Impact of exercise on vascular health during pregnancy.

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1. INTRODUCTION/SIGNIFICANCE

The purpose of this study is to identify the impact of a structured exercise program on sympathetic nervous system activity (SNA) and vascular function in pregnancy. Sympathetic hyperactivity and associated vascular dysfunction are closely tied to hypertension and cardiovascular disease(17) and may be an important mechanistic link between pregnancy complications and the development of future cardiovascular disease in both the mother and child(16). During normal pregnancy, sympathetic activity is elevated yet the transduction of this signal to a blood flow or vascular response is typically blunted (26). In pregnancies where women develop high blood pressure, sympathetic activity is even higher (11; 12; 21) and the transduction of this signal does not appear blunted (20; 29). In non-pregnant populations, exercise is considered an effective, low cost means to improve cardiovascular disease risk (1). Exercise training has been shown to reduce sympathetic activity in other clinical populations, improving cardiovascular function (2). Exercise is recommended to all healthy pregnant women, yet, the impact of exercise during pregnancy on vascular (peripheral and cerebral) and sympathetic function is virtually unknown. Understanding the role of exercise in modulating SNA and vascular function during pregnancy is important for the development of lifestyle recommendations and interventions to promote cardiovascular health in pregnant women. Therefore we goal of this study is to examine the potential benefits of prenatal exercise on neurovascular function.

2 STUDY OBJECTIVES

2.1. Specific Aims

1) *Determine the role of exercise in regulating sympathetic activity during pregnancy.*
2) *Determine the impact of exercise on vascular reactivity and function during pregnancy.*

2.2. Hypotheses

1) *We hypothesize that exercise training will blunt the increase in SNA which occurs with pregnancy.*
2) *We hypothesize that vascular reactivity to sympathetic stressors will be reduced in women who are taking part in a structured exercise program compared to those that have not.*

3 PATIENTS AND METHODS

3.1 Study Design

General Design

BASELINE (16-20 weeks), POST-INTERVENTION (34-36 weeks), AND POSTPARTUM (2 months): At each time-point during pregnancy, participants will report to the laboratory on two occasions. Women will report to the laboratory once in the postpartum.

Visit 1) Participants will complete a peak exercise test on the treadmill or bike to volitional fatigue. Instrumentation will include breathing through a mouthpiece attached to a metabolic cart (measures how and what the participant is breathing), heart rate, blood pressure and transcranial doppler ultrasound (TCD) to measure blood flow in the brain (middle cerebral artery). Resting diameter and flow of the internal carotid artery (ICA) will be assessed at rest. Lung capacity will be assessed at rest using spirometry. Participants will then warm up for 5 minutes at 3.4mph and 0% grade. After the warm up, the incline will increase in 1% increments until volitional fatigue. The test will be followed by a 5 minute cool down. Participants will hold the hand-rails through-out the peak test (5; 7). Exercise on the bike will follow a similar protocol of 5 minutes of rest, 5 minutes of warm-up at 0.5kpa. Exercise intensity will increase by 0.5kpa until volitional fatigue, followed by a 5 minutes of cool down and 5 minutes of rest.

***Visit 2*)** Participants will arrive after refraining from exercise, caffeine, alcohol or food for 12 h (overnight fast) and blood samples will be collected. Following centrifugation, aliquots will be frozen at -80°C until analysis. A finger poke will be used to measure glucose via glucometer and blood gases and metabolites (blood gas analyzer). Following the blood draw, anthropometrics will be recorded and a standardized breakfast will be provided. Participants will then undergo an assessment of reflex neurovascular control. Participants will rest in a dentist chair 30° above supine for the duration of testing to minimize discomfort of the pregnant women.

After instrumentation (described below) and an initial period of 20 min of rest, participants will undergo baseline testing which consists of arterial stiffness (pulse-wave velocity and ultrasound) and carotid distensibility. Next, participants will undergo 3 separate conditions separated by 20 minutes of quiet rest: **1)** 3 min with their hand in an ice bath (cold pressor test); **2)** Flow mediated dilation (~15 minutes) and **3)** an end expiratory breath hold (~15s). Following the cold pressor test a heating pad will be used to warm the hand to baseline temperature.

Postpartum Visit) Participants will arrive after refraining from exercise, caffeine, alcohol or food for 12 h (overnight fast) and blood samples will be collected. Following the blood draw, anthropometrics will be recorded and a standardized breakfast will be provided. Participants will then undergo an assessment of blood vessel health. After instrumentation (described below, but without microneurography), participants will undergo testing which consists of arterial stiffness (pulse-wave velocity and ultrasound) and carotid distensibility. Following this, participants will undergo an assessment of flow mediated dilation (described above).

Primary Outcome Variables: Basal SNA

SNA bursts will be visually identified as exhibiting pulse synchrony, having an amplitude two times the previous inter-burst period, and characteristic rising and falling slopes. SNA will quantified as burst frequency (bursts/min), burst occurrence (bursts/100 heartbeats), and burst amplitude (% of baseline).

Secondary Outcome Variables:

A) *Sympathetic reactivity and vascular transduction.* Basal vascular transduction and changes during the cold pressor test will be compared between groups to identify differences in vascular reactivity associated with exercise training. Femoral artery conductance will be calculated as blood flow divided by mean arterial pressure. Vascular transduction of sympathetic activity will be calculated for each heart beat that has a burst of SNA, as well as for the subsequent 15 beats. The time course of conductance following bursts of SNA (percent change) will be averaged and further stratified by burst amplitudes (9). Cerebrovascular reactivity to exercise (a major sympathetic stressor) will also be compared between groups to assess the impact of exercise training and on brain blood vessels reactivity.

B) *Arterial Stiffness (Pulse-wave velocity).* Basal arterial stiffness and changes during cold pressor test will be compared between groups to identify differences in vascular structure and function associated with normal and hypertensive pregnancies (6; 24).

C) *Sympathetic neurotransmitters.* Biochemical analyses will be conducted to determine plasma concentrations of epinephrine , norepinephrine, and neuropeptide-Y (ELISA) as secondary markers of sympathetic activation (24).

D) *Sex hormones.* Progesterone, estrogen, testosterone and their ratio influence basal SNA and vascular function (3; 15). As such, plasma sex hormones and the ratio between progesterone and estrogen will be determined (ELISA).

E) *Blood volume.* Vasopressin, aldosterone, plasma renin activity, plasma osmolality, Na, Cl, K.

F) *Respiratory measures.* Ventilation (l/min), and both inspired and expired Oxygen and Carbon dioxide will be measured during the initial baseline period. Lung capacity will also be measured prior to the exercise test (day 1).

Tertiary Outcome variables

A) *Insulin and glucose measurement*. Insulin has been shown to increase SNA in healthy and diabetic individuals (14). Vascular (both peripheral and cerebral) function is altered by these hormones. As insulin resistance increases during pregnancy, fasted measures of glucose and insulin (Day1) will be analyzed to

determine their relationship to the primary outcome measures. Insulin sensitivity will be determined using the HOMA equation (18).

B) *Arterial Stiffness and Endothelial-dependant Dilation.* Arterial stiffness and endothelial function will be assessed to determine the influence of exercise on other measures of vascular function.

3.2 Subject Selection and Withdrawal

One hundred pregnant women (>18yrs, with singleton pregnancies) will be recruited between 16-20 weeks gestation through physician and midwife referrals, newspaper advertisements and recruitment posters. Women diagnosed with multiple pregnancies will be excluded. Eligible women will be randomized to take part in an exercise intervention (n=50) or no intervention (n=50). The intervention will consist of aerobic exercise equivalent to 50-70% of heart rate reserve, 3-4 times per week until the end of the study (34-36 weeks). For initial baseline testing (16-20 weeks) and at the end of the intervention (34-36 weeks), participants will visit the laboratory twice. Following initial baseline testing, women will be randomly assigned to an exercise intervention or no intervention. Women will receive an opaque sequentially numbered envelop with their allocation. Allocation will be determined using a randomly generated allocation sequence by an individual not associated with the research study who will place and seal the allocation in the envelope.

3.2.1 Inclusion Criteria

Healthy, premenopausal, women, 18 years or older. Clearance to exercise in writing or using the PARmed-X for Pregnancy.

3.2.2 Exclusion Criteria

Women diagnosed with multiple pregnancies (twins etc). Contraindications to exercise/lack of physician clearance.

3.3 Study Procedures

The principle and co-investigators have expertise in and have previous experience with and have published manuscripts utilizing all of the proposed techniques.

Cold Pressor Test: The cold pressor test involves submersing the hand up to the wrist in an ice-bath (~0-4° C). The cold stimulus is simple to apply, familiar to most individuals and elicits a strong nociceptive (pain) reflex. This causes a large increase in SNA which causes an acute, yet robust increase in blood pressure(27). The participant can self-terminate the test at any point by removing their hand from the ice-bath. A heating pad will be used to re-warm the hand upon termination of the test.

Expiratory Breath-hold: Participants with breath normally (e.g. no preceeding hyperventilation), following a normal breath out they will hold their breath for as long as possible (usually < 30s). This will cause a near maximal activation of the sympathetic nervous system (22; 23).

Flow mediate dilation: Flow mediated dilation is a standard, non-invasive measure of endothelial function. Brachial artery diameters will be assessed at rest for 1-2 minutes (ultrasonography). A standard blood pressure cuff will then be inflated to supra-systolic pressure for 5 min. The return to flow following deflation of the cuff results in an increase stimulus in the artery leading to dilation. This dilation will be measured over 5 min following cuff deflation (25).

Sympathetic Nerve Activity

SNA directed at the vasculature within skeletal muscle will be assessed by microneurography(13). A sterile tungsten microelectrode (35 mm long; 200 μm in diameter; tapered to a 1- to 5-μm uninsulated tip) will be inserted transcutaneously into the fibular nerve posterior to the fibular head with a reference electrode positioned subcutaneously 1–3 cm away. SNA will be obtained by manually manipulating the microelectrode until a characteristic pulse-synchronous burst pattern(8). The raw SNA signal will be amplified, band-pass filtered (700–2,000 Hz), rectified and integrated to obtain a mean voltage neurogram (0.1-s time constant). SNA data will be sampled at 10,000 Hz and stored for off-line analysis (Powerlab Software, ADInstruments).

Cardiovascular Measures

The electrocardiogram (lead II) and blood pressure waveform (photoplethysmography; Finapres Medical Systems) will be recorded continuously to derive heart rate, mean, systolic, and diastolic blood pressures on a beat-by-beat basis. Blood pressure will validated by automatic sphygmomanometer measurements at the brachial artery. Cardiac output will be calculated on a beat-by-beat basis using the Model Flow Method(28).

Continuous common femoral artery blood flow velocity will be collected from the opposite leg as SNA (Doppler ultrasonography; GE Vivid-7, 14 MHz linear probe). B-mode images of the carotid and femoral artery will be recorded for measurement of diastolic and systolic diameters at baseline and the end of the hyperoxia and/or cold pressor tests. Blood flow velocity and diameters will be used to calculate volumetric flow. Uterine artery blood flow, resistance and pulsatility index will be assessed at rest.

Arterial stiffness

Pulse-wave velocity will be measured as a clinical index of arterial stiffness(19). Continuous carotid artery blood pressure waveforms will be collected 1–2 cm proximal to the carotid bifurcation using hand-held tonometery (Millar Instruments). Simultaneously, continuous common femoral artery blood pressure waveforms will be collected from the right leg for assessment of carotid-femoral pulse wave velocity which is the gold standard measure of central arterial stiffness (Millar Instruments)(19). Peripheral stiffness will be measured from carotid-finger pulse-wave velocity (tonometer to finger photoplethysmography).

Respiratory Measures:

Minute ventilation (l/min) will be determined using a heated pneumotachometer and spirometer. A gas line will be run from near the mouth to a gas analyzer to determine inspired and expired Oxygen and Carbon dioxide. This data will be collected by having the participant breath through a mouthpiece (similar to a snorkel) during the baseline period. They will wear a nose clip during this time.

Pulmonary function will be determined by performing three or four respiratory maneuvers. This involves taking a full breath all the way in (maximal inhalation) and breathing out as fast as they can for 6 seconds (maximal exhalation). This will be done in the seated position using the same mouthpiece as described above. This will allow us to determine their lung capacity.

Questionnaires and accelerometry (16-20 weeks; 26-28 weeks; 34-36 weeks, 2 months postpartum):

Participants will be given links to (or paper copies of) the following questionnaires: 1) Health History Questionnaire which asks about the medical history (completed at the first visit only; however, some questions will be updated at each subsequent visit); 2) Pregnancy Physical Activity Questionnaire; 3) The Pittsburgh Sleep Quality Index which gives us an idea about the quality of you sleep over the past month; 4) a questionnaire asking about attitudes to exercise during pregnancy; and 5) Edinburgh Depression Scale which gives us an index of depression levels during pregnancy. In order to complete a 3 day food intake record, participants will receive a special login that is linked to their study ID (your name will not be linked to the account) which can be accessed by their computer or smartphone. A hard copy can be provided and returned to us by mail upon request. Participants will also be sent home with (or mailed) a 7-day Sleep Log where they will be asked to record when they go to sleep and when they wake up over the course of a week and asked to return both forms. At each time point participants will also be given an accelerometer to measure their level of activity (24 hours per day) for 7 consecutive days. In the postpartum, women will also fill in three questionnaires related to baby: 1) Infant behavior questionnaire, 2) brief infant sleep questionnaire, and 3) infant ages & stages – 2 months.

3.4 Statistical Plan

Sample Size Determination

Our primary hypothesis is that exercise training will reduce resting SNA (burst/min) in pregnant women. Data from other clinical populations with high resting SNA indicates a ~23% decrease in resting SNA with exercise training (4). Previous data collected in our lab demonstrate a resting burst frequency of 40±11 burst/min. Based on previous findings we expect exercise training to reduce resting SNA to ~31±11 bursts/min (-23%). Using these data we estimate 22 pregnant women are required to observe a significant reduction in SNA following exercise training (80% power, $\alpha = 0.05$; G*Power v3.16 (10)). We expect an 80% success rate for obtaining an appropriate SNA signal and an 80% retention rate, requiring 43 women to be recruited into the

training arm of the study. To allow for additional variability and or decreased retention we will recruit 50 pregnant women to take part in exercise training and 50 pregnant women to take part as controls.

Statistical Methods

To assess our primary outcome (the difference in basal SNA between groups) a 2 way mixed model (group x time) ANOVA will be used. To assess the influence of exercise on our secondary outcomes (i.e. vascular function and blood markers) a similar 2 way mixed model design will be used. Tukey's post hoc analysis will be used to compare means when main effects are found to be significant.

The strength of the association between changes in SNA, vascular measures and blood markers within and across groups will be determined using the Pearson product-moment correlation coefficient. Statistical analyses will be performed using SPSS Version 16.0 (SPSS, Chicago, IL). Statistical significance will be assumed at $P < 0.05$.

4 Data Handling and Record Keeping

4.1 Confidentiality

After consenting to participate in the study, the participant will be given an identifying code which will be used for study identification from that point forward. Both name and date of birth will be retained on consent forms. Phone number, mailing address and email will be kept on subject information sheets. In addition, a master list linking participant identifiers with de-identified data will be retained in a locked cabinet within a locked room. Confidentiality agreements between the principal investigator and any other study personnel will be signed. All personnel will be made aware of the procedures for maintaining confidentiality and will be expected to not discuss the participants or results with any person not directly involved in the study.

4.2 Records Retention

No specific uses are planned for data beyond 5 years. All information related to personal identifiers will be destroyed at this time. However, non-identifiable data (including date of birth) will be kept indefinitely in the case that it may be informative in the future.

4.3 Regulatory Binder

A regulatory binder documenting all aspects (e.g. informed consent, questionnaires, adverse event forms, etc) will be maintained by the study investigators. This binder will be kept in a locked cabinet in a locked office to maintain confidentiality (see above).

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