**
Cardiac output (L/min)	6.1 ± 1.1	7.0 ± 1.5	0.07
Systemic vascular resistance (L/min/mmHg)	13.8 ± 1.9	11.5 ± 2.2	**0.003**
Blood pressure variability*			
SBP SD (mmHg)	3.2 ± 1.3	4.5 ± 1.8	**0.01**
DBP SD (mmHg)	2.5 ± 0.5	2.7 ± 1.0	0.73
MAP SD (mmHg)	2.6 ± 0.8	3.3 ± 1.2	0.20
Beat-by-beat SBP deviation (mmHg)	1.0 ± 0.4	1.2 ± 0.3	0.21
Beat-by-beat DBP deviation (mmHg)	0.8 ± 0.2	1.0 ± 0.8	0.18
Beat-by-beat MAP deviation (mmHg)	0.8 ± 0.3	1.0 ± 0.3	0.06
Resting sympathetic nerve activity**			
Number of women (n)	n=10	n=12	
Burst frequency (burst/minute)	27 ± 10	41 ± 11	**0.005**
Burst incidence (burst/100 heart beats)	38 ± 13	49 ± 15	0.09
Sympathetic BRG (burst probability slope)	-5.4 ± 1.3	-5.1 ± 2.0	0.62
Sympathetic BRG (total SNA slope)	-572 ± 144	-559 ± 248	0.89
MAP transduction slope (ΔMAP/ΔTotal SNA)	0.006 ± 0.003	0.004 ± 0.004	0.32

Values are mean ± standard deviation unless otherwise specified. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; BRG, baroreflex gain; BF, burst frequency; SNA, sympathetic nerve activity. *Data for blood pressure variability was not normally distributed (Shapiro-Wilks) and differences between groups were determined using Mann-Whitney test on ranks. **Mean burst amplitude at baseline was set to 100%, therefore total MSNA was equal to BFx100 and was not compared at rest.

We obtained adequate MSNA signals at rest in 12 pregnant and 10 non-pregnant women. MSNA BF was increased in pregnant compared to non-pregnant women (41 ± 11bursts/min vs 27 ± 10bursts/min, respectively, p=0.005). However, due to greater variability, MSNA BI was not statistically different between groups (38 ± 13 bursts/100heart beats vs 49 ± 15 bursts/100heart beats in non-pregnant and -pregnant women, respectively; p=0.09, d=0.76).

Contrary to previous reports (Usselman *et al.*, 2015a), we did not observe a difference in sympathetic BRG between third trimester pregnant and non-pregnant women (p=0.62). We also did not observe any group differences in the peak MAP response following burst sequences (main effect of group; p=0.955) or the nadir MAP response following non-bursts sequences (main effect of group; p=0.132) as shown in **Figure 2.1 A-B**. The gain (slope) of the transduction responses across quartiles of total SNA were compared in 10 non-pregnant and 10 pregnant women; data from two pregnant women did not meet the criteria for linearity as outlined in the methods. Slopes were determined to be linear ($p<0.001$; linear regression), and with at least a moderate effect ($R>0.3$; $R=0.79 \pm 0.10$ and $R=0.65 \pm 0.21$ for these non-pregnant and pregnant, respectively). MAP transduction slopes were not different between non-pregnant and pregnant women (0.006 ± 0.003mmHg/burst min^{-1} vs 0.004 ± 0.004mmHg/burst min^{-1}, respectively; p=0.32, see **Figure 2.1 C**).

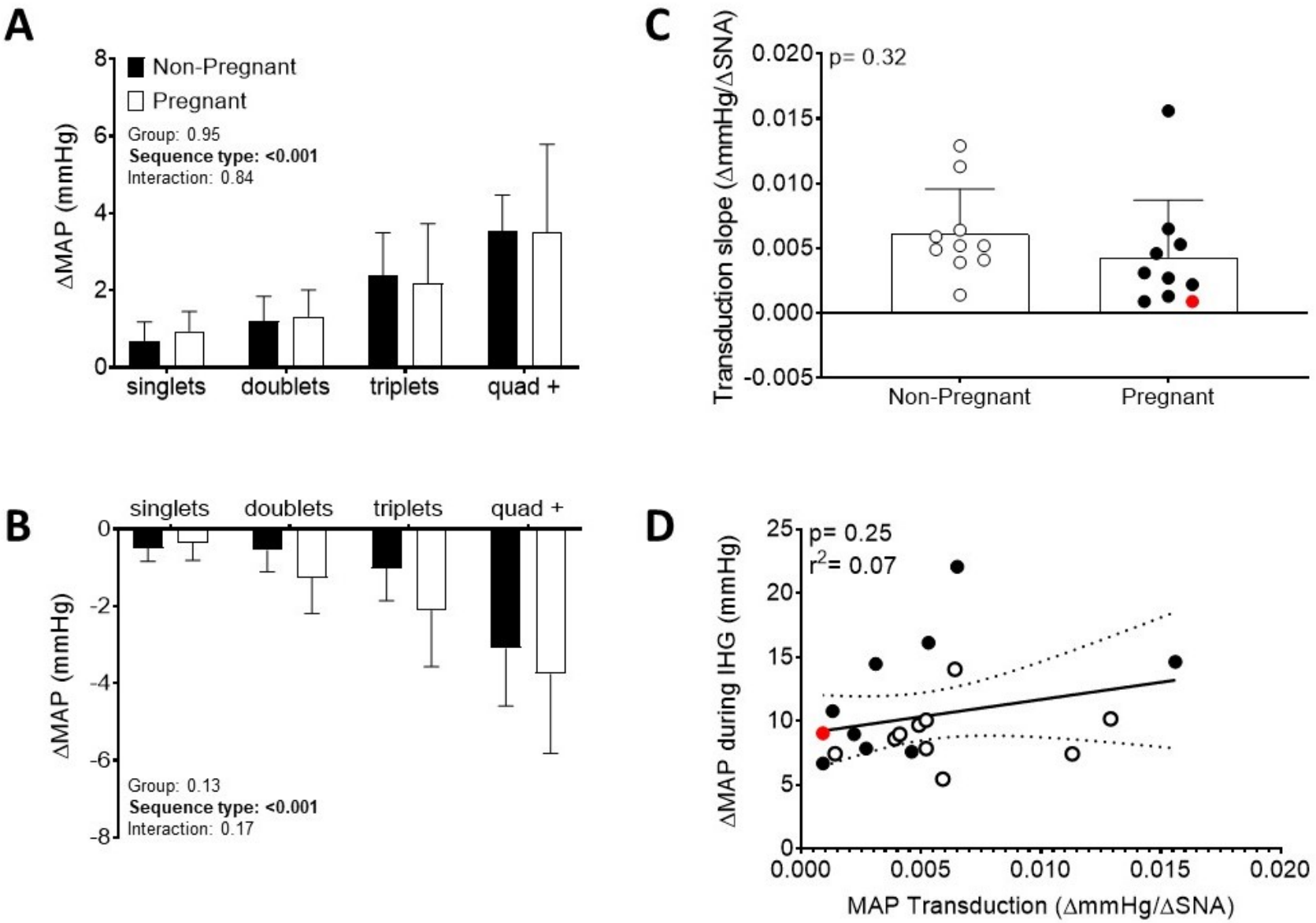

A
Non-Pregnant
Pregnant
Group: 0.95
Sequence type: <0.001
Interaction: 0.84
ΔMAP (mmHg)
8
6
4
2
0
singlets
doublets
triplets
quad +
B
singlets
doublets
triplets
quad +
0
-2
-4
-6
-8
ΔMAP (mmHg)
Group: 0.13
Sequence type: <0.001
Interaction: 0.17
C
p= 0.32
Transduction slope (ΔmmHg/ΔSNA)
0.020
0.015
0.010
0.005
0.000
-0.005
Non-Pregnant
Pregnant
D
p= 0.25
r2= 0.07
ΔMAP during IHG (mmHg)
25
20
15
10
5
0
0.000
0.005
0.010
0.015
0.020
MAP Transduction (ΔmmHg/ΔSNA)

Figure 2.1 Neurovascular transduction. A) increase in MAP during each of the burst sequence types (i.e., singlets, doublets, triplets, quadruplets+). There is no difference between non-pregnant (white bars) and pregnant (black bars) women in the rise in MAP following burst(s) of MSNA. **B)** decrease in MAP during each of the non-burst sequence types. There is no difference between non-pregnant and pregnant women in the decrease in MAP following non burst sequences. **C)** Comparison of neurovascular transduction slopes between non-pregnant (open circle) and pregnant (closed circles) women. The slopes represent the linear relationship between increases in mean arterial blood pressure (MAP) in response to increasing amounts of total sympathetic nerve activity (SNA). There is no difference between pregnant and non-pregnant women in the MAP transduction.; **D)** Correlation between the transduction slope and the MAP reactivity (i.e., peak increase in MAP) during isometric handgrip (IHG). There is no relationship between the MAP transduction slope and the rise in MAP during IHG. One pregnant participant was identified as an outlier in her peak MSNA response during IHG and is identified here in red symbols (see **Figure 2.2 B**).

2.3.3 Responses to isometric handgrip and post-exercise circulatory occlusion

Grip strength (MVC) was not different between groups (362 ± 68N vs 368 ± 84N for non-pregnant and pregnant women, respectively; p=0.84). Similarly, the %MVC achieved during the handgrip protocol was not different between pregnant and non-pregnant women (29.8 ± 1.1% MVC and 29.8 ± 1.1% MVC, respectively; p=0. 39). Lactate samples were successfully obtained in a subset of women (10 non-pregnant, 14 pregnant) and the change in lactate during handgrip and PECO did not differ between groups (+0.9 ± 1.1 mmol/L vs +0.9 ± 1.1 mmol/L respectively, p=0.92). Similarly, nadir deoxygenation within the blood (%; NIRS) was also successfully obtained in a subset of women (7 non-pregnant, 6 pregnant) and was not different between groups (12.5 ± 4.2% and 12.1 ± 6.3% in non-pregnant and pregnant women, respectively; p=0.90).

2.3.3.1 Hemodynamic responses

Hemodynamic responses to IHG and PECO are presented for all women (14 non-pregnant, 19 pregnant)**.** As expected, during IHG heart rate increased (main effect of time, p<0.001), but returned to baseline values during PECO. Heart rate was higher in pregnant women compared to non-pregnant women during baseline and throughout handgrip and PECO (main effect of group, p=0.01). However, there was no difference between groups in the heart rate response to handgrip or PECO (interaction effect, p=0.16). The peak increase in heart rate during IHG was 10 ± 5bpm and 8 ± 6bpm for non-pregnant and pregnant women, respectively (p=0.37). Mean arterial blood pressure (MAP) was increased during handgrip and PECO (main effect of time, p<0.0001), but was not different between groups (p=0.25), and there was no interaction (p=0.58). The peak increase in MAP during IHG was 10 ± 4mmHg vs 10 ± 5mmHg for non-pregnant and pregnant, respectively (p=0.85). SBP and DBP showed similar responses to MAP (increased during handgrip

and PECO, but no difference between groups or interaction; see ***Appendix A, Figure A2***). A representative tracing from one pregnant woman is show in ***Appendix A, Figure A3***.

2.3.3.2 Sympathetic responses

We obtained adequate MSNA signals in 9 non-pregnant and 9 pregnant women during IHG and PECO. MSNA was higher in pregnant women at rest and therefore, the change in MSNA BF, BI and total activity from baseline were compared for each minute of handgrip and PECO. The absolute changes in MSNA BF, BI and total activity are shown in **Figure 2.2, Panel A.** Briefly, MSNA increased during handgrip and remained increased during PECO, but there was no difference in the sympathetic response during the protocol between pregnant and non-pregnant groups (no main effect of group or interaction effect); the same was true if percent changes from baseline were analyzed (see **Figure 2.3**). We also evaluated the participant specific peak change in MSNA during IH and PECO between groups using an unpaired T-test. As shown in **Figure 2.2, panels B&C**, peak BF, BI and total MSNA were not different between groups during IHG or PECO. However, we did observe one negative responder in the pregnant group (i.e., outlier; indicated by red symbols). While we recognize that a negative response is physiologically possible (Incognito *et al.*, 2018), this particular incidence was a statistical outlier. After performing a sensitivity analysis removing this participant, the data suggests that the peak MSNA BF response was greater in pregnant women during IHG ($p=0.04$) but not during PECO ($p=0.24$). The peak change in MSNA BI was still not different between groups during IHG ($p=0.22$) or PECO ($p=0.89$), however, the peak change in total MSNA was higher in pregnant women ($p=0.07$, $d=0.93$ and $p=0.05$, $d=1.0$ during IHG and PECO, respectively). Her responses were within the 95% confidence intervals for all other data/ outcomes.

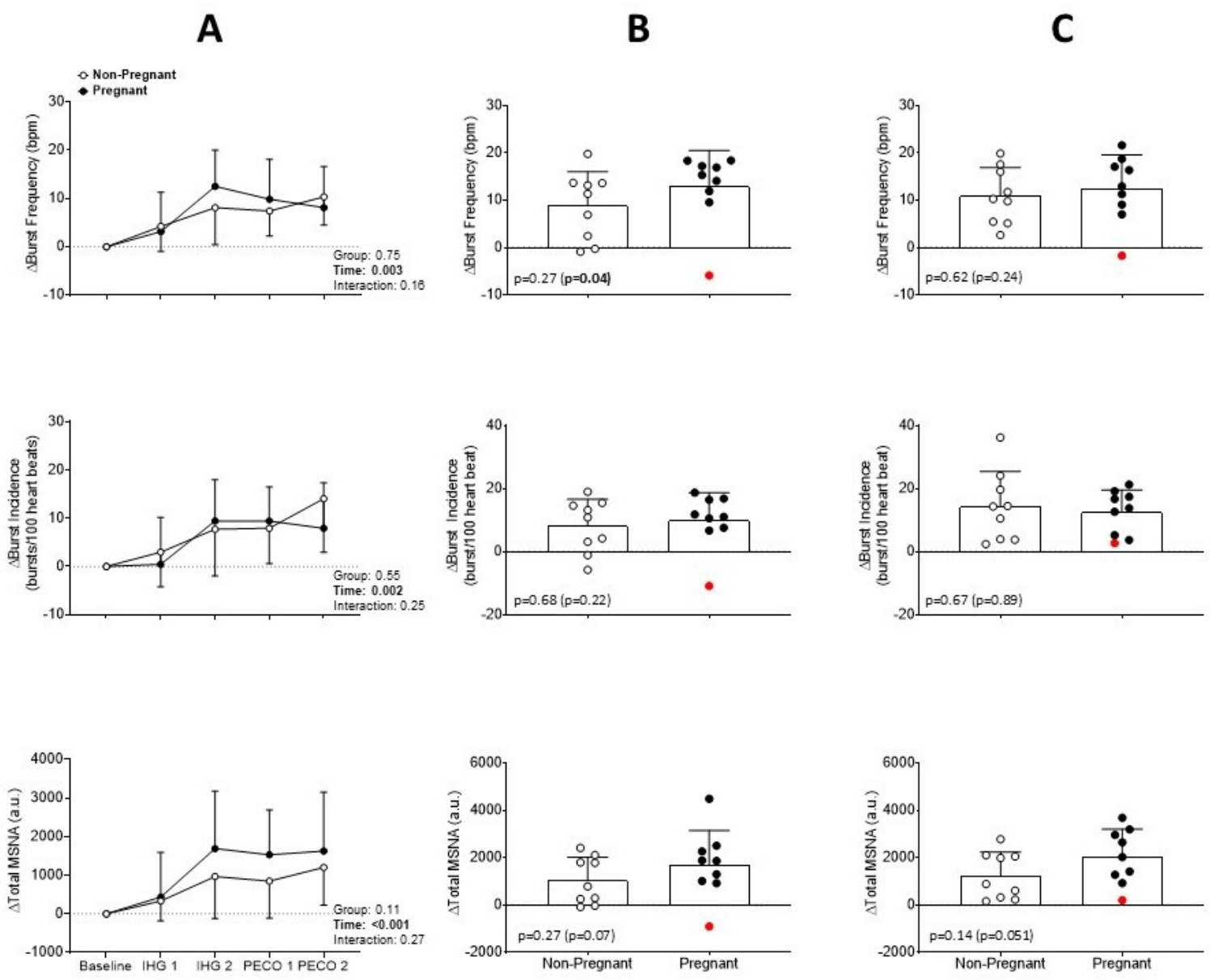

A
B
C
Non-Pregnant
Pregnant
ΔBurst Frequency (bpm)
Group: 0.75
Time: 0.003
Interaction: 0.16
p=0.27 (p=0.04)
p=0.62 (p=0.24)
ΔBurst Incidence (bursts/100 heart beats)
Group: 0.55
Time: 0.002
Interaction: 0.25
ΔBurst Incidence (burst/100 heart beat)
p=0.68 (p=0.22)
p=0.67 (p=0.89)
ΔTotal MSNA (a.u.)
Group: 0.11
Time: <0.001
Interaction: 0.27
Baseline
IHG 1
IHG 2
PECO 1
PECO 2
p=0.27 (p=0.07)
p=0.14 (p=0.051)
Non-Pregnant
Pregnant

Figure 2.2 Muscle sympathetic nerve activity (MSNA) responses during isometric handgrip (IHG) and post-exercise circulatory occlusion (PECO) for non-pregnant (open circles) and pregnant (filled circles) women. **Panel A)** MSNA burst frequency (BF; top), burst incidence (BI; middle) and total MSNA (bottom) responses during each minute of IHG and PECO. Data shown is mean ±SD. Statistics represent 2-way repeated measures ANOVA performed on absolute changes in MSNA during each minute of IHG and PECO (i.e., without using baseline data as they were zero). There is no difference in the sympathetic response to IHG or PECO between pregnant and non-pregnant women (no interaction effect). **Panel B)** Peak change in MSNA BF (top), BI (middle) and total MSNA (bottom) during IHG. Data represents each individual participant (symbols) and group mean (bars). There was no difference between non-pregnant and pregnant women in the peak MSNA response during IHG. One individual was identified as a negative response (significant outlier) and that data is identified in the red symbols. Sensitivity analysis removing her data revealed differences in the peak MSNA BF and total SNA response during IHG (sensitivity analysis p-values reported in parenthesis). **Panel C)** Peak change in MSNA BF (top), BI (middle) and total MSNA (bottom) during PECO. Data represents each individual participant (symbols) and group mean (bars). There was no difference between non-pregnant and pregnant women in the peak MSNA response during PECO. One individual who was identified as a negative response during IHG (significant outlier) is identified in the red symbols here as well. Sensitivity analysis removing her data revealed differences in the peak total SNA response during PECO (sensitivity analysis p-values reported in parenthesis).

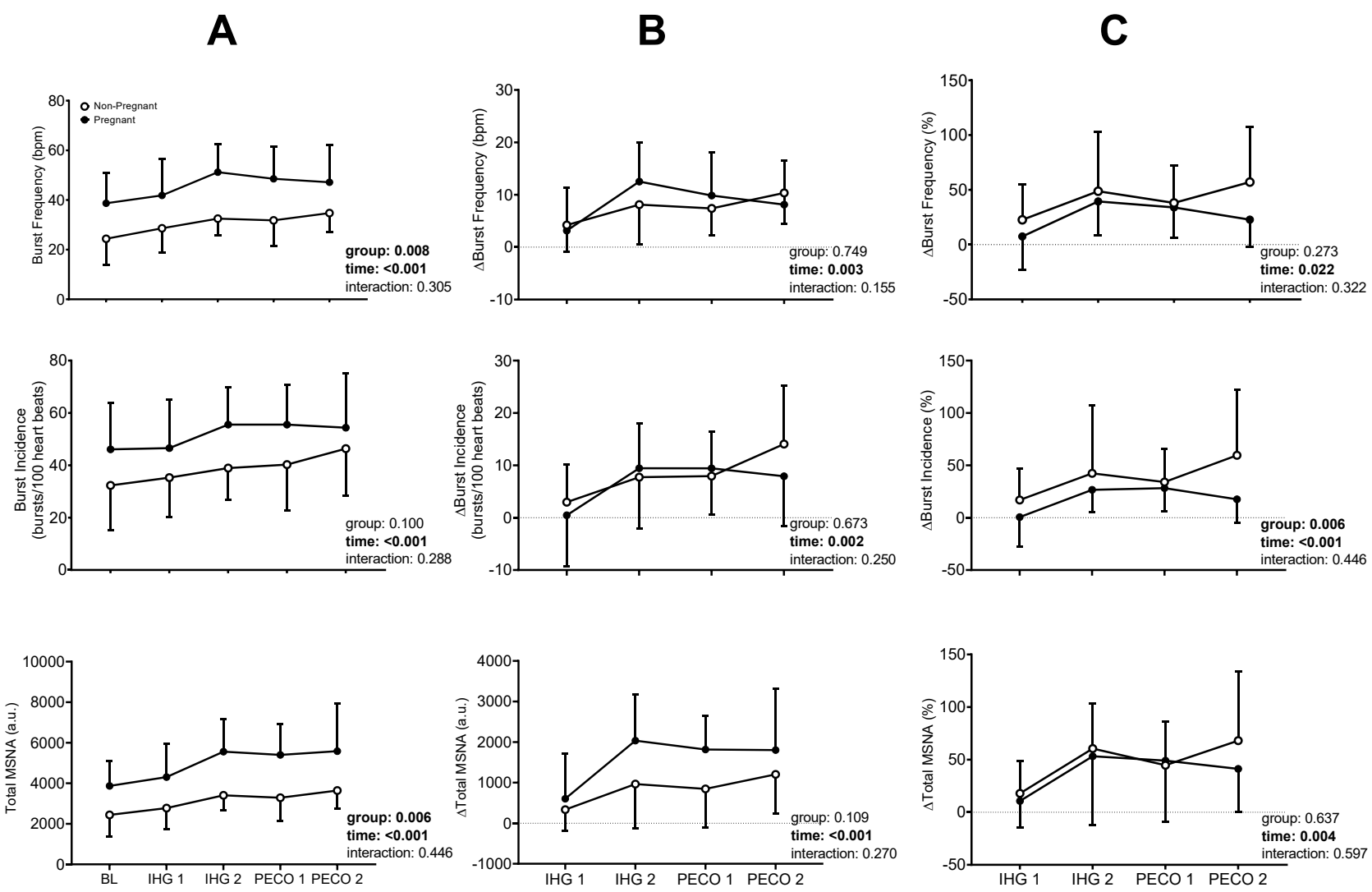

A
B
C
Non-Pregnant
Pregnant
Burst Frequency (bpm)
group: 0.008
time: <0.001
interaction: 0.305
ΔBurst Frequency (bpm)
group: 0.749
time: 0.003
interaction: 0.155
ΔBurst Frequency (%)
group: 0.273
time: 0.022
interaction: 0.322
Burst Incidence (bursts/100 heart beats)
group: 0.100
time: <0.001
interaction: 0.288
ΔBurst Incidence (bursts/100 heart beats)
group: 0.673
time: 0.002
interaction: 0.250
ΔBurst Incidence (%)
group: 0.006
time: <0.001
interaction: 0.446
Total MSNA (a.u.)
group: 0.006
time: <0.001
interaction: 0.446
ΔTotal MSNA (a.u.)
group: 0.109
time: <0.001
interaction: 0.270
ΔTotal MSNA (%)
group: 0.637
time: 0.004
interaction: 0.597
BL
IHG 1
IHG 2
PECO 1
PECO 2

Figure 2.3 Responses of muscle sympathetic nerve activity (MSNA) during isometric handgrip (IHG) and post exercise circulatory occlusion (PECO). Open circles indicate non-pregnant data, closed circles indicate pregnant data. All values are mean and error bars are standard deviation. **A)** Absolute values for MSNA burst frequency (BF; top), burst incidence (BI, middle) and total activity (bottom) at rest and during each minute of IHG and PECO. **B)** Absolute changes (relative to baseline) in MSNA BF (top), BI (middle) and total activity (bottom) during each minute of IHG and PECO. **C)** Percent change (relative to baseline) in MSNA BF (top), BI (middle) and total activity (bottom) during each minute of IHG and PECO. Statistics were performed using a 2-way mixed model (group x time) ANOVA in GraphPad Prism (v 9.0). Significance was set at $p<0.05$ and significant main effects are indicated in bold where applicable. MSNA BF, BI, and total activity increased during IHG and PECO (main effect of time). There were no group x time interactions for MSNA BF, BI, or total activity responses during IHG or PECO.

We evaluated the neurovascular reactivity as the relationship between the change in MSNA (BF and total) and the change in MAP or SVR during the second minute of IHG; these data are shown in **Figure 2.4**. Briefly, the relationship between changing blood pressure and MSNA was not different between non-pregnant and pregnant women ($p=0.71$ and $p=0.87$ for ΔMAP/ΔMSNA BF and ΔMAP/Δtotal MSNA, respectively). Further, the relationship between the changing SVR and MSNA was not different between non-pregnant and pregnant women ($p=0.17$ and $p=0.77$ for ΔSVR/ΔMSNA BF and ΔSVR/Δtotal MSNA, respectively).

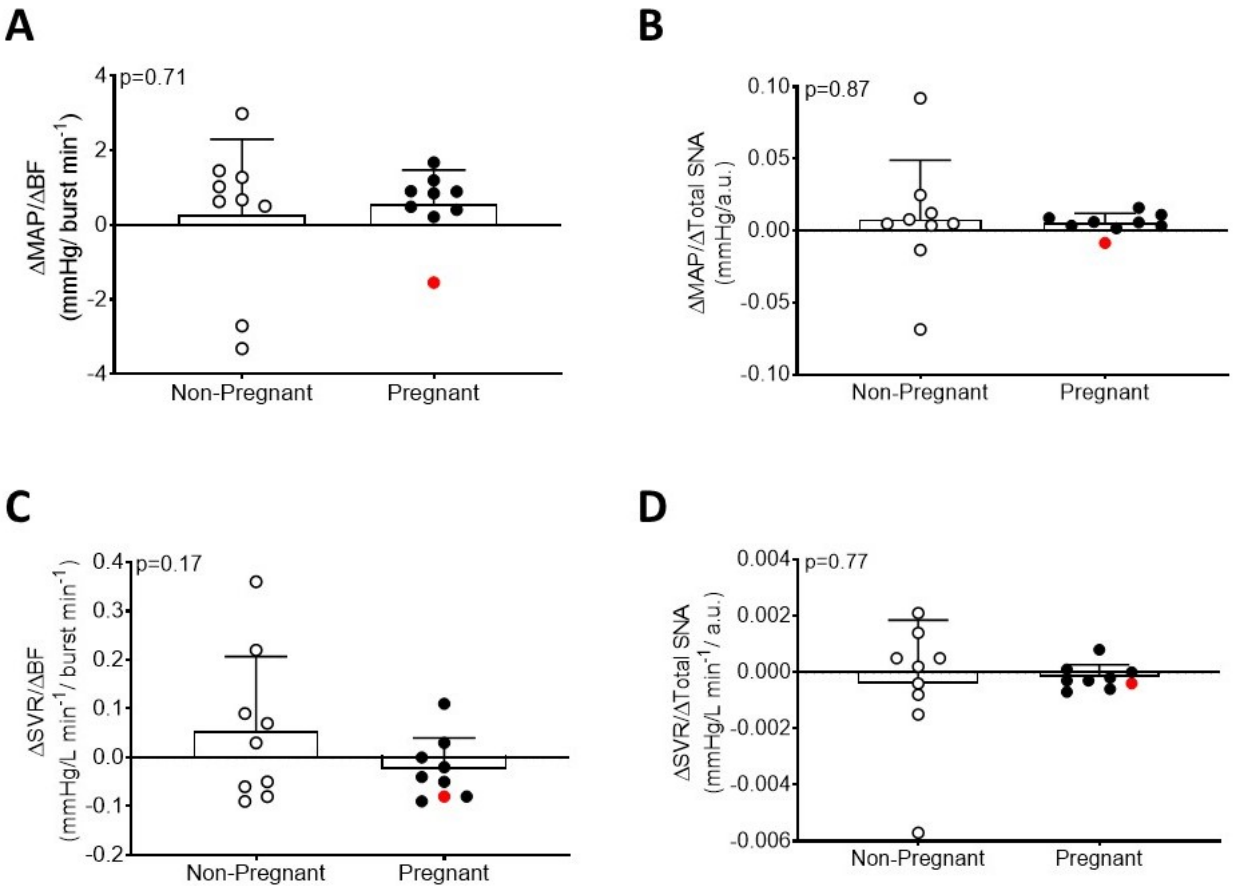


Figure 2.4 Neurovascular reactivity during isometric handgrip (IHG) minute two. A) The change in mean arterial blood pressure (MAP) relative to the change in muscle sympathetic nerve activity (MSNA) burst frequency (BF) during IHG between non-pregnant (open circles) and pregnant (closed circles) women. **B)** The change in MAP relative to change in total MSNA during IHG. **C)** The change in systemic vascular resistance (SVR) and MSNA BF during IHG. **D)** The change in SVR relative to the change in total MSNA during IHG. There are no differences between non-pregnant and pregnant women for the neurovascular reactivity response to IHG. One pregnant participant was identified as an outlier in her peak MSNA response during IHG and is identified here in red symbols (see **Figure 2.2 B**).

2.3.3.3 Catecholamine responses

There were no differences in the concentration of NE or EPI between groups during baseline (see **Table 2.2**). The NE response to IHG and PECO was determined in 8 non-pregnant and 9 pregnant women. Briefly, NE increased by 101 ± 178% in non-pregnant and 138 ± 148% in pregnant women during IHG and remained increased during PECO (p=0.07, main effect of time; d=0.97, large effect), but the increase was not different between non-pregnant and pregnant women (p=0.41, main effect of group) nor were there any group x time interactions (p=0.73 for interaction). The EPI response to IHG and PECO was determined in 7 non-pregnant and 13 pregnant women and demonstrates that EPI was not increased during IHG or PECO (p=0.65, main effect of time). There was a difference between the groups for the EPI response (p=0.09, main effect of group; d=0.88, large effect), however post-hoc multiple comparisons (Holm-Sidak) did not reveal between group differences during either IHG or PECO (p=0.35 and p=0.56, respectively; adjusted p-values). There was no group x time interaction for the EPI response (p=0.73 for interaction).

2.4 Discussion:

Our study demonstrated that sympathetic nervous system activation and control of blood pressure during IHG exercise (30% MVC) and PECO (i.e., muscle metaboreflex activation) is not different between non-pregnant women and normotensive pregnant women in TM3. Specifically, in the pregnancies whereby sympathetic BRG and NVT are maintained, there are not differences between the two groups. This data provides evidence that the sympathetic regulation of blood pressure during exercise is unlike non-exercise stressors (e.g. CPT) in that the relationship between the rise in MSNA and the rise in BP might be maintained during IHG exercise in normotensive pregnancy. However, there is large variability in the MSNA response to IHG as shown here and in the previously published study in pregnant women (Greenwood *et al.*, 1998) and future work is

necessary to elucidate the role of the muscle metaboreflex in the exercise pressor response in both healthy and complicated pregnancies.

2.4.1 Resting hemodynamics and sympathetic nerve activity

Resting prenatal hemodynamics have been extensively reported in the literature. Our data demonstrated the expected increase in resting heart rate and cardiac output, concurrent with decreased SVR such that blood pressure was similar between healthy normotensive late-pregnant and non-pregnant women. We and others have consistently reported increases in MSNA during the third trimester (reviewed in: Reyes *et al.*, 2018a; Hissen & Fu, 2020); here we also show similar increases in basal MSNA BF in TM3 compared to non-pregnant women. While we did not observe a significant difference in MSNA BI between pregnant and non-pregnant women ($p=0.09$), there was a large effect size between the groups ($d=0.76$) suggesting that the pregnant women did tend to have higher MSNA BI as well. Indeed, basal MSNA BI was on average 9 bursts/100 heart beats higher in the pregnant group compared to non-pregnant controls. We recruited women to be similar in age, height, pre-pregnant weight and BMI, and parity between the two groups; although, we have recently shown that these factors have a minimal influence on resting MSNA during pregnancy (Badrov *et al.*, 2020; Reyes *et al.*, 2020a). Nonetheless, there was a large variability in resting MSNA BF and heart rate in both the pregnant and non-pregnant women which may account for the lack of statistical difference in MSNA BI in the present study. We also report increases in resting sex hormones (estrogen, progesterone, and testosterone) and metabolic factors (fasting blood glucose and insulin) within the normal range for the third trimester (Kerlan *et al.*, 1994; Sonagra *et al.*, 2014) and there is no established reason why these increases would impact our resting sympathetic nerve activity results.

Contradictory to our original hypothesis and previous literature (Usselman *et al.*, 2015a), we did not observe blunting of the sympathetic baroreflex in our pregnant compared to non-pregnant women. While BRG during TM3 does not appear to be influenced by maternal age, pre-pregnancy BMI, gestational weight gain, or parity (Badrov *et al.*, 2020; Reyes *et al.*, 2020a), longitudinal assessments of BRG prior to and throughout healthy pregnancies suggest there is a large variation in the pattern of BRG between individuals (Hissen *et al.*, 2017; Reyes *et al.*, 2018b). Nonetheless, the values we obtained for both our pregnant and non-pregnant women fall within established ranges reported for both groups (Usselman *et al.*, 2015a; Hissen *et al.*, 2017; Reyes *et al.*, 2018b; Badrov *et al.*, 2020; Reyes *et al.*, 2020a). As previously mentioned, we did observe a wide range of self-reported and objectively measured physical activity and it is presently unclear if this may influence BRG during pregnancy. Previously, Incognito et al, (2018) showed no difference in BRG between those with the largest sympathetic responses to IHG and those with the smallest (or negative) responses, suggesting that sympathetic BRG does not influence the MSNA response during IHG in men. However, this does not address whether a blunted sympathetic BRG would result in differential reactivity during IHG or PECO. In considering the closed loop control of MSNA, we evaluated the relationship between sympathetic BRG and responses to HG and PECO but did not reveal any relationships between the two (see ***Appendix A, Table A2***).

Contrary to our previous work (Steinback *et al.*, 2019), the present study also did not show any difference between non-pregnant and pregnant women in their NVT. Recent work in our lab indicates that the increase in resting MSNA and blunting of the NVT during gestation in normotensive pregnancy might be influenced by physical activity (Skow *et al.*, 2020). Thus, we obtained objective measures of physical activity in the week immediately following the

assessments using accelerometry. We did not observe any differences between pregnant and non-pregnant women in the amount of MVPA they were performing. In the present study we did have over 20% of pregnant women who were meeting physical activity guidelines throughout their pregnancies (≥150minutes of MVPA per week; Mottola *et al.*, 2018); therefore, it is possible that the variability in physical activity levels may have contributed to the variability observed in the sympathetic nervous system measures. One major limitation of this study is that we did not measure VO_{2peak} as an objective measure of fitness, which has been recently shown to correlate with NVT in males (O'Brien *et al.*, 2020). It is also possible that the lack of observable blunting in neurovascular transduction in pregnant women in this study may have contributed to the lack of difference in the sympathetic response to IHG; however, we did not observe a correlation between the NVT slopes and the increase in MAP during IHG.

2.4.2 Responses to isometric handgrip and post-exercise circulatory occlusion

2.4.2.1 Hemodynamic responses

Here we show that the heart rate and blood pressure responses during two-minutes of IHG at 30% MVC and two-minutes PECO are not different between pregnant women in TM3 and non-pregnant women. The intensity and duration were chosen in line with previous reports of MSNA response during IHG in non-pregnant and pregnant women (Greenwood *et al.*, 1998; Shoemaker *et al.*, 2007). During IHG, MAP increased an average of 10mmHg which is 5-10mmHg lower than the values that have been previously reported in pregnant women (Nisell *et al.*, 1985; Eneroth-Grimfors *et al.*, 1988; Ekholm *et al.*, 1994; Greenwood *et al.*, 1998) and non-pregnant (Zygmunt & Stanczyk, 2010). However, previous investigations used protocols with longer durations (3-minutes; Nisell *et al.*, 1985; Eneroth-Grimfors *et al.*, 1988) or greater intensity (up to 40% MVC; Greenwood *et al.*, 1998) of IHG, which may account for subtle differences in outcomes.

Regardless, we observed that two-minutes at 30% MVC was sufficient to elicit significant change in blood pressure and MSNA, which is consistent with the literature from non-pregnant populations (Mark *et al.*, 1985). Whether higher intensity or longer durations might reveal differences in the pattern of sympathetic activation between pregnant and non-pregnant women is yet to be elucidated. Lastly, we recognize that exaggerated hemodynamic response to IHG in early pregnancy may be indicative of future cardiovascular disease risk (Meah *et al.*, 2018a); future work confirming these responses in the first and second trimester, and between high versus low risk women is necessary to determining the role of the sympathetic nervous system in contributing to the pathogenesis of the hypertensive disorders of pregnancy.

2.4.2.2 Sympathetic responses

Contrary to our hypothesis, here we show no difference in the sympathetic nervous system response to HG and PECO between pregnant women in TM3 and non-pregnant women. This is true if we evaluate the response using a time course (minute-by-minute) or peak reactivity. However, using the peak reactivity method, we identified one pregnant woman who was a significant outlier to the other responses observed. Removing this participant from the peak analysis resulted in differences between pregnant and non-pregnant women in terms of the absolute change in MSNA BF during IHG only (i.e., not during PECO). This alone might suggest that our hypothesis was indeed correct in that a larger sympathetic response would be required by pregnant women to elicit the same blood pressure response during IHG. However, this brings into question whether there are differences in muscle metaboreflex contribution of increasing MSNA during exercise between groups as we did not observe this during PECO. Nevertheless, removing this participant from the time-course analysis did not change the outcome (see ***Appendix A, Table A3***), further supporting that sympathetic reactivity during IHG and PECO is not different between

pregnant and non-pregnant women. Indeed, post-hoc sample size calculations (GPower 3.1) of peak MSNA response during IHG suggest that we would need 43 women per group to detect a difference (Faul *et al.*, 2007) In men, there has been an observed negative-responder / non-responder phenomenon (Incognito *et al.*, 2018), and therefore we believe that recruiting more women would reveal more negative responders in both groups. Indeed, large standard deviations in the peak MSNA response during IHG have been reported previously by Greenwood et al (1998), and would suggest that in both normotensive and hypertensive pregnancies there may be some negative responses.

2.4.2.3 Catecholamine responses

Data from the present study shows that NE, but not EPI, increases during IHG and PECO and is not different between non-pregnant and pregnant women. These data reinforce our findings that the sympathetic nerve activity reactivity is not different between groups. Nissel and colleagues (1985) measured NE and EPI during 3-minutes of handgrip in pregnant and postpartum (i.e., non-pregnant) women and showed that both catecholamines increased during IHG. Specifically, they found an ~30% increase in NE by the third minute of IHG that was not different between pregnant and postpartum assessments. Here we show an ~100% increase in NE during IHG and PECO in pregnant and non-pregnant women, however there was large variability in the responses. Unfortunately, we did not have measures of blood volume in our participants, which is known to increase in pregnancy and would influence measures of catecholamine concentrations within plasma (de Haas *et al.*, 2017). However, we used a within subject repeated measures design to compare responses and evaluated both absolute and relative (i.e., percent) changes in catecholamines during IHG and PECO and consistently show no difference between groups.

2.4.3 Future Directions

This study evaluated the IHG and PECO responses during TM3 compared to non-pregnant women. We hypothesized that normotensive pregnant women in their third trimester would experience an augmented increase in MSNA to achieve the same pressure response during IHG, and although the results from the present cohort suggest no differences, we must acknowledge that these particular pregnant women also do not exhibit blunting in sympathetic BRG or sympathetic NVT as discussed previously. We know that pregnant women meeting the recommended guideline for physical activity throughout pregnancy (≥150minutes MVPA per week; Mottola *et al.*, 2018) have improvements in their cardiovagal BRG (Sobierajski *et al.*, 2018) and endothelial function (measured by flow mediated dilation; Reyes *et al.*, 2020b). From this, we might expect that physical activity levels would influence other aspects of blood pressure regulation including that of the sympathetic nervous system. In total, there is a lot of work to be done in this area to fill in literature gaps that will help us explain blood pressure regulation in healthy and disordered pregnancies. Further studies including more women, in different trimesters, with different fitness levels, from different ethnicities, or in different risk categories (e.g., high vs low risk for hypertension) may help shed light on who may or may not have altered blood pressure regulation during exercise. Additionally, whether different exercise modes, intensities, or durations may impact the response is yet to be determined.

Sympathetic activation during exercise redistributes blood flow towards working muscles therefore we would expect vasoconstriction and increased resistance in non-exercising conduit and resistance arteries during IHG and PECO (reviewed in: Fadel, 2015). Following that NVT is typically blunted in pregnancy (Steinback *et al.*, 2019), it is postulated that there are alterations in neurotransmitter release or reuptake, or adrenergic receptor sensitivity and density in healthy pregnancy. There is no evidence to date suggesting that vascular adrenergic receptor density is

altered outside of the uterine artery, and thus is unlikely to play a large role in mediating the exercise pressor response in pregnancy. Alpha-adrenergic sensitivity, however, may be decreased during pregnancy as previously demonstrated in both humans (Landau *et al.*, 2002) and ewes (McLaughlin *et al.*, 1989). Thus, functional sympatholysis during exercise may be altered in pregnant women. One limitation of the current study is that we did not have measures of blood flow in exercising or non-exercising limbs to determine the contribution of the rise in MSNA to the changes in regional blood flow.

2.4.4 Perspectives

IHG and other functional hemodynamic tests (e.g., cold pressor test) have been utilized during mid pregnancy for their potential to detect gestational hypertension risk (Degani *et al.*, 1985a; Woisetschlager *et al.*, 2000; Meah *et al.*, 2018a). To date, only the work by Degani et al, (1985a) has used IHG to predict hypertension in pregnancy with 81% sensitivity and 86.5% specificity; however, they used 50% MVC for three minutes in women who were 28-32 weeks pregnant. Therefore, future studies evaluating IHG or other functional tests in early-to-mid-pregnancy are needed to understand if the mechanisms resulting in the rise in blood pressure (e,g, the sympathetic component) are altered in women who develop hypertension. Women who have already been diagnosed with gestational hypertension also do not appear to have different blood pressure or sympathetic responses to IHG (Nisell *et al.*, 1985; Eneroth-Grimfors *et al.*, 1988; Greenwood *et al.*, 1998) reinforcing our results showing that this reflex is less susceptible to change throughout gestation. However, to date only one study has evaluated the differences in the MSNA response to IHG between women with and without gestational hypertension (Greenwood *et al.*, 1998). Although non-significant, their data suggest that gestational hypertension may be associated with higher sympathetic reactivity during IHG as they observed increases in MSNA BF

during IHG of +23.6 ± 11.1 vs. +37.8 ±12.2 in normotensive and gestational hypertension, respectively (data is shown as mean ± SEM). The variability in the aforementioned study is incredibly high (standard deviation in the MSNA BF response was determined to be 40.0 and 40.4 in normotensive pregnant and gestational hypertension, respectively) which highlights the need for more research in this area to confirm their findings.

In conclusion, the data presented in this study indicate that in healthy, normotensive, euglycemic pregnant women in their third trimester, the muscle metaboreflex regulation of sympathetic nerve activity and blood pressure during pregnancy does not appear to be altered. We acknowledge that there is large variability in resting blood pressure regulation in the third trimester (i.e., BRG and NVT) which may have contributed to our findings, but nonetheless the data here demonstrated consistency in a maintenance of sympathetic coupling to cardiovascular responses in this cohort. These data are the first to show that compared to other sympathetic stressors (e.g., CPT), the communication between the sympathetic nervous system and blood pressure is maintained during IHG exercise in pregnant compared to non-pregnant women.

2.5 References

Badrov MB, Yoo JK, Steinback CD, Davenport MH & Fu Q. (2020). Influence of multiparity on sympathetic nerve activity during normal pregnancy. American journal of physiology Heart and circulatory physiology 318, H816-h819.

Chasan-Taber L, Schmidt MD, Roberts DE, Hosmer D, Markenson G & Freedson PS. (2004). Development and validation of a Pregnancy Physical Activity Questionnaire. Med Sci Sports Exerc 36, 1750-1760.

Cohen J. (1992). A power primer. Psychological bulletin 112, 155-159.

de Haas S, Ghossein-Doha C, van Kuijk SM, van Drongelen J & Spaanderman ME. (2017). Physiological adaptation of maternal plasma volume during pregnancy: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 49, 177-187.

Degani S, Abinader E, Eibschitz I, Oettinger M, Shapiro I & Sharf M. (1985). Isometric exercise test for predicting gestational hypertension. Obstet Gynecol 65, 652-654.

Delius W, Hagbarth KE, Hongell A & Wallin BG. (1972). General characteristics of sympathetic activity in human muscle nerves. Acta Physiologica Scandinavica 84, 65-81.

Ekholm E, Erkkola R & Hartiala J. (1994). Comparison of cardiovascular reflex tests and blood pressure measurement in prediction of pregnancy-induced hypertension. Eur J Obstet Gynecol Reprod Biol 54, 37-41.

Eneroth-Grimfors E, Bevegård S & Nilsson BA. (1988). Evaluation of three simple physiologic tests as predictors of pregnancy-induced hypertension. A pilot study. Acta Obstet Gynecol Scand 67, 109-113.

Fadel PJ. (2015). Reflex control of the circulation during exercise. Scandinavian Journal of Medicine & Science in Sports 25 Suppl 4, 74-82.

Faul F, Erdfelder E, Lang AG & Buchner A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods 39, 175-191.

Freedson PS, Melanson E & Sirard J. (1998). Calibration of the Computer Science and Applications, Inc. accelerometer. Medicine and science in sports and exercise 30, 777-781.

Garg R, Malhotra V, Dhar U & Tripathi Y. (2013). The isometric handgrip exercise as a test for unmasking hypertension in the offsprings of hypertensive parents. Journal of clinical and diagnostic research: JCDR 7, 996-999.

Greenwood JP, Stoker JB, Walker JJ & Mary DA. (1998). Sympathetic nerve discharge in normal pregnancy and pregnancy-induced hypertension. Journal of hypertension 16, 617-624.

Hissen SL, El Sayed K, Macefield VG, Brown R & Taylor CE. (2017). Muscle sympathetic nerve activity peaks in the first trimester in healthy pregnancy: a longitudinal case study. Clinical autonomic research : official journal of the Clinical Autonomic Research Society 27, 401-406.

Hissen SL & Fu Q. (2020). Neural control of blood pressure during pregnancy in humans. Clin Auton Res.

Incognito AV, Doherty CJ, Lee JB, Burns MJ & Millar PJ. (2018). Interindividual variability in muscle sympathetic responses to static handgrip in young men: evidence for sympathetic responder types? American journal of physiologyRegulatory, integrative and comparative physiology 314, R121.

Jansen JR, Schreuder JJ, Mulier JP, Smith NT, Settels JJ & Wesseling KH. (2001). A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. British journal of anaesthesia 87, 212-222.

Jarvis SS, VanGundy TB, Galbreath MM, Shibata S, Okazaki K, Reelick MF, Levine BD & Fu Q. (2011). Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans. American journal of physiology Regulatory, integrative and comparative physiology 301, R193-200.

Kienbaum P, Karlssonn T, Sverrisdottir YB, Elam M & Wallin BG. (2001). Two sites for modulation of human sympathetic activity by arterial baroreceptors? *J Physiol* **531,** 861-869.

Kerlan V, Nahoul K, Le Martelot MT & Bercovici JP. (1994). Longitudinal study of maternal plasma bioavailable testosterone and androstanediol glucuronide levels during pregnancy. Clinical endocrinology 40, 263-267.

Landau R, Dishy V, Wood AJ, Stein CM & Smiley RM. (2002). Disproportionate decrease in alpha- compared with beta-adrenergic sensitivity in the dorsal hand vein in pregnancy favors vasodilation. Circulation 106, 1116-1120.

Lenhard W & Lenhard A. (2016). Calculation of Effect Sizes. Dettelbach (Germany).

Mark AL, Victor RG, Nerhed C & Wallin BG. (1985). Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. Circulation research 57, 461-469.

McLaughlin MK, Keve TM & Cooke R. (1989). Vascular catecholamine sensitivity during pregnancy in the ewe. Am J Obstet Gynecol 160, 47-53.

Meah VL, Backx K & Davenport MH. (2018). Functional hemodynamic testing in pregnancy: recommendations of the International Working Group on Maternal Hemodynamics. Ultrasound Obstet Gynecol 51, 331-340.

Meah VL, Busch SA, Jones KE, Davenport MH & Steinback CD. (2019). A review of acute responses, after-effects and chronic complications related to microneurography. Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology 130, 1781-1788.

Melanson EL & Freedson PS. (1995). Validity of the Computer Science and Applications, Inc. (CSA) activity monitor. Medicine and science in sports and exercise 27, 934-940.

Mottola MF, Davenport MH, Ruchat SM, Davies GA, Poitras VJ, Gray CE, Jaramillo Garcia A, Barrowman N, Adamo KB, Duggan M, Barakat R, Chilibeck P, Fleming K, Forte M, Korolnek J, Nagpal T, Slater LG, Stirling D & Zehr L. (2018). 2019 Canadian guideline for physical activity throughout pregnancy. British journal of sports medicine 52, 1339-1346.

Nisell H, Hjemdahl P, Linde B & Lunell NO. (1985). Sympatho-adrenal and cardiovascular reactivity in pregnancy-induced hypertension. I. Responses to isometric exercise and a cold pressor test. British journal of obstetrics and gynaecology 92, 722-731.

O'Brien MW, Ramsay D, Johnston W & Kimmerly DS. (2020). Aerobic fitness and sympathetic responses to spontaneous muscle sympathetic nerve activity in young males. Clin Auton Res.

Purdy GM, James MA, Wakefield PK, Skow RJ, Van Diepen S, May LE, Davenport MH & Steinback CD. (2019). Maternal cardioautonomic responses during and following exercise throughout pregnancy. Appl Physiol Nutr Metab 44, 263-270.

Reyes LM, Badrov MB, Fu Q, Steinback CD & Davenport MH. (2020a). Age, body mass index and weight gain do not increase sympathetic activity during pregnancy. Appl Physiol Nutr Metab.

Reyes LM, Farooq SM, Skow RJ, Busch SA, Pyke KE, Khurana R, Chari RS, Stickland MK, Devolin M, Davidge ST, Sobierajski F, Lugg A, Steinback CD & Davenport MH. (2020b). Physical Activity in Pregnancy Is Associated with Increased Flow-mediated Dilation. Med Sci Sports Exerc 52, 801-809.

Reyes LM, Usselman CW, Davenport MH & Steinback CD. (2018a). Sympathetic Nervous System Regulation in Human Normotensive and Hypertensive Pregnancies. Hypertension (Dallas, Tex: 1979).

Reyes LM, Usselman CW, Skow RJ, Charkoudian N, Staab JS, Davenport MH & Steinback CD. (2018b). Sympathetic neurovascular regulation during pregnancy: A longitudinal case series study. Experimental physiology 103, 318-323.

Sady SP, Carpenter MW, Thompson PD, Sady MA, Haydon B & Coustan DR. (1989). Cardiovascular response to cycle exercise during and after pregnancy. Journal of applied physiology (Bethesda, Md: 1985) 66, 336-341.

Shoemaker JK, Mattar L, Kerbeci P, Trotter S, Arbeille P & Hughson RL. (2007). WISE 2005: stroke volume changes contribute to the pressor response during ischemic handgrip exercise in women. Journal of applied physiology (Bethesda, Md: 1985) 103, 228-233.

Skow RJ, Fraser G, Steinback CD & Davenport MH. (2020). Prenatal Exercise and Cardiovascular Health (PEACH) Study: Impact on Muscle Sympathetic Nerve (Re)activity. Med Sci Sports Exerc.

Sobierajski FM, Purdy GM, Usselman CW, Skow RJ, James MA, Chari RS, Khurana R, Stickland MK, Davidge ST, Devolin M, Steinback CD & Davenport MH. (2018). Maternal Physical Activity Is Associated With Improved Blood Pressure Regulation During Late Pregnancy. The Canadian journal of cardiology 34, 485-491.

Sonagra AD, Biradar SM, K D & Murthy DSJ. (2014). Normal pregnancy- a state of insulin resistance. Journal of clinical and diagnostic research: JCDR 8, Cc01-03.

Steinback CD, Fraser GM, Usselman CW, Reyes LM, Julian CG, Stickland MK, Chari RS, Khurana R, Davidge ST & Davenport MH. (2019). Blunted sympathetic neurovascular transduction during normotensive pregnancy. J Physiol 597, 3687-3696.

Usselman CW, Skow RJ, Matenchuk BA, Chari RS, Julian CG, Stickland MK, Davenport MH & Steinback CD. (2015a). Sympathetic baroreflex gain in normotensive pregnant women. Journal of applied physiology (Bethesda, Md: 1985) 119, 468-474.

Usselman CW, Wakefield PK, Skow RJ, Stickland MK, Chari RS, Julian CG, Steinback CD & Davenport MH. (2015b). Regulation of sympathetic nerve activity during the cold pressor test in normotensive pregnant and nonpregnant women. Hypertension (Dallas, Tex: 1979) 66, 858-864.

Woisetschlager C, Waldenhofer U, Bur A, Herkner H, Kiss H, Binder M, Laggner AN & Hirschl MM. (2000). Increased blood pressure response to the cold pressor test in pregnant women developing pre-eclampsia. Journal of hypertension 18, 399-403.

Zygmunt A & Stanczyk J. (2010). Methods of evaluation of autonomic nervous system function. Archives of medical science: AMS 6, 11-18.

3 Prenatal exercise and cardiovascular health (PEACH) study: impact of aerobic exercise training on muscle sympathetic nerve (re)activity

3.1 Introduction

Blood pressure regulation during pregnancy is multifaceted and involves coordinated interactions between the sympathetic nervous system and vasculature. Despite rapid and progressive blood volume expansion (de Haas *et al.*, 2017) and increases in cardiac output (Meah *et al.*, 2016), arterial vasodilation and an increase in muscle sympathetic nervous system activity (MSNA) act to maintain normal blood pressure (Sanghavi & Rutherford, 2014; Reyes *et al.*, 2018a; Hissen & Fu, 2020). Evidence shows that MSNA is elevated as early as 6 weeks gestation (Jarvis *et al.*, 2012; Okada *et al.*, 2015; Hissen *et al.*, 2017) with greater sympathetic activation in the third trimester (TM3) approaching 200% of non-pregnant values (Reyes *et al.*, 2018a). Combined with minimal or no differences in resting blood pressure in the third trimester (Meah *et al.*, 2016; Green *et al.*, 2020), these indicate a shift in blood pressure regulation throughout healthy pregnancy. Indeed, we have shown that sympathetic baroreflex gain (BRG; changes in MNSA in response to fluctuations in blood pressure) and neurovascular transduction (NVT; changes in blood pressure resulting from fluctuations in MSNA) are both blunted in TM3 compared to non-pregnant controls (Usselman *et al.*, 2015a; Steinback *et al.*, 2019). Further, the sympathetic nervous system response to stress (e.g. cold pressor test; CPT) is either not changed (Schobel *et al.*, 1996), or augmented (Usselman *et al.*, 2015b; Reyes *et al.*, 2018b) in TM3 in healthy pregnant women.

Conversely, maladaptation of sympathetic cardiovascular control, specifically an augmented response to sympathetic stress during pregnancy, may be an important determinant of hypertensive disorders of pregnancy (Degani *et al.*, 1985b; Woisetschlager *et al.*, 2000). Coupled with heightened basal MSNA prior to (Badrov *et al.*, 2019) and after the development of

gestational hypertension (Reyes *et al.*, 2018a), this would suggest that NVT is further blunted in women who later develop hypertension in their pregnancies. This highlights a unique period (i.e., before 20 weeks) during which the impairments in blood pressure regulation could be influenced in order to prevent hypertensive disorders of pregnancy. Understanding how to effectively intervene to prevent the development of hypertensive disorders of pregnancy is still elusive, however prenatal exercise has been shown to reduce the odds of developing hypertensive disorders of pregnancy by 40% . The mechanisms by which exercise confers its benefits in pregnancy are unclear; however, exercise consistently demonstrates a reduction in both resting and reflex reactivity in non-pregnant populations who have sympathetic hyperactivity (e.g. hypertension; Carter & Ray, 2015). Together, these data indicate plasticity of the sympathetic nervous system which may be modified by aerobic exercise; however, this has not been evaluated in pregnancy. Determining the effects of exercise intervention on sympathetic blood pressure regulation otherwise healthy pregnant women is an important first step in this line of inquiry.

The primary aim of this study was to determine the influence of a prenatal exercise intervention on basal MSNA, BRG, and NVT in normotensive pregnant women between TM2 and TM3. Secondary aims of this study were to determine the effects of a prenatal exercise intervention on the MSNA and blood pressure responses to CPT. We hypothesized that an exercise intervention would attenuate the rise in resting MSNA and blood pressure, and the blunting in sympathetic BRG and NVT that occurs between the second and third trimester. We further hypothesized that women who were randomized to the exercise group would have blunted MSNA and blood pressure responses to CPT compared to non-exercising controls.

3.2 Methodology

This study was approved by the University of Alberta Research Ethics Board (Pro00061045) and conforms to the standards set by the latest version of the *Declaration of Helsinki*. Out of the 230 pregnant women who contacted the *Program for Pregnancy and Postpartum Health for information regarding this study,* one hundred women attended an in-person screening and information session regarding participation in a randomized controlled trial investigating the effects of aerobic exercise on cardiovascular health (Prenatal Exercise and Cardiovascular Health [PEACH] study, NCT02948439), and 64 consented to participate in the study; reasons for not participating/exclusion are given in **Figure 3.1.**

Prior to participation, all participants provided written, informed consent and their maternity health care provider (obstetrician, mid-wife etc.) signed the *PARmed-X for Pregnancy* (Wolfe & Mottola, 2015a). Women were included if they were over 18 years of age, had a singleton pregnancy, and were without contraindication to exercise as outlined in the *PARmed-X for Pregnancy* (Wolfe & Mottola, 2015a). Potential participants were excluded if they engaged in more than 60-minutes of moderate-to-vigorous physical activity (MVPA) per week (self-report at initial meeting), or had a pre-existing cardiovascular, respiratory, or nervous system disorder (e.g. hypertension). A sample of the screening questions related to eligibility can be found in Appendix A, Figure A1.

Assessments of neurovascular health were conducted at baseline (16-20 weeks gestation) and repeated post-intervention (34-36 weeks gestation). At each time point, women were given a tri-axial accelerometer (WGT3X-BT; Actigraph, Florida, USA) to measure their level of activity for 7 consecutive days. Additionally, at the mid-point of the study (26-28 weeks) women in both groups wore the accelerometer for a 7-day period. Following completion of baseline assessments, women received an opaque envelope with their allocation (exercise or control group). Prior to the

start of the study, an individual not associated with the study pre-filled sequentially numbered envelopes with group allocation using an online randomizer (www.sealedenvelope.com/simple-randomiser/v1) which randomized in blocks of four to eight.

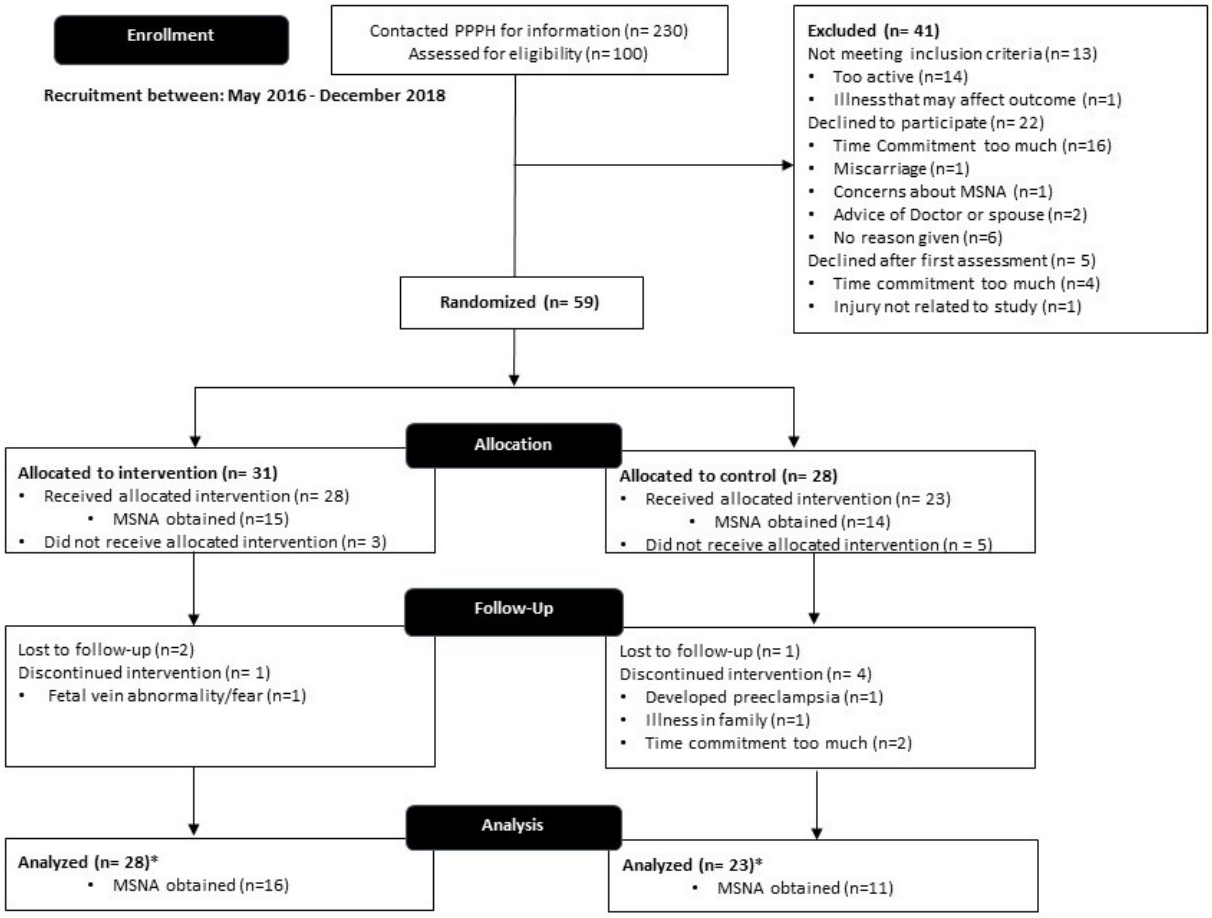


Figure 3.1 CONSORT diagram outlining flow of participants through the study. Randomization occurred after baseline neurovascular assessment was complete. PPPH, *Program for Pregnancy and Postpartum Health* www.exerciseandpregnancy.ca, MSNA, muscle sympathetic nerve activity. *These values represent the number of women who completed both assessments. Analysis was complete as intent-to-treat and included all women who were randomized (including dropouts). Specific n for analysis varies by outcome and is reported wherever it is less than the group n.

3.2.1 Neurovascular Assessment

Women arrived after a 12 hour (overnight) fast, during which they were instructed to avoid all food and drink, except water (including caffeine, alcohol, vitamins, and over the counter pain medications). Women were provided a standard breakfast after arrival in the lab. Women were also asked to refrain from doing any exercise for at least 12 hours before their assessment. Following breakfast, the woman's height (cm; stadiometer) and weight (kg; calibrated scale) were measured. Women then sat in a semi-reclined chair in a temperature-controlled room and were instrumented to measure heart rate (ECG; lead II; ADInstruments, Colorado Springs, USA), blood pressure using finger photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, the Netherlands), oxygen saturation (pulse oximeter, Nellcor Oximax N-600X, Medtronic, USA). Cardiac output (Q; derived model flow) was determined using the Finometer and used to calculated systemic vascular resistance (SVR) as MAP/Q * 80 (dynes/L·min^{-1}).

Following instrumentation, MSNA was recorded from the peroneal (fibular) nerve using microneurography (662C-3; University of Iowa Bioengineering, Iowa). Briefly, a small, tungsten microelectrode (35 mm long, 200 μm in diameter; tapered to a 1-5μm un-insulated tip) was inserted just under the skin below the knee. A second reference electrode was inserted subcutaneously 1-3cm away to allow for background noise to be filtered out of the signal. Adequate MSNA signals were determined according to standard criteria (Delius *et al.*, 1972). The raw MSNA signal will be amplified (100,000x), band-pass filtered (700-2000Hz), rectified and integrated (0.1-s time constant) to obtain a mean voltage neurogram according to standard practice. Raw MSNA data was be sampled at 10,000 Hz and stored for off-line analysis (Lab Chart).

Once the MSNA signal was found we performed three automated blood pressure measurements (BP785; Omron Healthcare, Toronto, Canada) to calibrate the Finometer followed by a 10-minute baseline. The baseline was followed by a three-minute CPT (submerging hand up

to the wrist in ice water; 4ºC). During this protocol, heart rate, arterial blood pressure, and MSNA were continuously recorded. Following CPT, women's hands were re-warmed using a heating pad. All variables were exported into Lab Chart 8 via Powerlab 16/35 data acquisition system (PL3516, ADInstruments) and stored for offline analysis.

3.2.2 Exercise Intervention

Women randomized to the exercise intervention were prescribed aerobic exercise three to four times per week with a target heart rate of 50-70% HRR as per the *2003 Canadian Clinical Practice Guideline* for exercise during pregnancy (ACOG, 2015). The first week began with 25 minutes (5-minute warmup, 15 minutes at target heart rate, and 5-minute cool-down) and increased in duration by 2 minutes per week until 40 minutes per session is achieved (i.e. 30 minutes at target heart rate; Ruchat *et al.*, 2012). The intervention started at 18-21 weeks gestation and continued until 33-36 weeks gestation. Women were required to attend a minimum of one supervised exercise session per week and were given logbooks to track their unsupervised sessions. Exercise modality included any aerobic exercise (e.g. treadmill, stationary bicycle, elliptical). Every session began with 5 minutes of quiet, seated rest where heart rate and RPE were recorded. Heart rate and RPE were recorded twice during the exercise sessions (at approximately 1/3 and 2/3 of the duration). Immediately following the cool down, women sat for 5 minutes and all baseline measures were repeated.

3.2.3 Data analysis

All data was de-identified prior to analysis (i.e., blinded to the person doing the analysis) to decrease the risk of bias. Hemodynamic data were collected during the last five minutes of quiet rest. Bursts of MSNA were detected using a semi-automated peak detection algorithm (Chart 8.1.3; ADInstruments) and bursts were confirmed by a trained observer (RS) based on a pulse-

synchronous pattern which was observed in both the raw and integrated neurograms. MSNA was expressed as integrated burst frequency (BF; bursts/min), burst incidence (BI; bursts/100 cardiac cycles), and total activity (a.u.). MSNA BI was used to account for differences in resting heart rate between subjects and burst amplitude was used to determine total activity for comparisons to baseline during CPT. Burst amplitude was normalized by setting the mean burst amplitude during the baseline period to 100%. All other burst amplitude measures during the protocol were expressed as a percentage compared to this.

Spontaneous weighted sympathetic BRG was determined as previously described (Usselman *et al.*, 2015a) and confirmed by two trained observers (RS and CDS). Briefly, the BRG was determined during quiet rest as the slope of the linear relationship between the probability of bursts occurring (BI) at any given diastolic blood pressure. Diastolic blood pressure was determined in 2mmHg bins, and burst probability was the percentage of heart beats with bursts occurring/ those without bursts. To correct for differences in the number of data points at each diastolic bin, we performed weighted regressions using SPSS (v26; IBM, USA). BRG was also determined using total activity (amplitude x incidence) as the dependent variable.

For NVT, MSNA and hemodynamic variables were extracted on a beat-by-beat basis during the resting period (duration was 10.4 ± 3.5 minutes), saved to Excel spreadsheets and subsequently analyzed using custom software written in MATLAB (The MathWorks, Natick, Massachusetts) as previously described (Steinback *et al.*, 2019). Briefly, burst sequences were determined as sequence of heart beats with burst(s) of activity occurring preceded by one heartbeat without a burst and proceeded by one heartbeat without a burst. Non-bursts sequences represent the opposite (sequences of heartbeats without MSNA) and give information about the vascular changes in the absence of neural activity. The magnitude of change in mean arterial blood pressure

(MAP) was determined following both burst and non-burst sequences according to the sequence type (e.g. singlets, doublets, triplets, quadruplet+). NVT slopes were determined using a weighted linear regression (SPSS v26; IBM) comparing the increase in MAP to the total MSNA defined according to the mean sum of burst amplitudes within a sequence type which was subsequently separated by quartiles (i.e., 16 data points of increasing total activity). For these, the average amplitude of the lowest quartile within the singlet sequence type was set to 100, and all other quartiles/ sequences were scaled according to this (i.e.,, increasing in total activity). Slopes were deemed adequate if the linear regression was significant ($p<0.05$) and $r>0.5$ (intermediate and large effect size) (Cohen, 1992). CPT reactivity was determined as the increase in MSNA from baseline to the maximal point during the CPT. This was determined as the one-minute segment with the greatest change in MSNA during CPT (Usselman *et al.*, 2015b). Accelerometry was analyzed using Actigraph (LLC, Pensacola, Fl) by determining the amount of activity occurring in the moderate-to-vigorous intensity category if it occurred for at least 10-minutes (Freedson *et al.*, 1998b). The total time in these 10-minute bouts (but not all MVPA) were averaged over a 7-day period (i.e., per week) and compared between groups at each time-point.

3.2.4 Statistical Analysis

For all outcome variables we performed intent-to-treat analysis including all women who were randomized. The number of women included in each outcome analysis is reported when it is less than the full group n. All data is reported as mean ± standard deviation, unless otherwise specified. All statistical analysis was performed using GraphPad Prism (v8.4.3). 2-way (group x gestational age) mixed effects ANOVA were used to assess statistical differences for resting heart rate, blood pressure, basal MSNA, sympathetic BRG, and NVT slopes. This allowed us to determine if there was a main effect of group (i.e., that control and exercise groups were different from one another),

main effect of gestational age (i.e., that there are differences from pre-to-post intervention or between TM2 and TM3), or an interaction effect (i.e., that exercise modified the response across gestation). Post-hoc Sidak's multiple comparison test was used to determine between group differences at each time-point where applicable. The change in MAP for each burst and non-burst sequence type (i.e., singlets, doublets, triplets, and quadruplets+) were compared using a 3-way mixed effects ANOVA in GraphPad Prism to compare differences in NVT between sequence type, gestational age, and group; the group x gestational age interaction was also determined from this. Pearson correlations between the change in NVT slopes and the change in resting MSNA BF were completed using GraphPad Prism for all women who had adequate measures at each time-point (control, n=7; exercise, n=11). CPT reactivity analysis was performed using a 3-way mixed effects ANOVA in GraphPad Prism including time (i.e., minute of CPT), gestational age (i.e., pre-post intervention), and group (control vs. exercise) as factors; the group x gestational age interaction was also determined from this. CPT reactivity was further assessed using blood pressure and MSNA data from the peak 1-minute bin and compared using 2-way mixed effects ANOVA in GraphPad Prism. $p<0.05$ was considered statistically significant for all outcome variables. Where $p\geq0.05$ but less than 0.1, effect size was determined and reported according to https://www.psychometrica.de/effect_size.html; $d>0.8$ (large effect size) was interpreted to be a meaningful difference regardless of statistical significance (Cohen, 1992; Lenhard & Lenhard, 2016).

3.2.5 a-priori sample size calculation

My primary hypothesis is that exercise training will reduce resting MSNA (burst/min) in pregnant women. Data from healthy (mostly male) and clinical populations (with elevated resting MSNA) indicates an ~18% decrease in resting MSNA with aerobic exercise training (Carter & Ray, 2015). Data from all published cohorts in the third trimester of pregnancy suggests that burst frequency be ~28 ± 7. Based on these values, we expect exercise training to reduce resting SNA to ~23 ± 5.7 bursts/min (-18% reduction). Using these data, we estimate 21 pregnant women are required in each group to observe a significant reduction in SNA following exercise training (80% power, $\alpha = 0.05$; G*Power v3.16; Faul *et al.*, 2009). Further, MSNA is shown to increase 50-150% in the third trimester compared to non-pregnant values and is not different during the second trimester compared to non-pregnant values (Reyes *et al.*, 2018a). On this basis, we would expect resting MSNA to increase at least 50% from the second to third trimester. Therefore, the sample size calculated based on exercise training differences should be sufficient to detect the differences across gestation. I expected a ~60% success rate for obtaining an appropriate MSNA signal and an 80% retention rate, requiring 44 women to be recruited into the training arm of the study. To allow for additional variability and/or decreased retention I planned to recruit 50 pregnant women to take part in the exercise training and 50 pregnant women to take part as controls. We performed an interim analysis once we had half of the data (i.e., 11 women minimum per time point with MSNA) and found our primary outcome to be significantly affected, therefore we discontinued enrollment at that time.

3.3 Results

A detailed consort diagram is shown in **Figure 3.1**. Briefly, 59 women completed baseline assessments and were randomized into control (n=28) or exercise conditions (n=31). Similar numbers of women dropped out of either group; 51 women completed both assessments for the

study (n=23 control, n=28 exercise). Five women in the control group and three women in the exercise group dropped out prior to their 34-36week assessment. Reasons for drop out are shown in **Figure 3.1**, and none were specifically related to the intervention. All women delivered healthy babies (i.e., no fetal complications).

3.3.1 Participant demographics

Participant demographics (except maternal height), parity, and pre-pregnancy physical activity were not different between groups at the beginning of the study (see **Table 3.1**). Briefly, women were in their thirties (78%), were non-obese before becoming pregnant (88%), and approximately half had other children in the home (57%). Women in this study were predominantly Caucasian (76%), have post-secondary education (95%), and have a partner living at home (90%). One woman in the exercise group had gestational hypertension in a previous pregnancy. There were no differences between the groups with respect to any of the demographics at enrollment.

Women in the exercise group were enrolled in the intervention for an average of 14±1weeks (range: 11-16weeks). Twenty-four out of 28 women (86%) who completed the study in the exercise group were compliant (completed at least 75% of the prescribed exercise). Compliance to the exercise intervention was set at achieving at least 75% of prescribed exercise sessions. Using this criteria, four women out of 28 who completed the exercise intervention were non-compliant. It is important to note that only three women out of the 24 compliant women fell below 80% compliance. No woman was achieving more than 40 minutes of activity on four days per week (upper limit of the prescription).

Compliance to the control condition was determined using accelerometry at three time points (pre-intervention, 16-20weeks; mid-intervention, 26-28 weeks; post-intervention, 34-36 weeks).

Women were deemed to be non-compliant to the control condition if they participated in at least 20 minutes of moderate-to-vigorous activity (MVPA) on at least 3 days of the week during each week measured (i.e., >60minutes MVPA per week). Using these criteria, two women in the control group were determined to be non-compliant (i.e., 91% compliance).

Regardless, intention-to-treat analysis including data from all women who were randomized was complete for the outcomes from this study. Reasons for drop out are indicated in the consort diagram (see **Figure 3.1**) and are not related to the intervention per say. However, it should be noted that the same results are present when considering only those women who were compliant to and completed the intervention (see ***Appendix B, Tables B1 and B2, and Figures B1 and B2)***. At the mid-point (26-28weeks), data from accelerometry shows that women in the control group had decreased their MVPA an average of 41 ± 69 minutes while women in the exercise group increased their MVPA an average of 21 ± 86 minutes (p=0.011) over pre-intervention levels.

Table 3.1 *Participant demographics, parity, and pre-pregnancy physical activity levels.*

	CONTROL	EXERCISE	p-value
Participant demographics, mean (SD)			
Number of women	28	31	
Age (years)	32 (4)	31 (2)	0.251
Height (cm)	168 (6)	165 (8)	**0.034**
Pre-pregnancy weight (kg)	70 (14)	73 (25)	0.536
Pre-pregnancy BMI (kg/m^2)	24.6 (4.8)	26.8 (7.8)	0.215
Parity, n (%)			
Number of women	27	31	0.974
Nulliparous	12 (44)	13 (42)	
Primiparous	8 (30)	10 (32)	
Multiparous	7 (26)	8 (26)	
Pre-pregnancy physical activity levels, n (%)			
Number of women	25	31	0.311
Sedentary	8 (32)	10 (32)	
Moderately active	9 (36)	6 (19)	
Active	8 (32)	15 (48)	
Ethnicity			
Caucasian; n (%)	22 (79)	23 (74)	0.621
Asian; n (%)	2 (7)	3 (10)	
Black; n (%)	0 (0)	1 (3)	
Hispanic; n (%)	2 (7)	1 (3)	
Middle Eastern; n (%)	1 (3.5)	0 (0)	
Aboriginal/Metis; n (%)	0 (0)	2 (6)	
Mixed race*; n (%)	1 (3.5)	1 (3)	
Highest level of education complete**			
High School; n (%)	1 (4)	2 (6)	0.544
College/ Certificate; n (%)	3 (12)	4 (12)	

Bachelors; n (%)	11 (42)	18 (58)	
Masters; n (%)	8 (31)	4 (12)	
PhD or MD; n (%)	3 (12)	3 (10)	
Marital status**			
Married; n (%)	20 (77)	26 (84)	0.792
Common-Law; n (%)	4 (15)	3 (10)	
Divorced; n (%)	1 (4)	0 (0)	
Single; n (%)	1 (4)	2 (6)	

Data is presented as mean (SD) for anthropometric data and as n (%) for categorical data (parity and pre-pregnancy physical activity levels). BMI, body mass index. Pre-pregnancy data were determined from self-report at 16-20 weeks gestation. Pre-pregnancy physical activity category was determined using the Godin score (Godin, 2011). Statistical analysis was performed using GraphPad Prism (v8.4.3). Anthropometric data was compared between groups using an unpaired t-test. Parity, pre-pregnancy physical activity, and demographic data were compared using Chi-squared test. *Two women described their ethnicity as mixed (Control, Hispanic / Caucasian; Exercise, Chinese / German). **Two women in the control group did not report their marital status or highest level of education.

3.3.2 Resting hemodynamics

All values (mean +/- SD) for resting hemodynamics are presented in **Table 3.2**. As expected, resting heart rate increased with gestation (main effect of gestational age, $p<0.0001$); however, this increase was attenuated in the exercise group ($p=0.002$, interaction effect). Resting blood pressure (SBP, DBP, and MAP) was not different between groups at any time point. MAP and DBP were shown to increase (main effect of gestational age, $p<0.0001$), but SBP was not different between TM2 and TM3 (main effect of gestational age, $p=0.248$). Contrary to our hypothesis, there was no interaction between gestational age and group (i.e., no effect of intervention) on resting blood pressure (interaction effect $p=0.241$, 0.175, 0.149 for SBP, DBP, and MAP, respectively). All women, at all assessments, were normotensive. There were no differences between groups for resting Q (main effect of group, $p=0.508$) and there was a intermediate effect ($d=0.565$) for an increase in Q during the study (main effect of gestational age, $p=0.055$), however there was no interaction between group and gestational age ($p=0.959$). SVR was not different across gestation ($p=0.541$, main effect of gestational age) or between groups (main effect of group, $p=0.114$) and there was no interaction between group and gestational age ($p=0.494$).

Table 3.2 *Summary of data for all basal hemodynamic variables and sympathetic baroreflex gain.*

	Control Group				**Exercise Group**				**P-values**		
	Pre-intervention		Post-intervention		Pre-intervention		Post-intervention				
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	Group	Gestational Age	Interaction
Gestational Age (wks)	28	18 (1)	23	35 (2)	31	18 (2)	28	34 (1)	n/a		
Heart rate (bpm)	28	76 (7)	23	88 (11)	31	82 (8)	28	86 (8)	0.238	**<0.0001**	**0.002**
SBP (mmHg)	28	108 (11)	23	111 (12)	31	110 (11)	28	110 (9)	0.739	0.248	0.241
DBP (mmHg)	28	61 (7)	23	68 (7)	31	65 (7)	28	62 (8)	0.160	**<0.0001**	0.175
MAP (mmHg)	28	79 (8)	23	85 (7)	31	83 (7)	28	86 (8)	0.222	**<0.0001**	0.149
Q (L/min)	28	7.8 (1.8)	22	8.2 (1.8)	31	7.5 (1.6)	28	7.8 (1.6)	0.508	0.055	0.959
SVR (mmHg/L/min)	28	837 (133)	22	859 (151)	31	909 (172)	28	910 (156)	0.114	0.541	0.494
BRG (probability slope)	14	-5.29 (2.54)	11	-5.17 (1.82)	15	-5.51 (1.99)	16	-4.83 (2.13)	0.429	0.369	0.762
BRG (Total MSNA slope)	14	-506 (218)	11	-552 (185)	15	-581 (258)	16	-530 (302)	0.902	0.966	0.461

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; Q, cardiac output; SVR, systemic vascular resistance; BRG, baroreflex gain; MSNA, muscle sympathetic nerve activity. Statistics determined as an intent-to-treat analysis using mixed-effects ANOVA in GraphPad Prism (v8.4.3).

3.3.3 Basal Sympathetic Activity

We successfully obtained 56 adequate MSNA recordings from women in this study (out of a total of 109 assessments). The women in whom we obtained MSNA were not different in maternal age, height, pre-pregnancy weight or BMI compared to women who did not have MSNA recordings (see ***Appendix C, Table C1***). Data is presented as intent-to-treat analysis including all available assessments (i.e., all women who were randomized). A representative trace of data during baseline is shown in ***Appendix C, Figure C1 (top)***.

The changes in basal MSNA during the intervention are shown in **Figure 3.2**. Briefly, there was a main effect of gestational age such that MSNA BF and BI were increased in TM3 compared to TM2 (main effect of gestational age; $p=0.002$ and $p<0.001$, respectively). There was no main effect of group for MSNA BF or BI ($p=0.097$ and $p=0.197$, respectively). However, Sidak's multiple comparisons post-hoc test revealed that the groups were different at the pre-intervention timepoint for both BF and BI ($p=0.009$, and $p=0.027$, respectively), such that women in the exercise group had higher resting MSNA than the control group pre-intervention but were not different post-intervention ($p=0.999$ and $p=0.996$, respectively). In line with our hypothesis, we did observe an interaction between group and gestational age such that women randomized to the exercise group had smaller increases in MSNA BF and BI during the intervention period (i.e., from TM2 to TM3) compared to the control group (interaction effect; $p=0.010$ and $p=0.002$, respectively). Baseline burst amplitude was set to 100% for all women (see **Data analysis**), therefore neither resting burst amplitude or total MSNA (amplitude x BF; a.u.) were compared.

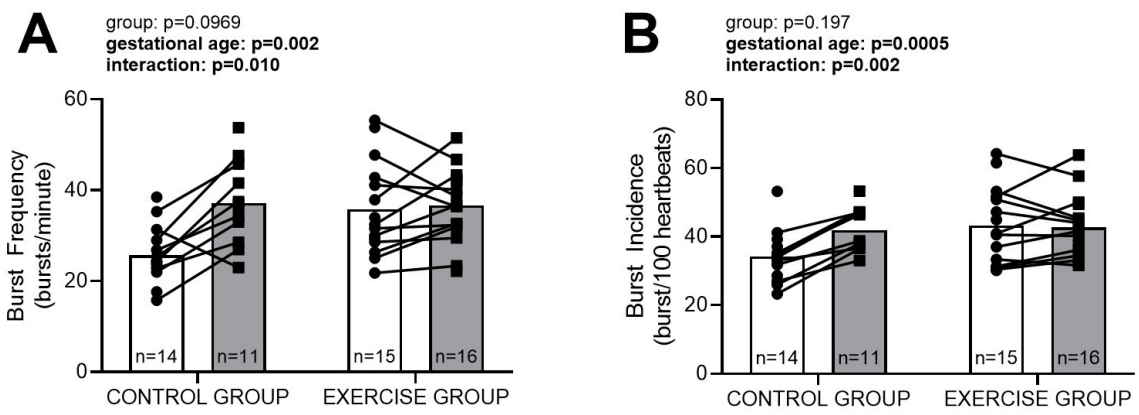


Figure 3.2 Basal muscle sympathetic nerve activity (MSNA). Pre-intervention data (trimester 2) is shown in white bars and post-intervention data (trimester 3) is shown in grey bars. Individual data is presented with lines connecting women in whom we have obtained MSNA signal at both time pints (n=9 control group, n=12 exercise group); the n is shown for each group/time-point within the bars. A) Basal MSNA burst frequency (BF). The rise in MSNA BF across gestation is attenuated in women in the exercise group. B) Basal MSNA burst incidence (BI). The rise in MSNA BI across gestation is attenuated in women in the exercise group.

Contrary to our hypothesis, sympathetic BRG, assessed as weighted probability slope (Usselman *et al.*, 2015a) was not different across gestation (main effect of gestational age, p=0.300) or between groups (main effect of group, p=0.258), nor was there a group x gestational age interaction (i.e., effect of the exercise intervention; p=0.691, interaction effect). The same was true if sympathetic BRG was assessed using total MSNA as the dependent variable (see **Table 3.2**).

3.3.4 Neurovascular Transduction

3.3.4.1 Burst sequences

There were no differences between groups in the percentage of bursts occurring in each of the sequence types (singlet, doublet, triplet, quadruplet+; main effect of group p=0.974). As expected, there was a main effect of sequence type (p<0.001) such that there was a greater proportion of smaller sequences (i.e., more singlets). There were no group x gestational age interactions for the distribution of bursts (p=0.994).

There was a main effect of sequence type for the peak increase in MAP following bursts sequences (p<0.0001) such that larger burst sequences were associated with larger increases in MAP. There was no effect of group or gestation on the magnitude of the MAP response when considering sequences of SNA bursts (p=0.5988 and p=0.676, respectively, see **Figure 3.3A, top**). There was also no interaction between group and gestational age (i.e., no impact of exercise on the response to advancing gestation, p=0.575) for the peak MAP response for each sequence type.

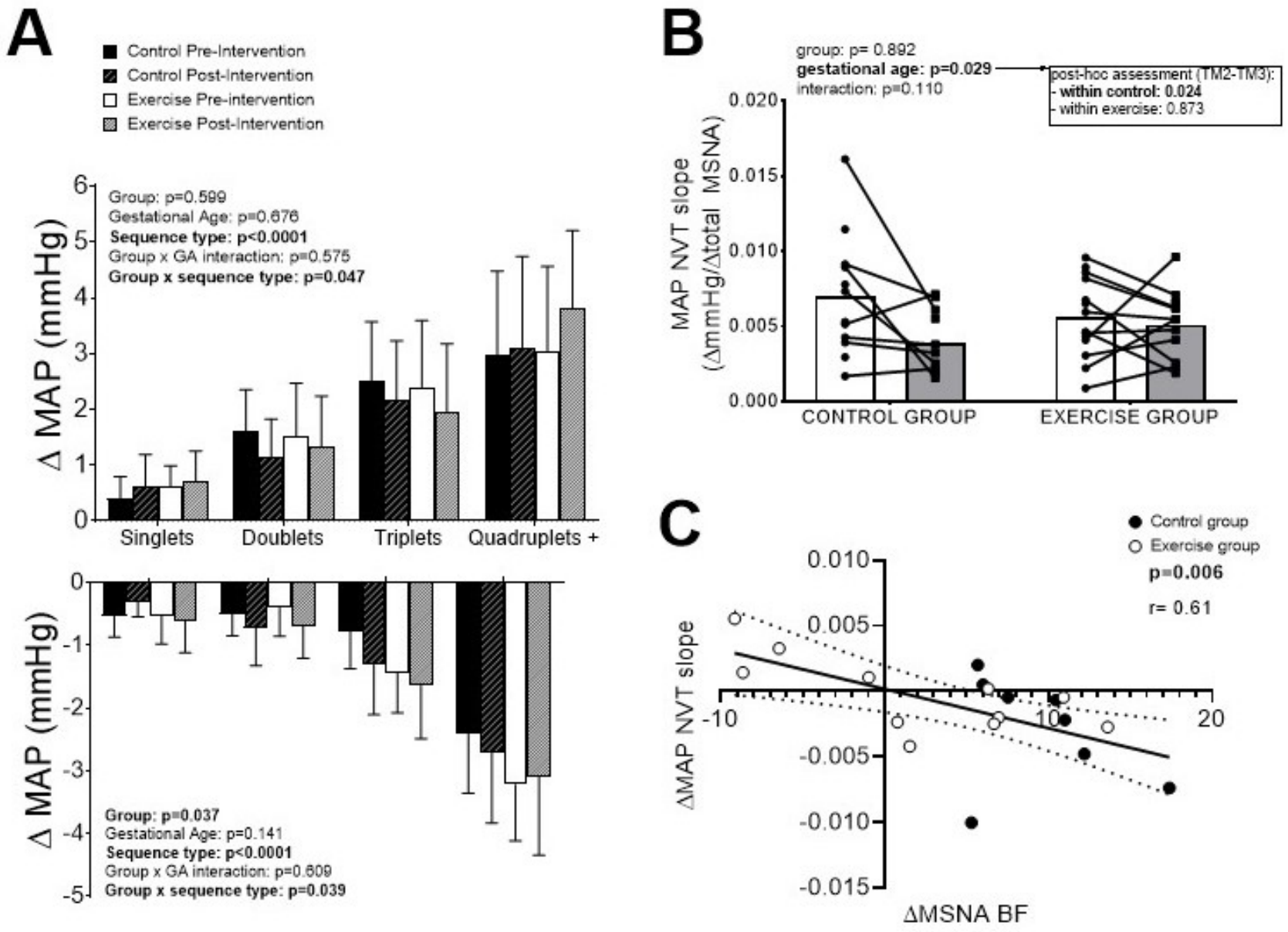

A
Control Pre-Intervention
Control Post-Intervention
Exercise Pre-intervention
Exercise Post-Intervention
Group: p=0.599
Gestational Age: p=0.676
Sequence type: p<0.0001
Group x GA interaction: p=0.575
Group x sequence type: p=0.047
Δ MAP (mmHg)
Singlets
Doublets
Triplets
Quadruplets +
Group: p=0.037
Gestational Age: p=0.141
Sequence type: p<0.0001
Group x GA interaction: p=0.609
Group x sequence type: p=0.039
B
group: p= 0.892
gestational age: p=0.029
interaction: p=0.110
post-hoc assessment (TM2-TM3):
- within control: 0.024
- within exercise: 0.873
MAP NVT slope
(ΔmmHg/Δtotal MSNA)
CONTROL GROUP
EXERCISE GROUP
C
Control group
Exercise group
p=0.006
r= 0.61
ΔMAP NVT slope
ΔMSNA BF

Figure 3.3 Neurovascular Transduction (NVT). **A)** Changes in mean arterial blood pressure (MAP) following bursts **(top)** and non-bursts **(bottom)** of muscle sympathetic nerve activity (MSNA). MAP response to singlets, doublets, triplets, and sequences with four or more bursts (quadruplets+). Bars represent the mean (SD) response for each group. Pre-intervention (trimester 2; TM2) data is shown in solid bars for control and exercise groups (control group, black; exercise group, white) and post-intervention (trimester 3; TM3) data is shown with hatches (control group, thick black and grey lines; exercise group, white with thin black lines). B) Individual and mean changes in NVT during the intervention. Control group is shown in the left, and exercise group is shown on the right. Bars represent the group mean (white, pre-intervention, TM2; grey, post-intervention, TM3). Symbols represent individual data points with lines connecting women who had measures at both pre-and post-intervention (circles and squares, respectively). There was an observed blunting across gestation for the MAP NVT slope. **C)** Correlation between the change in MSNA burst frequency (BF) and the change in the MAP NVT slope. The control group is shown in filled black circles and the exercise group is shown in open white circles. The X-axis represents the absolute change in MSNA BF from pre-to-post-intervention whereby positive values (right side of axis) represent an increase in MSNA BF across gestation and negative values (left side of axis axis) represent a decrease in MSNA BF across gestation. The Y-axis represents the absolute change in the MAP NVT slope from pre-to-post intervention whereby a negative value (below axis) represents a blunting of the NVT slope (i.e., lesser gain) with advancing gestation and positive values (above axis) represents an increase in NVT slope (i.e., higher gain) with advancing gestation. Pearson correlation showed a significant correlation between the two measures.

3.3.4.2 Non-burst sequences

There were no group or gestational age differences in the distribution of non-bursts sequences across the four sequence types (p= 0.728 and p=0.780, respectively). There was a main effect of sequence type (p<0.0001) which shows a greater proportion of non-bursts occurring in quadruplets+ sequences. Similar to bursts, there was a main effect of sequence type for the nadir MAP response following a non-burst sequence (p<0.0001) such that larger sequences resulted in greater drops in MAP (**see Figure 3.3A, bottom**). There was no effect of gestational age (p=0.141), but there was a main effect of group (p=0.037) for the nadir MAP following non-burst sequences such that women in the exercise group (at both time points) had larger drops in MAP following non-bursts sequences. However, there was no interaction between group and gestational age (p=0.609) suggesting no effect of exercise on this observation.

3.3.4.3 Neurovascular transduction slopes

NVT slopes were determined evaluating the linear relationship between the increase in total MSNA and MAP. Using this approach, we observed a blunting of the NVT gain (slope) in TM3 (p=0.029, main effect of gestational age, see **Figure 3.3B**). However, Sidak's multiple comparisons post-hoc test revealed that this was only true in the control group (p=0.024), and that there was no difference in the NVT slope between TM2 and TM3 in the exercise group (p=0.873). Previously we showed an inverse relationship between basal SNA and transduction slope in TM3 (Steinback *et al.*, 2019). In the current study, we demonstrate the change in MSNA BF with gestation is correlated with the concurrent change in NVT slope (**Figure 3.3C**). Regardless of group or compliance, women who increased their MSNA BF were more likely to have a decrease in NVT slope (i.e., blunted) as shown in the lower right quadrant of **Figure 3.3C**. Similarly, women

who had a reduction in MSNA BF during their pregnancy increased their NVT slope as shown in the upper left quadrant of **Figure 3.3C**.

3.3.5 Cold pressor test response

CPT data was analyzed using a 3-way (group x gestational age x time) mixed effects ANOVA using raw data (i.e., absolute values). Peak changes during CPT (i.e., the minute with the largest change) were also analyzed using a 2-way mixed effects ANOVA to account for baseline differences in outcome variables. Heart rate was increased during CPT (main effect of time, $p<0.0001$), and was higher in the post-intervention time point (main effect of gestational age, $p<0.0001$), but was not different between control and exercise groups at either time point (main effect of group, $p=0.160$), nor was there an interaction between group x time ($p=0.127$). The peak increase in HR during CPT was not different between groups ($p=0.862$) or across gestation ($p=0.248$), nor was there a group x gestational age interaction ($p=0.131$).

The group MAP responses to the CPT are shown in **Figure 3.4A**. Briefly, there is a main effect of time such that MAP is increased during the CPT ($p<0.0001$). Here we show augmentation in this response between pre-to-post intervention (main effect of gestational age, $p<0.0001$), which is echoed in the peak MAP analysis (main effect of gestational age, $p=0.011$; see **Figure 3.4B**). That is to say, the increase in MAP during CPT is larger in TM3 compared to TM2. However, there was no difference between control and exercising women (main effect of group, $p=0.259$) and no interaction between group, time, and gestational age (i.e., no effect of the exercise intervention on the pattern of the response, $p=0.509$). The peak responses (defined as the minute with highest MAP on an individual basis) are shown in **Figure 3.4B**. These data also show that there is no effect of group ($p=0.295$) or interaction ($p=0.980$) for the MAP response to CPT. SBP and DBP showed similar responses to CPT (i.e., no effect of group of interaction) and are therefore

not shown. A representative tracing of the MSNA response during CPT is shown in ***Appendix C, Figure C1*** (bottom).

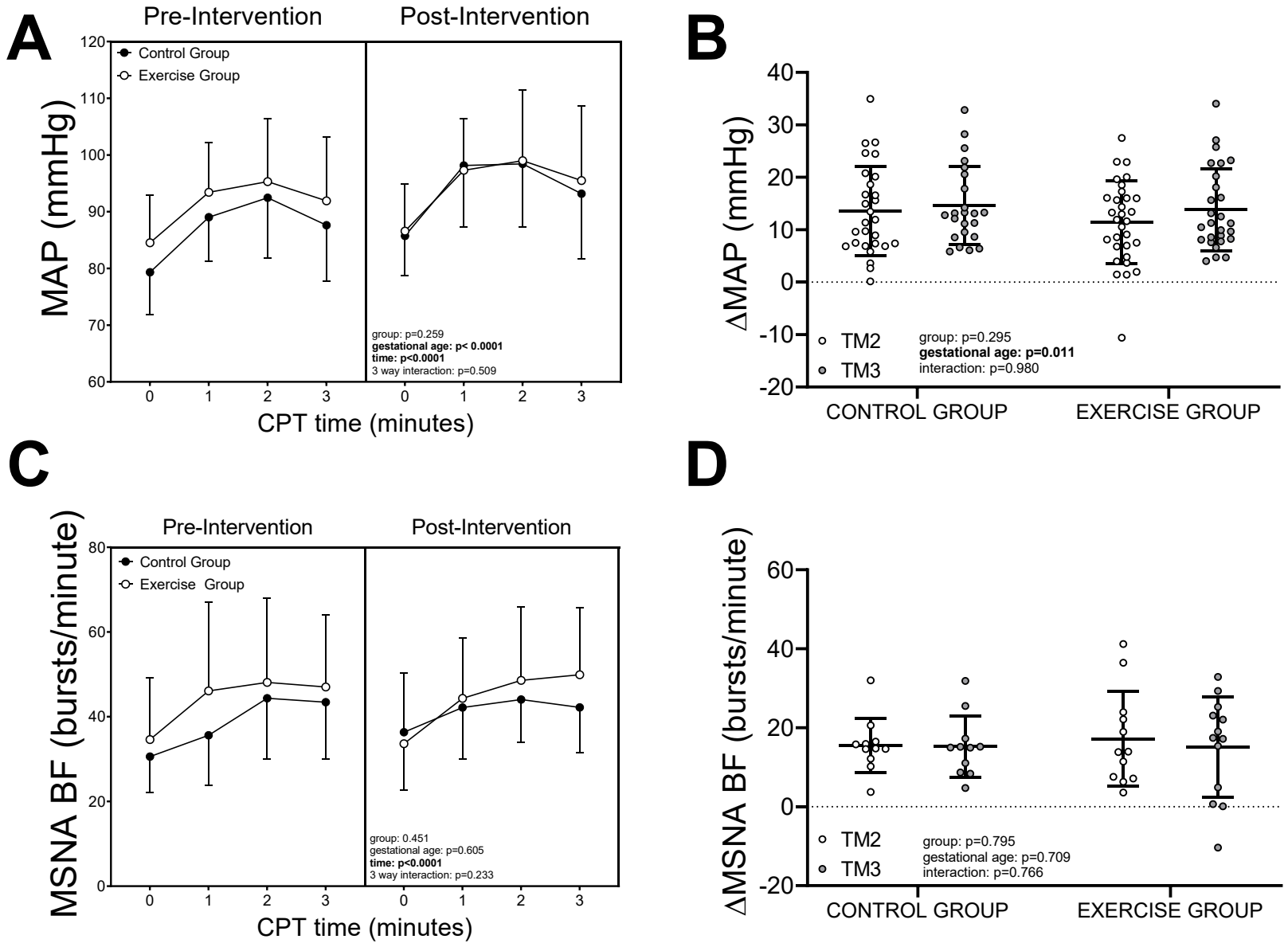

A
Pre-Intervention
Post-Intervention
Control Group
Exercise Group
MAP (mmHg)
CPT time (minutes)
group: p=0.259
gestational age: p< 0.0001
time: p<0.0001
3 way interaction: p=0.509
B
ΔMAP (mmHg)
TM2
TM3
group: p=0.295
gestational age: p=0.011
interaction: p=0.980
CONTROL GROUP
EXERCISE GROUP
C
Pre-Intervention
Post-Intervention
Control Group
Exercise Group
MSNA BF (bursts/minute)
CPT time (minutes)
group: 0.451
gestational age: p=0.605
time: p<0.0001
3 way interaction: p=0.233
D
ΔMSNA BF (bursts/minute)
TM2
TM3
group: p=0.795
gestational age: p=0.709
interaction: p=0.766
CONTROL GROUP
EXERCISE GROUP

Figure 3.4 Cold pressor test responses. A) Minute-by-minute responses in mean arterial blood pressure (MAP) during the cold pressor test (CPT). Data represent the mean (SD) responses in each group at pre- and post-intervention. Control group is shown in black symbols and exercise group in open circles. The pre-intervention (trimester 2, TM2) is shown in the left-hand box and the post-intervention (trimester 3; TM3) is in the right-hand box. There is a main effect of time such that MAP is increased during CPT, and the increase in MAP during CPT is augmented in TM3 but there is no effect of exercise on the MAP response to CPT. **B)** Data represents the individual peak responses during the CPT (i.e., the minute with the largest change in MAP during CPT on an individual basis) from pre-intervention (TM2, mean response shown in white circles) to post-intervention (TM3, mean response shown in grey circles). There is an effect of gestational age such that the peak increase in MAP during CPT was larger in the third trimester (i.e., post-intervention). **C**) minute-by minutes responses in muscle sympathetic nervous system activity (MSNA) burst frequency (BF) during CPT. The symbols are the same as the MAP response. There is a main effect of time such that MSNA is increased during the CPT but no effect of group, gestational age, or interactions. **D)** MSNA BF peak responses during CPT. There was no significant effect of group, gestational age, or interaction for the peak MSNA response during CPT.

The sympathetic nervous system response to CPT was evaluated in a subset of women and was also analyzed as intent-to-treat including all data from women who were randomized. The minute-by-minute group mean MSNA BF responses during CPT are shown in **Figure 3.4C.** Briefly, there was a main effect of time ($p<0.0001$) such that MSNA was increased during CPT. However, there was no effect of gestational age ($p=0.605$), group ($p=0.451$), or a group x gestational age x time interaction for MSNA BF ($p=0.233$). Similarly, the peak MSNA BF response during CPT is shown in **Figure 3.4D** and show no effect of group ($p=0.795$), gestational age ($p=0.709$), or an interaction between the three (group x gestational age x time; $p=0.766$). MSNA BI and Total MSNA showed similar responses to CPT and are therefore not shown (see ***Appendix C, Figure C2***).

3.4 Discussion

Prenatal exercise has been associated with a 40% reduction in the odds of developing hypertension during pregnancy (Davenport *et al.*, 2018c). As hypertensive disorders of pregnancy affect up to 10% of the population (Magee *et al.*, 2014) and result in long term cardiovascular consequences for both mother and child (Mosca *et al.*, 2011a), understanding how best to prevent them is of utmost importance. We conducted a randomized controlled trial investigating sympathetic neural control of blood pressure; our data suggest that structured prenatal exercise attenuates the normative increase in basal MSNA with gestation, without impacting blood pressure or BRG. Women who were randomized to engage in structured aerobic exercise during their second and third trimesters had smaller increases in MSNA throughout pregnancy and less blunting of NVT (i.e., the functional reactivity of peripheral blood vessels to sympathetic stimulation). Therefore, prenatal exercise may act through the sympathetic nervous system to elicit neurovascular adaptations that promote cardiovascular health.

3.4.1 Exercise intervention

We prescribed pregnant women in the exercise group to participate in three to four moderate intensity aerobic exercise sessions for the duration of the intervention (~14weeks) based on the *2003 Canadian Clinical Practice Guideline* (ACOG, 2015) and heart rate zones outlined in the *PARmed-X for Pregnancy* (Wolfe & Mottola, 2015a). Since then, the *2019 Canadian Guideline for Physical Activity throughout Pregnancy* was developed (Mottola *et al.*, 2018), which recommends 150-minutes of moderate intensity aerobic exercise per week. We recognize that the prescription in the present study falls below these new guidelines, and that we may have observed greater changes in resting hemodynamics if we had prescribed according to the new guideline. However, the threshold to alter sympathetic nervous system regulation may be lower. First, we have recently shown that as little as 260 MET-minutes of exercise per week (i.e., 60 min moderate intensity walking) can reduce the odds of preeclampsia in pregnancy by at least 25% (Davenport *et al.*, 2018c). Second, in non-pregnant populations with sympathetic hyperactivity (e.g., patients with myocardial ischemia), as little at 4 weeks of aerobic exercise training (walking at 60% HRpeak, 160 minutes per week) has been shown to lower MSNA and improve sympathetic BRG (Mimura *et al.*, 2005). In keeping with these previous studies, we believe that our prescribed exercise (achieving 50-70% HRR for 120-160 minutes per week) was sufficient to elicit measurable sympathetic adaptation. Further, in the present randomized controlled trial, 51 out of 59 women completed the study (86% retention rate; drop out similar between groups) and 86% of the women in the exercise group achieved 75% of the prescribed exercise.

In the present study we also observed a decrease in physical activity in women in the control group. Importantly, these participants were not advised to reduce their physical activity levels, but rather did so of their own volition. It is estimated that as many as 85% of pregnant women do not meet the guideline for physical activity throughout pregnancy (Hesketh & Evenson, 2016), and as

such, this was not unexpected. However, it may have accentuated our findings by increasing the difference in physical activity between the two groups to a greater extent that the exercise prescription alone. Regardless, these data mimic realistic effects of current prenatal care practices, and as such do represent realistic responses of the sympathetic nervous system to normal pregnancy in the absence of physical activity prescription.

It is important to recognize that exercise interventions may exert influences via parallel adaptations. In otherwise healthy women with obesity, 12-weeks of aerobic training (40 minutes cycling, 3 days/week) has been shown to lower resting MSNA and MSNA reactivity during static hand grip or mental stress (Carter & Ray, 2015). However, in these aforementioned studies, weight loss without exercise also reduced resting MSNA, highlighting the importance of accounting for differences in weight-gain/loss trajectories. In the present study, none of the participants lost weight during the course of the exercise intervention, and gestational weight gain did not differ between study groups at any time point. Therefore, we expect the results we have observed are the result of the exercise intervention and not any parallel changes in body weight.

3.4.2 Resting hemodynamics

As expected, we observed an overall increase in heart rate and blood pressure between TM2 and TM3. The increase in heart rate was attenuated in the women who participated in the exercise intervention, which is a well-documented effect of exercise training that extends into pregnancy (Cai *et al.*, 2020a). Interestingly, there was no interaction of group and gestational age with respect to resting blood pressure indicating women in both groups had similar increases in blood pressure from mid-to-late pregnancy. Two women randomized to the control group developed hypertensive disorders of pregnancy which aligns with population estimates (Magee *et al.*, 2014). In contrast, no woman in the exercise group developed hypertensive disorders of pregnancy which aligns with

a recent systematic review and meta analysis demonstrating that prenatal physical activity reduced odds of developing hypertensive disorders of pregnancy (Davenport *et al.*, 2018c). Therefore, although we did not observe a statistically significant interaction effect with respect to MAP, there was a moderate effect size (Cohen, 1992) and post-hoc sample size calculations (G-Power3; Faul *et al.*, 2007) suggest that there would be differences in MAP between exercise and control women with a larger sample size (78 women per group needed; i.e., likely to occur at the population level). It is worth noting that exercise did not impact resting SVR or cardiac output, which may indicate functional adaptations of the vasculature (i.e., vasodilation). Further, this may indicate changes in cardiac function, which have been previously reported to change both with respect to advancing gestation (Meah *et al.*, 2016; Meah *et al.*, 2019a) and with exercise training (Wundersitz *et al.*, 2020). However, in the present study cardiac output and SVR were determined using the model flow algorithm from the Finometer and are not validated in pregnancy (Bijl *et al.*, 2019); therefore, our interpretation of these data is limited.

3.4.3 Resting MSNA

We know very little about the changes in MSNA during the first half of pregnancy. To date there have been measures of MSNA in only 22 women in the TM2 (Reyes *et al.*, 2018a), and existing evidence would suggest that MSNA may not be different from the non-pregnant state at this point despite observations that MSNA is increased during early (6 weeks) gestation (Jarvis *et al.*, 2012; Hissen *et al.*, 2017; Reyes *et al.*, 2018b). In the present study, there were apparent differences between the control and exercise groups in resting MSNA BF in the second trimester that were unexpected and the result of randomization. Nonetheless, we have shown that the increase in MSNA during the intervention (i.e., from TM2 to TM3) was blunted in women who engage in structured aerobic exercise program.

The present study adds significantly to the limited literature on MSNA in TM2 and highlights the variability in the responses in the first half of pregnancy. Here we show that resting MSNA in TM2 ranged from 16 up to 55 bursts per minute. There is no evidence that this variability is due to differences in maternal age, pre-pregnancy BMI or gestational weight gain (GWG) as they were not different between study groups. Further these factors (age, BMI, GWG) were recently shown have no impact on MSNA BF in TM3 (Reyes *et al.*, 2020a). While Badrov et al. (2020) have recently reported that MSNA BF may be increased with increasing parity (i.e., higher in the second pregnancy compared to the first), there were no differences between our two study groups, and likely not the cause of the disparity. Based on the findings that aerobic exercise intervention attenuates the increase in MSNA between TM2 and TM3, physical activity levels (including pre-pregnancy physical activity levels) may have played a role in the changes in basal MSNA prior to the initiation of this intervention. Future studies that are able to include longitudinal measures starting at pre-pregnancy will be of utmost importance in continuing to answer these questions.

It is possible that the higher MSNA BF at TM2 may have led to the smaller increase in MSNA across gestation; however, we do not feel that this is the case for two reasons. First that MSNA BF in TM3 ranged from 22 to 54 bursts per minute, which is in line with previous measures in the third trimester (Reyes *et al.*, 2018a). Second, burst incidence measures in TM3 ranged from 32-64 bursts per 100 heart beats; a maximal value for this measure would be 100, and reports would suggest values >75 have been measured in human pregnancy (Greenwood *et al.*, 2003) indicating that the measures in this study are not maximal (i.e., could still have increased further). Further, MSNA BI was not different between the groups at TM2, but was still influenced by our intervention. Regardless, the aim of this study was to determine the impact of exercise on the change in MSNA from TM2 to TM3, which was attenuated in the exercise group in this study.

Therefore, evidence from this study suggests that physical activity has an important influence on basal MSNA during pregnancy. Future studies investigating chronic physical activity patterns on the MSNA responses in pregnancy are needed to elucidate the reasons for the large variability that we are seeing as early as 16weeks.

While it is unclear how exercise is altering the normative changes in MSNA in human pregnancy, it may also be that changes are occurring at other locations along the neurovascular pathway (i.e., not just MSNA per se). These include potential changes in neurotransmitter release and reuptake, alpha-adrenergic receptor sensitivity and density. Indeed, normal pregnancy has been shown to increase neurotransmitter concentrations parallel to the increase in MSNA (Barron *et al.*, 1986; Jarvis *et al.*, 2012) and therefore, we may expect neurotransmitter release to be lesser in women who exercise. Normotensive pregnancy may be also associated with an increase in alpha- and beta-adrenergic receptor density and sensitivity (Aune *et al.*, 2000), however this evidence is limited to the uterine artery. Further, sex hormone concentration may be correlated to basal MSNA during pregnancy (Reyes *et al.*, 2018a) and have been shown to be modified by physical activity (Ennour-Idrissi *et al.*, 2015). Lastly, an increase in soluble-fms-like tyrosine kinase to placental growth factor (sFlt-1:PlGF) is implicated in the pathogenesis of gestational hypertension (Akhter *et al.*, 2016). Pregnant women who are more physically active have been previously shown to have lower ratio of sFlt-1:PlGF indicating better angiogenic balance (Weissgerber *et al.*, 2010). Therefore, we suspect the attenuated rise in MSNA to be multifaceted and likely a combination of the aforementioned factors. Future studies are needed to evaluate these mechanisms in more detail.

3.4.4 Baroreflex gain

Contrary to previous reports, we did not observe a blunting of the weighted probability slope for the sympathetic baroreflex with advancing gestation. However, previous reports compared TM3 to non-pregnant women (Usselman *et al.*, 2015a), and thus BRG may have already been blunted by the second trimester. We measured sympathetic BRG (based on burst probability) in the same manner as our previous report (Usselman *et al.*, 2015a) and show a similar average slope in TM3. Longitudinal case study reports on three women (Hissen *et al.*, 2017; Reyes *et al.*, 2018b) suggest that there are interindividual differences in the pattern of the sympathetic BRG response to pregnancy, however from these no discernible pattern could be elucidated. Within the context of this study we suggest that sympathetic BRG is not altered between TM2 and TM3, and also that exercise occurring between mid-to-late pregnancy does not impact the change in BRG during this time. Here we also report BRG based on total MSNA in pregnancy. This measure has been reported once in pregnancy (Badrov *et al.*, 2020); however, a comparison of BRG based on total MSNA between pregnant and non-pregnant women, or between trimesters has not been evaluated and therefore our results are difficult to interpret in the context of changes during a normal pregnancy. One limitation of this method is that we used spontaneous BRG assessment rather than the gold-standard modified oxford procedure (i.e., injections of blood pressure lowering and raising medications) as it is not recommended in pregnancy (Jarvis *et al.*, 2012). However, the spontaneous BRG method is validated against the modified oxford protocol (Hart *et al.*, 2010), and thus we do not feel that our results would have been altered by a different methodology in this instance. In both healthy and unhealthy (e.g. hypertension) non-pregnant populations, aerobic exercise interventions have shown improvement in BRG compared to controls (Carter & Ray, 2015). Future studies need to investigate baroreflex function starting with pre-pregnancy measures

and continue longitudinally throughout pregnancy to determine the full impact of both advancing gestational age and exercise on BRG in pregnancy.

3.4.5 Neurovascular transduction

Similar to our previous work (Steinback *et al.*, 2019), we found that sympathetic NVT was blunted in TM3 compared to TM2; this blunting was most pronounced in the control group. We acknowledge here that our sample size is relatively small, partially due to the high incidence of pre-syncope during MSNA search in pregnant women (Meah *et al.*, 2019b) and obtaining adequate quality repeated measures MSNA signals. A post-hoc sample sized calculation based on our data would suggest 25 women per group would be needed to detect a significant difference in NVT slop change across gestation. In addition, we have previously shown that the relationship between basal SVR and MSNA is blunted in TM3 (Usselman *et al.*, 2015b) and possibly in early & mid pregnancy (Fu & Levine, 2009; Reyes *et al.*, 2018b). Combined with the data from the present study, these would suggest that the blunting of NVT is related to the increase in MSNA that occurs in typical normotensive pregnancy which may be attenuated by aerobic exercise. Indeed, here we show that changes in NVT were correlated with changes MSNA BF which supports this notion. We have also previously shown that the decrease in MAP following non-burst sequences is unaltered in TM3 compared to non-pregnant controls (Steinback *et al.*, 2019) which was attributed to no difference in vasodilatory mechanisms between pregnant and non-pregnant women. The fact that we see no difference in the decrease in MAP following sequences without bursts in the present study suggests that the underlying vasoconstrictor tone is similar between conditions (i.e., with respect to both gestation and exercise) and complements the previous literature. Together, these data are important as it suggests that an increase in MSNA during pregnancy is not necessary to

maintain the same blood pressure. Thus, we posit that augmented MSNA is a consequence of pregnancy per se and not reflex engagement to counter lower vascular resistance.

In the current study we evaluated NVT based on MAP. Although the communication between MSNA and the vasculature occurs within the resistance vessels, we feel that within a clinical context MAP may be a better and more relevant representation than peripheral (forearm or femoral) vascular conductance as it represents the systemic/ total body effects. In non-pregnant populations where transduction has been evaluated in terms of both local and systemic vasoconstriction (or decrease in conductance), the interpretation of the results remains the same if only MAP is considered (Fairfax *et al.*, 2013). Further, for some populations, differences between groups may only exist if MAP is considered (e.g. black vs white males; Vranish *et al.*, 2018) To date there have been no observations that forearm or calf vascular resistance is altered in pregnancy. Despite this, Jarvis and colleagues (2012) showed that the ratio between resting forearm vascular resistance/MSNA is decreased in the first trimester. Therefore, future studies should investigate the potential differences in local versus systemic vascular transduction in pregnancy. This would be of especial importance in women who are at high risk for hypertension as they may have altered sympathetic regulation prior to diagnosis (Badrov *et al.*, 2019).

3.4.6 Reactivity to CPT

In the current study, there was no effect of gestational age or exercise on the sympathetic or blood pressure response to CPT. The CPT is a safe and effective tool to evaluate hemodynamic and sympathetic responsiveness during pregnancy and has been utilized in both healthy and hypertensive pregnancies (Meah *et al.*, 2018b). A larger blood pressure response to CPT in mid pregnancy has been previously shown to precede the development of preeclampsia (Woisetschlager *et al.*, 2000); however, this elevated blood pressure responsiveness to CPT has

not been observed in cross-sectional studies comparing pregnant women with and without hypertension (Schobel *et al.*, 1996; Greenwood *et al.*, 1998). In non-pregnant populations, aerobic exercise has been shown reduce the responsiveness of the sympathetic nervous system to mental stress and handgrip/knee extension exercise, but not CPT, head-up-tilt, or Valsalva (Carter & Ray, 2015). The pathways which activate MSNA during CPT (i.e., nociceptors) are different from those in the aforementioned studies (e.g., metaboreceptors) which may explain the differences in response/ lack of response to aerobic exercise. Further, we did not control for respiration at rest or during the CPT in the present study. Advanced gestation is associated with increases in minute ventilation which can influence MSNA (Pernoll *et al.*, 1975). Changes in ventilation during the CPT (e.g., deep breathing or hyperventilating) could potentially impact our results such that deep breathing may lower the MSNA response, whereas hyperventilating may increase the response (Dempsey *et al.*, 2002). Future work should measure and control for ventilation when performing CPT or other reflex manoeuvres in pregnancy. Lastly, CPT reactivity was a secondary (exploratory) outcome, and the study sample size was not determined in order to detect significant changes in the MSNA or BP response to CPT. Using the data obtained from the current study, we estimate 218 women per group would be needed to detect a difference in the peak MSNA BF response during CPT. Therefore, we are uncertain whether aerobic exercise would impact CPT reactivity in otherwise healthy pregnant women. Future studies investigating this in women who are at high risk for gestational hypertension (e.g., with obesity), or who have gestational hypertension might be able to ascertain if there are populations whose reactivity to stress (e.g., CPT) can be positively altered by aerobic exercise intervention.

3.4.7 Future directions

Within the current study we examined the role of a structured prenatal aerobic exercise program on basal and reflex MSNA, sympathetic baroreflex, and the NVT (MAP response). Exercise during pregnancy may oppose the abnormal remodelling that occurs in gestational hypertension and pre-eclampsia through the indirect actions at the level of the vasculature (Skow *et al.*, 2017). Whether the longitudinal changes across gestation result in differences in forearm or femoral blood flow, reactivity, or transduction is yet to be elucidated. Aerobic exercise may alter blood pressure regulation through improvements in vascular function (i.e. enhanced endothelial function; Green, 2009). Data from our non-burst sequences would suggest that basal vasodilatory status is not altered by prenatal physical activity; however, the ability to dilate in response to increases in shear stress may be enhanced with aerobic exercise (Green & Smith, 2018). Prenatal physical activity in the third trimester is associated with increases in normalized flow mediated dilation (Reyes *et al.*, 2020b), and one randomized controlled trial showed that aerobic exercise intervention may increase flow mediated dilation in pregnancy (Ramírez-Vélez *et al.*, 2011). Further, normal pregnancy is associated with a curvilinear change in arterial stiffness that mirrors SVR and blood pressure (Skow *et al.*, 2017). Exercise is associated with decreases in arterial stiffness in non-pregnant (Tanaka & Safar, 2005) and recently in pregnant populations (Kawabata *et al.*, 2012) and may influence blood pressure control through the direct actions on the vasculature (i.e., remodelling). As reviewed by Green and colleagues (2018), exercise exerts positive cardiovascular benefit through flow-dependent and endothelium meditated dilation and remodelling; the sympathetic nervous system plays a role in centrally mediating the functional changes which result in structural adaptations. However, functional, or structural changes in specific peripheral vascular beds (e.g. forearm or femoral) may be occurring in response to aerobic exercise during pregnancy and have not yet been considered. Future work in both humans and

animal models addressing blood vessel specificity and reactivity will help determine the mechanisms by which prenatal exercise improves cardiovascular health and reduces hypertensive risk. Specifically determining the effects of aerobic exercise on sympathetic neurotransmitter receptor density and sensitivity would help fill in some of the current gaps in our knowledge.

Despite the overwhelming evidence that prenatal exercise is beneficial for most women, 85% of pregnant women are not meeting exercise guidelines (Evenson & Wen, 2011). Moreover, up to 10% of pregnant women will develop hypertensive disorders of pregnancy (Magee *et al.*, 2014). Gestational hypertension is associated with sympathetic hyperactivity, as such the data from our exercise intervention provides some insight into ways by which prenatal exercise might oppose this (i.e., by reducing the increase in MSNA across gestation). Prenatal exercise has been shown to reduce the odds of developing hypertension and preeclampsia by up to 40% (Davenport *et al.*, 2018c), and therefore is of importance when considering the long-term cardiovascular health of these women and their children. These data provide a potential mechanism underlying the reduced odds of developing hypertensive disorders of pregnancy in physically active pregnant women. Specifically, that the communication between the nervous system and the blood vessels (i.e., NVT) is less changed in women who participated in the structured aerobic exercise program despite the attenuation of the normal increase in MSNA during a normotensive pregnancy. Decreasing the amount of sympathetic hyperactivity while not disrupting blood pressure control (e.g. sympathetic baroreflex and CPT reactivity) may also facilitate long term vascular adaptations that promote lifelong cardiovascular health. However, more research is needed to fully elucidate the mechanisms behind which the risk of gestational hypertension is reduced by prenatal exercise including the vascular component. Specifically, more research is needed involving women who

are at high risk for developing gestational hypertension (e.g. women with prior history), as the improvements may be greater in this population.

3.4.8 Conclusion

The aim of the present study was to determine whether a structured aerobic exercise program could alter the normative responses of the sympathetic nervous system during pregnancy. Here we show that prenatal aerobic exercise attenuates the increase in MSNA and possibly the decrease in NVT between the second and third trimesters in low-risk, normotensive pregnant women. Thus, the communication between the nervous system and the blood vessels appears to be less changed at rest in women who performed aerobic exercise. Interestingly, resting blood pressure and CPT reactivity were not altered in this study; however, all women were normotensive. Importantly, we know that gestational hypertension risk is reduced by prenatal exercise (Davenport *et al.*, 2018c), and therefore the sympathetic regulation of blood pressure may be one mechanism behind this observation; more work is needed to verify this hypothesis in high risk women.

3.5 References

ACOG. (2015). ACOG Committee Opinion No. 650: Physical Activity and Exercise During Pregnancy and the Postpartum Period. Obstetrics and gynecology 126, 135.

Akhter T, Wikstrom AK, Larsson M, Larsson A, Wikstrom G & Naessen T. (2016). Association between angiogenic factors and signs of arterial aging in women with pre-eclampsia. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology.

Aune B, Vårtun A, Oian P & Sager G. (2000). Evidence of dysfunctional beta2-adrenoceptor signal system in pre-eclampsia. Bjog 107, 116-121.

Badrov MB, Park SY, Yoo JK, Hieda M, Okada Y, Jarvis SS, Stickford AS, Best SA, Nelson DB & Fu Q. (2019). Role of Corin in Blood Pressure Regulation in Normotensive and Hypertensive Pregnancy. Hypertension 73, 432-439.

Badrov MB, Yoo JK, Steinback CD, Davenport MH & Fu Q. (2020). Influence of multiparity on sympathetic nerve activity during normal pregnancy. American journal of physiology Heart and circulatory physiology 318, H816-h819.

Barron WM, Mujais SK, Zinaman M, Bravo EL & Lindheimer MD. (1986). Plasma catecholamine responses to physiologic stimuli in normal human pregnancy. Am J Obstet Gynecol 154, 80-84.

Bijl RC, Valensise H, Novelli GP, Vasapollo B, Wilkinson I, Thilaganathan B, Stöhr EJ, Lees C, van der Marel CD & Cornette JMJ. (2019). Methods and considerations concerning cardiac output measurement in pregnant women: recommendations of the International Working Group on Maternal Hemodynamics. Ultrasound Obstet Gynecol 54, 35-50.

Cai C, Ruchat SM, Sivak A & Davenport MH. (2020). Prenatal Exercise and Cardiorespiratory Health and Fitness: A Meta-analysis. Med Sci Sports Exerc.

Carter JR & Ray CA. (2015). Sympathetic neural adaptations to exercise training in humans. Autonomic Neuroscience : Basic & Clinical 188, 36-43.

Cohen J. (1992). A power primer. Psychological bulletin 112, 155-159.

Davenport MH, Ruchat SM, Poitras VJ, Garcia AJ, Gray CE, Barrowman N, Skow RJ, Meah VL, Riske L, Sobierajski F, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater L, Weeks A, Adamo KB, Davies GA, Barakat R & Mottola MF. (2018). Prenatal exercise for

the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. British Journal of Sports Medicine.

de Haas S, Ghossein-Doha C, van Kuijk SM, van Drongelen J & Spaanderman ME. (2017). Physiological adaptation of maternal plasma volume during pregnancy: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 49, 177-187.

Degani S, Abinader E, Eibschitz I, Oettinger M, Shapiro I & Sharf M. (1985). Isometric exercise test for predicting gestational hypertension. Obstetrics and gynecology 65, 652-654.

Delius W, Hagbarth KE, Hongell A & Wallin BG. (1972). General characteristics of sympathetic activity in human muscle nerves. Acta Physiologica Scandinavica 84, 65-81.

Dempsey JA, Sheel AW, St Croix CM & Morgan BJ. (2002). Respiratory influences on sympathetic vasomotor outflow in humans. Respir Physiol Neurobiol 130, 3-20.

Ennour-Idrissi K, Maunsell E & Diorio C. (2015). Effect of physical activity on sex hormones in women: a systematic review and meta-analysis of randomized controlled trials. Breast cancer research : BCR 17, 139.

Evenson KR & Wen F. (2011). Prevalence and correlates of objectively measured physical activity and sedentary behavior among US pregnant women. Preventive medicine 53, 39-43.

Fairfax ST, Padilla J, Vianna LC, Davis MJ & Fadel PJ. (2013). Spontaneous bursts of muscle sympathetic nerve activity decrease leg vascular conductance in resting humans. American journal of physiologyHeart and circulatory physiology 304, 759.

Faul F, Erdfelder E, Lang AG & Buchner A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods 39, 175-191.

Freedson PS, Melanson E & Sirard J. (1998). Calibration of the Computer Science and Applications, Inc. accelerometer. Med Sci Sports Exerc 30, 777-781.

Fu Q & Levine BD. (2009). Autonomic circulatory control during pregnancy in humans. Semin Reprod Med 27, 330-337.

Green DJ. (2009). Exercise training as vascular medicine: direct impacts on the vasculature in humans. Exercise and sport sciences reviews 37, 196-202.

Green DJ & Smith KJ. (2018). Effects of Exercise on Vascular Function, Structure, and Health in Humans. Cold Spring Harbor perspectives in medicine 8.

Green LJ, Mackillop LH, Salvi D, Pullon R, Loerup L, Tarassenko L, Mossop J, Edwards C, Gerry S, Birks J, Gauntlett R, Harding K, Chappell LC & Watkinson PJ. (2020). Gestation-Specific Vital Sign Reference Ranges in Pregnancy. Obstet Gynecol 135, 653-664.

Greenwood JP, Scott EM, Walker JJ, Stoker JB & Mary DA. (2003). The magnitude of sympathetic hyperactivity in pregnancy-induced hypertension and preeclampsia. American journal of hypertension 16, 194-199.

Greenwood JP, Stoker JB, Walker JJ & Mary DA. (1998). Sympathetic nerve discharge in normal pregnancy and pregnancy-induced hypertension. Journal of hypertension 16, 617-624.

Hart EC, Joyner MJ, Wallin BG, Karlsson T, Curry TB & Charkoudian N. (2010). Baroreflex control of muscle sympathetic nerve activity: a nonpharmacological measure of baroreflex sensitivity. American journal of physiology Heart and circulatory physiology 298, H816-822.

Hesketh KR & Evenson KR. (2016). Prevalence of U.S. Pregnant Women Meeting 2015 ACOG Physical Activity Guidelines. American journal of preventive medicine 51, e87-89.

Hissen SL, El Sayed K, Macefield VG, Brown R & Taylor CE. (2017). Muscle sympathetic nerve activity peaks in the first trimester in healthy pregnancy: a longitudinal case study. Clinical autonomic research : official journal of the Clinical Autonomic Research Society 27, 401-406.

Hissen SL & Fu Q. (2020). Neural control of blood pressure during pregnancy in humans. Clin Auton Res.

Jarvis SS, Shibata S, Bivens TB, Okada Y, Casey BM, Levine BD & Fu Q. (2012). Sympathetic activation during early pregnancy in humans. The Journal of physiology 590, 3535-3543.

Kawabata I, Nakai A, Sekiguchi A, Inoue Y & Takeshita T. (2012). The effect of regular exercise training during pregnancy on postpartum brachial-ankle pulse wave velocity, a measure of arterial stiffness. Journal of sports science & medicine 11, 489-494.

Lenhard W & Lenhard A. (2016). Calculation of Effect Sizes. Dettelbach (Germany).

Magee LA, Pels A, Helewa M, Rey E & von Dadelszen P. (2014). Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. Journal of

obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC 36, 575-576.

Meah VL, Backx K, Cockcroft JR, Shave RE & Stöhr EJ. (2019a). Left ventricular mechanics in late second trimester of healthy pregnancy. Ultrasound Obstet Gynecol 54, 350-358.

Meah VL, Backx K, Davenport MH, Bruckmann A, Cockcroft J, Cornette J, Duvekot JJ, Ferrazzi E, Foo FL, Ghossein-Doha C, Gyselaers W, Khalil A, McEniery CM, Lees C, Meah V, Novelli GP, Spaanderman M, Stohr E, Tay J, Thilaganathan B, Valensise H & Wilkinson I. (2018). Functional hemodynamic testing in pregnancy: recommendations of the International Working Group on Maternal Hemodynamics. Ultrasound in Obstetrics & Gynecology 51, 331-340.

Meah VL, Busch SA, Jones KE, Davenport MH & Steinback CD. (2019b). A review of acute responses, after-effects and chronic complications related to microneurography. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 130, 1781-1788.

Meah VL, Cockcroft JR, Backx K, Shave R & Stohr EJ. (2016). Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. Heart (British Cardiac Society) 102, 518-526.

Mimura J, Yuasa F, Yuyama R, Kawamura A, Iwasaki M, Sugiura T & Iwasaka T. (2005). The effect of residential exercise training on baroreflex control of heart rate and sympathetic nerve activity in patients with acute myocardial infarction. Chest 127, 1108-1115.

Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Piña IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC, Jr., Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V & Wenger NK. (2011). Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. Circulation 123, 1243-1262.

Mottola MF, Davenport MH, Ruchat SM, Davies GA, Poitras VJ, Gray CE, Jaramillo Garcia A, Barrowman N, Adamo KB, Duggan M, Barakat R, Chilibeck P, Fleming K, Forte M, Korolnek J, Nagpal T, Slater LG, Stirling D & Zehr L. (2018). 2019 Canadian guideline for physical activity throughout pregnancy. British journal of sports medicine 52, 1339-1346.

Okada Y, Best SA, Jarvis SS, Shibata S, Parker RS, Casey BM, Levine BD & Fu Q. (2015). Asian women have attenuated sympathetic activation but enhanced renal-adrenal responses during pregnancy compared to Caucasian women. The Journal of physiology 593, 1159-1168.

Pernoll ML, Metcalfe J, Kovach PA, Wachtel R & Dunham MJ. (1975). Ventilation during rest and exercise in pregnancy and postpartum. Respiration physiology 25, 295-310.

Ramírez-Vélez R, Aguilar de Plata AC, Escudero MM, Echeverry I, Ortega JG, Salazar B, Rey JJ, Hormiga C & López-Jaramillo P. (2011). Influence of regular aerobic exercise on endothelium-dependent vasodilation and cardiorespiratory fitness in pregnant women. J Obstet Gynaecol Res 37, 1601-1608.

Reyes LM, Badrov MB, Fu Q, Steinback CD & Davenport MH. (2020a). Age, body mass index and weight gain do not increase sympathetic activity during pregnancy. Appl Physiol Nutr Metab.

Reyes LM, Farooq SM, Skow RJ, Busch SA, Pyke KE, Khurana R, Chari RS, Stickland MK, Devolin M, Davidge ST, Sobierajski F, Lugg A, Steinback CD & Davenport MH. (2020b). Physical Activity in Pregnancy Is Associated with Increased Flow-mediated Dilation. Med Sci Sports Exerc 52, 801-809.

Reyes LM, Usselman CW, Davenport MH & Steinback CD. (2018a). Sympathetic Nervous System Regulation in Human Normotensive and Hypertensive Pregnancies. Hypertension (Dallas, Tex: 1979).

Reyes LM, Usselman CW, Skow RJ, Charkoudian N, Staab JS, Davenport MH & Steinback CD. (2018b). Sympathetic neurovascular regulation during pregnancy: A longitudinal case series study. Experimental physiology 103, 318-323.

Ruchat SM, Davenport MH, Giroux I, Hillier M, Batada A, Sopper MM, Hammond JA & Mottola M. (2012). Walking program of low or vigorous intensity during pregnancy confers an aerobic benefit. International Journal of Sports Medicine 33, 661-666.

Sanghavi M & Rutherford JD. (2014). Cardiovascular physiology of pregnancy. Circulation 130, 1003-1008.

Schobel HP, Fischer T, Heuszer K, Geiger H & Schmieder RE. (1996). Preeclampsia -- a state of sympathetic overactivity. The New England journal of medicine 335, 1480-1485.

Skow RJ, King EC, Steinback CD & Davenport MH. (2017). The influence of prenatal exercise and pre-eclampsia on maternal vascular function. Clinical science (London, England : 1979) 131, 2223-2240.

Steinback CD, Fraser GM, Usselman CW, Reyes LM, Julian CG, Stickland MK, Chari RS, Khurana R, Davidge ST & Davenport MH. (2019). Blunted sympathetic neurovascular transduction during normotensive pregnancy. J Physiol 597, 3687-3696.

Tanaka H & Safar ME. (2005). Influence of lifestyle modification on arterial stiffness and wave reflections. American journal of hypertension 18, 137-144.

Usselman CW, Skow RJ, Matenchuk BA, Chari RS, Julian CG, Stickland MK, Davenport MH & Steinback CD. (2015a). Sympathetic baroreflex gain in normotensive pregnant women. Journal of applied physiology (Bethesda, Md: 1985) 119, 468-474.

Usselman CW, Wakefield PK, Skow RJ, Stickland MK, Chari RS, Julian CG, Steinback CD & Davenport MH. (2015b). Regulation of sympathetic nerve activity during the cold pressor test in normotensive pregnant and nonpregnant women. Hypertension (Dallas, Tex: 1979) 66, 858-864.

Vranish JR, Holwerda SW, Young BE, Credeur DP, Patik JC, Barbosa TC, Keller DM & Fadel PJ. (2018). Exaggerated Vasoconstriction to Spontaneous Bursts of Muscle Sympathetic Nerve Activity in Healthy Young Black Men. Hypertension 71, 192-198.

Weissgerber TL, Davies GA & Roberts JM. (2010). Modification of angiogenic factors by regular and acute exercise during pregnancy. Journal of applied physiology (Bethesda, Md: 1985) 108, 1217-1223.

Woisetschlager C, Waldenhofer U, Bur A, Herkner H, Kiss H, Binder M, Laggner AN & Hirschl MM. (2000). Increased blood pressure response to the cold pressor test in pregnant women developing pre-eclampsia. Journal of hypertension 18, 399-403.

Wolfe L & Mottola M. (2015). Physical activity readiness medical examination for pregnancy: PARmed-X for pregnancy.

Wundersitz DWT, Gordon BA, Lavie CJ, Nadurata V & Kingsley MIC. (2020). Impact of endurance exercise on the heart of cyclists: A systematic review and meta-analysis. Progress in cardiovascular diseases.

4 Prenatal exercise and cardiovascular health (PEACH) study: impact of aerobic exercise training on vascular health

4.1 Introduction

In healthy pregnancy, there are immediate and progressive hemodynamic changes associated with adaptations in vascular structure and function. Specifically, during normotensive pregnancy there are increases in blood vessel diameter, improvements in endothelial function and arterial stiffness, and a shift towards a more pro-angiogenic state (Skow *et al.*, 2017). These adaptations interact to result in minimal changes in mean arterial blood pressure (MAP) throughout a healthy pregnancy (Green *et al.*, 2020). Conversely, in women with pregnancy-specific hypertensive disorders (e.g., preeclampsia), there are known maladaptations of the cardiovascular system, including increases arterial stiffness and higher levels of anti-angiogenic factors (Skow *et al.*, 2017). Preeclampsia is the leading cause of maternal and fetal mortality and affects 8-15% of pregnancies and is associated with future cardiovascular risk (Mosca *et al.*, 2011a; Magee *et al.*, 2014). Yet, it is unclear what mechanisms underpin the development of cardiovascular maladaptations in these women and contribute to increased cardiovascular risk. Therefore, understanding the mechanisms by which blood pressure regulation can be optimized during pregnancy is of critical importance. Impressively, exercise prior to and during pregnancy has been shown to reduce the odds of developing hypertensive disorders of pregnancy by 40% (Davenport *et al.*, 2018d). The mechanisms by which exercise confers its benefits are presently unclear, but prenatal exercise may contribute to enhanced vascular adaptations which facilitate improvements in blood pressure (Skow *et al.*, 2017).

In non-pregnant populations, exercise reduces cardiovascular risk through improvements in vascular structure and function (Green, 2009; Carter & Ray, 2015). Specifically, in healthy trained

populations, vascular conductance is increased (lower resistance) by vascular remodeling (increased diameter) to accommodate an increase in resting cardiac output and sheer stress (Green, 2009). Moreover, exercise training also increases carotid artery distensibility (decrease stiffness; Bjarnegård *et al.*, 2019), independent of a change in cardiorespiratory fitness (Hetherington-Rauth *et al.*, 2020).

We do know that prenatal exercise augments endothelial function (Ramírez-Vélez *et al.*, 2011) indicating the capacity for further improvement in vascular health during pregnancy. In non-pregnant women, aerobic exercise training decreases arterial stiffness by up to 50% (Tanaka et al., 1998). Similarly, aerobic exercise training initiated at 16 weeks gestation was associated with a 10% reduction in PWV at one month postpartum (Kawabata *et al.*, 2012). However, the impact of exercise training on metrics of arterial stiffness has not been studied during pregnancy. Improvements in vascular function with prenatal exercise may be related to improvements in angiogenic balance. Specifically, women who are active during pregnancy have a lower ratio of soluble fms-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PlGF) in their third trimester (Weissgerber *et al.*, 2010). However, it is unclear if this shift in sFlt-1:PlGF ratio is associated with an altered trajectory of vascular adaptations.

Using data collected during the Prenatal Exercise and Cardiovascular Health (PEACH) randomized control trail (NCT 02948439), we aimed to determine the influence of aerobic exercise training on vascular adaptation and underlying angiogenic balance during normotensive pregnancy. We hypothesized that aerobic exercise training would be associated with augmentation of peripheral vascular dilation, compliance, and distensibility. Secondary to this, we hypothesized smaller increases in PWV and sFlt-1:PlGF ratio from TM2 to TM3 in women randomized to the exercise group compared to controls.

4.2 Methodology

This study has been approved by the University of Alberta Research Ethics Board (Pro00061045) and conforms to the standards set by the latest version of the Declaration of Helsinki. Between May 2016 and December 2018, 230 women contacted the Program for Pregnancy and Postpartum Health regarding participation in this study. Of these, one hundred pregnant women attended in-person recruitment interviews to assess eligibility for participation in our randomized controlled trial investigating the effects of aerobic exercise on cardiovascular health. Sixty-four women consented to participation in the intervention, however five dropped out prior to randomization leaving 59 women randomized to exercise (n=31) or control (n=28) conditions. Three women in the exercise group and five women in the control group dropped out between the initial and follow-up assessments (adherence of 90% and 82% for exercise and control, respectively). A complete description of reasons for exclusion and drop out are included in our previous publication reporting on sympathetic nervous system adaptations to exercise in this cohort (Skow *et al.*, 2020).

Women were included if they were over 18 years of age, had a singleton pregnancy, and did not have an absolute contraindication to exercise as outlined on the PARmed-X for Pregnancy (Wolfe & Mottola, 2015b). Prior to participation, individuals provided written, informed consent and their maternity health care provider (obstetrician, mid-wife etc.) provided medical clearance that they could participate in an exercise program (signed the PARmed-X for Pregnancy form). Women were excluded if they had any cardiovascular, respiratory, or nervous system disorder which may impact the results of the study (e.g., Raynauds). Women were also excluded if they were engaging in more than 45 minutes of moderate-to-vigorous intensity aerobic activity per week (self-report at initial meeting). This cut-off was chosen because it was the lower limit of the

Canadian Clinical Practice Guidelines for exercise during pregnancy which was the current guideline when the trial was started (aerobic exercise for at least 15 minutes, 3 times per week)(Davies *et al.*, 2003). Baseline assessments occurred between 16-20 weeks of pregnancy, and post-intervention assessments occurred between 34-36 weeks of pregnancy. At each of these timepoints, women completed assessments of vascular function.

Prior to commencement of the study, an online randomization program (https://www.sealedenvelope.com/simple-randomiser/v1/lists) was used to randomly assign values from 1 to 100 to either exercise or control groups in random blocks of 4, 6, & 8. Randomization and filling of the opaque envelopes were completed prior to the initiation of the study by a person affiliated with the *Program for Pregnancy and Postpartum Health* but not involved with the present study. Participant randomization occurred following their baseline pre-intervention assessment.

4.2.1 Vascular Assessment

The results presented in this paper have not been reported previously but were part of a larger assessment on neurovascular health (see **Chapter 3**). Briefly, women arrived after a 12 hour (overnight) fast and had their blood sampled from an antecubital vein by a trained person (RS) to measure sex hormones (estrogen, progesterone, testosterone), metabolic markers (fasting glucose and insulin) and vascular growth factors (VEGF, PlGF, and sFlt-1). Fasted blood samples were sent to a local commercial laboratory (DynaLIFE, Edmonton, AB, Canada) for the determination of glucose (hexokinase, Seimens Advia 1800), insulin (chemiluminescence microparticle immunoassay, Abbott Architect i2000), estradiol (electrochemiluniscence, Roche Cobas), progesterone (chemilunimescence competitive immunoassay, Siemens Centaur), and testosterone (two-site sandwich chemiluninescence, Siemens Centaur) concentrations. Insulin resistance was

determined using HOMA-IR calculated from https://www.dtu.ox.ac.uk/. Enzyme-linked immunosorbent assay (ELISA) was used to determine concentrations of VEGF, PlGF, and sFlt-1 (R&D Systems; Human VEGF Immunoassay DVE00, Human PlGF immunoassay DPG00, and Human VEGFRI/Flt-1 Immunoassay DVR100C, respectively). Following the blood draw, women ate a standard breakfast (multigrain bagel, jam, and juice).

After breakfast, the woman's height (cm) and current weight (kg) were recorded using a stadiometer and a calibrated scale. Women then sat in a semi-reclined chair in a temperature-controlled room and were instrumented to measure heart rate (ECG; lead II; ADInstruments, Colorado Springs, USA), blood pressure using finger photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, the Netherlands), and pulse at toe (pulse transducer; TN1012; ADInstruments). Systolic, diastolic blood pressure (SBP and DBP, respectively) were calibrated offline (in LabChart) using three recordings taken by an automated blood pressure cuff (Omron). Cardiac output (derived model flow) was determined using the Finometer (Bijl *et al.*, 2019) and SVR was determined as the ratio between MAP/cardiac output.

Following instrumentation, we assessed PWV and resting blood flow using doppler ultrasound (12Mhz linear array probe; Vivid 7; General Electric) on the right carotid artery and right superficial femoral artery for at least one minute each in succession. PWV was assessed in order to determine central (aortic) and peripheral (upper and lower limb) arterial stiffness. Video files of the ultrasound screen were saved using video capture software (DVI2USB 3.0, Epiphan Video, Ottawa, Canada) and stored for offline analysis in Vascular Research Tools (Medical Imaging Applications LLC, Iowa, USA). Mean blood velocity waveforms were collected continuously in LabChart using a QDAT for offline analysis (Herr *et al.*, 2010).

Central PWV was determined as the ratio of distance between the carotid and femoral artery recording sites and the transit time of the carotid and femoral pulse (determined offline using LabChart threshold detection to identify the foot of the carotid and femoral pulse) according to the following equation:

Central PWV = (distance between femoral site – carotid site)/ (femoral artery - carotid artery transit time)

The distance to the carotid artery was determined as the distance from the sternal notch to the center of the recording window from the ultrasound. The distance to the femoral artery recording site was measured along the lateral side of the body to accommodate the growing abdomen. Upper limb pulse wave velocity was determined using the transit time to the pulse detected at the finger by the Finometer as:

Upper limb PWV = (distance to finger-carotid)/ (transit time to finger-carotid)

Distance was taken from the sternal notch to the location of the pulse transducer (Finometer) on the middle or index finger of the left hand. Lower limb PWV was determined using the transit time from the foot of the superficial femoral artery waveform to the foot of the pressure waveform from the toe pulse transducer placed on the big toe of the right foot:

Lower limb PWV = (distance from femoral artery to toe) / (transit time from femoral artery to toe)

Distance was determined as the length from the location of the ultrasound signal on the superficial femoral artery to the big toe of the right foot.

We used simultaneous systolic and diastolic diameter and blood pressure and carotid systolic and diastolic vessel diameter data from at least ten consecutive heartbeats to determine the coefficients for carotid compliance (CC) and distensibility (DC). CC and DC are measures of vessel wall buffering function and elastic properties, respectively and are the reciprocal of arterial stiffness. CC and DC were determined according to the equations provided by Van Bortel *et al.* (2002):

$$CC = \Delta A / \Delta P$$

$$DC = (\Delta A / A) / \Delta P$$

where A represents the cross-sectional area of the carotid artery in mm^2 and ΔP represents the pulse pressure (converted to kPa). DC was multiplied by a factor of 1000 to give final measures in the units 10^{-3}/kPa. Carotid distensibility measures were determined from ten consecutive heart beats according to the equations found in the publications by Gamble *et al.* (1994); Godia *et al.* (2007). In addition to CC and DC we also measured other carotid artery distensibility metrics including strain (%), stiffness (β), distensibility (1/β), and Elastic modulus (E) to allow for a more complete picture of arterial stiffness changes throughout gestation and with respect to exercise intervention as these measures are not always well corelated with one another (Tanaka, 2018). Briefly, strain (%) is a measure of the relative deformation of the blood vessel throughout the cardiac cycle, measures as:

$$\text{Strain (\%)} = (\text{systolic diameter} - \text{diastolic diameter}) / \text{diastolic diameter}$$

whereas β-Stiffness is a measure of the stiffness of the artery and distensibility (1/β) is the reciprocal:

$$\text{Stiffness } (\beta) = \ln(\text{SBP} / \text{DBP})/ \text{ strain}$$

$$\text{Distensibility} = 1/\beta$$

More specifically, distensibility measures the ability of the artery to expand and contract during systole and diastole that considers the arterial blood pressure within the vessel in addition to the changes in vessel diameter. Lastly, the elastic modulus is an estimate of the stiffness of the artery that is independent of the wall properties (i.e., thickness of the wall). In this manuscript, we specifically calculated this as Petersons Elastic modulus as we were unable to control for carotid intima media thickness (IMT):

$$\text{Elastic modulus } (E) = (\text{SBP-DBP})/ \text{ strain}$$

Arterial blood flow was determined from artery diameter and blood velocity, where mean artery diameter (cm) was determined as the average of one minute of continuous diameter measurements offline in Vascular Research Tools (Medical Imaging Applications LLC, Iowa, USA) brachial analyzer (version 5.6.5). Briefly, this software uses edge detection software and determines the diameter of the blood vessel on a frame-by-frame basis. Concurrent, blood velocity (cm/s) was determined as a 1 min average from the beat-by-beat integrated weighted mean waveform collected simultaneously in LabChart.

We determined superficial femoral vascular conductance (FVC) and resistance (FVR) according to the following equations:

FVC= femoral blood flow/ MAP

FVR= MAP/femoral blood flow

Women sat for three to five minutes of quiet rest, followed by a three-minute cold pressor test (submerging hand up to the wrist in ice water; 4ºC). During this protocol, heart rate, arterial blood pressure, cardiac output, and blood flow in the superficial femoral artery were continuously recorded. Following CPT, women's hands were re-warmed using a heating pad. All variables were exported into Lab Chart 8 via Powerlab 16/35 data acquisition system (PL3516, ADInstruments) and stored for offline analysis. Superficial femoral artery blood flow, FVC and FVR during the CPT were determined in one-minute-bins, concurrent with HR and blood pressure.

4.2.2 Exercise Intervention

Women in the intervention group were prescribed an aerobic exercise intervention that conformed to the current Canadian Clinical Practice Guidelines for exercise during pregnancy at the time of study conception (Davies *et al.*, 2003). The intervention has been reported in detail elsewhere (see **Chapter 3.2.2**); briefly, women in the exercise group were instructed to exercise 3-4 times per week at a moderate intensity of exercise (50-70% HRR; determined the first week of their program) initiated immediately following their baseline pre-intervention assessment (17-21weeks), continuing until their post-intervention follow-up assessment 34-36 weeks gestation. The average intervention length was 14 ± 1 weeks. Women attended a minimum of one supervised exercise session per week, and when exercising on their own, they recorded their heart rate using a pulse count or available heart rate monitor. Women in the control group were not prescribed any physical activities.

4.2.3 Statistical Analysis

All statistical analyses were determined using GraphPad Prism (v8.3.4). Resting hematological values from baseline assessments and subject demographics were compared using an unpaired t-test. For these data, normality was tested using a Shapiro-Wilks test, and when data were not normally distributed a Mann-Whitney test on ranks was used to determine differences. 2-way repeated measures (group x gestational age) mixed effects ANOVA were used to assess statistical differences between the exercise intervention and control group from pre-to-post-intervention for all resting measures. For CPT responses, a three-way (group x gestational age x time) mixed effects ANOVA was used. If applicable, post-hoc Holm-Sidak multiple comparisons test was used to determine the if there were differences between the groups in the change in outcome measure between trimesters. Significance was set at $p<0.05$; however, effect size was reported if $p>0.05$ but less than 0.1 and $d>0.8$ (large effect size) was interpreted to be a meaningful difference (Cohen, 1992; Lenhard & Lenhard, 2016). No separate sample size calculation was performed for this manuscript as the sample size was determined based on the sympathetic nerve activity measures as the primary outcome (see **Chapter 3.2.5**).

4.3 Results

4.3.1 Subject demographics

Subject demographics have been previously reported (see **Chapter 3.3.1**). Briefly, there were no differences at study entry between groups for maternal age or their pre-pregnancy BMI. Gestational weight gain is shown in **Table 4.1**. There was no difference between groups for total gestational weight gain or weekly weight gain during the intervention ($p=0.508$ and $p=0.728$, respectively). Gestational weight gain categories were based on pre-pregnancy BMI category (i.e., normal weight, overweight, or obese) and the *2009 Institute of Medicine Guideline*, and there were

no differences in the number of women in each group who gained inadequately, adequately, or excessively ($p=0.155$).

Table 4.1 *Gestational weight gain and birth outcomes*

	Control Group	Exercise Group	p=value
Gestational age at delivery (weeks)	39 ± 1	39 ± 1	0.221
Gestational weight gain*			
Number of women	23	23	
Total gestational weight gain (kg)	14.2 ± 5.7	15.2 ± 4.7	0.508
Inadequate; n (%) Adequate; n (%) Excessive; n (%)	6 (22) 11 (41) 10 (37)	2 (8) 7 (29) 15 (63)	0.155
Weekly weight gain during the intervention period (kg)**	0.54 ± 0.16	0.52 ± 0.29	0.728
Birth weight			
Number of women	27	29	
Fetal birth weight (g)	3432 ± 508	3456 ± 454	0.849
Microsomia (<2500g); n (%)[#] Normal (2500-4000g); n (%) Macrosomia (>4000g); n (%)	0 (0) 25 (93) 2 (7)	(0) 26 (90) 3 (10)	0.700
Delivery mode[$]			
Vaginal; n (%) C-section; n (%)	20 (74) 7 (26)	22 (78) 6 (22)	0.695

Data are represented as mean ± SD or n (%). One woman in the control group and two women in the exercise group did not provide delivery information. *Gestational weight gain was determined in a subset of women due to inadequate patient recall (i.e., they did not weigh themselves or remember their final weight). Pre-pregnancy weight was determined from participant recall at the initial meeting (<20weeks). Final weight was determined from participant recall in the postpartum (<2months) and used if the last maternal weight recorded was within 2 weeks of delivery. **Weekly weight gain was measured in the 51 women who completed the study (23 control, 28 exercise). [#]No women had babies <2500g, therefore, this category could not be included in the chi squared test. [$]Delivery mode was not reported for one woman in the exercise group (n=28).

Two women in the control group and three women in the exercise group developed gestational diabetes (8.7% vs 10.7% incidence, respectively). Women who developed gestational diabetes mellitus in the exercise group continued their exercise program and all women followed advice from their health care providers for disease management. One woman in the control group developed gestational hypertension at delivery, and one woman in the control group dropped out after the first assessment due to the development of preeclampsia, but no women experienced hypertension while enrolled in the study.

Birth outcomes are shown in **Table 4.1**. There were no differences between exercise and control groups for gestational age at delivery ($p=0.221$), birth weight ($p=0.849$) or mode of delivery ($p=0.695$). One woman in the control group delivered her infant prior to 37 weeks (at 36+2 weeks; developed preeclampsia). Two women in the control group and three women in the exercise group delivered infants >4000g; no women delivered an infant <2500g.

4.3.2 Resting measures in the carotid and femoral arteries.

The baseline hemodynamic measures (heart rate, SBP, DBP, MAP, cardiac output, and SVR) have been previously published (Skow *et al.*, 2020). Briefly, the increase in heart rate across gestation was attenuated in women in the exercise group. However, the increase in blood pressure with advancing gestational age was not impacted by exercise. Cardiac output and SVR were not impacted by gestational age or exercise in this study.

Carotid and superficial femoral artery measurements at rest are presented in **Table 4.2.** A representative tracing and ultrasound image sample for both resting carotid and superficial femoral artery are shown in ***Appendix C, Figure C3*** (top & middle). Overall, there were no group differences at either trimester for all outcome variables. Carotid artery diameter was increased from TM2 to TM3 (main effect of gestational age, $p<0.0001$). Carotid artery strain (%) decreased from TM2 to TM3 (main effect of gestational age, $p=0.0003$). Similar to the carotid artery, the superficial femoral artery diameter increased from TM2 to TM3 (main effect of gestational age, $p=0.004$). Blood flow in the superficial femoral artery was in line with previous literature in non-pregnant women (Holland *et al.*, 1998; Olver *et al.*, 2012). All other variables were not impacted by gestational age (see **Table 4.2**). None of our outcome measures were impacted by exercise. Although an intent-to-treat analysis was used to determine the impact of a structured aerobic intervention on these measures, compliance did not impact the interpretation of any of our results (see ***Appendix B, Table B3 and B4***).

Table 4.2 *Summary of resting variables in the common carotid and superficial femoral arteries.*

	Control Group				**Exercise Group**				**P-values**		
	Pre-intervention		Post-intervention		Pre-intervention		Post-intervention				
	n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	Group	G.A	Interaction
Gestational Age (wks)	28	18 ± 1	23	35 ± 2	31	18 ± 2	28	34 ± 1	n/a		
Resting Hemodynamics											
Heart rate (bpm)	28	76 ± 7	23	88 ± 11	31	82 ± 8	28	86 ± 8	0.238	**<0.001**	**0.002**
SBP (mmHg)	28	108 ± 11	23	111 ± 12	31	110 ± 11	28	110 ± 9	0.739	0.248	0.241
DBP (mmHg)	28	61 ± 7	23	68 ± 7	31	65 ± 7	28	62 ± 8	0.160	**<0.001**	0.175
MAP (mmHg)	28	79 ± 8	23	85 ± 7	31	83 ± 7	28	86 ± 8	0.222	**<0.001**	0.149
Common Carotid Artery											
Diameter (mm)	25	6.58 ± 0.57	16	6.88 ± 0.56	26	6.63 ± 0.58	21	6.90 ± 0.48	0.899	**<0.001**	0.321
Blood flow (ml/min)	24	583 ± 160	16	542 ± 131	26	588 ± 151	21	567 ± 177	0.789	0.417	0.783
CC (mm^2/kPa)	23	1.08 ± 0.32	15	1.12 ± 0.23	24	1.07 ± 0.32	20	1.08 ± 0.38	0.650	0.743	0.605
DC (10^{-3}/kPa)	23	35.5 ± 12.9	15	33.9 ± 7.8	24	34.3 ± 9.7	20	31.6 ± 11.2	0.521	0.146	0.772
Strain (%)	23	10.0 ± 3.0	15	8.6 ± 1.9	24	9.9 ± 2.3	20	8.2 ± 1.7	0.626	**<0.001**	0.978
Stiffness (β)	23	6.0 ± 1.9	15	5.6 ± 1.3	24	5.8 ± 1.2	20	7.4 ± 6.1	0.291	0.432	0.176
Distensibility (1/β)	23	0.18 ± 0.07	15	0.19 ± 0.04	24	0.18 ± 0.05	20	0.17 ± 0.06	0.537	0.587	0.618
Elastic modulus (E)	23	66.3 ± 22.2	15	64.6 ± 15.1	24	65.1 ± 15.0	20	75.6 ± 31.9	0.363	0.229	0.149
Superficial Femoral Artery											

Diameter (mm)	26	5.34 ± 0.72	18	5.53 ± 0.64	24	5.47 ± 0.69	21	5.78 ± 0.84	0.434	**0.004**	0.477
Blood flow (ml/min)	23	76.3 ± 36.2	19	84.4 ± 49.9	20	96.8 ± 46.8	20	91.1 ± 50.3	0.257	0.745	0.494
FVC (ml/min/mmHg)	23	0.95 ± 0.47	19	0.99 ± 0.53	20	1.13 ± 0.54	20	1.08 ± 0.63	0.344	0.941	0.943
FVR (mmHg/ml/min)	23	1.52 ± 1.38	19	1.28 ± 0.68	20	1.09 ± 0.55	20	1.33 ± 0.92	0.366	0.986	0.271

All data is presented as mean ± SD. Resting hemodynamics has been published previously. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; CC, compliance coefficient; DC, distensibility coefficient; FVC, femoral vascular conductance; FVR, femoral vascular resistance.

4.3.3 Pulse wave velocity

PWV measures are shown in **Table 4.3**. Central PWV measured as carotid-femoral velocity was not altered across the gestation (main effect of gestational age p=0.719), nor was it influenced by exercise (interaction effect, p=0.943). Upper PWV measured as carotid-finger velocity was not different between TM2 and TM3 (main effect of gestational age, p=0.756), nor was it influenced by exercise (interaction effect, p=0.231). Similarly, lower PWV measured as femoral-toe velocity not different in TM3 compared to TM2 (main effect of gestational age, p=0.101), nor was it impacted by exercise (interaction effect p=0.962).

Table 4.3 *Summary of central and peripheral pulse wave velocity measures.*

	Control Group				**Exercise Group**				**P-values**		
	Pre-intervention		Post-intervention		Pre-intervention		Post-intervention				
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	Group	G.A	Interaction
Central PWV (m/s)	20	4.92 ± 0.87	17	4.88 ± 0.54	26	4.97 ± 1.15	23	4.92 ± 1.16	0.759	0.719	0.943
Upper limb PWV (m/s)	23	6.13 ± 1.01	20	6.39 ± 0.95	28	6.25 ± 1.15	24	6.19 ± 0.81	0.796	0.335	0.231
Lower limb PWV (m/s)	16	7.54 ± 1.85	17	7.95 ± 1.03	21	7.19 ± 1.62	18	7.72 ± 1.25	0.311	0.101	0.962

All data is shown as mean ± SD. PWV, pulse wave velocity. Central PWV was determined as carotid to femoral. Upper PWV was determined from the finger pulse on the Finometer and corrected using the carotid ultrasound signal. Lower PWV was determined from the pulse transducer on the big toe and the ultrasound at the femoral artery.

4.3.4 Cold pressor test response

We have previously published the heart rate and blood pressure responses to the CPT (see **Chapter 3.3.5**). Briefly, there is no effect of gestational age or exercise on the heart rate response to CPT. However, there was an effect of gestational age on the blood pressure response to CPT such that women in the third trimester have a larger increase in blood pressure during CPT compared to the second trimester. The change in blood pressure response across gestation was not influenced by our exercise intervention.

Here we evaluated the blood flow responses in the superficial femoral artery during 3-minutes CPT. These data are shown in **Figure 4.1** and a representative trace of blood velocity and vessel diameter is shown in ***Appendix C, Figure C3*** (bottom). As previously mentioned, the diameter in the superficial femoral artery was larger in TM3 compared to TM2, this observation persisted throughout the CPT (main effect of gestational age, $p=0.044$). However, there was no change in diameter during the CPT (main effect of time, $p=0.305$), nor was there an interaction with respect to the intervention (group x gestational age x time interaction, $p=0.740$). Blood flow in the superficial femoral artery was increased during the CPT (main effect of time, $p<0.0001$) but there were no differences in the blood flow response across pregnancy (gestational age x time interaction, $p=0.621$) nor was there an effect of exercise (group x gestational age x time interaction, $p=0.397$). Taking concurrent changes in blood pressure into account, FVC was increased and FVR was decreased throughout the CPT (main effect of time, $p<0.001$ for both); however, this was not influenced by gestational age or exercise. Compliance did not affect any of these results (see ***Appendix B, Figure B2***). We also determined the individualized peak response (minute with largest deviation from baseline) for each femoral blood flow, FVC, and FVR. For each of these,

there was no main effect of group or gestational age, nor a group x gestational age interaction, as shown in **Figure 4.2**.

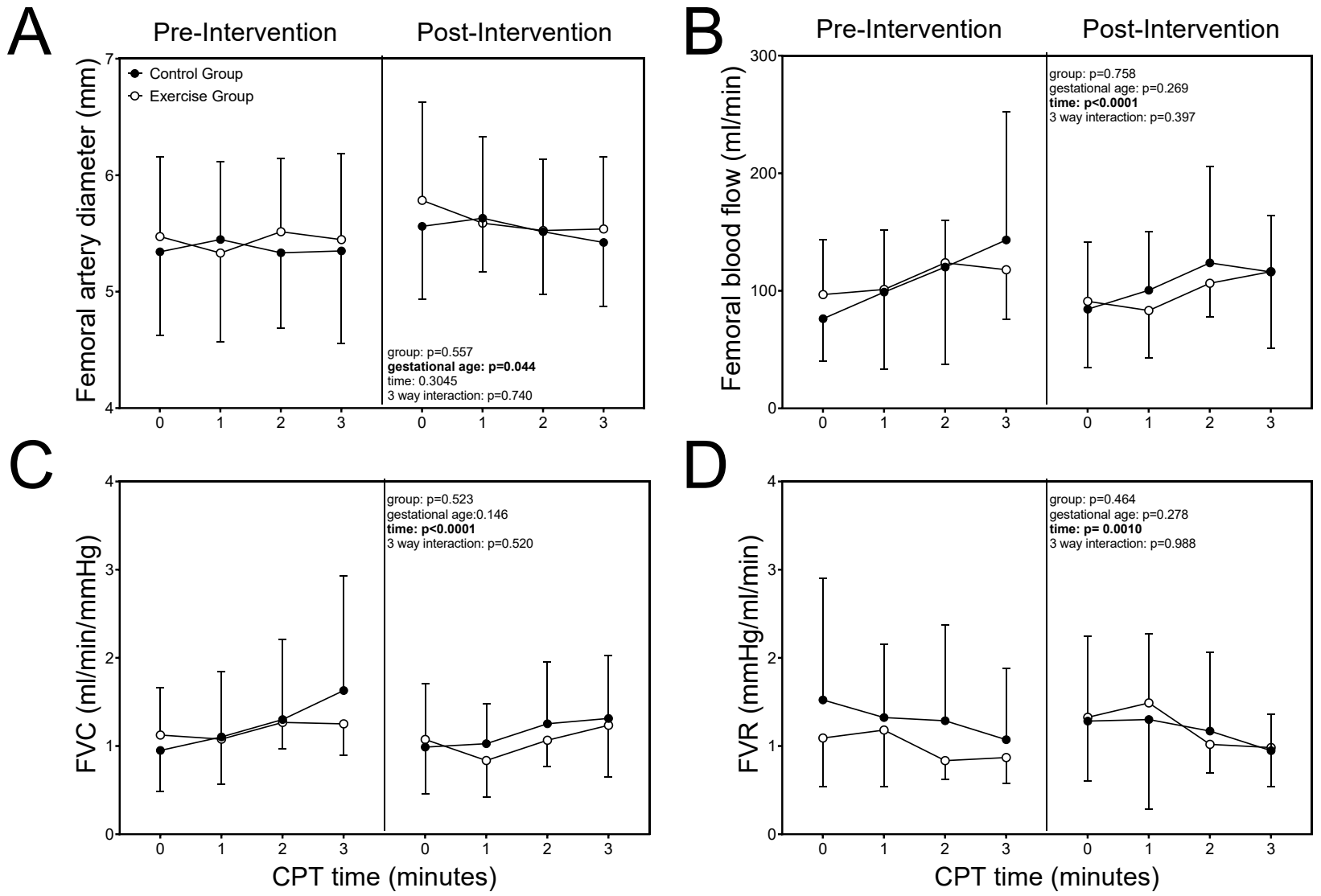

A
Pre-Intervention
Post-Intervention
Control Group
Exercise Group
Femoral artery diameter (mm)
group: p=0.557
gestational age: p=0.044
time: 0.3045
3 way interaction: p=0.740
B
Pre-Intervention
Post-Intervention
Femoral blood flow (ml/min)
group: p=0.758
gestational age: p=0.269
time: p<0.0001
3 way interaction: p=0.397
C
FVC (ml/min/mmHg)
group: p=0.523
gestational age:0.146
time: p<0.0001
3 way interaction: p=0.520
CPT time (minutes)
D
FVR (mmHg/ml/min)
group: p=0.464
gestational age: p=0.278
time: p= 0.0010
3 way interaction: p=0.988
CPT time (minutes)

Figure 4.1. Cold pressure test (CPT) responses in the superficial femoral artery. All data is mean ± SD. A) superficial femoral artery diameter in control (filled circles) and exercise (open circles) groups at rest (minute 0) and during each minute of the CPT at pre-intervention (left) and post-intervention (right). There was no change in diameter of the superficial femoral artery during the CPT. B) Blood flow (ml/min) in the superficial artery during each minute of CPT. Blood flow increased during the CPT, but the response was not different across gestation or with respect to exercise intervention. C) Femoral vascular conductance (FVC) in the superficial femoral artery during each minute of CPT. FVC increased during the CPT, but the response was not different across gestation or with respect to exercise intervention. D) Femoral vascular resistance (FVR) in the superficial femoral artery during each minute of CPT. FVR decreased during the CPT, but the response was not different across gestation or with respect to exercise intervention.

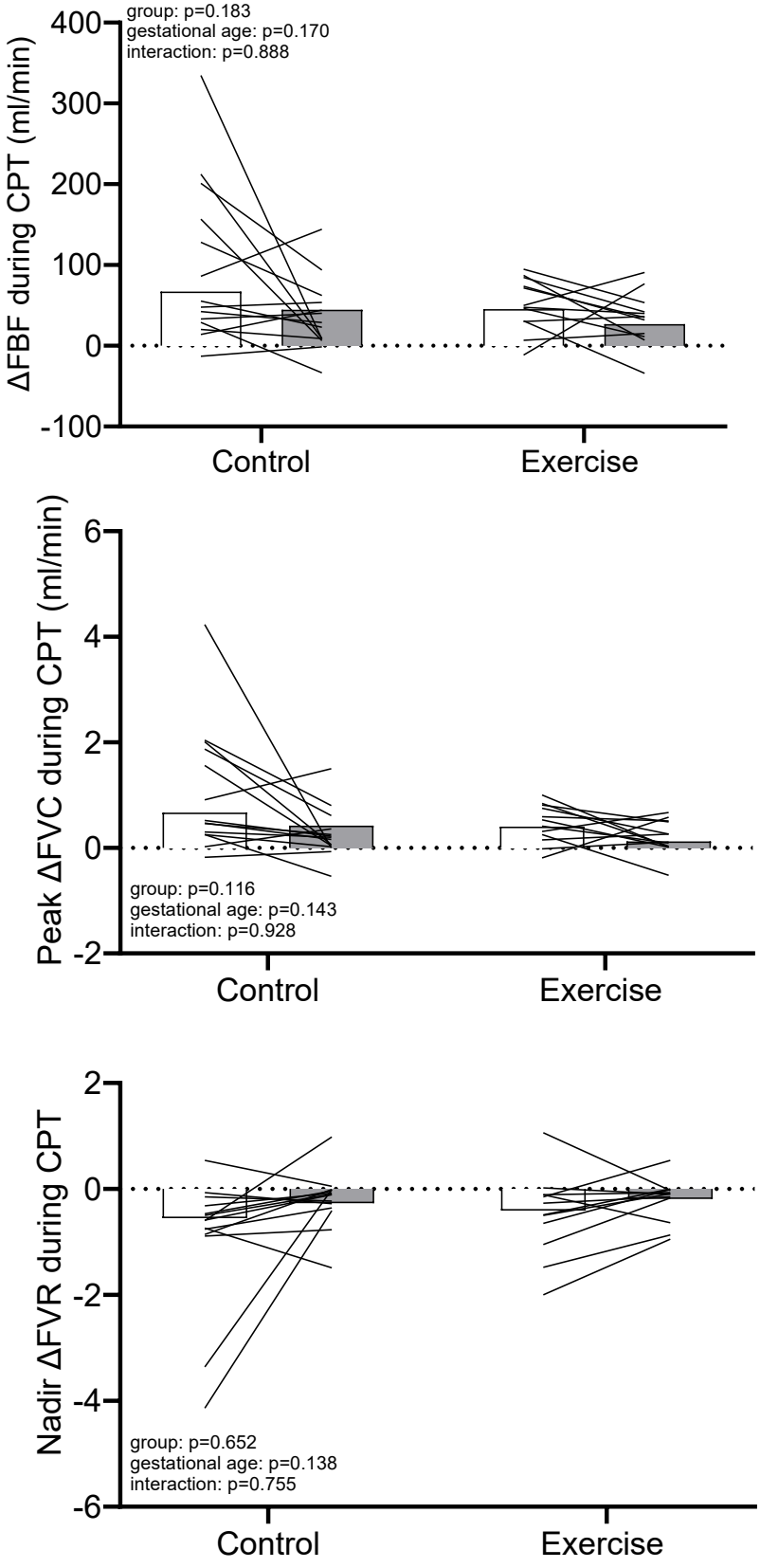

group: p=0.183
gestational age: p=0.170
interaction: p=0.888
ΔFBF during CPT (ml/min)
400
300
200
100
0
-100
Control
Exercise
Peak ΔFVC during CPT (ml/min)
6
4
2
0
-2
group: p=0.116
gestational age: p=0.143
interaction: p=0.928
Control
Exercise
Nadir ΔFVR during CPT
2
0
-2
-4
-6
group: p=0.652
gestational age: p=0.138
interaction: p=0.755
Control
Exercise

Figure 4.2 Peak response in superficial femoral blood flow (FBF), femoral vascular conductance (FVC), and resistance (FVR) during three minutes of cold pressor test (CPT). Top) The peak change (increase) in FBF (ml/min) during CPT was not affected by gestational age or exercise. **Middle)** The peak change (increase) in FVC (mmHg/ml/min) during CPT was not affected by gestational age or exercise. **Bottom)** The peak change (decrease) in FVR (ml/min/mmHg) during CPT was not affected by gestational age or exercise.

4.3.5 Vascular growth factors, sex hormones, and metabolic markers.

Blood analysis results are shown in **Table 4.4.** VEGF was not different between TM2 and TM3 (main effect of gestational age, p=0.291), nor was there an effect of exercise (interaction effect, p=0.560). PlGF was increased between TM2 and TM3 (main effect of gestational age, p<0.0001) but again there was no effect of exercise (interaction effect, p=0.817). Similarly, sFlt-1 was increased with gestational age (p<0.0001) but was not impacted by exercise (interaction effect, p=0.496). The sFlt-1:PlGF ratio was normal in all women (<38.0; Zeisler *et al.*, 2016), and did not change across gestation (p=0.083; d=0.58), nor with exercise (interaction effect, p=0.903). To determine the relationship between changing angiogenic status (i.e., sFlt-1:PlGF ratio) and vascular outcomes, we performed correlations between the sFlt-1:PlGF ratio and carotid artery distensibility, arterial stiffness, and vascular reactivity measures. There were no significant correlations between angiogenic status and any vascular outcome (**see *Appendix C, Table C2***).

Sex hormones estrogen, progesterone, and testosterone were increased across pregnancy (main effect of gestational age; p<0.001) but there was no impact of exercise on this response (interaction effect p=0.241, 0.304, and 0.387, respectively). Fasting blood glucose was determined after 12 hours fast and was not different across gestation or impacted by exercise. Insulin levels increased with advancing gestation (main effect of gestational age, <0.0001) but was also not impacted by exercise (interaction effect; p=0.586). Insulin resistance (HOMA-IR) was also higher in TM3 compared to TM2 (main effect of gestational age; p=0.0003) but was not impacted by exercise (interaction effect, p=0.971). There were significant correlations between estrogen and some of the measures of carotid distensibility in the TM3 (see ***Appendix C, Table C2***).

Table 4.4 *Summary of vascular growth factors, sex hormones, and metabolic markers.*

	CONTROL				EXERCISE				p-value		
	n	TM2	n	TM3	n	TM2	n	TM3	Group	G.A.	Interaction
Vascular growth factors											
VEGF (pg/ml)	23	12 ± 10	19	15 ± 17	28	22 ± 34	25	30 ± 61	0.177	0.291	0.560
PlGF (pg/ml)	27	163 ± 77	20	477 ± 413	30	160 ± 72	26	452 ± 374	0.794	**<0.0001**	0.817
sFlt-1 (pg/ml)	27	971 ± 749	17	1610 ± 933	29	941 ± 495	24	1497 ± 792	0.587	**<0.0001**	0.496
sFlt-1/PlGF	27	7.3 ± 6.7	17	5.3 ± 5.4	29	6.5 ± 3.3	24	4.9 ± 4.2	0.580	0.083	0.903
Sex hormones and fasted blood markers											
Estrogen (pmol/L)	27	28068 ± 11352	19	69438 ± 21897	30	25175 ± 11175	26	62781 ± 24692	0.246	**<0.0001**	0.241
Progesterone (nmol/L)	27	169 ± 40	19	619 ± 167	30	163 ± 45	27	662 ± 210	0.514	**<0.0001**	0.304
Testosterone (nmol/L)	27	1.9 ± 1.0	20	2.1 ± 1.1	30	2.1 ± 1.3	27	2.7 ± 1.7	0.318	**0.0002**	0.387
Glucose (mmol/L)	27	4.3 ± 0.4	19	4.4 ± 0.5	30	4.5 ± 0.4	26	4.4 ± 0.5	0.510	0.813	0.219
Insulin (pmol/L)	27	40.4 ± 19.4	17	66.0 ± 33.4	30	53.5 ± 40.1	26	68.9 ± 49.0	0.274	**<0.0001**	0.586
HOMA IR	24	0.79 ± 0.34	17	1.16 ± 0.63	29	0.99 ± 0.74	26	1.25 ± 0.90	0.290	**0.0003**	0.971

All data is shown as mean ± SD. Blood samples were taken in the fasted state (12 hour) and centrifuged and frozen for analysis at study completion. VEGF, vascular endothelial growth factor; PlGF, placental growth factor; sFLT-1, soluble fms-like tyrosine kinase-1; IR, insulin resistance

4.4 Discussion

Significant evidence indicates prenatal physical activity confers clear benefits to both mother and child (Davenport *et al.*, 2018a; Davenport *et al.*, 2018b; Davenport *et al.*, 2018e; Ruchat *et al.*, 2018), including the reduction in the risk of developing pregnancy complications such as hypertension (Davenport *et al.*, 2018d). These benefits are mediated through multiple pathways including a shift in autonomic balance (Skow *et al.*, 2020), improvements in fasting blood glucose (Davenport *et al.*, 2018e), and improvements in endothelial function (Ramírez-Vélez *et al.*, 2011). Therefore, we hypothesized that a structured physical activity program would result in positive adaptations in vascular structure and function, linked to improved angiogenic balance. We conducted robust assessments of peripheral and central vascular structure and function, including vessel diameters, indices of vascular stiffness, and resting and reflex changes in blood flow and resistance. While common carotid and superficial femoral arteries increased in diameter across gestation, this was not associated with altered flow. Further, these changes were not impacted by our exercise intervention. Similarly, we also did not observe differences in central or peripheral PWV, fasting blood glucose, or angiogenic factors in response to our intervention. Lastly, we observed no impact of gestational age or exercise intervention on the vascular responses during a three-minute CPT. The culmination of these results leads us to conclude that: a) the reduced cardiovascular risk imparted by prenatal exercise may not stem from altered macrovascular structure or function; b) prenatal exercise may not lead to observable changes in macrovascular function and structure in otherwise healthy women; and c) future work in high-risk women is needed.

4.4.1 Gestational weight gain and birth outcomes

Exercise during pregnancy reduces GWG (Ruchat *et al.*, 2018); however, we did not observe differences in total GWG. While both groups (63% in exercisers and 37% in controls) gained in excess of other randomized trials (34%) (Ruchat *et al.*, 2018) and the *2009 Institute of Medicine* recommendation, this weight gain occurred outside of the intervention period as noted by non-significant differences in weekly GWG. This highlights the importance of early pregnancy intervention. While excessive GWG is associated with larger infants (Wang *et al.*, 2020); exercise during pregnancy has been shown to reduce the odds of having a baby with macrosomia (>4000g) (Davenport *et al.*, 2018b). We had similar numbers of women in each group with infants with macrosomia (7% in controls and 10% in exercisers), and these values fell below our provincial average of 12.4% (Dubois *et al.*, 2007).

4.4.2 Carotid artery structure and function

In the current study we measured macrovascular structure and function including the diameter, blood flow, and function (CC, DC, strain, stiffness, and Youngs elastic modulus) of the common carotid artery. In line with previous literature, we showed a progressive increase in carotid artery diameter such that it was largest in TM3 compared to TM2 (Skow *et al.*, 2017). Similar to the common carotid artery, here we also showed an increase in basal superficial femoral artery diameter, but not flow, with gestation; this was also not impacted by exercise. The observations from this study would suggest that these increases in artery diameter did not support a higher blood flow, but rather served to reduce artery strain while maintaining blood pressure. Decreased carotid artery distensibility (CC and DC) are associated with poorer health outcomes (Yuan *et al.*, 2016) and thus we hypothesized that aerobic exercise would increase the distensibility metrics relative to no exercise during pregnancy. We did not show an effect of exercise on any of our carotid function measures; however, the values we obtained for CC and DC were similar to those which

have been previous reported in pregnancy (Yuan *et al.*, 2013b). Specifically, Yuan et al., (2013b) showed that carotid DC and CC are increased in TM3 of normal pregnancy (compared to non-pregnant controls), but that these differences disappeared when they controlled for BMI, gestational age, heart rate, and blood pressure. Similar changes in distensibility and stiffness of the carotid artery were reported in a cross- sectional study by Kärkkäinen *et al.* (2014) in that women in TM3 had increased stiffness and decreases CC compared to women the non-pregnant state. However, in the aforementioned study and others (Visontai *et al.*, 2002; Mersich *et al.*, 2005) the measures of carotid function were not different between the second and third trimester indicating that the time-points we chose may have been too similar to detect a difference. This highlights the need for earlier interventions. These findings are taken in combination with negative correlations between carotid diameter and DC ($p=0.002$) suggests that larger diameter vessels display qualities of increased stiffness even in an otherwise healthy circulation (i.e., no evidence of increased peripheral or central PWV).

Here we observed a decrease in strain (the percent change in carotid diameter from diastole to systole) in TM3 compared to TM2. This implies that the carotid wall is deformed to a lesser amount for each heartbeat and suggests a stiffer artery. Visontai and colleagues (2002) observed a similar increase in stiffness (measured by the DC) in both TM2 and TM3 compared to the first trimester. Although this may be related to the increase in diameter in the carotid artery observed, as larger diameter arteries may not be able to deform as much, it is more likely to be related to the ratio between systolic and diastolic diameters or the changing pulse pressure. Indeed, if pulse pressure is lower, then measures of stiffness, distensibility, and Elastic modulus would also be lower. Visontai et al., (Visontai *et al.*, 2002) observed a correlation between DC and cardiovagal baroreflex sensitivity (which was decreased in TM2 and TM3 compared to the first trimester or

postpartum), and therefore attributed the increase in stiffness to the decrease in cardiovagal BRG that they observed during pregnancy. We have shown that cardiovagal BRG is positively correlated with both self-reported physical activity and objectively measured physical activity (i.e., accelerometry) in pregnancy (Sobierajski *et al.*, 2018) suggesting that there is a potential for exercise to influence these measures. Future work in this area is needed to determine the mechanisms for exercise-related changes in cardiovascular health in pregnancy.

4.4.3 Pulse wave velocity

Central PWV assesses aortic stiffness and is linked with cardiovascular health and mortality (Willum-Hansen *et al.*, 2006), and is known to be influenced by exercise intervention (Huang *et al.*, 2016). Contrary to our hypothesis, we did not observe changes in central PWV across gestation, nor with exercise. This finding is corroborated by the recent work of Brislane and colleagues (Brislane *et al.*, 2020). However, they reported that PWV was lower in TM2 compared to TM3, which is consistent with previous literature (reviewed in: Skow *et al.*, 2017). The data from the present study reflect similar values in TM2 compared to the work by Brislane and colleagues (2020). Yuan et al (2013b) showed that PWV was related to SBP in pregnancy, and while we replicated those findings here, the change in SBP across gestation was not correlated with the change in central PWV across gestation ($p=0.260$). Peripheral PWV is an indicator of the stiffness of the muscular arteries in the peripheral circulation (i.e., limbs), but does not appear to predict future cardiovascular risk (Laurent *et al.*, 2006). Previous literature in non-pregnant individuals with hypertension and obesity suggest that the greatest changes in response to aerobic exercise training occur in peripheral PWV rather than central PWV (Huang *et al.*, 2016). While studies in healthy non-pregnant populations show no change in lower PWV (femoral-ankle PWV) (Yoshizawa *et al.*, 2009), no studies have looked at upper limb PWV and exercise training in

healthy persons that we know of. In our study we demonstrated that upper and lower PWV were unchanged with exercise; however, our study included mainly healthy, normotensive pregnant women. Therefore, it remains possible that aerobic exercise may be more effective at reducing arterial stiffness women who are at high risk for developing hypertension (i.e., who already might have high PWV).

4.4.4 Reactivity during cold pressor stress

Previous literature has shown increased blood pressure reactivity during CPT in women who subsequently developed preeclampsia (Woisetschlager *et al.*, 2000), and exercise training has been shown to increase vasoreactivity during both dilatory and constrictor stressors in non-pregnant populations (Welsch *et al.*, 2013). While no study has reported on the changes in femoral artery conductance/resistance during a cold pressor test in pregnancy, work from Miller and colleagues (2019) suggests that women (and not men) are more likely to vasodilate during CPT stress despite large increases in blood pressure and sympathetic nervous system activation. Thus, we hypothesized that prenatal exercise would result in a larger femoral vasodilation (increased conductance). We observed a similar increase in FVC during CPT in pregnant women suggesting vasodilation in downstream resistance vessels. This aligns with reduced NVT evident in pregnant women (Usselman *et al.*, 2015b; Steinback *et al.*, 2019). However, we did not observe an impact of exercise on the vascular response to CPT. Future research in women at high risk for developing gestational hypertension is needed.

4.4.5 Vascular growth factors, sex hormones, and metabolic markers.

In the current study we investigated the impact of our exercise intervention on vascular structure and function as well potential molecular mechanism, in particular vascular growth factors (angiogenic factors) VEGF, PlGF and sFlt-1. sFlt-1 is higher in women with preeclampsia; sFlt-1

inactivated PlGF and thus impairs angiogenesis. Moreover, having a ratio of sFlt-1:PlGF ≥38.0 has been shown as a positive predictor for preeclampsia development (Zeisler *et al.*, 2016). Importantly, none of the women in this study had sFlt-1:PlGF ≥38 at any time point, thus all women (exercise or control group) had normal angiogenic status. Cross-sectional evidence from Weissgerber *et al.,* (Weissgerber *et al.*, 2010) showed that the ratio of sFlt-1:PlGF was lower in women who engaged in at least three hours of moderate intensity activity per week compared to less active women. The data from the current study did not support this finding; however, the volume of exercise was lower (2 hours per week) and may not have been sufficient to elicit changes in angiogenic balance.

Estrogen is especially important for some of the vascular changes (e.g., increase in diameter) that are occurring throughout pregnancy. Estrogen and progesterone are up to 40% lower (Berkane *et al.*, 2017; Wan *et al.*, 2018), and testosterone is up to 60% higher (Salamalekis *et al.*, 2006) in women who have preeclampsia. Therefore, we hypothesized that exercise might influence the change in sex hormone concentration across gestation. Estrogen and testosterone have both been observed to increase immediately following 40-minutes of moderate intensity cycling in healthy females (Copeland *et al.*, 2002), which return to basal levels within 30-minutes. Whether repeated elevations in sex hormone concentrations (i.e., chronic exercise) could elicit long term changes or cause vascular responses (similar to sheer stress) is less clear. It is more likely that sex hormones are already elevated prior to or early in pregnancy and that the timing of our intervention was insufficient to elicit changes. For example, estrogen and progesterone have been shown previously to be increased in women with higher BMI at their first prenatal visit (Troisi *et al.*, 2008). Similarly, higher testosterone has been observed in pregnant women with high BMI (Kallak *et al.*, 2017) or PCOS (Caanen *et al.*, 2016). In the present study one women who was in the control

group dropped out due to the development of preeclampsia; her sex hormone levels were indeed high in TM2. Therefore, interventions targeting improved health pre-pregnancy may be more important at changing the sex hormone levels/ trajectory than once pregnancy has commenced. One strength of our intervention was that we included women at high risk for gestational hypertension (e.g., women categorized as having obesity, Black, history of hypertension); however, the current study did not have enough data to separate these women into subgroups for the analysis. Future work investigating the impact of exercise on sex hormones in pregnancy needs to include high risk women.

Previous literature would suggest that prenatal exercise is associated with reductions in fasting blood glucose (Davenport *et al.*, 2018e). The aforementioned meta-analysis showed that exercise-only interventions such as the present study, resulted in a decrease of 0.48mmol/L (n=12 studies, 2244 women). While we were unable to replicate those findings within the context of the present study, we might expect to see the greatest changes in blood glucose and other biomarkers of insulin resistance in women who are at risk for and have been diagnosed with GDM. Development of GDM during pregnancy is associated with poorer vascular outcomes in the postpartum including lower carotid and brachial artery distensibility (Davenport *et al.*, 2012). Therefore, knowing the impact of an exercise intervention during pregnancy on postpartum vascular health is of utmost importance for future research. Further, a recent retrospective study showed a reduction in the prenatal increase in insulin, but no change in HOMA-IR gestation in women who had participated in an exercise intervention (3x per week; 57-58minutes; moderate intensity) from 16weeks of pregnancy through to delivery (McDonald *et al.*, 2020). We also recently showed that women who were active prior to pregnancy (Godin score), or whom had a higher sport-score on the PPAQ (i.e. purposeful physical activity during pregnancy) had lower HOMA-IR in late pregnancy (Cai *et al.*,

2020b). We expected to see an effect of exercise intervention; however, the data here did not support that. It may be that a higher volume of exercise, or a longer duration intervention is needed to show change in a group of relatively healthy pregnant women.

4.4.6 Strengths and limitations

The major strength of this study is the randomized controlled trial design and comprehensive assessment of vascular structure and function. We also used non-invasive, reliable, and repeatable methods for determining vessel health (Hoeks *et al.*, 1999; Godia *et al.*, 2007). Specifically, we looked at structure and function of the common carotid and superficial femoral arteries at rest and including an assessment of vascular reactivity to a non-exercise stressor (CPT). Here we used beat-by-beat edge detection software and synchronous arterial blood pressure waveforms in at least ten-consecutive heart beats to determine a multitude of distensibility metrics. Lastly, a strength of our analysis is that we used intent -to- treat analysis to determine the effectiveness of the intervention in the general population.

We included any woman who did not have an absolute contraindication to exercise (Mottola *et al.*, 2018), which allowed for more generalizable study results, but also may have confounded them. By including some women who were deemed high risk (e.g., having obesity or a history of gestational hypertension) we may have introduced larger variability into our sample which may have resulted in the null responses we observed. We did not have enough high-risk women to stratify our results based on risk, however we did have a sample size more than twice that of the recently published pilot study (non-randomized trial; Brislane *et al.*, 2020).

One limitation to our measures was that we were unable to determine carotid intima-media thickness (IMT); the IMT is an important determinant of vessel stiffness. The carotid IMT has

been previous shown to be increased (Visontai *et al.*, 2002), decreased (Iacobaeus *et al.*, 2017) or unchanged in pregnancy (Mersich *et al.*, 2005; Yuan *et al.*, 2013a; Brislane *et al.*, 2020). Future randomized controlled trials investigating cardiovascular health in pregnancy should take the IMT into account when characterizing arterial remodeling. Another limitation of these findings is that due to the growing abdomen and the semi-reclined position of our participants, we were unable to obtain reliable measures of common femoral artery blood flow. We would expect greater diameter and flow in the common femoral artery and therefore, a greater ability to detect changes. However, we did not observe differences in the heart rate, blood pressure, or sympathetic nerve activity reactivity to CPT (see **Chapter 3.3**) the results for the superficial femoral artery here are in line with those observations.

4.4.7 Considerations/ future directions

Although the results from this randomized controlled trial appear to tell the story that prenatal aerobic exercise has little influence on conduit blood vessels, the present study was conducted in normotensive pregnant women who were relatively healthy (i.e., normotensive) and overall, at low risk for developing gestational hypertension. Therefore, these findings cannot be extended beyond healthy pregnancy. Although it is possible that our exercise intervention may have been too short, or too low in volume to elicit changes within this population, we still have much research to do to address the potential for aerobic exercise to mitigate the negative vascular consequences of developing complications or pregnancy. In this sense, future studies recruiting women in high and low risk categories are needed to see if change can be elicited in those who need it most. We already know that prenatal exercise helps to reduce the risk of developing gestational hypertension and preeclampsia at that cardiovascular health markers like arterial stiffness are altered in these disorders (Skow *et al.*, 2017); therefore, determining which clinical outcomes (especially non-

invasive ones) to target that are adaptable is of utmost importance. Lastly, as pre-pregnancy physical activity has been shown to predict prenatal health outcomes, studies initiated at this time-point are necessary to address whether exercise can shift the gestational trajectory of these vascular outcomes, especially in women who are at high-risk for developing complications.

4.5 References

Berkane N, Liere P, Oudinet JP, Hertig A, Lefèvre G, Pluchino N, Schumacher M & Chabbert-Buffet N. (2017). From Pregnancy to Preeclampsia: A Key Role for Estrogens. Endocrine reviews 38, 123-144.

Bijl RC, Valensise H, Novelli GP, Vasapollo B, Wilkinson I, Thilaganathan B, Stöhr EJ, Lees C, van der Marel CD & Cornette JMJ. (2019). Methods and considerations concerning cardiac output measurement in pregnant women: recommendations of the International Working Group on Maternal Hemodynamics. Ultrasound Obstet Gynecol 54, 35-50.

Bjarnegård N, Hedman K & Länne T. (2019). Vascular Adaptation to Indoor Cycling Exercise in Premenopausal Women. Int J Sports Med 40, 245-252.

Brislane Á, Jones H, Holder SM, Low DA & Hopkins ND. (2020). The Effect of Exercise During Pregnancy on Maternal and Offspring Vascular Outcomes: a Pilot Study. Reproductive sciences (Thousand Oaks, Calif).

Caanen MR, Kuijper EA, Hompes PG, Kushnir MM, Rockwood AL, Meikle WA, Homburg R & Lambalk CB. (2016). Mass spectrometry methods measured androgen and estrogen concentrations during pregnancy and in newborns of mothers with polycystic ovary syndrome. European journal of endocrinology 174, 25-32.

Cai C, Zhang Z, Mcdonald S, Cody S, May LE, Steinback CD & Davenport MH. (2020). Greater leisure-time physical activity before and during pregnancy is associated with improved insulin resistance in late pregnancy. Applied Physiology, Nutrition, and Metabolism 45, S290-291.

Carter JR & Ray CA. (2015). Sympathetic neural adaptations to exercise training in humans. Autonomic Neuroscience : Basic & Clinical 188, 36-43.

Cohen J. (1992). A power primer. Psychological bulletin 112, 155-159.

Copeland JL, Consitt LA & Tremblay MS. (2002). Hormonal responses to endurance and resistance exercise in females aged 19-69 years. The journals of gerontology Series A, Biological sciences and medical sciences 57, B158-165.

Davenport MH, Goswami R, Shoemaker JK & Mottola MF. (2012). Influence of hyperglycemia during and after pregnancy on postpartum vascular function. American journal of physiology Regulatory, integrative and comparative physiology 302, R768-775.

Davenport MH, McCurdy AP, Mottola MF, Skow RJ, Meah VL, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, Riske L, Sobierajski F, James M, Nagpal T, Marchand AA, Nuspl M, Slater LG, Barakat R, Adamo KB, Davies GA & Ruchat SM. (2018a). Impact of prenatal exercise on both prenatal and postnatal anxiety and depressive symptoms: a systematic review and meta-analysis. British journal of sports medicine 52, 1376-1385.

Davenport MH, Meah VL, Ruchat SM, Davies GA, Skow RJ, Barrowman N, Adamo KB, Poitras VJ, Gray CE, Jaramillo Garcia A, Sobierajski F, Riske L, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Barakat R & Mottola MF. (2018b). Impact of prenatal exercise on neonatal and childhood outcomes: a systematic review and meta-analysis. British journal of sports medicine 52, 1386-1396.

Davenport MH, Ruchat SM, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, Skow RJ, Meah VL, Riske L, Sobierajski F, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Adamo KB, Davies GA, Barakat R & Mottola MF. (2018c). Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. British journal of sports medicine 52, 1367-1375.

Davenport MH, Sobierajski F, Mottola MF, Skow RJ, Meah VL, Poitras VJ, Gray CE, Jaramillo Garcia A, Barrowman N, Riske L, James M, Nagpal TS, Marchand AA, Slater LG, Adamo KB, Davies GA, Barakat R & Ruchat SM. (2018d). Glucose responses to acute and chronic exercise during pregnancy: a systematic review and meta-analysis. British journal of sports medicine 52, 1357-1366.

Davies GA, Wolfe LA, Mottola MF, MacKinnon C, Society of O & gynecologists of Canada SCPOC. (2003). Joint SOGC/CSEP clinical practice guideline: exercise in pregnancy and the postpartum period. Canadian journal of applied physiology = Revue canadienne de physiologie appliquee 28, 330-341.

Dubois L, Girard M & Tatone-Tokuda F. (2007). Determinants of high birth weight by geographic region in Canada. Chronic diseases in Canada 28, 63-70.

Gamble G, Zorn J, Sanders G, MacMahon S & Sharpe N. (1994). Estimation of arterial stiffness, compliance, and distensibility from M-mode ultrasound measurements of the common carotid artery. *Stroke* **25,** 11-16.

Godia EC, Madhok R, Pittman J, Trocio S, Ramas R, Cabral D, Sacco RL & Rundek T. (2007). Carotid artery distensibility: a reliability study. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 26, 1157-1165.

Green DJ. (2009). Exercise training as vascular medicine: direct impacts on the vasculature in humans. Exercise and sport sciences reviews 37, 196-202.

Green LJ, Mackillop LH, Salvi D, Pullon R, Loerup L, Tarassenko L, Mossop J, Edwards C, Gerry S, Birks J, Gauntlett R, Harding K, Chappell LC & Watkinson PJ. (2020). Gestation-Specific Vital Sign Reference Ranges in Pregnancy. Obstet Gynecol 135, 653-664.

Herr MD, Hogeman CS, Koch DW, Krishnan A, Momen A & Leuenberger UA. (2010). A real-time device for converting Doppler ultrasound audio signals into fluid flow velocity. American journal of physiology Heart and circulatory physiology 298, H1626-1632.

Hetherington-Rauth M, Magalhães JP, Júdice PB, Melo X & Sardinha LB. (2020). Vascular improvements in individuals with type 2 diabetes following a 1 year randomised controlled exercise intervention, irrespective of changes in cardiorespiratory fitness. Diabetologia 63, 722-732.

Hoeks AP, Brands PJ, Willigers JM & Reneman RS. (1999). Non-invasive measurement of mechanical properties of arteries in health and disease. Proceedings of the Institution of Mechanical Engineers Part H, Journal of engineering in medicine 213, 195-202.

Holland CK, Brown JM, Scoutt LM & Taylor KJ. (1998). Lower extremity volumetric arterial blood flow in normal subjects. Ultrasound Med Biol 24, 1079-1086.

Huang C, Wang J, Deng S, She Q & Wu L. (2016). The effects of aerobic endurance exercise on pulse wave velocity and intima media thickness in adults: A systematic review and meta-analysis. Scand J Med Sci Sports 26, 478-487.

Iacobaeus C, Andolf E, Thorsell M, Bremme K, Jorneskog G, Ostlund E & Kahan T. (2017). Longitudinal study of vascular structure and function during normal pregnancy. Ultrasound in obstetrics & gynecology: the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 49, 46-53.

Kallak TK, Hellgren C, Skalkidou A, Sandelin-Francke L, Ubhayasekhera K, Bergquist J, Axelsson O, Comasco E, Campbell RE & Sundström Poromaa I. (2017). Maternal and female fetal testosterone levels are associated with maternal age and gestational weight gain. European journal of endocrinology 177, 379-388.

Kärkkäinen H, Saarelainen H, Valtonen P, Laitinen T, Raitakari OT, Juonala M, Kähönen M, Hutri-Kähönen N, Heinonen S & Laitinen T. (2014). Carotid artery elasticity decreases during pregnancy - the Cardiovascular Risk in Young Finns study. *BMC Pregnancy Childbirth* **14,** 98

Kawabata I, Nakai A, Sekiguchi A, Inoue Y & Takeshita T. (2012). The effect of regular exercise training during pregnancy on postpartum brachial-ankle pulse wave velocity, a measure of arterial stiffness. Journal of sports science & medicine 11, 489-494.

Kawasaki T, Sasayama S, Yagi S, Asakawa T & Hirai T. (1987). Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. Cardiovasc Res 21, 678-687.

Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I & Struijker-Boudier H. (2006). Expert consensus document on arterial stiffness: methodological issues and clinical applications. European heart journal 27, 2588-2605.

Lenhard W & Lenhard A. (2016). Calculation of Effect Sizes. Dettelbach (Germany).

Magee LA, Pels A, Helewa M, Rey E & von Dadelszen P. (2014). Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC 36, 575-576.

McDonald SM, Strom C, Remchak MM, Chaves A, Broskey NT, Isler C, Haven K, Newton E, DeVente J, Acosta-Manzano P, Aparicio VA & May LE. (2020). The effects of aerobic exercise on markers of maternal metabolism during pregnancy. Birth defects research.

Mersich B, Rigo J, Besenyei C, Lenard Z, Studinger P & Kollai M. (2005). Opposite changes in carotid versus aortic stiffness during healthy human pregnancy. Clinical science (London, England : 1979) 109, 103-107.

Miller AJ, Cui J, Luck JC, Sinoway LI & Muller MD. (2019). Age and sex differences in sympathetic and hemodynamic responses to hypoxia and cold pressor test. Physiological reports 7, e13988.

Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Piña IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR,

Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC, Jr., Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V & Wenger NK. (2011). Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. Circulation 123, 1243-1262.

Mottola MF, Davenport MH, Ruchat SM, Davies GA, Poitras VJ, Gray CE, Jaramillo Garcia A, Barrowman N, Adamo KB, Duggan M, Barakat R, Chilibeck P, Fleming K, Forte M, Korolnek J, Nagpal T, Slater LG, Stirling D & Zehr L. (2018). 2019 Canadian guideline for physical activity throughout pregnancy. British journal of sports medicine 52, 1339-1346.

Olver TD, Hazell TJ, Hamilton CD, Shoemaker JK & Lemon PW. (2012). Impaired superficial femoral artery vasodilation and leg blood flow in young obese women following an oral glucose tolerance test. *Appl Physiol Nutr Metab* **37,** 176-183.

Ramírez-Vélez R, Aguilar de Plata AC, Escudero MM, Echeverry I, Ortega JG, Salazar B, Rey JJ, Hormiga C & López-Jaramillo P. (2011). Influence of regular aerobic exercise on endothelium-dependent vasodilation and cardiorespiratory fitness in pregnant women. J Obstet Gynaecol Res 37, 1601-1608.

Ruchat SM, Mottola MF, Skow RJ, Nagpal TS, Meah VL, James M, Riske L, Sobierajski F, Kathol AJ, Marchand AA, Nuspl M, Weeks A, Gray CE, Poitras VJ, Jaramillo Garcia A, Barrowman N, Slater LG, Adamo KB, Davies GA, Barakat R & Davenport MH. (2018). Effectiveness of exercise interventions in the prevention of excessive gestational weight gain and postpartum weight retention: a systematic review and meta-analysis. British journal of sports medicine 52, 1347-1356.

Salamalekis E, Bakas P, Vitoratos N, Eleptheriadis M & Creatsas G. (2006). . Eur J Obstet Gynecol Reprod Biol 126, 16-19.

Skow RJ, Fraser G, Steinback CD & Davenport MH. (2020). Prenatal Exercise and Cardiovascular Health (PEACH) Study: Impact on Muscle Sympathetic Nerve (Re)activity. Med Sci Sports Exerc.

Skow RJ, King EC, Steinback CD & Davenport MH. (2017). The influence of prenatal exercise and pre-eclampsia on maternal vascular function. Clinical science (London, England : 1979) 131, 2223-2240.

Sobierajski FM, Purdy GM, Usselman CW, Skow RJ, James MA, Chari RS, Khurana R, Stickland MK, Davidge ST, Devolin M, Steinback CD & Davenport MH. (2018). Maternal Physical Activity Is Associated With Improved Blood Pressure Regulation During Late Pregnancy. The Canadian journal of cardiology 34, 485-491.

Steinback CD, Fraser GM, Usselman CW, Reyes LM, Julian CG, Stickland MK, Chari RS, Khurana R, Davidge ST & Davenport MH. (2019). Blunted sympathetic neurovascular transduction during normotensive pregnancy. J Physiol 597, 3687-3696.

Tanaka H. (2018). Various Indices of Arterial Stiffness: Are They Closely Related or Distinctly Different? *Pulse (Basel, Switzerland)* **5,** 1-6.

Troisi R, Hoover RN, Thadhani R, Hsieh CC, Sluss P, Ballard-Barbash R & Potischman N. (2008). Maternal, prenatal and perinatal characteristics and first trimester maternal serum hormone concentrations. British journal of cancer 99, 1161-1164.

Usselman CW, Wakefield PK, Skow RJ, Stickland MK, Chari RS, Julian CG, Steinback CD & Davenport MH. (2015). Regulation of sympathetic nerve activity during the cold pressor test in normotensive pregnant and nonpregnant women. Hypertension (Dallas, Tex: 1979) 66, 858-864.

Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J, Kaiser DR & Thuillez C. (2002). Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. Am J Hypertens 15, 445-452.

Visontai Z, Lenard Z, Studinger P, Rigo J & Kollai M. (2002). Impaired baroreflex function during pregnancy is associated with stiffening of the carotid artery. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 20, 364-369.

Wan J, Hu Z, Zeng K, Yin Y, Zhao M, Chen M & Chen Q. (2018). The reduction in circulating levels of estrogen and progesterone in women with preeclampsia. Pregnancy Hypertens 11, 18-25.

Wang Y, Ma H, Feng Y, Zhan Y, Wu S, Cai S, Shi Y, Chen Y, Ma L & Jiang Y. (2020). Association among pre-pregnancy body mass index, gestational weight gain and neonatal birth weight: a prospective cohort study in China. BMC Pregnancy Childbirth 20, 690.

Weissgerber TL, Davies GA & Roberts JM. (2010). Modification of angiogenic factors by regular and acute exercise during pregnancy. Journal of applied physiology (Bethesda, Md: 1985) 108, 1217-1223.

Welsch MA, Blalock P, Credeur DP & Parish TR. (2013). Comparison of brachial artery vasoreactivity in elite power athletes and age-matched controls. PLoS One 8, e54718.

Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H & Jeppesen J. (2006). Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation 113, 664-670.

Woisetschlager C, Waldenhofer U, Bur A, Herkner H, Kiss H, Binder M, Laggner AN & Hirschl MM. (2000). Increased blood pressure response to the cold pressor test in pregnant women developing pre-eclampsia. Journal of hypertension 18, 399-403.

Wolfe LA & Mottola M. (2015). PARmed-X FOR PREGNANCY Physical Activity Readiness Medical Examination. Canadian Society for Exercise Physiology.

Yoshizawa M, Maeda S, Miyaki A, Misono M, Saito Y, Tanabe K, Kuno S & Ajisaka R. (2009). Effect of 12 weeks of moderate-intensity resistance training on arterial stiffness: a randomised controlled trial in women aged 32-59 years. British journal of sports medicine 43, 615-618.

Yuan C, Wang J & Ying M. (2016). Predictive Value of Carotid Distensibility Coefficient for Cardiovascular Diseases and All-Cause Mortality: A Meta-Analysis. PLoS One 11, e0152799.

Yuan LJ, Xue D, Duan YY, Cao TS, Yang HG & Zhou N. (2013a). Carotid arterial intima-media thickness and arterial stiffness in pre-eclampsia: analysis with a radiofrequency ultrasound technique. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 42, 644-652.

Yuan LJ, Xue D, Duan YY, Cao TS & Zhou N. (2013b). Maternal carotid remodeling and increased carotid arterial stiffness in normal late-gestational pregnancy as assessed by radio-frequency ultrasound technique. BMC Pregnancy Childbirth 13, 122.

Zeisler H, Llurba E, Chantraine F, Vatish M, Staff A, Sennström M, Olovsson M, Brennecke S, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M & Verlohren S. (2016). Predictive Value of the sFlt-1: PlGF Ratio in Women With Suspected Preeclampsia. Obstetrical & gynecological survey 71, 273-274.

5 Discussion

The overall aim of my thesis was to explore the relationship between prenatal exercise and the sympathetic nervous system control of blood pressure. First, we identified that sympathetic nervous system activation and control of blood pressure during IHG exercise (30% MVC) and PECO (i.e., muscle metaboreflex activation) is not different between non-pregnant women and normotensive pregnant women in TM3. This observation was different than the previously reported augmented MSNA reactivity during CPT in TM3 (Usselman *et al.*, 2015b). However, this cross-sectional study conducted as part of this thesis was completed in a group of pregnant and non-pregnant women in whom BRG and NVT were not different at rest, which appears counter to previous reports. Nonetheless, this is the first report of metaboreflex control of MSNA within the literature, and further study will be required to assess if sympathetic reactivity is or isn't different between stressors. My second study (PEACH) sought to determine whether prenatal exercise could influence resting MSNA, BRG, NVT, or reactivity to CPT stress (more commonly reported in this population) using a randomized controlled trial. We determined that a structured aerobic exercise intervention initiated during mid-pregnancy could attenuate the pregnancy-associated rise in resting MSNA and blunting of NVT without impacting resting blood pressure, BRG, or the neurovascular response to CPT (**Chapter 3**). We also investigated whether resting measures of arterial structure and function (e.g., PWV) could be altered in response to an exercise intervention and found no effect of exercise intervention (**Chapter 4**) on our measured variables. My work directly aligns with a recently published pilot study by another research group (Brislane *et al.*, 2020). Both studies were conducted in normotensive pregnant women who were relatively healthy and overall, at low risk for developing gestational hypertension. Therefore, I believe that positive pregnancy-related vascular adaptations may not be further enhanced by prenatal exercise. However, within this population the sympathetic control of the vasculature at rest can be altered.

Therefore, findings from this thesis should underpin future research in women at high-risk for developing hypertension during pregnancy to determine if aerobic exercise can positively influence blood vessel health in women who may need it the most.

5.1 Resting measures of blood pressure regulation in pregnancy

5.1.1 Hemodynamics

Resting prenatal hemodynamics have been extensively reported in the literature. Across studies, my data demonstrated the expected increase in resting heart rate and cardiac output, concurrent with decreased SVR such that blood pressure was similar between normotensive late-pregnant and non-pregnant women. We also showed an expected rise in heart rate and blood pressure between TM2 and TM3 in normotensive pregnant women who participated in the exercise intervention regardless of their group allocation. However, the expected pregnancy-associated increase in heart rate was attenuated in the women who participated in the exercise intervention (see **Chapter 3.3.2**), which is a well-documented effect of exercise training that extends into pregnancy (Cai *et al.*, 2020a). Of interest, resting heart rate in TM3 was not significantly different between studies which we attribute to the recruitment of a wider range of exercise engagement in women from the metaboreflex study. However, MAP was lower in the women in the metaboreflex study (see **Chapter 2**) compared to PEACH groups (**Chapter 3**; see ***Appendix D, Table D2***) which aligns with the previous suggestion that the women in the metaboreflex study had higher physical activity levels throughout their pregnancies. Blood pressure has been reported to be decreased in TM2, returning to pre-pregnant levels at term (Meah *et al.*, 2016) or not changed throughout pregnancy (Green *et al.*, 2020). Although there was no interaction of group and gestational age with respect to resting blood pressure in our intervention study, our data suggest that exercise during pregnancy can help to attenuate the rise in blood pressure within a larger sample. This is

supported by a recent meta-analysis showing that prenatal exercise interventions are associated with a reduction in both systolic and diastolic blood pressure (Cai *et al.*, 2020a).

5.1.2 Hormones and blood markers of vascular health

Sex hormone concentrations are positively correlated to basal MSNA during pregnancy (Reyes *et al.*, 2018a) and have been shown to be modified by physical activity in non-pregnant populations (Ennour-Idrissi *et al.*, 2015). Specifically, estrogen is especially important for some of the vascular changes (e.g., increase in diameter) that are occurring throughout pregnancy and the increase in estrogen throughout pregnancy is suspected to contribute to the blunting of NVT observed (Reyes *et al.*, 2018a). We failed to observe a relationship between the change in sex hormones from TM2 to TM3 and basal MSNA BF or NVT; but did observe a correlation between estrogen and carotid artery diameter, DC, stiffness, distensibility, and Young's elastic modulus in TM3 (see ***Appendix C, Table C2***). While there is an established relationship between estrogen and outward remodeling (i.e., vasodilation) of the arteries in pregnancy (Berkane *et al.*, 2017), we interpret these relationships with caution as neither sex hormones nor carotid distensibility measures were impacted by our intervention. However, there is evidence that sex hormones do play a role in the pathogenesis of preeclampsia (Berkane *et al.*, 2017). Therefore, future work determining the role of exercise and sex hormones on sympathetic and vascular outcomes in pregnancy are needed.

The same was true regarding our measures of metabolic health (e.g., no changes in basal glucose and insulin). However, we expect to see the greatest changes in blood glucose and other biomarkers of insulin resistance in women who are at risk for and have been diagnosed with GDM. The current study did not have enough data to separate women into subgroups based on their risk classification or either GDM or gestational hypertension; future work investigating the impact of exercise on sex hormones in pregnancy needs to include high risk women. Also of importance will

be classifying women based on their angiogenic status as an indicator of cardiovascular risk; having a ratio of sFlt-1:PlGF ≥38.0 has been shown as a positive predictor for the development of preeclampsia (Zeisler *et al.*, 2016). Cross-sectional evidence from Weissgerber *et al.,* (Weissgerber *et al.*, 2010) showed that the ratio of sFlt-1:PlGF was lower in women who engaged in at least three hours of moderate intensity activity per week compared to less active women. The data from the current study did not support this finding; however, the volume of exercise was lower (2 hours per week) and may not have been sufficient to elicit changes in angiogenic balance. Moreover, none of the women in this study had sFlt-1:PlGF ≥38.0 at any time point, thus all women (exercise or control group) had normal angiogenic status and further improvement may not be possible.

5.2 Vascular structure and function

We conducted a comprehensive assessment of vascular structure and function in the common carotid and superficial femoral arteries. In line with previous literature (reviewed in: Skow *et al.*, 2017), we showed a progressive increase in carotid and superficial femoral artery diameters and a decrease in carotid artery strain (%) and distensibility (1/β) which were in line with previous literature (Visontai *et al.*, 2002; Yuan *et al.*, 2013b). There was no effect of exercise on any of our carotid or femoral artery measures; however, carotid artery distensibility (1/β) was reduced with advancing gestation in the exercise group, but not the control group. Two reasons exist why we may have not observed an interaction between many of our vascular measures and exercise in the present study; first that we tested relatively healthy, normotensive pregnant women, and second, that our exercise intervention may have been too short, or too low in volume to elicit changes within this population. Future studies in high-risk women or intervening earlier in pregnancy are needed.

5.3 Pulse wave velocity

Central PWV is linked with cardiovascular health and mortality (Willum-Hansen *et al.*, 2006), and is known to be influenced by an aerobic exercise intervention (Huang *et al.*, 2016). Peripheral PWV is an indicator of the stiffness of the muscular arteries and resistance arterioles in the peripheral circulation (i.e., limbs), but does not appear to predict future cardiovascular risk (Laurent *et al.*, 2006). Nonetheless, previous literature in non-pregnant individuals with hypertension and obesity suggest that the greatest changes in response to aerobic exercise training occur in peripheral PWV rather than central PWV (Huang *et al.*, 2016). Contrary to our hypothesis, we did not observe changes in central or peripheral PWV across gestation, nor with exercise. This finding is corroborated by the recent work of Brislane and colleagues (2020). Again, our study included mainly healthy, low risk, normotensive pregnant women. Therefore, aerobic exercise may be especially important for reducing arterial stiffness in women who are at high risk for developing hypertension (i.e., who already might have elevated PWV).

5.4 Muscle sympathetic nerve activity

We and others have consistently reported increases in MSNA during TM3 of normotensive pregnancy (reviewed in: Reyes *et al.*, 2018a; Hissen & Fu, 2020); here we also show similar increases in basal MSNA BF in TM3 compared to non-pregnant women (see **Figure 5.1**). However, we know much less about the changes in MSNA during the first half of pregnancy. To date there have been measures of MSNA in only 23 women in the TM2 (Reyes *et al.*, 2018a), and existing evidence would suggest that MSNA may not be different from the non-pregnant state at this point despite observations that MSNA is increased during early (6 weeks) gestation (Jarvis *et al.*, 2012; Hissen *et al.*, 2017; Reyes *et al.*, 2018b). This research adds a substantial amount of data to the literature from women in TM2 and shows that MSNA is lower in TM2 compared to

TM3. However, we recognize that there was a large variability in resting MSNA BF measured here, especially within our non-pregnant and TM2 populations. There is no evidence that this variability is due to differences in maternal age, pre-pregnancy BMI, parity, or gestational weight gain (GWG) as they were recently shown have no impact on MSNA BF in TM3 (Badrov *et al.*, 2020; Reyes *et al.*, 2020a). Regardless, there is large interindividual variability in basal MSNA in the general population (Charkoudian & Wallin, 2014) and also the other studies in pregnancy (reviewed in: Reyes *et al.*, 2018a; Hissen & Fu, 2020). Moreover, the variability in non-pregnant and TM2 women highlights the need for earlier interventions, as MSNA can already be high at pre- to mid-pregnancy.

While it is unclear how exercise is altering the normative changes in MSNA in human pregnancy, it is important to recognize that exercise interventions may exert influences via parallel adaptations (i.e., at other locations along the neurovascular cascade). These include potential changes in neurotransmitter release and reuptake, alpha-adrenergic receptor sensitivity and density. Indeed, neurotransmitter concentrations have been shown to parallel the increase in MSNA in healthy pregnancy (Barron *et al.*, 1986; Jarvis *et al.*, 2012) and therefore, we may expect the rise in neurotransmitter release to be lesser in women who exercise. Within our cross-sectional study, we only have basal MSNA and neurotransmitter data from nine women in TM3 and thus cannot reasonably determine anything meaningful from correlations or lack thereof; this should be considered in future work.

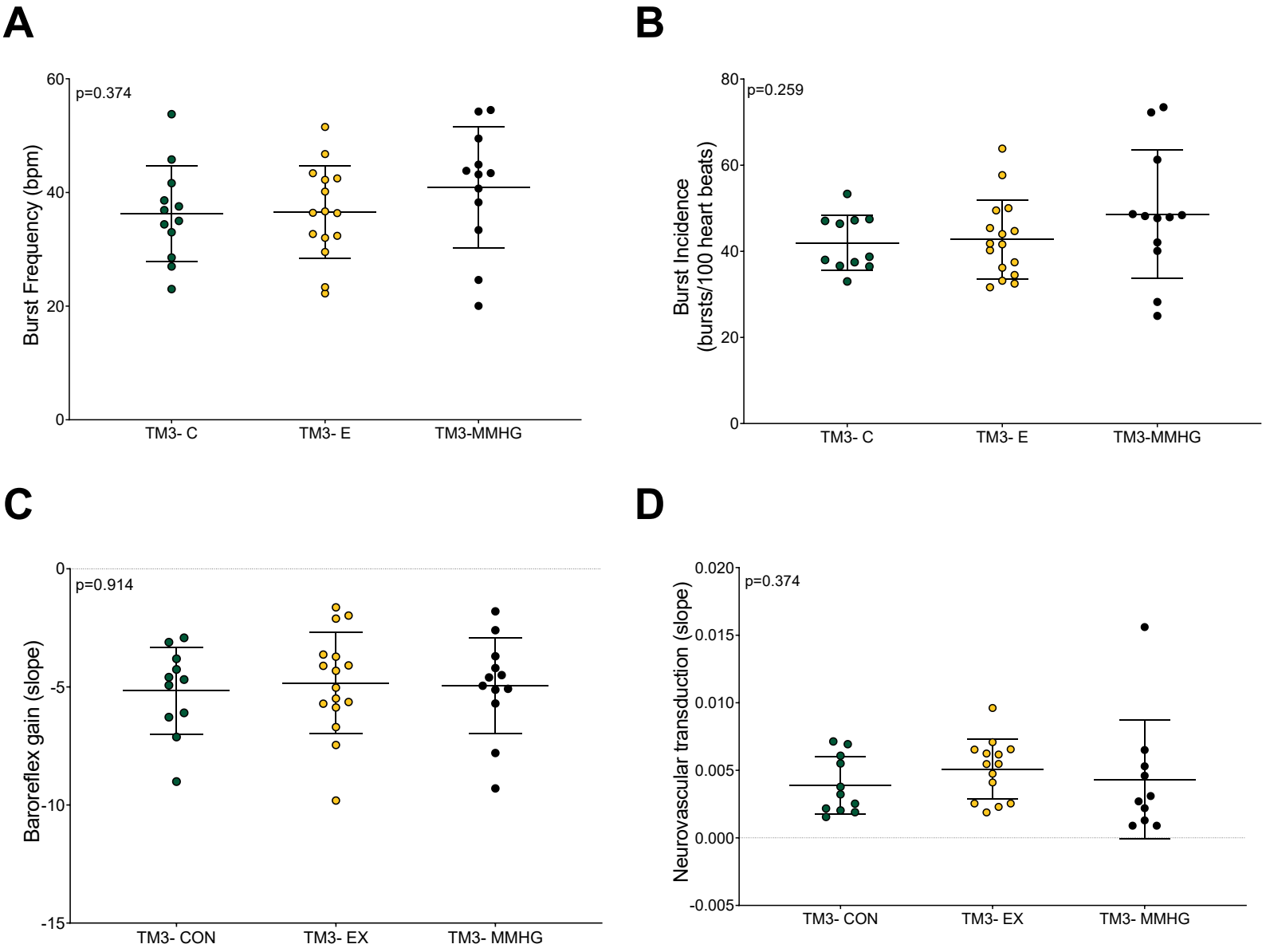

A
p=0.374
Burst Frequency (bpm)
60
40
20
0
TM3- C
TM3- E
TM3-MMHG
B
p=0.259
Burst Incidence (bursts/100 heart beats)
80
60
40
20
0
TM3- C
TM3- E
TM3-MMHG
C
p=0.914
Baroreflex gain (slope)
0
-5
-10
-15
TM3- CON
TM3- EX
TM3- MMHG
D
p=0.374
Neurovascular transduction (slope)
0.020
0.015
0.010
0.005
0.000
-0.005
TM3- CON
TM3- EX
TM3- MMHG

Figure 5.1 Comparisons between study 1 (metaboreflex study) and study 2 (PEACH study) for basal sympathetic nervous system activity in the third trimester (TM3). Data is presented as mean and standard deviation with individual data points represented by filled circles (green, PEACH control group; yellow, PEACH study exercise group; black, metaboreflex study pregnant group). **A)** Muscle sympathetic nerve activity (MSNA) burst frequency between groups was not different. **B)** MSNA burst incidence was not different between groups. **C)** Sympathetic baroreflex gain was not different between groups. **D)** Neurovascular transduction slopes were not different between groups. Comparisons between groups were performed using a one-way ANOVA in GraphPad Prism (v9.0). Significance was set at $p<0.05$ and indicated in bold where applicable.

5.5 Sympathetic baroreflex gain

Contradictory to our original hypothesis and previous literature (Usselman *et al.*, 2015a), we did not observe blunting of the sympathetic baroreflex in our pregnant compared to non-pregnant women (see **Chapter 2**). We also did not observe a blunting of the weighted probability slope for the sympathetic baroreflex between TM2 and TM3 (see **Chapter 3**). The values we obtained for both our pregnant and non-pregnant women fall within established ranges reported for both groups (Usselman *et al.*, 2015a; Hissen *et al.*, 2017; Reyes *et al.*, 2018b; Badrov *et al.*, 2020; Reyes *et al.*, 2020a). While baroreflex gain during TM3 does not appear to be influenced by maternal age, pre-pregnancy BMI, gestational weight gain, or parity (Badrov *et al.*, 2020; Reyes *et al.*, 2020a), longitudinal assessments of BRG prior to and throughout healthy pregnancies suggest there is a large variation in the pattern of BRG between individuals (Hissen *et al.*, 2017; Reyes *et al.*, 2018b).

Within the context of this study we suggest that sympathetic BRG is not altered between TM2 and TM3, and also that exercise training between mid-to-late pregnancy does not impact the change in BRG during this time. In both healthy and unhealthy (e.g. hypertension) non-pregnant populations, aerobic exercise interventions have shown improvement in BRG compared to controls (Carter & Ray, 2015). Future studies investigating baroreflex function starting with pre-pregnancy measures and continue longitudinally throughout pregnancy are needed to determine the full impact of both advancing gestational age and exercise on BRG in pregnancy.

5.6 Neurovascular transduction

Contrary to our previous work (Steinback *et al.*, 2019), the present cross-sectional study did not show differences between non-pregnant and pregnant women in their NVT. In this instance, we attribute the lack of difference to the variability in our populations and recognise that future research is needed to determine what might influence resting NVT in either population. Our

exercise intervention indicated that the increase in resting MSNA and blunting of the NVT during gestation in normotensive pregnancy might be influenced by physical activity. We did not measure VO_{2peak} as an objective measure of fitness in either of our studies here, but VO_{2max} has been recently shown to be correlated with NVT in males (O'Brien *et al.*, 2020). We did, however, find that sympathetic NVT was blunted in TM3 compared to TM2 suggesting that similar to the increase in MSNA, NVT is progressively blunted across gestation. Indeed, here we also show that changes in NVT were correlated with changes MSNA BF which supports this notion that these two measures of basal sympathetic activity are not mutually exclusive. Therefore, interventions which alter resting MSNA also affect NVT.

5.7 Reactivity to stress

5.7.1 Reactivity to IHG and PECO

Isometric handgrip is a non-invasive assessment often used to discern the reflex response of blood pressure and sympathetic nerve activity in a variety of populations (Meah *et al.*, 2018a). In the metaboreflex study (**Chapter 2**) we found that the heart rate and blood pressure responses during two-minutes of IHG at 30% MVC and two-minutes PECO are not different between pregnant women in TM3 and non-pregnant women. We observed that two-minutes at 30% MVC was sufficient to elicit a significant change in blood pressure and MSNA, which is consistent with the literature from non-pregnant populations (Mark *et al.*, 1985). This finding was reinforced by no significant different between pregnant and non-pregnant women in the NE or EPI response during IHG or PECO. We therefore conclude here that in pregnancies where sympathetic BRG and NVT are maintained (i.e. not different from non-pregnant), there are not differences in the metaboreflex reactivity between the two groups. These data provide evidence that the sympathetic regulation of blood pressure during exercise is unlike non-exercise stressors (e.g. CPT) in that the

relationship between the rise in MSNA and the rise in blood pressure might be maintained during IHG exercise in normotensive pregnancy. However, we recognize that exaggerated hemodynamic response to IHG in early pregnancy may be indicative of future cardiovascular disease risk (Meah *et al.*, 2018a); future work confirming these responses in the first and second trimester, and between high versus low risk women is necessary to follow up this work in healthy pregnancy..

5.7.2 Reactivity to CPT

The CPT is a safe and effective tool to evaluate hemodynamic and sympathetic responsiveness during pregnancy and has been utilized in both healthy and hypertensive pregnancies (Meah *et al.*, 2018b). A larger blood pressure response to CPT in mid pregnancy has been previously shown to precede the development of preeclampsia (Woisetschlager *et al.*, 2000). In the PEACH study, there was an increased blood pressure response to CPT in TM3 compared to TM2, but this occurred without changing the MSNA response to CPT. This is in contrast with the blunting of basal NVT observed across gestation here and the blunting of reflex NVT during CPT previously shown in TM3 compared to non-pregnant women (Usselman *et al.*, 2015b; Steinback *et al.*, 2019). This highlights the need for more longitudinal studies to decern the changes from the non-pregnant state through pregnancy. However, the change in CPT response across gestation was shown to occur independent of exercise (i.e., no effect of exercise on the response to CPT), which is supported by data in healthy men (Sheldahl *et al.*, 1994) but has not yet been explored in populations with sympathetic hyperactivity. Exercise training has been shown to alter the sympathetic reactivity to other stressors (e.g., IHG, mental stress) in healthy and hypertensive populations (Carter & Ray, 2015) and has been shown to increase vasoreactivity during both dilatory and constrictor stressors (Welsch *et al.*, 2013). We measured the vasoreactivity of the superficial femoral artery (blood flow, FVC, and FVR) and were unable to replicate these findings

suggesting that in normotensive pregnant women, there may be limited "room for improvement" in their vascular health. Future studies investigating this in women who are at high risk for gestational hypertension (e.g., have obesity) might be useful to ascertain if there are populations whose reactivity to stress can be positively altered by aerobic exercise intervention.

5.8 Limitations

All studies have inherent limitations; and within the research presented here we saw results that did not support our hypotheses, or which contradicted the literature. Although we stand by our results and the methods by which we determined them, it is possible that we were unable to account for confounding variables that may have affected our data. First, within the context of the metaboreflex study (**Chapter 2**) study, we were unable to replicate the documented decrease in BRG and NVT in TM3 compared to non-pregnant women. We suspect this observation may have been due to higher variability of prenatal physical activity levels in our pregnant cohort in the metaboreflex study (**Chapter 2**) compared to previous literature. This is supported by the result from our intervention showing attenuated blunting of NVT in the exercise group (see **Chapter 3.3.4**), and also by a recent study in men showing that fitness (VO_{2max}) was correlated with NVT (O'Brien *et al.*, 2020).

Similarly, determining changes in fitness in the intervention study (**Chapter 3**) would have been helpful in testing the efficacy of our intervention. A recent meta-analysis showed that prenatal exercise intervention is associated with improved fitness, lower resting heart rate, and blood pressure (Cai *et al.*, 2020a). We did observe a smaller increase in resting heart rate in the exercise compared to the control group but did not observe any differences for blood pressure. We also did not observe differences between groups for GWG (during the intervention period, or total). In fact, 54% of women in the intervention study gained above current recommendations, which is higher

than the 34% reported by a recent meta-analysis (Ruchat *et al.*, 2018) but in line with Canadian statistics (44%; Lowell & Miller, 2010).

One other limitation is that we prescribed pregnant women in the exercise group to participate in three to four moderate intensity aerobic exercise sessions for the duration of the intervention (~14weeks) based on the *2003 Canadian Clinical Practice Guideline* (ACOG, 2015) and heart rate zones outlined in the *PARmed-X for Pregnancy* (Wolfe & Mottola, 2015a). Since then, the *2019 Canadian Guideline for Physical Activity throughout Pregnancy* was developed (Mottola *et al.*, 2018), which recommends 150-minutes of moderate intensity aerobic exercise per week. We recognize that the prescription in the present study falls below these new guidelines, and that we may have observed greater changes if we had prescribed according to the new guideline. However, the threshold to alter sympathetic nervous system regulation may be lower. First, we have recently shown that as little as 260 MET-minutes of exercise per week (i.e., 60 min moderate intensity walking) can reduce the odds of preeclampsia in pregnancy by at least 25% (Davenport *et al.*, 2018c). Second, in non-pregnant populations with sympathetic hyperactivity (e.g. patients with myocardial ischemia), as little at 4 weeks of aerobic exercise training (walking at 60% HRpeak, 160 minutes per week) has been shown to lower MSNA and improve sympathetic BRG (Mimura *et al.*, 2005). In keeping with these previous studies, and in line with our results, we believe that our prescribed exercise (achieving 50-70% HRR for 120-160 minutes per week) was sufficient to elicit measurable sympathetic adaptation. Further, in the present randomized controlled trial, 51 out of 59 women completed the study (86% retention rate; drop out similar between groups) and 86% of the women in the exercise group achieved 75% of the prescribed exercise. Regardless, this thesis provides evidence that exercise does alter the rise in resting MSNA and NVT in healthy

pregnancy providing further rational for performing these studies in women who are at risk for the development of gestational hypertension.

Lastly, we included any woman who did not have an absolute contraindication to exercise (Mottola *et al.*, 2018), which allowed for more generalizable study results, but also may have confounded them. By including some women who were deemed high risk (e.g., having obesity or a history of gestational hypertension) we introduced larger variability into our sample which may have resulted in some of the null responses we observed (e.g., PWV). Indeed, meta-analysis evidence from the non-pregnant literature shows a positive correlation between resting MSNA and BMI (Grassi *et al.*, 2019). However, women included in these studies were all normotensive and the majority were at low risk for developing gestational hypertension, which does limit our generalizability as well. Unfortunately, we did not have enough high-risk women to stratify our results based on risk; however, we did have a sample size more than twice that of the recently published pilot study investigating prenatal exercise and cardiovascular health (non-randomized trial; Brislane *et al.*, 2020). Future research in high-risk women is critical to continue to answer these questions.

5.9 Future Directions

We know that pregnant women meeting the recommended guideline for physical activity throughout pregnancy (≥150minutes MVPA per week; Mottola *et al.*, 2018) have improvements in their cardiovagal BRG (Sobierajski *et al.*, 2018), endothelial function (measured by flow mediated dilation; Reyes *et al.*, 2020b), and glucose homeostasis (Cai *et al.*, 2020b). From the data in this thesis, we might expect that aerobic exercise training would influence other aspects of blood pressure regulation during acute exercise. However, the data related to neurovascular control during pregnancy remains limited. Further studies needed that include more women, across all

trimesters, with different fitness levels, from different ethnicities, or in different risk categories (e.g., high vs low risk for hypertension). Studies along these lines may help shed light on who may or may not develop hypertension or have altered blood pressure regulation during normal stressors such as exercise. Additionally, much work is required to determine whether different exercise modes, intensities, or durations may impact the MSNA and blood pressure.

Exercise during pregnancy may oppose the abnormal remodelling that occurs in gestational hypertension and pre-eclampsia through the indirect actions at the level of the vasculature (Skow *et al.*, 2017). Although we did not observe an effect of exercise on carotid artery distensibility, PWV, or FVR, they are many other vascular outcomes that could be explored that may help address this. For instance, data from our non-burst sequences (i.e., NVT data) would suggest that basal vasodilatory status is not altered by prenatal physical activity. However, Green and colleagues (2018) would suggest that exercise exerts positive cardiovascular benefit through flow-dependent and endothelium meditated dilation and remodelling. Indeed, prenatal physical activity in the third trimester is associated with increases in normalized flow mediated dilation (Reyes *et al.*, 2020b), and one randomized controlled trial showed that aerobic exercise intervention may increase flow mediated dilation in pregnancy (Ramírez-Vélez *et al.*, 2011). Thus future work including flow mediated dilation or other measures of endothelial function are needed to determine the role of exercise during pregnancy on functional changes in the vasculature. Similarly, it is postulated that there are alterations in neurotransmitter release or reuptake, or adrenergic receptor sensitivity and density in healthy pregnancy. Alpha-adrenergic sensitivity may also be decreased during pregnancy as previously demonstrated in both humans (Landau *et al.*, 2002) and ewes (McLaughlin *et al.*, 1989). Thus, functional sympatholysis during exercise may be altered in pregnant women. Measures of blood flow in exercising or non-exercising limbs to determine the

contribution of the rise in MSNA to the changes in regional blood flow and NVT. Future work (in both humans and animal models) addressing blood vessel specificity and reactivity will help determine the mechanisms by which prenatal exercise improves cardiovascular health and reduces hypertensive risk. Specifically determining the effects of aerobic exercise on sympathetic neurotransmitter receptor density and sensitivity would help fill in some of the current gaps in our knowledge.

5.10 Perspective

Despite the overwhelming evidence that prenatal exercise is beneficial for most women, 85% of pregnant women are not meeting exercise guidelines (Evenson & Wen, 2011). Moreover, up to 10% of pregnant women will develop hypertensive disorders of pregnancy (Magee *et al.*, 2014). Gestational hypertension is associated with sympathetic hyperactivity, as such the data from our exercise intervention provides some insight into ways by which prenatal exercise might oppose this (i.e., by reducing the increase in MSNA across gestation) in low risk, normotensive women. Prenatal exercise has been shown to reduce the odds of developing hypertension and preeclampsia by up to 40%, and therefore is of importance when considering the long-term cardiovascular health of these women and their children. These data provide a potential mechanism underlying the reduced odds of developing hypertensive disorders of pregnancy in physically active pregnant women. Specifically, that the communication between the nervous system and the blood vessels (i.e., NVT) is less changed in women who participated in the structured aerobic exercise program despite the attenuation of the normal increase in MSNA during a normotensive pregnancy. Decreasing the amount of sympathetic hyperactivity while not disrupting blood pressure control (e.g. sympathetic baroreflex and CPT reactivity) may also facilitate long term vascular adaptations that promote lifelong cardiovascular health. However, more research is needed to fully elucidate

the mechanisms behind which the risk of gestational hypertension is reduced by prenatal exercise including the vascular component. Specifically, more research is needed involving women who are at high risk for developing gestational hypertension (e.g. women with prior history), as the improvements may be greater in this population.

5.11 Conclusion

The aim of the present research was to determine how acute IHG exercise or structured aerobic exercise affected the normative responses of the sympathetic nervous system during healthy pregnancy. We demonstrated that the MSNA response to IHG and PECO was not different between TM3 and non-pregnant participants which highlights the diversity in reflex responses to different sympathetic stressors. We also showed that the MSNA response across pregnancy (between TM2 and TM3) can be attenuated by a structured aerobic exercise program. However, within a set of low risk, normotensive pregnant women aerobic exercise did not impact sympathetic baroreflex, vascular outcomes (e.g., PWV) or the reflex reactivity during CPT. We conclude that prenatal exercise may act through the sympathetic nervous system to elicit neurovascular adaptations that may decrease hypertension risk and promote cardiovascular health. We still have much research to do to address the potential for aerobic exercise to mitigate the potential negative vascular consequences of developing complications or pregnancy. In this sense, future studies recruiting women in high and low risk categories are needed to see if change can be elicited in those who need it most. We already know that prenatal exercise helps to reduce the risk of developing gestational hypertension and preeclampsia; therefore, determining other clinical outcomes (especially non-invasive ones) to target that are adaptable is of utmost importance.

Reference List

ACOG. (2015). ACOG Committee Opinion No. 650: Physical Activity and Exercise During Pregnancy and the Postpartum Period. *Obstetrics and gynecology* **126,** 135.

Akhter T, Wikstrom AK, Larsson M, Larsson A, Wikstrom G & Naessen T. (2016). Association between angiogenic factors and signs of arterial aging in women with pre-eclampsia. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*.

Antunes-Correa LM, Kanamura BY, Melo RC, Nobre TS, Ueno LM, Franco FG, Roveda F, Braga AM, Rondon MU, Brum PC, Barretto AC, Middlekauff HR & Negrao CE. (2012). Exercise training improves neurovascular control and functional capacity in heart failure patients regardless of age. *European journal of preventive cardiology* **19,** 822-829.

Antunes-Correa LM, Melo RC, Nobre TS, Ueno LM, Franco FG, Braga AM, Rondon MU, Brum PC, Barretto AC, Middlekauff HR & Negrao CE. (2010). Impact of gender on benefits of exercise training on sympathetic nerve activity and muscle blood flow in heart failure. *European journal of heart failure* **12,** 58-65.

Aune B, Vårtun A, Oian P & Sager G. (2000). Evidence of dysfunctional beta2-adrenoceptor signal system in pre-eclampsia. *Bjog* **107,** 116-121.

Badrov MB, Park SY, Yoo JK, Hieda M, Okada Y, Jarvis SS, Stickford AS, Best SA, Nelson DB & Fu Q. (2019). Role of Corin in Blood Pressure Regulation in Normotensive and Hypertensive Pregnancy. *Hypertension* **73,** 432-439.

Badrov MB, Yoo JK, Steinback CD, Davenport MH & Fu Q. (2020). Influence of multiparity on sympathetic nerve activity during normal pregnancy. *American journal of physiology Heart and circulatory physiology* **318,** H816-h819.

Barron WM, Mujais SK, Zinaman M, Bravo EL & Lindheimer MD. (1986). Plasma catecholamine responses to physiologic stimuli in normal human pregnancy. *Am J Obstet Gynecol* **154,** 80-84.

Beldekas JC, Smith B, Gerstenfeld LC, Sonenshein GE & Franzblau C. (1981). Effects of 17 beta-estradiol on the biosynthesis of collagen in cultured bovine aortic smooth muscle cells. *Biochemistry* **20,** 2162-2167.

Bellamy L, Casas JP, Hingorani AD & Williams DJ. (2007). Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ (Clinical research ed)* **335,** 974.

Berkane N, Liere P, Oudinet JP, Hertig A, Lefèvre G, Pluchino N, Schumacher M & Chabbert-Buffet N. (2017). From Pregnancy to Preeclampsia: A Key Role for Estrogens. *Endocrine reviews* **38,** 123-144.

Bijl RC, Valensise H, Novelli GP, Vasapollo B, Wilkinson I, Thilaganathan B, Stöhr EJ, Lees C, van der Marel CD & Cornette JMJ. (2019). Methods and considerations concerning cardiac output measurement in pregnant women: recommendations of the International Working Group on Maternal Hemodynamics. *Ultrasound Obstet Gynecol* **54,** 35-50.

Bjarnegård N, Hedman K & Länne T. (2019). Vascular Adaptation to Indoor Cycling Exercise in Premenopausal Women. *Int J Sports Med* **40,** 245-252.

Brislane Á, Jones H, Holder SM, Low DA & Hopkins ND. (2020). The Effect of Exercise During Pregnancy on Maternal and Offspring Vascular Outcomes: a Pilot Study. *Reproductive sciences (Thousand Oaks, Calif)*.

Caanen MR, Kuijper EA, Hompes PG, Kushnir MM, Rockwood AL, Meikle WA, Homburg R & Lambalk CB. (2016). Mass spectrometry methods measured androgen and estrogen concentrations during pregnancy and in newborns of mothers with polycystic ovary syndrome. *European journal of endocrinology* **174,** 25-32.

Cai C, Ruchat SM, Sivak A & Davenport MH. (2020a). Prenatal Exercise and Cardiorespiratory Health and Fitness: A Meta-analysis. *Med Sci Sports Exerc*.

Cai C, Zhang Z, Mcdonald S, Cody S, May LE, Steinback CD & Davenport MH. (2020b). Greater leisure-time physical activity before and during pregnancy is associated with improved insulin resistance in late pregnancy. *Applied Physiology, Nutrition, and Metabolism* **45,** S290-291.

Carter JR & Ray CA. (2015). Sympathetic neural adaptations to exercise training in humans. *Autonomic Neuroscience : Basic & Clinical* **188,** 36-43.

Carthy ER. (2014). Autonomic dysfunction in essential hypertension: A systematic review. *Annals of medicine and surgery (2012)* **3,** 2-7.

Charkoudian N & Wallin BG. (2014). Sympathetic neural activity to the cardiovascular system: integrator of systemic physiology and interindividual characteristics. *Compr Physiol* **4,** 825-850.

Chasan-Taber L, Schmidt MD, Roberts DE, Hosmer D, Markenson G & Freedson PS. (2004). Development and validation of a Pregnancy Physical Activity Questionnaire. *Med Sci Sports Exerc* **36,** 1750-1760.

Choi JW, Im MW & Pai SH. (2002). Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. *Annals of Clinical and Laboratory Science* **32,** 257-263.

Cohen J. (1992). A power primer. *Psychological bulletin* **112,** 155-159.

Copeland JL, Consitt LA & Tremblay MS. (2002). Hormonal responses to endurance and resistance exercise in females aged 19-69 years. *The journals of gerontology Series A, Biological sciences and medical sciences* **57,** B158-165.

Davenport MH, Goswami R, Shoemaker JK & Mottola MF. (2012). Influence of hyperglycemia during and after pregnancy on postpartum vascular function. *American journal of physiology Regulatory, integrative and comparative physiology* **302,** R768-775.

Davenport MH, McCurdy AP, Mottola MF, Skow RJ, Meah VL, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, Riske L, Sobierajski F, James M, Nagpal T, Marchand AA, Nuspl M, Slater LG, Barakat R, Adamo KB, Davies GA & Ruchat SM. (2018a). Impact of prenatal exercise on both prenatal and postnatal anxiety and depressive symptoms: a systematic review and meta-analysis. *British journal of sports medicine* **52,** 1376-1385.

Davenport MH, Meah VL, Ruchat SM, Davies GA, Skow RJ, Barrowman N, Adamo KB, Poitras VJ, Gray CE, Jaramillo Garcia A, Sobierajski F, Riske L, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Barakat R & Mottola MF. (2018b). Impact of prenatal exercise on neonatal and childhood outcomes: a systematic review and meta-analysis. *British journal of sports medicine* **52,** 1386-1396.

Davenport MH, Ruchat SM, Poitras VJ, Garcia AJ, Gray CE, Barrowman N, Skow RJ, Meah VL, Riske L, Sobierajski F, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater L, Weeks A, Adamo KB, Davies GA, Barakat R & Mottola MF. (2018c). Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *British Journal of Sports Medicine*.

Davenport MH, Ruchat SM, Poitras VJ, Jaramillo Garcia A, Gray CE, Barrowman N, Skow RJ, Meah VL, Riske L, Sobierajski F, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Adamo KB, Davies GA, Barakat R & Mottola MF. (2018d). Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *British journal of sports medicine* **52,** 1367-1375.

Davenport MH, Skow RJ & Steinback CD. (2016). Maternal Responses to Aerobic Exercise in Pregnancy. *Clinical obstetrics and gynecology* **59,** 541-551.

Davenport MH, Sobierajski F, Mottola MF, Skow RJ, Meah VL, Poitras VJ, Gray CE, Jaramillo Garcia A, Barrowman N, Riske L, James M, Nagpal TS, Marchand AA, Slater LG, Adamo KB, Davies GA, Barakat R & Ruchat SM. (2018e). Glucose responses to acute and chronic exercise during pregnancy: a systematic review and meta-analysis. *British journal of sports medicine* **52,** 1357-1366.

Davies GA, Wolfe LA, Mottola MF, MacKinnon C, Society of O & gynecologists of Canada SCPOC. (2003). Joint SOGC/CSEP clinical practice guideline: exercise in pregnancy and the postpartum period. *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee* **28,** 330-341.

de Haas S, Ghossein-Doha C, van Kuijk SM, van Drongelen J & Spaanderman ME. (2017). Physiological adaptation of maternal plasma volume during pregnancy: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* **49,** 177-187.

de Mello Franco FG, Santos AC, Rondon MU, Trombetta IC, Strunz C, Braga AM, Middlekauff H, Negrao CE & Pereira Barretto AC. (2006). Effects of home-based exercise training on neurovascular control in patients with heart failure. *European journal of heart failure* **8,** 851-855.

Degani S, Abinader E, Eibschitz I, Oettinger M, Shapiro I & Sharf M. (1985a). Isometric exercise test for predicting gestational hypertension. *Obstet Gynecol* **65,** 652-654.

Degani S, Abinader E, Eibschitz I, Oettinger M, Shapiro I & Sharf M. (1985b). Isometric exercise test for predicting gestational hypertension. *Obstetrics and gynecology* **65,** 652-654.

Delius W, Hagbarth KE, Hongell A & Wallin BG. (1972). General characteristics of sympathetic activity in human muscle nerves. *Acta Physiologica Scandinavica* **84,** 65-81.

Dempsey JA, Sheel AW, St Croix CM & Morgan BJ. (2002). Respiratory influences on sympathetic vasomotor outflow in humans. *Respir Physiol Neurobiol* **130,** 3-20.

Dubois L, Girard M & Tatone-Tokuda F. (2007). Determinants of high birth weight by geographic region in Canada. *Chronic diseases in Canada* **28,** 63-70.

Edouard DA, Pannier BM, London GM, Cuche JL & Safar ME. (1998). Venous and arterial behavior during normal pregnancy. *The American Journal of Physiology* **274,** 1605.

Ekholm E, Erkkola R & Hartiala J. (1994). Comparison of cardiovascular reflex tests and blood pressure measurement in prediction of pregnancy-induced hypertension. *Eur J Obstet Gynecol Reprod Biol* **54,** 37-41.

Eneroth-Grimfors E, Bevegård S & Nilsson BA. (1988). Evaluation of three simple physiologic tests as predictors of pregnancy-induced hypertension. A pilot study. *Acta Obstet Gynecol Scand* **67,** 109-113.

Ennour-Idrissi K, Maunsell E & Diorio C. (2015). Effect of physical activity on sex hormones in women: a systematic review and meta-analysis of randomized controlled trials. *Breast cancer research : BCR* **17,** 139.

Evenson KR & Wen F. (2011). Prevalence and correlates of objectively measured physical activity and sedentary behavior among US pregnant women. *Preventive medicine* **53,** 39-43.

Fadel PJ. (2015). Reflex control of the circulation during exercise. *Scandinavian Journal of Medicine & Science in Sports* **25 Suppl 4,** 74-82.

Fairfax ST, Padilla J, Vianna LC, Davis MJ & Fadel PJ. (2013). Spontaneous bursts of muscle sympathetic nerve activity decrease leg vascular conductance in resting humans. *American journal of physiologyHeart and circulatory physiology* **304,** 759.

Faul F, Erdfelder E, Buchner A & Lang AG. (2009). Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behavior research methods* **41,** 1149-1160.

Faul F, Erdfelder E, Lang AG & Buchner A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods* **39,** 175-191.

Fraga R, Franco FG, Roveda F, de Matos LN, Braga AM, Rondon MU, Rotta DR, Brum PC, Barretto AC, Middlekauff HR & Negrao CE. (2007). Exercise training reduces sympathetic nerve activity in heart failure patients treated with carvedilol. *European journal of heart failure* **9,** 630-636.

Freedson PS, Melanson E & Sirard J. (1998a). Calibration of the Computer Science and Applications, Inc. accelerometer. *Medicine and science in sports and exercise* **30,** 777-781.

Freedson PS, Melanson E & Sirard J. (1998b). Calibration of the Computer Science and Applications, Inc. accelerometer. *Med Sci Sports Exerc* **30,** 777-781.

Fu Q. (2018). Hemodynamic and Electrocardiographic Aspects of Uncomplicated Singleton Pregnancy. *Advances in experimental medicine and biology* **1065,** 413-431.

Fu Q & Levine BD. (2009). Autonomic circulatory control during pregnancy in humans. *Semin Reprod Med* **27,** 330-337.

Gamble G, Zorn J, Sanders G, MacMahon S & Sharpe N. (1994). Estimation of arterial stiffness, compliance, and distensibility from M-mode ultrasound measurements of the common carotid artery. *Stroke* **25,** 11-16.

Garg R, Malhotra V, Dhar U & Tripathi Y. (2013). The isometric handgrip exercise as a test for unmasking hypertension in the offsprings of hypertensive parents. *Journal of clinical and diagnostic research : JCDR* **7,** 996-999.

Godia EC, Madhok R, Pittman J, Trocio S, Ramas R, Cabral D, Sacco RL & Rundek T. (2007). Carotid artery distensibility: a reliability study. *Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine* **26,** 1157-1165.

Godin G. (2011). The Godin-Shephard Leisure-Time Physical Activity Questionnaire. *Health and Fitness Journal of Canada* **4,** 18-22.

Grassi G, Biffi A, Seravalle G, Trevano FQ, Dell'Oro R, Corrao G & Mancia G. (2019). Sympathetic Neural Overdrive in the Obese and Overweight State. *Hypertension* **74,** 349-358.

Grassi G, Seravalle G, Calhoun DA & Mancia G. (1994). Physical training and baroreceptor control of sympathetic nerve activity in humans. *Hypertension (Dallas, Tex: 1979)* **23,** 294-301.

Green DJ. (2009). Exercise training as vascular medicine: direct impacts on the vasculature in humans. *Exercise and sport sciences reviews* **37,** 196-202.

Green DJ & Smith KJ. (2018). Effects of Exercise on Vascular Function, Structure, and Health in Humans. *Cold Spring Harbor perspectives in medicine* **8**.

Green LJ, Mackillop LH, Salvi D, Pullon R, Loerup L, Tarassenko L, Mossop J, Edwards C, Gerry S, Birks J, Gauntlett R, Harding K, Chappell LC & Watkinson PJ. (2020). Gestation-Specific Vital Sign Reference Ranges in Pregnancy. *Obstet Gynecol* **135,** 653-664.

Greenwood JP, Scott EM, Walker JJ, Stoker JB & Mary DA. (2003). The magnitude of sympathetic hyperactivity in pregnancy-induced hypertension and preeclampsia. *American journal of hypertension* **16,** 194-199.

Greenwood JP, Stoker JB, Walker JJ & Mary DA. (1998). Sympathetic nerve discharge in normal pregnancy and pregnancy-induced hypertension. *Journal of hypertension* **16,** 617-624.

Hart EC, Joyner MJ, Wallin BG, Karlsson T, Curry TB & Charkoudian N. (2010). Baroreflex control of muscle sympathetic nerve activity: a nonpharmacological measure of baroreflex sensitivity. *American journal of physiology Heart and circulatory physiology* **298,** H816-822.

Herr MD, Hogeman CS, Koch DW, Krishnan A, Momen A & Leuenberger UA. (2010). A real-time device for converting Doppler ultrasound audio signals into fluid flow velocity. *American journal of physiology Heart and circulatory physiology* **298,** H1626-1632.

Hesketh KR & Evenson KR. (2016). Prevalence of U.S. Pregnant Women Meeting 2015 ACOG Physical Activity Guidelines. *American journal of preventive medicine* **51,** e87-89.

Hetherington-Rauth M, Magalhães JP, Júdice PB, Melo X & Sardinha LB. (2020). Vascular improvements in individuals with type 2 diabetes following a 1 year randomised controlled exercise intervention, irrespective of changes in cardiorespiratory fitness. *Diabetologia* **63,** 722-732.

Hissen SL, El Sayed K, Macefield VG, Brown R & Taylor CE. (2017). Muscle sympathetic nerve activity peaks in the first trimester in healthy pregnancy: a longitudinal case study. *Clinical autonomic research : official journal of the Clinical Autonomic Research Society* **27,** 401-406.

Hissen SL & Fu Q. (2020). Neural control of blood pressure during pregnancy in humans. *Clin Auton Res*.

Hoeks AP, Brands PJ, Willigers JM & Reneman RS. (1999). Non-invasive measurement of mechanical properties of arteries in health and disease. *Proceedings of the Institution of Mechanical Engineers Part H, Journal of engineering in medicine* **213,** 195-202.

Holland CK, Brown JM, Scoutt LM & Taylor KJ. (1998). Lower extremity volumetric arterial blood flow in normal subjects. *Ultrasound Med Biol* **24,** 1079-1086.

Hollegaard B, Lykke JA & Boomsma JJ. (2017). Time from pre-eclampsia diagnosis to delivery affects future health prospects of children. *Evolution, medicine, and public health* **2017,** 53-66.

Huang C, Wang J, Deng S, She Q & Wu L. (2016). The effects of aerobic endurance exercise on pulse wave velocity and intima media thickness in adults: A systematic review and meta-analysis. *Scand J Med Sci Sports* **26,** 478-487.

Iacobaeus C, Andolf E, Thorsell M, Bremme K, Jorneskog G, Ostlund E & Kahan T. (2017). Longitudinal study of vascular structure and function during normal pregnancy. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* **49,** 46-53.

Incognito AV, Doherty CJ, Lee JB, Burns MJ & Millar PJ. (2018). Interindividual variability in muscle sympathetic responses to static handgrip in young men: evidence for sympathetic responder types? *American journal of physiologyRegulatory, integrative and comparative physiology* **314,** R121.

Jansen JR, Schreuder JJ, Mulier JP, Smith NT, Settels JJ & Wesseling KH. (2001). A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. *British journal of anaesthesia* **87,** 212-222.

Jarvis SS, Shibata S, Bivens TB, Okada Y, Casey BM, Levine BD & Fu Q. (2012). Sympathetic activation during early pregnancy in humans. *The Journal of physiology* **590,** 3535-3543.

Jarvis SS, VanGundy TB, Galbreath MM, Shibata S, Okazaki K, Reelick MF, Levine BD & Fu Q. (2011). Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans. *American journal of physiology Regulatory, integrative and comparative physiology* **301,** R193-200.

Kallak TK, Hellgren C, Skalkidou A, Sandelin-Francke L, Ubhayasekhera K, Bergquist J, Axelsson O, Comasco E, Campbell RE & Sundström Poromaa I. (2017). Maternal and female fetal testosterone levels are associated with maternal age and gestational weight gain. *European journal of endocrinology* **177,** 379-388.

Kärkkäinen H, Saarelainen H, Valtonen P, Laitinen T, Raitakari OT, Juonala M, Kähönen M, Hutri-Kähönen N, Heinonen S & Laitinen T. (2014). Carotid artery elasticity decreases during pregnancy - the Cardiovascular Risk in Young Finns study. *BMC Pregnancy Childbirth* **14,** 98.

Kasawara KT, do Nascimento SL, Costa ML, Surita FG & e Silva JL. (2012). Exercise and physical activity in the prevention of pre-eclampsia: systematic review. *Acta Obstetricia et Gynecologica Scandinavica* **91,** 1147-1157.

Kawabata I, Nakai A, Sekiguchi A, Inoue Y & Takeshita T. (2012). The effect of regular exercise training during pregnancy on postpartum brachial-ankle pulse wave velocity, a measure of arterial stiffness. *Journal of sports science & medicine* **11,** 489-494.

Kerlan V, Nahoul K, Le Martelot MT & Bercovici JP. (1994). Longitudinal study of maternal plasma bioavailable testosterone and androstanediol glucuronide levels during pregnancy. *Clinical endocrinology* **40,** 263-267.

Kienbaum P, Karlssonn T, Sverrisdottir YB, Elam M & Wallin BG. (2001). Two sites for modulation of human sympathetic activity by arterial baroreceptors? *J Physiol* **531,** 861-869.

Landau R, Dishy V, Wood AJ, Stein CM & Smiley RM. (2002). Disproportionate decrease in alpha- compared with beta-adrenergic sensitivity in the dorsal hand vein in pregnancy favors vasodilation. *Circulation* **106,** 1116-1120.

Laterza MC, de Matos LD, Trombetta IC, Braga AM, Roveda F, Alves MJ, Krieger EM, Negrao CE & Rondon MU. (2007). Exercise training restores baroreflex sensitivity in never-treated hypertensive patients. *Hypertension (Dallas, Tex: 1979)* **49,** 1298-1306.

Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I & Struijker-Boudier H. (2006). Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European heart journal* **27,** 2588-2605.

Lenhard W & Lenhard A. (2016). Calculation of Effect Sizes. Dettelbach (Germany).

Longo LD. (1983). Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control. *The American Journal of Physiology* **245,** 720.

Lowell H & Miller DC. (2010). Weight gain during pregnancy: Adherence to Health Canada's guidelines. *Health Reports* **21,** 1-6.

Magee LA, Pels A, Helewa M, Rey E & von Dadelszen P. (2014). Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. *Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC* **36,** 575-576.

Mark AL, Victor RG, Nerhed C & Wallin BG. (1985). Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circulation research* **57,** 461-469.

McDonald SM, Strom C, Remchak MM, Chaves A, Broskey NT, Isler C, Haven K, Newton E, DeVente J, Acosta-Manzano P, Aparicio VA & May LE. (2020). The effects of aerobic exercise on markers of maternal metabolism during pregnancy. *Birth defects research*.

McLaughlin MK, Keve TM & Cooke R. (1989). Vascular catecholamine sensitivity during pregnancy in the ewe. *Am J Obstet Gynecol* **160,** 47-53.

Meah VL, Backx K, Cockcroft JR, Shave RE & Stöhr EJ. (2019a). Left ventricular mechanics in late second trimester of healthy pregnancy. *Ultrasound Obstet Gynecol* **54,** 350-358.

Meah VL, Backx K & Davenport MH. (2018a). Functional hemodynamic testing in pregnancy: recommendations of the International Working Group on Maternal Hemodynamics. *Ultrasound Obstet Gynecol* **51,** 331-340.

Meah VL, Backx K, Davenport MH, Bruckmann A, Cockcroft J, Cornette J, Duvekot JJ, Ferrazzi E, Foo FL, Ghossein-Doha C, Gyselaers W, Khalil A, McEniery CM, Lees C, Meah V, Novelli GP, Spaanderman M, Stohr E, Tay J, Thilaganathan B, Valensise H & Wilkinson I. (2018b). Functional hemodynamic testing in pregnancy: recommendations of the International Working Group on Maternal Hemodynamics. *Ultrasound in Obstetrics & Gynecology* **51,** 331-340.

Meah VL, Busch SA, Jones KE, Davenport MH & Steinback CD. (2019b). A review of acute responses, after-effects and chronic complications related to microneurography. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **130,** 1781-1788.

Meah VL, Cockcroft JR, Backx K, Shave R & Stohr EJ. (2016). Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. *Heart (British Cardiac Society)* **102,** 518-526.

Melanson EL & Freedson PS. (1995). Validity of the Computer Science and Applications, Inc. (CSA) activity monitor. *Medicine and science in sports and exercise* **27,** 934-940.

Mersich B, Rigo J, Besenyei C, Lenard Z, Studinger P & Kollai M. (2005). Opposite changes in carotid versus aortic stiffness during healthy human pregnancy. *Clinical science (London, England : 1979)* **109,** 103-107.

Miller AJ, Cui J, Luck JC, Sinoway LI & Muller MD. (2019). Age and sex differences in sympathetic and hemodynamic responses to hypoxia and cold pressor test. *Physiological reports* **7,** e13988.

Mimura J, Yuasa F, Yuyama R, Kawamura A, Iwasaki M, Sugiura T & Iwasaka T. (2005). The effect of residential exercise training on baroreflex control of heart rate and sympathetic nerve activity in patients with acute myocardial infarction. *Chest* **127,** 1108-1115.

Mora S, Cook N, Buring JE, Ridker PM & Lee IM. (2007). Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation* **116,** 2110-2118.

Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Piña IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC, Jr., Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V & Wenger NK. (2011a). Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. *Circulation* **123,** 1243-1262.

Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC, Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V, Wenger NK & American Heart A. (2011b). Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American Heart Association. *Journal of the American College of Cardiology* **57,** 1404-1423.

Mottola MF, Davenport MH, Ruchat SM, Davies GA, Poitras VJ, Gray CE, Jaramillo Garcia A, Barrowman N, Adamo KB, Duggan M, Barakat R, Chilibeck P, Fleming K, Forte M, Korolnek J, Nagpal T, Slater LG, Stirling D & Zehr L. (2018). 2019 Canadian guideline for physical activity throughout pregnancy. *British journal of sports medicine* **52,** 1339-1346.

Nardone M, Floras JS & Millar PJ. (2020). Sympathetic neural modulation of arterial stiffness in humans. *American journal of physiology Heart and circulatory physiology* **319,** H1338-h1346.

Nisell H, Hjemdahl P, Linde B & Lunell NO. (1985). Sympatho-adrenal and cardiovascular reactivity in pregnancy-induced hypertension. I. Responses to isometric exercise and a cold pressor test. *British journal of obstetrics and gynaecology* **92,** 722-731.

O'Brien MW, Ramsay D, Johnston W & Kimmerly DS. (2020). Aerobic fitness and sympathetic responses to spontaneous muscle sympathetic nerve activity in young males. *Clin Auton Res*.

Okada Y, Best SA, Jarvis SS, Shibata S, Parker RS, Casey BM, Levine BD & Fu Q. (2015). Asian women have attenuated sympathetic activation but enhanced renal-adrenal responses during pregnancy compared to Caucasian women. *The Journal of physiology* **593,** 1159-1168.

Olver TD, Hazell TJ, Hamilton CD, Shoemaker JK & Lemon PW. (2012). Impaired superficial femoral artery vasodilation and leg blood flow in young obese women following an oral glucose tolerance test. *Appl Physiol Nutr Metab* **37,** 176-183.

Pernoll ML, Metcalfe J, Kovach PA, Wachtel R & Dunham MJ. (1975). Ventilation during rest and exercise in pregnancy and postpartum. *Respiration physiology* **25,** 295-310.

Purdy GM, James MA, Wakefield PK, Skow RJ, Van Diepen S, May LE, Davenport MH & Steinback CD. (2019). Maternal cardioautonomic responses during and following exercise throughout pregnancy. *Appl Physiol Nutr Metab* **44,** 263-270.

Ramírez-Vélez R, Aguilar de Plata AC, Escudero MM, Echeverry I, Ortega JG, Salazar B, Rey JJ, Hormiga C & López-Jaramillo P. (2011). Influence of regular aerobic exercise on endothelium-dependent vasodilation and cardiorespiratory fitness in pregnant women. *J Obstet Gynaecol Res* **37,** 1601-1608.

Ray CA. (1999). Sympathetic adaptations to one-legged training. *Journal of applied physiology (Bethesda, Md: 1985)* **86,** 1583-1587.

Ray CA & Carter JR. (2010). Effects of aerobic exercise training on sympathetic and renal responses to mental stress in humans. *American journal of physiologyHeart and circulatory physiology* **298,** 229.

Reyes LM, Badrov MB, Fu Q, Steinback CD & Davenport MH. (2020a). Age, body mass index and weight gain do not increase sympathetic activity during pregnancy. *Appl Physiol Nutr Metab*.

Reyes LM, Farooq SM, Skow RJ, Busch SA, Pyke KE, Khurana R, Chari RS, Stickland MK, Devolin M, Davidge ST, Sobierajski F, Lugg A, Steinback CD & Davenport MH. (2020b). Physical Activity in Pregnancy Is Associated with Increased Flow-mediated Dilation. *Med Sci Sports Exerc* **52,** 801-809.

Reyes LM, Usselman CW, Davenport MH & Steinback CD. (2018a). Sympathetic Nervous System Regulation in Human Normotensive and Hypertensive Pregnancies. *Hypertension (Dallas, Tex: 1979)*.

Reyes LM, Usselman CW, Skow RJ, Charkoudian N, Staab JS, Davenport MH & Steinback CD. (2018b). Sympathetic neurovascular regulation during pregnancy: A longitudinal case series study. *Experimental physiology* **103,** 318-323.

Roberts JM, August PA, Bakris G, Barton JR, Bernstein IM, Druzin M, Gaiser RR, Granger JP, Jeyabalan A, Johnson DD, Karumanchi S, Lindheimer M, Owens MY, Saade GR, Sibai BM, Spong CY, Tsigas E, Joseph GF, O'Reilly N, Politzer A, Son S & Ngaiza K. (2013). Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstetrics and gynecology* **122,** 1122.

Roveda F, Middlekauff HR, Rondon MU, Reis SF, Souza M, Nastari L, Barretto AC, Krieger EM & Negrao CE. (2003). The effects of exercise training on sympathetic neural activation in advanced heart failure: a randomized controlled trial. *Journal of the American College of Cardiology* **42,** 854-860.

Ruchat SM, Davenport MH, Giroux I, Hillier M, Batada A, Sopper MM, Hammond JA & Mottola M. (2012). Walking program of low or vigorous intensity during pregnancy confers an aerobic benefit. *International Journal of Sports Medicine* **33,** 661-666.

Ruchat SM, Mottola MF, Skow RJ, Nagpal TS, Meah VL, James M, Riske L, Sobierajski F, Kathol AJ, Marchand AA, Nuspl M, Weeks A, Gray CE, Poitras VJ, Jaramillo Garcia A, Barrowman N, Slater LG, Adamo KB, Davies GA, Barakat R & Davenport MH. (2018). Effectiveness of exercise interventions in the prevention of excessive gestational weight gain and postpartum weight retention: a systematic review and meta-analysis. *British journal of sports medicine* **52,** 1347-1356.

Sady SP, Carpenter MW, Thompson PD, Sady MA, Haydon B & Coustan DR. (1989). Cardiovascular response to cycle exercise during and after pregnancy. *Journal of applied physiology (Bethesda, Md: 1985)* **66,** 336-341.

Salamalekis E, Bakas P, Vitoratos N, Eleptheriadis M & Creatsas G. (2006). . *Eur J Obstet Gynecol Reprod Biol* **126,** 16-19.

Sanghavi M & Rutherford JD. (2014). Cardiovascular physiology of pregnancy. *Circulation* **130,** 1003-1008.

Schobel HP, Fischer T, Heuszer K, Geiger H & Schmieder RE. (1996). Preeclampsia -- a state of sympathetic overactivity. *The New England journal of medicine* **335,** 1480-1485.

Sheldahl LM, Ebert TJ, Cox B & Tristani FE. (1994). Effect of aerobic training on baroreflex regulation of cardiac and sympathetic function. *Journal of applied physiology (Bethesda, Md: 1985)* **76,** 158-165.

Shoemaker JK, Mattar L, Kerbeci P, Trotter S, Arbeille P & Hughson RL. (2007). WISE 2005: stroke volume changes contribute to the pressor response during ischemic handgrip exercise in women. *Journal of applied physiology (Bethesda, Md: 1985)* **103,** 228-233.

Skow RJ, Fraser G, Steinback CD & Davenport MH. (2020). Prenatal Exercise and Cardiovascular Health (PEACH) Study: Impact on Muscle Sympathetic Nerve (Re)activity. *Med Sci Sports Exerc*.

Skow RJ, King EC, Steinback CD & Davenport MH. (2017). The influence of prenatal exercise and pre-eclampsia on maternal vascular function. *Clinical science (London, England : 1979)* **131,** 2223-2240.

Sobierajski FM, Purdy GM, Usselman CW, Skow RJ, James MA, Chari RS, Khurana R, Stickland MK, Davidge ST, Devolin M, Steinback CD & Davenport MH. (2018). Maternal Physical Activity Is Associated With Improved Blood Pressure Regulation During Late Pregnancy. *The Canadian journal of cardiology* **34,** 485-491.

Sonagra AD, Biradar SM, K D & Murthy DSJ. (2014). Normal pregnancy- a state of insulin resistance. *Journal of clinical and diagnostic research : JCDR* **8,** Cc01-03.

Steinback CD, Fraser GM, Usselman CW, Reyes LM, Julian CG, Stickland MK, Chari RS, Khurana R, Davidge ST & Davenport MH. (2019). Blunted sympathetic neurovascular transduction during normotensive pregnancy. *J Physiol* **597,** 3687-3696.

Svedenhag J, Wallin BG, Sundlof G & Henriksson J. (1984). Skeletal muscle sympathetic activity at rest in trained and untrained subjects. *Acta Physiologica Scandinavica* **120,** 499-504.

Tanaka H. (2018). Various Indices of Arterial Stiffness: Are They Closely Related or Distinctly Different? *Pulse (Basel, Switzerland)* **5,** 1-6.

Tanaka H & Safar ME. (2005). Influence of lifestyle modification on arterial stiffness and wave reflections. *American journal of hypertension* **18,** 137-144.

Thijssen DH, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MT & Green DJ. (2010). Impact of inactivity and exercise on the vasculature in humans. *European journal of applied physiology* **108,** 845-875.

Tonacio AC, Trombetta IC, Rondon MU, Batalha LT, Kuniyoshi FH, Laterza MC, Suzuki PH, Gowdak MM, Barretto AC, Halpern A, Villares SM & Negrao CE. (2006). Effects of diet and exercise training on neurovascular control during mental stress in obese women. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas* **39,** 53-62.

Troisi R, Hoover RN, Thadhani R, Hsieh CC, Sluss P, Ballard-Barbash R & Potischman N. (2008). Maternal, prenatal and perinatal characteristics and first trimester maternal serum hormone concentrations. *British journal of cancer* **99,** 1161-1164.

Trombetta IC, Batalha LT, Rondon MU, Laterza MC, Kuniyoshi FH, Gowdak MM, Barretto AC, Halpern A, Villares SM & Negrao CE. (2003). Weight loss improves neurovascular and muscle metaboreflex control in obesity. *American journal of physiologyHeart and circulatory physiology* **285,** 974.

Ueno LM, Drager LF, Rodrigues AC, Rondon MU, Braga AM, Mathias W, Krieger EM, Barretto AC, Middlekauff HR, Lorenzi-Filho G & Negrao CE. (2009). Effects of exercise training in patients with chronic heart failure and sleep apnea. *Sleep* **32,** 637-647.

Usselman CW, Skow RJ, Matenchuk BA, Chari RS, Julian CG, Stickland MK, Davenport MH & Steinback CD. (2015a). Sympathetic baroreflex gain in normotensive pregnant women. *Journal of applied physiology (Bethesda, Md: 1985)* **119,** 468-474.

Usselman CW, Wakefield PK, Skow RJ, Stickland MK, Chari RS, Julian CG, Steinback CD & Davenport MH. (2015b). Regulation of sympathetic nerve activity during the cold pressor test in normotensive pregnant and nonpregnant women. *Hypertension (Dallas, Tex: 1979)* **66,** 858-864.

Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J, Kaiser DR & Thuillez C. (2002). Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. *Am J Hypertens* **15,** 445-452.

Visontai Z, Lenard Z, Studinger P, Rigo J & Kollai M. (2002). Impaired baroreflex function during pregnancy is associated with stiffening of the carotid artery. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* **20,** 364-369.

Vranish JR, Holwerda SW, Young BE, Credeur DP, Patik JC, Barbosa TC, Keller DM & Fadel PJ. (2018). Exaggerated Vasoconstriction to Spontaneous Bursts of Muscle Sympathetic Nerve Activity in Healthy Young Black Men. *Hypertension* **71,** 192-198.

Wan J, Hu Z, Zeng K, Yin Y, Zhao M, Chen M & Chen Q. (2018). The reduction in circulating levels of estrogen and progesterone in women with preeclampsia. *Pregnancy Hypertens* **11,** 18-25.

Wang Y, Ma H, Feng Y, Zhan Y, Wu S, Cai S, Shi Y, Chen Y, Ma L & Jiang Y. (2020). Association among pre-pregnancy body mass index, gestational weight gain and neonatal birth weight: a prospective cohort study in China. *BMC Pregnancy Childbirth* **20,** 690.

Watanabe K, Naruse K, Tanaka K, Metoki H & Suzuki Y. (2013). Outline of Definition and Classification of "Pregnancy induced Hypertension (PIH)". *Hypertension Research in Pregnancy* **1,** 3-4.

Weissgerber TL, Davies GA & Roberts JM. (2010). Modification of angiogenic factors by regular and acute exercise during pregnancy. *Journal of applied physiology (Bethesda, Md: 1985)* **108,** 1217-1223.

Welsch MA, Blalock P, Credeur DP & Parish TR. (2013). Comparison of brachial artery vasoreactivity in elite power athletes and age-matched controls. *PLoS One* **8,** e54718.

Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H & Jeppesen J. (2006). Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* **113,** 664-670.

Woisetschlager C, Waldenhofer U, Bur A, Herkner H, Kiss H, Binder M, Laggner AN & Hirschl MM. (2000). Increased blood pressure response to the cold pressor test in pregnant women developing pre-eclampsia. *Journal of hypertension* **18,** 399-403.

Wolfe L & Mottola M. (2015a). Physical activity readiness medical examination for pregnancy: PARmed-X for pregnancy.

Wolfe LA & Mottola M. (2015b). PARmed-X FOR PREGNANCY Physical Activity Readiness Medical Examination. Canadian Society for Exercise Physiology.

Wundersitz DWT, Gordon BA, Lavie CJ, Nadurata V & Kingsley MIC. (2020). Impact of endurance exercise on the heart of cyclists: A systematic review and meta-analysis. *Progress in cardiovascular diseases*.

Yoshizawa M, Maeda S, Miyaki A, Misono M, Saito Y, Tanabe K, Kuno S & Ajisaka R. (2009). Effect of 12 weeks of moderate-intensity resistance training on arterial stiffness: a randomised controlled trial in women aged 32-59 years. *British journal of sports medicine* **43,** 615-618.

Yuan C, Wang J & Ying M. (2016). Predictive Value of Carotid Distensibility Coefficient for Cardiovascular Diseases and All-Cause Mortality: A Meta-Analysis. *PLoS One* **11,** e0152799.

Yuan LJ, Xue D, Duan YY, Cao TS, Yang HG & Zhou N. (2013a). Carotid arterial intima-media thickness and arterial stiffness in pre-eclampsia: analysis with a radiofrequency ultrasound technique. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* **42,** 644-652.

Yuan LJ, Xue D, Duan YY, Cao TS & Zhou N. (2013b). Maternal carotid remodeling and increased carotid arterial stiffness in normal late-gestational pregnancy as assessed by radio-frequency ultrasound technique. *BMC Pregnancy Childbirth* **13,** 122.

Zeisler H, Llurba E, Chantraine F, Vatish M, Staff A, Sennström M, Olovsson M, Brennecke S, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M & Verlohren S. (2016). Predictive Value of the sFlt-1: PlGF Ratio in Women With Suspected Preeclampsia. *Obstetrical & gynecological survey* **71,** 273-274.

Zygmunt A & Stanczyk J. (2010). Methods of evaluation of autonomic nervous system function. *Archives of medical science : AMS* **6,** 11-18.

Appendices

Appendix A: The effects of pregnancy on blood pressure control during handgrip exercise. Additional data.

Table A1. *Participant characteristics and resting hemodynamics in women with and without MSNA.*

Outcome	Non-Pregnant			Pregnant		
	No MSNA (n=4)	MSNA (n=10)	p-value	No MSNA (n=7)	MSNA (n=12) *	p-value
Participant characteristics						
Age (years)	30 ± 3	31 ± 5	0.656	35 ± 3	32 ± 3	**0.04**
Pre/non- pregnant BMI (kg/m^2)	25 ± 2	25 ± 3	0.662	24 ± 5	24 ± 5	0.959
Resting Hemodynamics						
Heart rate (bpm)	72 ± 5	71 ± 8	0.909	81 ± 16	86 ± 10	0.444
SBP (mmHg)	102 ± 9	105 ± 7	0.482	98 ± 5	101 ± 10	0.500
DBP (mmHg)	66 ± 8	70 ± 9	0.399	66 ± 4	64 ± 9	0.492
MAP (mmHg)	82 ± 7	84 ± 8	0.595	78 ± 4	78 ± 8	0.754
Q (L/min)	5.9 ± 0.8	6.2 ± 1.2	0.594	6.9 ± 1.4	7.1 ± 1.6	0.790
SVR (mmHg/L/min)	14 ± 1	14 ± 2	0.899	12 ± 3	11 ± 2	0.592

All values are mean ± SD unless otherwise indicated. Comparisons between women with and without MSNA made using a student t-test in GraphPad Prism (v 9.0). Significance was set at $p<0.05$ and indicated in bold text. MSNA, muscle sympathetic nerve activity; n=number of women; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; Q, cardiac output; SVR, systemic vascular resistance. *pre-pregnancy BMI, Q, and SVR are missing in one pregnant woman with MSNA (n=11).

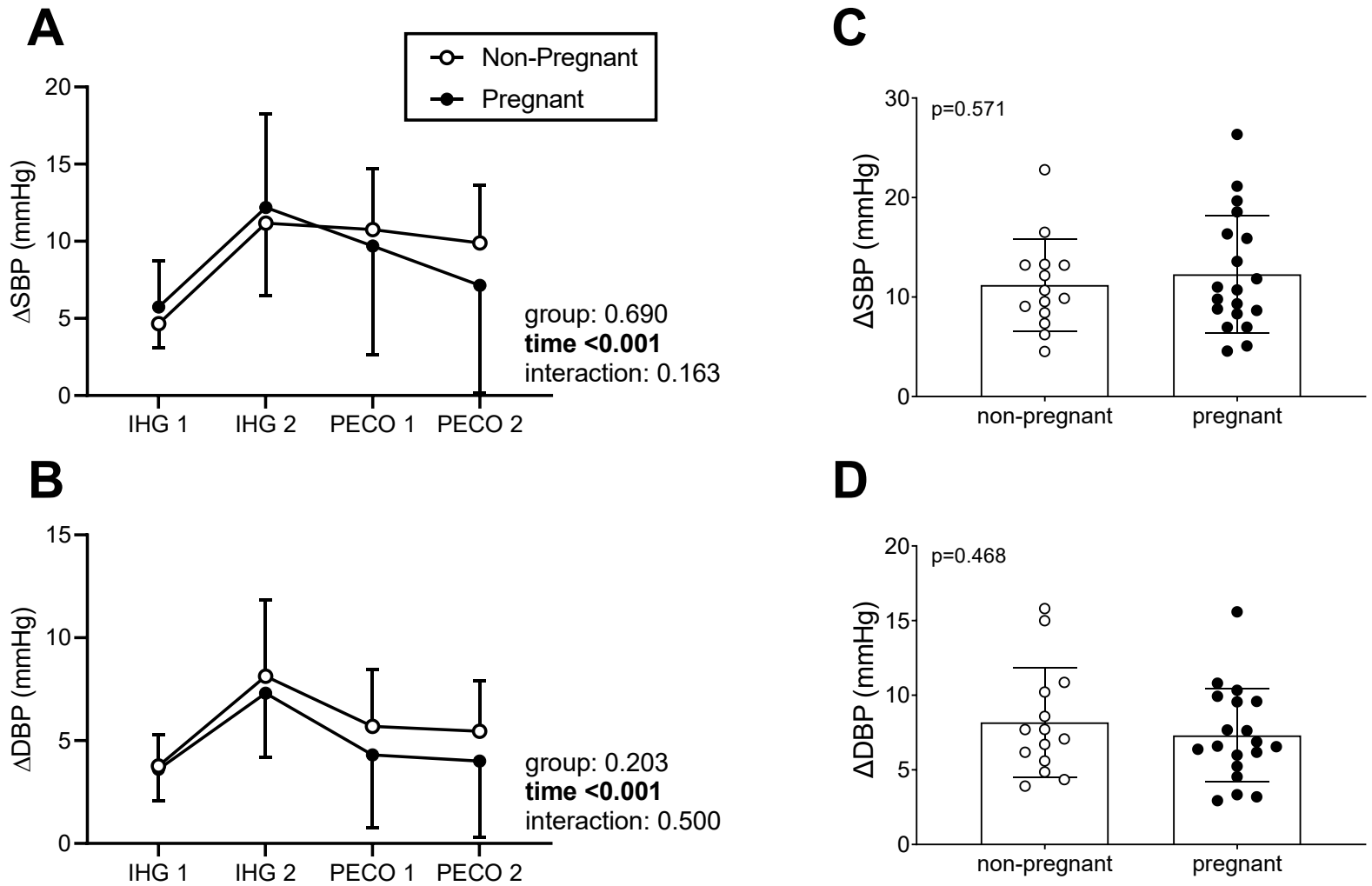

A
Non-Pregnant
Pregnant
ΔSBP (mmHg)
20
15
10
5
0
IHG 1
IHG 2
PECO 1
PECO 2
group: 0.690
time <0.001
interaction: 0.163
B
ΔDBP (mmHg)
15
10
5
0
IHG 1
IHG 2
PECO 1
PECO 2
group: 0.203
time <0.001
interaction: 0.500
C
p=0.571
ΔSBP (mmHg)
30
20
10
0
non-pregnant
pregnant
D
p=0.468
ΔDBP (mmHg)
20
15
10
5
0
non-pregnant
pregnant

Figure A2. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) responses during 2-minutes of isometric handgrip (IHG) and 2-minutes of post exercise circulatory occlusion (PECO). Non-pregnant women are depicted as open circles and pregnant women are depicted as closed circles. Statistics for the response were performed using a two-way mixed model ANOVA in GraphPad Prism (v9.0) using absolute raw values for SBP and DBP. Significance was set at $p<0.05$ and indicated by bold text where applicable. **A)** Absolute changes in SBP during each minute of IHG and PECO. SBP is increased during IHG and PECO compared to baseline (main effect of time) but does not differ between groups. **B)** Peak change in SBP during IHG was not different between groups. Individual data is shown by the symbols and the bar represents the group mean (SD) changes. **C)** Absolute changes in DBP during each minute of IHG and PECO. DBP is increased during IHG and PECO compared to baseline (main effect of time) but does not differ between groups. **D)** Peak change in DBP during IHG was not different between groups.

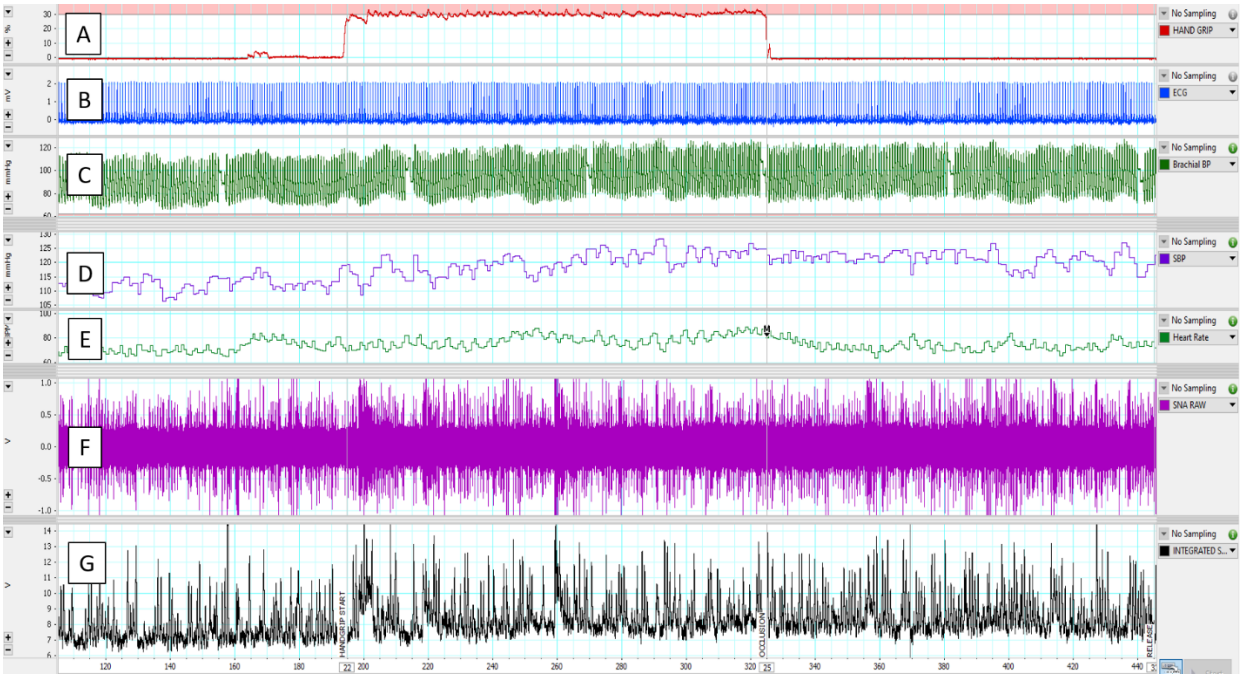


Figure A3. *Representative tracing from one pregnant woman. Channels are as follows (in order from top to bottom): A) hand grip (% maximal voluntary contraction; MVC); B) ECG (mV); C) Finometer derived brachial blood pressure (mmHg); D) Systolic blood pressure (SBP) calculated from the Finometer tracing; E) Heart rate (calculated from ECG channel); F) Raw MSNA signal; G) Integrated MSNA signal (unlabeled). Protocol involved 3 minutes of baseline (rest) followed by two minutes of isometric handgrip (IHG) at 30% MVC and 2-minutes of post exercise circulatory occlusion (PECO).*

Table A2. *Correlations between sympathetic baroreflex gain and the peak sympathetic response during two-minutes of isometric handgrip.*

	p-value	**R-squared**	**effect size (R)**
ΔBF (bursts/ minute)	0.855	0.002	0.046
ΔBI (bursts/100 heartbeats)	0.874	0.002	0.040
ΔTotal activity (a.u.)	0.574	0.020	0.142

BF, burst frequency; BI, burst incidence. Data is presented as a correlation for all women (pregnant and non-pregnant) and was completed in GraphPad Prism (v 9.0). Significance was set at $p<0.05$. A moderate effect size ($R>0.5$) was used to determine a meaningful relationship. There were no correlations between resting sympathetic baroreflex and the reflex reactivity of the sympathetic nervous system during isometric handgrip.

Table A3. *Difference in statistics for 2-way ANOVA comparing the sympathetic response (time-course response) to two-minutes of isometric handgrip and two-minutes of post-exercise circulatory occlusion with and without "outlier" data.*

	Group	**Time**	**Interaction**
Brust Frequency (bursts/minute)			
All data	**0.008**	**<0.001**	0.305
Outlier removed	**0.012**	**<0.001**	0.095
Brust Incidence (bursts/ 100 heartbeats)			
All data	0.100	**<0.001**	0.288
Outlier removed	0.160	**<0.001**	0.159
Total Activity (a.u.)			
All data	**0.006**	**<0.001**	0.446
Outlier removed	**0.008**	**<0.001**	0.136

Outlier data occurred in the peak sympathetic response to isometric handgrip (negative response) in one pregnant woman. Removing the outlier data from the time-course analysis did not change our interpretation of the data.

Appendix B: Exercise and neurovascular function in pregnancy: compliance data.

Compliance to the exercise intervention was set at achieving at least 75% of prescribed exercise sessions. Using this criteria, four women out of 28 who completed the exercise intervention were non-compliant. It is important to note that only three women out of the 24 compliant women fell below 80% compliance. No woman was achieving more than 40 minutes of activity on four days per week (upper limit of the prescription).

Women were non-compliant due to (anecdotal report):

1) Second trimester bleeding (resulting in 3 weeks of no-exercise while waiting for clearance from Dr. to continue) and then a fear of workout out to hard and not reaching HR targets during sessions.
2) Pubic symphysis and extreme discomfort when exercising.
3) Did not return their logbook at the end of the intervention. Likely was compliant (based off conversations throughout supervised sessions) but cannot confirm without logbook.
4) Sciatic and foot pain resulting in extreme discomfort during exercise.

Compliance to the control condition was determined using accelerometry at three time points (pre-intervention, 16-20weeks; mid-intervention, 26-28 weeks; post-intervention, 34-36 weeks). Women were deemed to be non-compliant to the control condition if they participated in at least 20 minutes of moderate-to-vigorous activity (MVPA) on at least 3 days of the week during each week measured (i.e., >60minutes MVPA per week). Using these criteria, two women in the control group were determined to be non-compliant.

All women who dropped out of the study (5 control, 3 exercise) were also not included in this analysis. Reasons for drop out are indicated in the consort diagram (see **Figure 3.1**) and are not related to the intervention per say.

Table B1. *Resting hemodynamics in women who were compliant to the exercise intervention group and control group.*

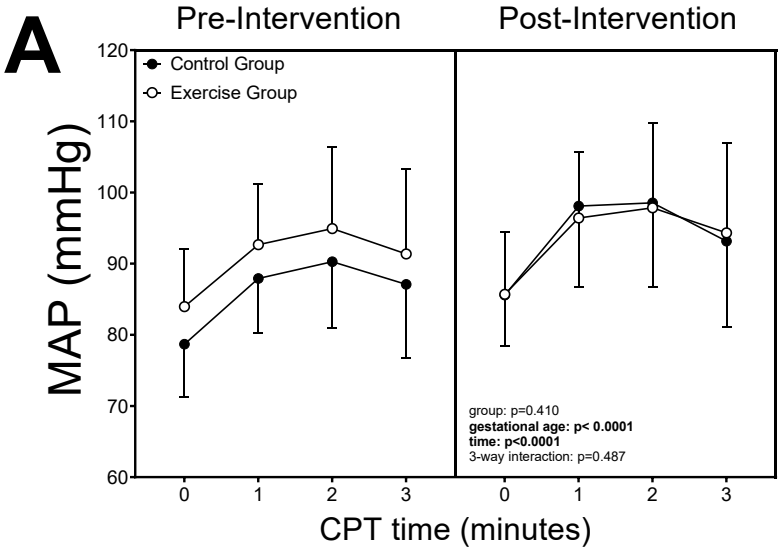
	Control Group				**Exercise Group**				**P-values**		
	Pre-intervention		Post-intervention		Pre-intervention		Post-intervention				
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	Group	G.A.	Interaction
G.A. (wks)	21	18 (1)	21	35 (1)	24	18 (2)	24	34 (1)	n/a		
Heart rate (bpm)	21	76 (7)	21	88 (11)	24	82 (7)	24	85 (8)	0.497	**<0.001**	**0.003**
SBP (mmHg)	21	108 (10)	21	111 (12)	24	111 (12)	24	109 (9)	0.795	0.594	0.075
DBP (mmHg)	21	60 (7)	21	68 (7)	24	64 (6)	24	68 (8)	0.166	**<0.001**	0.107
MAP (mmHg)	21	78 (7)	21	85 (8)	24	82 (8)	24	85 (8)	0.291	**<0.001**	0.069
Q (L/min)	21	7.9 (2.0)	20	8.1 (1.8)	24	7.5 (1.7)	24	7.7 (1.7)	0.443	0.190	0.856
SVR (dynes)	21	820 (125)	20	863 (156)	24	915 (187)	24	911 (164)	0.107	0.353	0.262

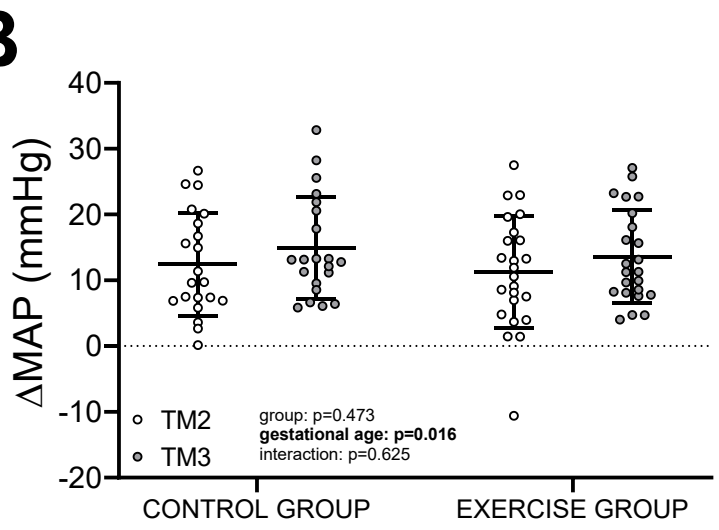
n, number of women; SD, standard deviation; G.A., gestational weeks; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; Q, cardiac output; SVR, systemic vascular resistance. Statistics determined using mixed model ANOVA in GraphPad Prism (v9.0) on data from women who were compliant to the intervention/control. Significance was set at $p<0.05$ and indicated in bold text.

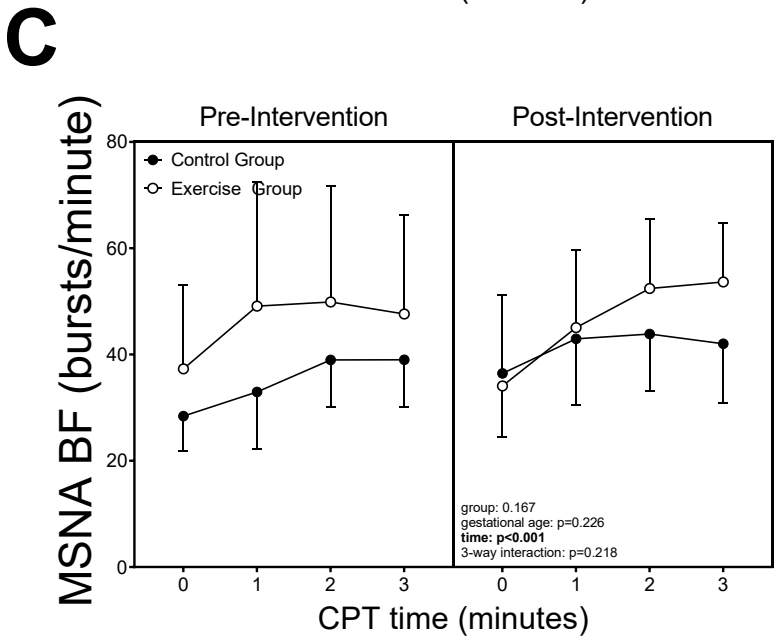
Table B2. *Resting sympathetic nerve activity, baroreflex gain, and neurovascular transduction in women who were compliant to the exercise intervention.*

	Control Group				**Exercise Group**				**P-values**		
	Pre-intervention		Post-intervention		Pre-intervention		Post-intervention				
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	Group	G.A.	Interaction
BF (bursts/min)	10	24 (5)	10	36 (9)	11	38 (11)	13	36 (8)	**0.043**	**0.012**	**0.008**
BI (bursts/100 heart beats)	10	32 (5)	10	41 (7)	11	46 (12)	13	43 (9)	**0.045**	**0.002**	**<0.001**
BRG (probability slope)	10	-4.7 (2.1)	10	-5.2 (1.9)	11	-5.9 (2.1)	13	-5.0 (2.0)	0.834	0.424	0.472
BRG (Total MSNA slope)	10	-446 (193)	10	-557 (195)	22	-631 (272)	13	-544 (309)	0.454	0.926	0.244
NVT (MAP slope)	10	0.007 (0.004)	10	0.005 (0.003)	8	0.004 (0.002)	11	0.005 (0.002)	0.261	0.129	0.140

n, number of women; SD, standard deviation; G.A., gestational age; BF, burst frequency; BI, burst incidence; NVT, neurovascular transduction; BRG, baroreflex gain; MSNA, muscle sympathetic nerve activity; MAP, mean arterial blood pressure. Statistics determined using mixed model ANOVA in GraphPad Prism (v9.0) on data from women who were compliant to the intervention/control. Significance was set at $p<0.05$ and indicated in bold text.


A
Pre-Intervention
Post-Intervention
Control Group
Exercise Group
MAP (mmHg)
120
110
100
90
80
70
60
group: p=0.410
gestational age: p< 0.0001
time: p<0.0001
3-way interaction: p=0.487
0
1
2
3
CPT time (minutes)


B
ΔMAP (mmHg)
40
30
20
10
0
-10
-20
TM2
TM3
group: p=0.473
gestational age: p=0.016
interaction: p=0.625
CONTROL GROUP
EXEXERCISE GROUP


C
Pre-Intervention
Post-Intervention
Control Group
Exercise Group
MSNA BF (bursts/minute)
80
60
40
20
0
group: 0.167
gestational age: p=0.226
time: p<0.001
3-way interaction: p=0.218
0
1
2
3
CPT time (minutes)

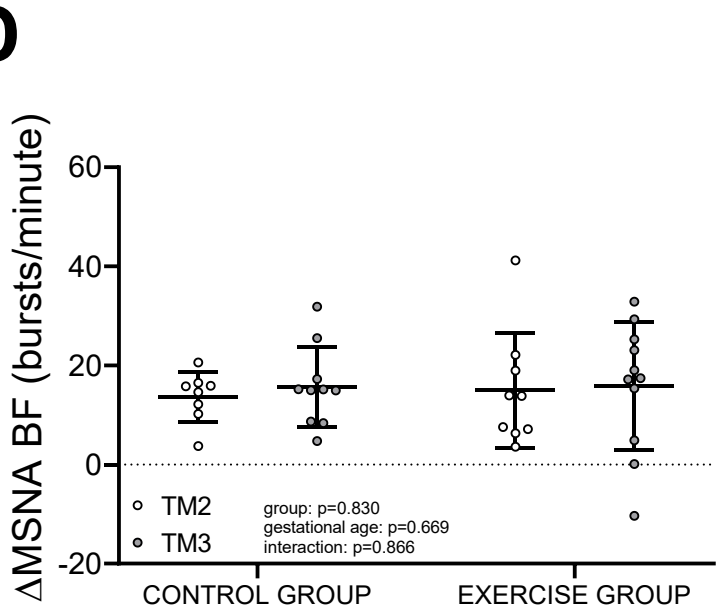

D
ΔMSNA BF (bursts/minute)
60
40
20
0
-20
TM2
TM3
group: p=0.830
gestational age: p=0.669
interaction: p=0.866
CONTROL GROUP
EXERCISE GROUP

Figure B1. Mean arterial blood pressure (MAP) and muscle sympathetic burst frequency (MSNA BF) responses during the cold pressor test in women who were compliant to the exercise intervention. A) Minute-by-minute responses in mean arterial blood pressure (MAP) during the cold pressor test (CPT). Data represent the mean (SD) responses in each group at pre- and post-intervention. Control group is shown in black symbols and exercise group in open circles. The pre-intervention (trimester 2, TM2) is shown in the left-hand box and the post-intervention (trimester 3; TM3) is in the right-hand box. There is a main effect of time such that MAP is increased during CPT, and the increase in MAP during CPT is augmented in TM3 but there is no effect of exercise on the MAP response to CPT. **B)** Data represents the individual peak responses during the CPT (i.e., the minute with the largest change in MAP during CPT on an individual basis) from pre-intervention (TM2, mean response shown in white circles) to post-intervention (TM3, mean response shown in grey circles). There is an effect of gestational age such that the peak increase in MAP during CPT was larger in the third trimester (i.e., post-intervention). **C**) minute-by minutes responses in muscle sympathetic nervous system activity (MSNA) burst frequency (BF) during CPT. The symbols are the same as the MAP response. There is a main effect of time such that MSNA is increased during the CPT but no effect of group, gestational age, or interactions. **D)** MSNA BF peak responses during CPT. There was no significant effect of group, gestational age, or interaction for the peak MSNA response during CPT.

Table B3. *Resting vascular measures in women who were compliant to the exercise intervention.*

	Control Group				**Exercise Group**				**P-values**		
	Pre-intervention		Post-intervention		Pre-intervention		Post-intervention				
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	Group	G.A.	Interaction
Carotid diameter (mm)	20	6.6 (0.6)	14	6.8 (0.6)	20	6.6 (0.6)	18	6.9 (0.5)	0.923	**0.002**	0.741
Carotid blood flow (ml/min)	19	577 (161)	14	535 (139)	20	582 (150)	18	531 (145)	0.991	0.213	0.916
CC (mm^2/kPa)	18	1.1 (0.3)	13	1.1 (0.2)	19	1.0 (0.2)	17	1.1 (0.4)	0.499	0.492	0.889
DC (10^{-3} /kPa)	18	35 (13)	13	34 (8)	19	32 (8)	17	32 (11)	0.407	0.323	0.896
Strain (%)	18	10.3 (2.8)	13	8.7 (2.0)	19	9.5 (1.7)	17	8.1 (1.7)	0.229	**<0.001**	0.732
Stiffness (β)	18	6.3 (2.0)	13	5.6 (1.3)	19	5.8 (1.2)	17	5.8 (1.5)	0.825	0.601	0.178
Distensibility (1/ β)	18	0.18 (0.07)	13	0.19 (0.04)	19	0.18 (0.05)	17	0.18 (0.05)	0.974	0.915	0.713
Elastic modulus (E)	18	69 (23)	13	64 (15)	19	65 (16)	17	68 (20)	0.908	0.782	0.240
Femoral diameter (mm)	19	5.3 (0.6)	16	5.5 (0.6)	19	5.5 (0.7)	18	5.8 (0.9)	0.356	**0.002**	0.390
Femoral blood flow (ml/min)	17	77 (42)	17	84 (53)	17	101 (49)	17	88 (54)	0.332	0.964	0.387
FVC (ml/min/mmHg)	17	0.98 (0.54)	17	0.98 (0.56)	17	1.18 (0.56)	17	1.06 (0.69)	0.422	0.778	0.648
FVR (mmHg/ml/min)	17	1.64 (1.59)	17	1.32 (0.71)	17	1.05 (0.56)	17	1.41 (0.97)	0.327	0.949	0.184

Central PWV (m/s)	16	4.9 (0.9)	16	4.9 (0.6)	23	4.9 (1.0)	19	4.7 (0.6)	0.785	0.842	0.665
Upper PWV (m/s)	18	6.2 (1.1)	19	6.4 (1.0)	24	6.1 (1.1)	20	6.0 (0.6)	0.402	0.490	0.312
Lower PWV (m/s)	12	7.1 (1.7)	17	8.0 (1.0)	18	7.1 (1.7)	15	7.7 (1.4)	0.644	**0.049**	0.697

G.A., gestational age; CC, compliance coefficient; DC, distensibility coefficient; FVC, femoral vascular conductance; FVR, femoral vascular resistance; PWV, pulse wave velocity. Statistics determined using mixed model ANOVA in GraphPad Prism (v9.0) on data from women who were compliant to the intervention/control. Significance was set at $p<0.05$ and indicated in bold text.

Table B4. *Angiogenic factors, sex hormones, and metabolic markers in women who were compliant to the exercise intervention.*

	Control Group				**Exercise Group**				**P-values**		
	Pre-intervention		Post-intervention		Pre-intervention		Post-intervention				
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	Group	G.A.	Interaction
VEGF (pg/ml)	17	11.0 (10.3)	17	12.3 (11.3)	22	24.2 (37.1)	22	33.2 (64.2)	0.145	0.392	0.446
PlGF (pg/ml)	20	174 (79)	18	450 (418)	23	172 (78)	23	422 (363)	0.811	**<0.001**	0.806
sFlt-1 (pg/ml)	20	1055 (819)	15	1639 (942)	22	919 (539)	21	1455 (785)	0.345	**<0.001**	0.476
sFlt-1:PlGF	20	7.3 (7.0)	15	5.7 (5.5)	22	6.0 (3.6)	21	5.2 (4.4)	0.442	0.305	0.820
Estrogen (pmol/L)	20	26445 (10578)	17	69738 (21801)	23	26614 (11954)	23	63794 (25057)	0.665	**<0.001**	0.106
Progesterone (nmol/L)	20	168 (40)	17	606 (158)	23	166 (45)	24	652 (201)	0.514	**<0.001**	0.355
Testosterone (nmol/L)	20	1.6 (1.0)	18	2.1 (1.1)	23	2.2 (1.5)	24	2.8 (1.8)	0.099	**<0.001**	0.644
Glucose (mmol/L)	20	4.4 (0.4)	17	4.4 (0.6)	23	4.5 (0.4)	23	4.4 (0.5)	0.743	0.507	0.226
Insulin (pmol/L)	20	43 (22)	15	62 (30)	23	50 (34)	23	70 (51)	0.460	**<0.001**	0.799
HOMA-IR	17	0.86 (0.37)	15	1.10 (0.59)	23	0.91 (0.69)	23	1.26 (0.94)	0.545	**0.002**	0.354

G.A., gestational age; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; HOMA-IR, homeostatic model assessment of insulin resistance. No value for sFlt-1:PlGF was >38.0 at any assessment. Statistics determined using mixed model ANOVA in GraphPad Prism (v9.0) on data from women who were compliant to the intervention/control. Significance was set at $p<0.05$ and indicated in bold text where applicable.

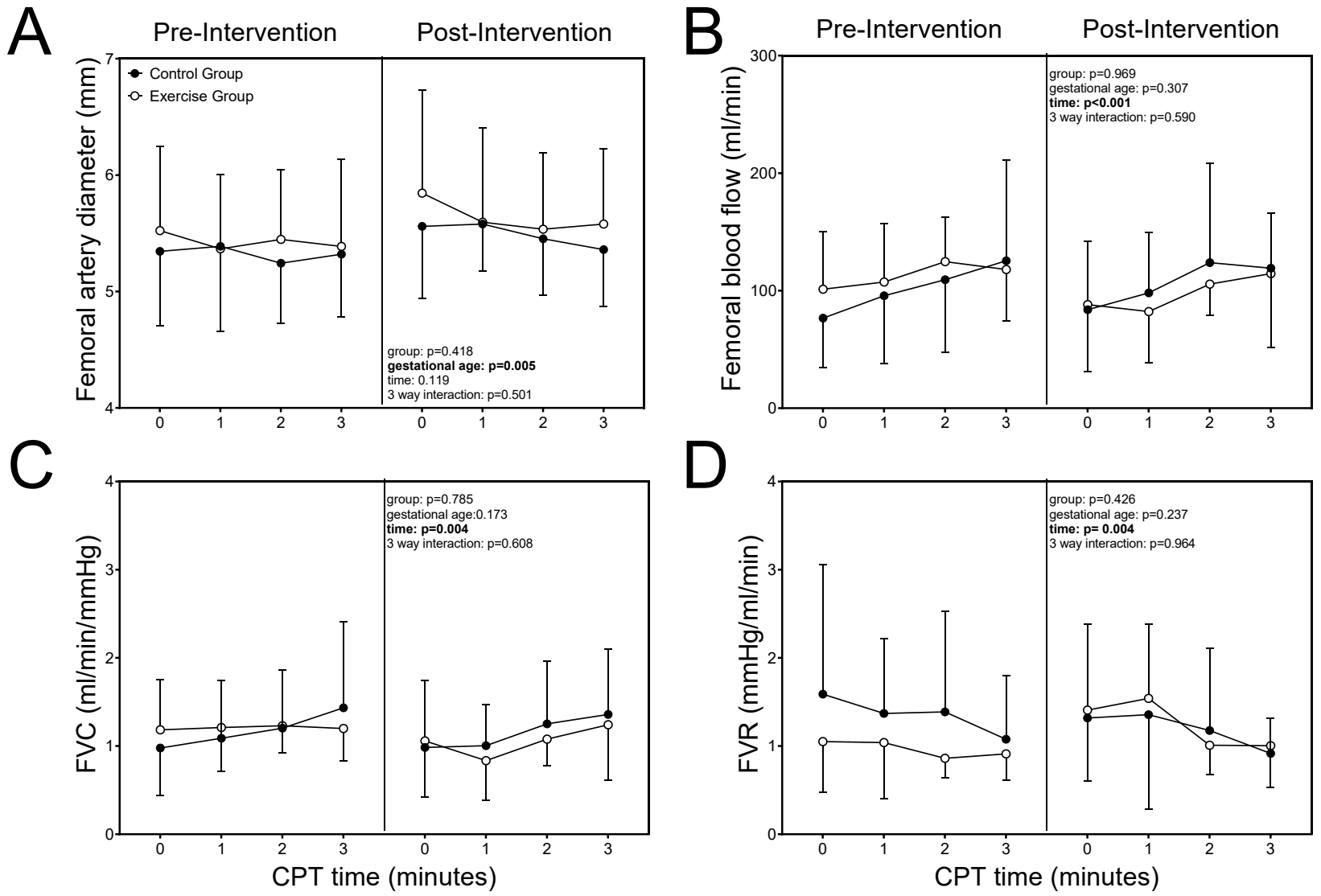

A
Pre-Intervention
Post-Intervention
Control Group
Exercise Group
Femoral artery diameter (mm)
group: p=0.418
gestational age: p=0.005
time: 0.119
3 way interaction: p=0.501
B
Pre-Intervention
Post-Intervention
Femoral blood flow (ml/min)
group: p=0.969
gestational age: p=0.307
time: p<0.001
3 way interaction: p=0.590
C
FVC (ml/min/mmHg)
group: p=0.785
gestational age:0.173
time: p=0.004
3 way interaction: p=0.608
CPT time (minutes)
D
FVR (mmHg/ml/min)
group: p=0.426
gestational age: p=0.237
time: p= 0.004
3 way interaction: p=0.964
CPT time (minutes)

Figure B2. Vascular responses during the cold pressor test in women who were compliant to the exercise intervention. All data is mean ± SD. A) superficial femoral artery diameter in control (filled circles) and exercise (open circles) groups at rest (minute 0) and during each minute of the CPT at pre-intervention (left) and post-intervention (right). There was no change in diameter of the superficial femoral artery during the CPT. B) Blood flow (ml/min) in the superficial artery during each minute of CPT. Blood flow increased during the CPT, but the response was not different across gestation or with respect to exercise intervention. C) Femoral vascular conductance (FVC) in the superficial femoral artery during each minute of CPT. FVC increased during the CPT, but the response was not different across gestation or with respect to exercise intervention. D) Femoral vascular resistance (FVR) in the superficial femoral artery during each minute of CPT. FVR decreased during the CPT, but the response was not different across gestation or with respect to exercise intervention. Significance was set at $p<0.05$ and indicated in bold where applicable.

Appendix C: Exercise and neurovascular function. Additional data

Table C1. *Participant characteristics in women with MSNA at only.*

	No MSNA	MSNA	p-value
Participant demographics, mean (SD)			
Number of women	24	35	n/a
Age (years)	31 (3)	32 (4)	0.360
Height (cm)	166 (8)	166 (7)	0.879
Pre-pregnancy weight (kg)	75 (28)	70 (14)	0.357
Pre-pregnancy BMI (kg/m^2)	26 (9)	25 (5)	0.518

MSNA, muscle sympathetic nerve activity; BMI, body mass index; n, number of women. MSNA was collected on 21 women twice (9 control, 12 exercise) and on 14 women once. Of these, 8 had MSNA measures during trimester two only (5 control, 3 exercise) and 6 had MSNA measures during trimester 3 only (2 control, 4 exercise). Pre-pregnancy weight, BMI, and physical activity levels are all determined from a self-report. Significance was set at $p<0.05$ and indicated in bold where applicable.

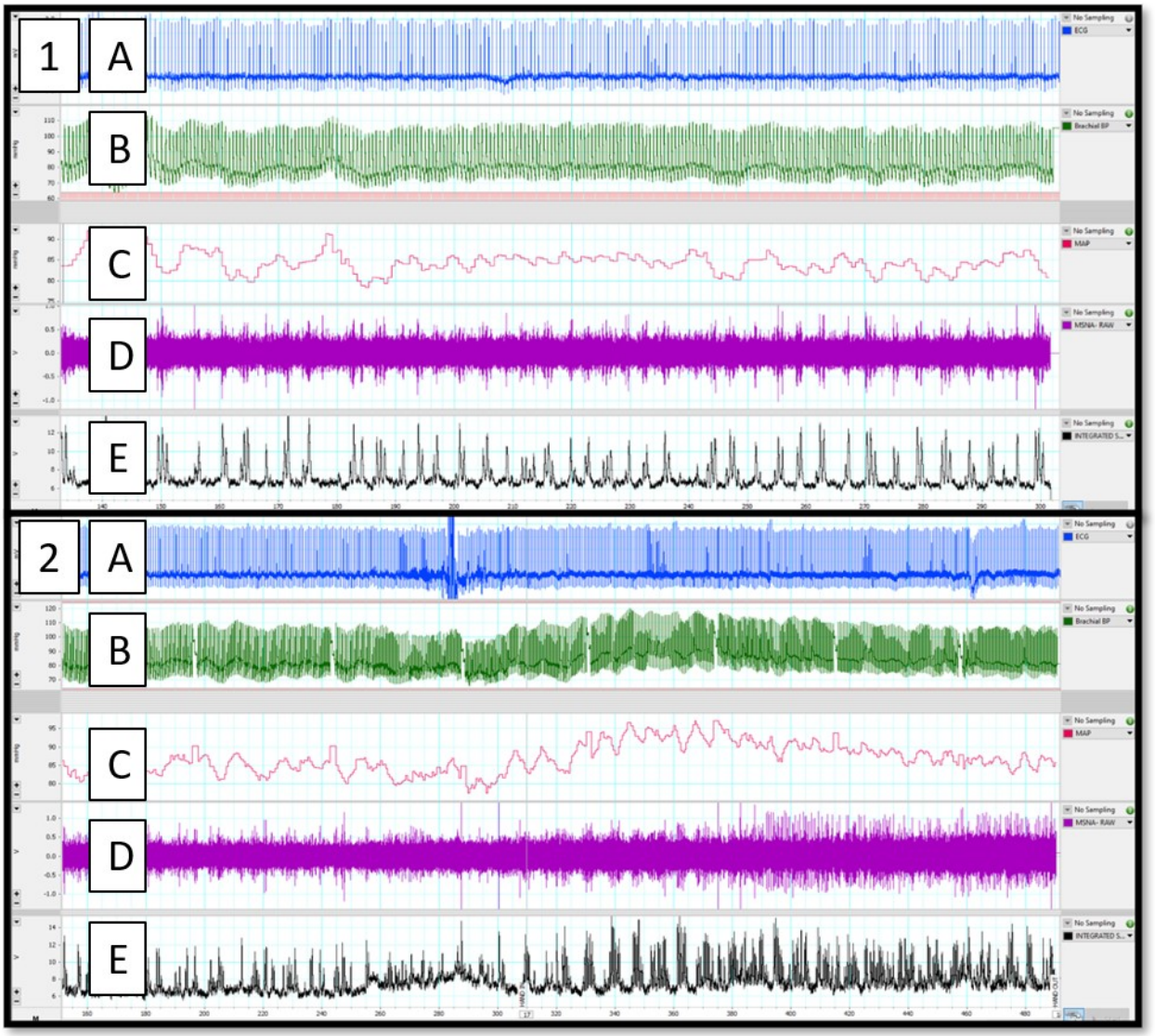


Figure C1. **Representative tracing from one pregnant woman in the second trimester (19wks; exercise group).** Data was sampled during rest (**panel 1**) and during cold pressor test (CPT; **panel 2**) baseline and CPT (bottom; 3 minutes of rest and 3 minutes with hand in ice water). Channels are as follows for both tracings (in order from top to bottom): A) ECG (mV); B) Finometer derived brachial blood pressure (mmHg); C) Mean arterial blood pressure (MAP; mmHg) calculated from the Finometer tracing; D) Raw MSNA signal (V); E) Integrated MSNA signal (bursts unlabeled).

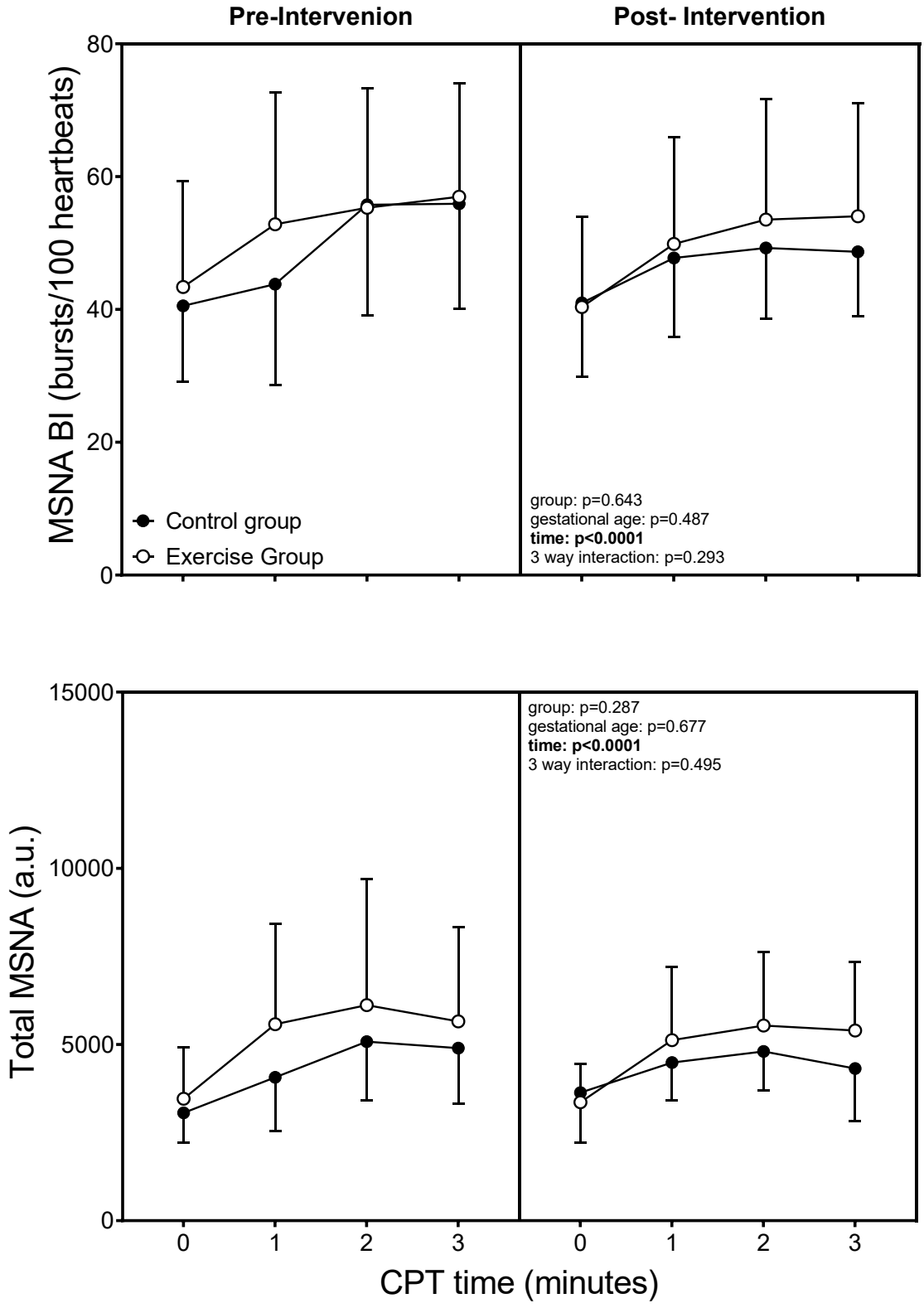

Pre-Intervenion
Post- Intervention
MSNA BI (bursts/100 heartbeats)
80
60
40
20
0
Control group
Exercise Group
group: p=0.643
gestational age: p=0.487
time: p<0.0001
3 way interaction: p=0.293
Total MSNA (a.u.)
15000
10000
5000
0
group: p=0.287
gestational age: p=0.677
time: p<0.0001
3 way interaction: p=0.495
0
1
2
3
0
1
2
3
CPT time (minutes)

Figure C2. Muscle sympathetic nerve activity (MSNA) burst incidence (BI) and total activity response to cold pressor test. Control group depicted by closed circles and exercise group is depicted by the open circles. Data represents mean and standard deviation. **Top)** MSNA BI response during three minutes of CPT pre- (left) and post- (right) intervention. MSNA BI increased during CPT but there was no difference across gestation age, between groups, nor was there an interaction. **Bottom)** Total MSNA response during three minutes of CPT pre- (left) and post- (right) intervention. Total MSNA increased during CPT but there was no difference across gestation age, between groups, nor was there an interaction. All statistics were performed using a three way (group x gestational age x time) mixed effects ANOVA in GraphPad Prism (v9/0). Significance was set at $p<0.05$ and indicated in bold where applicable.

Table C2. *Correlations between angiogenic status (sFlt-1:PlGF) or estrogen and vascular outcomes in the third trimester.*

	Angiogenic Status (sFlt-1:PlGF)				**Estrogen**			
	n	p-value	R squared	R-value	n	p-value	R squared	R-value
Common carotid artery- resting measures								
Carotid diameter (mm)	27	0.0824	0.317	0.100	27	**0.005**	0.462	0.214
Carotid blood flow (ml/min)	27	0.7451	0.061	0.004	27	0.812	0.042	0.002
DC (10^{-3}/kPa)	24	0.6064	0.098	0.010	23	**0.002**	0.520	0.270
CC (mm^{-2}/kPa)	24	0.3447	0.179	0.032	23	0.103	0.289	0.083
Strain (%)	24	0.3001	0.196	0.038	23	0.235	0.212	0.045
Stiffness (β)	24	0.845	0.001	0.037	23	**0.025**	0.151	0.389
Distensibility (1/β)	24	0.686	0.006	0.077	23	**0.002**	0.268	0.517
Elastic modulus (E)	24	0.992	0.000	0.002	23	**0.001**	0.282	0.531
Pulse wave velocity								
Central PWV (m/s)	31	0.635	0.087	0.008	31	0.244	0.199	0.040
Upper limb PWV (m/s)	37	0.856	0.032	0.001	38	0.572	0.093	0.009
Lower limb PWV (m/s)	22	0.764	0.059	0.004	22	0.649	0.084	0.007

Superficial femoral artery- resting measures								
Femoral diameter (mm)	31	0.705	0.070	0.005	32	**0.030**	0.358	0.128
Femoral blood flow (ml/min)	30	0.182	0.242	0.059	30	0.754	0.054	0.003
FVC (ml/min/mmHg)	30	0.111	0.287	0.082	30	0.547	0.104	0.011
FVR (mmHg/ml/min)	30	0.059	0.338	0.114	30	0.993	0.002	0.000
Superficial femoral artery- reactivity measures								
ΔFemoral blood flow (ml/min)	22	0.413	0.158	0.025	20	0.864	0.032	0.001
ΔFVC (ml/min/mmHg)	22	0.276	0.209	0.044	20	0.426	0.148	0.022
ΔFVR (mmHg/ml/min)	22	0.220	0.235	0.055	20	0.762	0.057	0.003

sFlt-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; n= number of women; DC, distensibility coefficient; CC, compliance coefficient; PWV, pulse wave velocity; FVC, femoral vascular conductance; FVR, femoral vascular resistance. Central PWV was determined from the carotid artery to femoral artery, upper PWV was determined from carotid artery to the finger (Finometer), and lower PWV was determined from the femoral artery to the toe (pulse transducer). All correlations were performed in GraphPad Prism (v9.0) using a linear regression model. Blood samples, or vascular data were not obtained in all women at each assessment. Reasons for missing data are not related to the study and are considered missing at random. Number of women with both data sets for each outcome (i.e., having data for both blood marker and vascular outcome) are presented.

Appendix D: Comparisons between Study 1 (metaboreflex study) and Study 2 (PEACH study).

Table D1. *Comparisons between metaboreflex study and PEACH study for participant characteristics in the third trimester.*

	Metaboreflex	**PEACH-control**	**PEACH-exercise**	**p-value**
Number of women	19	23	28	n/a
Age (years)	33 (3)	32 (4)	32 (3)	0.090
Gestational Age (weeks)	32 (3)	35 (1)	34 (1)	**<0.001**
Height (cm)	167 (7)	169 (6)	165 (7)	0.145
Pre-pregnancy weight (kg)	67.2 (14.7)	70.0 (14.9)	73.7 (25.6)	0.551
Pre-pregnancy BMI (kg/m^2)	24.1 (4.8)	24.5 (5.0)	26.7 (7.9)	0.314
Weight at assessment (kg)	77.7 (15.4)	82.2 (14.6)	85.5 (22.2)	0.362
GWG at assessment (kg)	10.4 (3.2)	12.2 (5.0)	11.9 (6.7)	0.541
Excessive GWG at assessment; n (%)	14 (50)	8 (42)	9 (39)	0.779
Nulliparous; n (%)	11 (39)	12 (63)	13 (56)	0.230
MVPA per week (minutes)*	68 (92)	65 (76)	79 (106)	0.875
Meeting guideline (>150min MVPA /wk); n (%)*	4 (24)	3 (16)	4 (15)	0.694

BMI, body mass index; GWG, gestational weight gain; n, number of women; MVPA, moderate-to-vigorous physical activity. Data is mean (SD) unless otherwise stated. MVPA was determined using accelerometry in the week following the assessment in the third trimester. Minutes of MVPA per week were determined using Freedson activity counts which only includes MVPA activity if it occurs in a bout of at least ten-minutes. *Data was missing for accelerometry in 4 women in the PEACH control group, 1 woman in the PEACH exercise group, and 2 women in the metaboreflex group. Comparisons between groups were performed using a one-way ANOVA in GraphPad Prism (v9.0). Comparisons for categorical data were completed using a using a chi square test. Significance was set at $p<0.05$ and indicated by bold text where applicable.

Table D2. *Comparisons between metaboreflex study and PEACH study for resting hemodynamics in the third trimester.*

	Metaboreflex	**PEACH-control**	**PEACH-exercise**	**p-value**
Number of women	19	23*	28	n/a
Heart rate (bpm)	84 (12)	88 (11)	86 (8)	0.496
SBP (mmHg)	100 (9)	111 (12)	110 (9)	**0.001**
DBP (mmHg)	64 (7)	68 (7)	69 (8)	0.075
MAP (mmHg)	78 (7)	85 (7)	86 (8)	**0.002**
Cardiac output (L/min)	7.0 (1.5)	8.2 (1.8)	7.8 (1.6)	0.090
SVR (dynes)	918 (179)	859 (151)	910 (156)	0.428

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; SVR, systemic vascular resistance. *Data for cardiac output and SVR are missing from one woman in the PEACH control group (n=22). Comparisons between groups were performed using a one-way ANOVA in GraphPad Prism (v9.0). Significance was set at $p<0.05$ and indicated in bold where applicable.