Placental Mitochondria Response to Different Modes of Exercise During Pregnancy

By

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ABSTRACT

Previous research shows exercise provides health benefits, and this is true in the context of pregnancy. However, little research has been done to examine placenta adaptations to exercise that mediate maternal and fetal health benefits. **Purpose:** The aim of this study was to determine if exercise during pregnancy increases oxidative phosphorylation protein expression in placental mitochondria. Furthermore, does the type of exercise performed during pregnancy influence placental mitochondria changes. **Methods:** Healthy women with a low-risk singleton pregnancy participated in an exercise intervention from 13 – 16 weeks gestation through delivery. Women were randomized to one of four groups: Aerobic only, Resistance only, Combination of Aerobic + Resistance, Stretching/Breathing Controls. Each participant completed 50 minutes, 3 times each week of either moderate-intensity (Aerobic, Resistance, Combination) or light-intensity stretching/breathing. Placenta tissue was collected after delivery. Villous tissue samples were obtained within 24 hours of delivery. Mitochondria content was determined by western blotting. Placentae from women diagnosed with GDM were excluded. **Results:** Placenta tissue was collected from 42 healthy females (13=Aerobic, 9= Resistance, 10=Combination, 10=Control). Aerobic training significantly increased complex III

protein expression compared to the resistance group (p=0.02), and a near significant increase compared to the control group (p=0.056). Additionally, the aerobic group showed a significant increase in complex IV expression compared to the combination group (p = 0.036). **Conclusions:** Results suggest the mode of exercise performed during pregnancy could impact the placenta differently. Placenta protein expression of electron transport chain complexes III and IV in women who participated in aerobic training during pregnancy were significantly higher than those who did resistance training and combination training, respectively. Complex IV protein expression was significantly lower in the combination training group compared to aerobic trained individuals (p=0.04). Future research should investigate placental mitochondria function to understand how different types of exercise during pregnancy may impact the placenta and thus facilitate maternal-fetal health benefits.

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by

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LIST OF ABBREVIATIONS

Normal Weight (NW) = a pre-pregnancy BMI of 18.5 – 24.9 in the adult population

Overweight (OW) = a pre-pregnancy BMI of 25 – 29.9 in the adult population

Obese (OB) = a pre-pregnancy BMI of 30 – 34.9; class 1 obesity per ACSM classification

Exercise Testing = a single date/visit during which treadmill and 1 repetition maximum testing occurs in order to set a target heart rate zone and external load for exercise training.

Exercise Training = exercise program incorporating FITT principle (frequency, intensity, type, and time of activity) over an extended time period (i.e., 25 weeks)

Aerobic Training (AT) = cardiovascular exercises completed on aerobic exercise machines with a pre-determined resistance and speed settings for achieving and maintaining an individual's target heart rate zone

Resistance Training (RT) = strength exercise completed on Cybex machines, with resistance bands, or dumbbell weights

Combination Training (CT) = a combination of aerobic and resistance training programs into one inclusive exercise regimen

Oxidative phosphorylation (OXPHOS) = process by which mitochondria consume oxygen and generate ATP utilizing the electron transport chain

Electron transport chain (ETC) = a series of five protein complexes (CI- CV) that transfer protons across the mitochondrial membrane as a part of OXPHOS to generate ATP

Gestational Age (GA) = defines how far along a woman is in her pregnancy

Maternal Obesity (MO) = a pregnant woman with obesity

Gestational Diabetes Mellitus (GDM) = a condition characterized by an elevated level of blood glucose during pregnancy

Complex I (CI) = first enzyme in the electron transport chain. CI is also called NADH-ubiquinone oxidoreductase and is the largest multisubunit enzyme complex in the ETC

Complex II (CII) = second enzyme in the electron transport chain; CII, succinate dehydrogenase is a component of the Krebs cycle as well as the ETC; thus, it serves as a link between metabolism and OXPHOS

Complex III (CIII) = third enzyme in the electron transport chain; CIII is commonly referred to as a cytochrome bcl complex, or CoQ-cytochrome c reductase. CIII transfers electrons to cytochrome c

Complex IV (CIV) = fourth enzyme in the electron transport chain; CIV, cytochrome c oxidase, transfers electrons from cytochrome c to the terminal electron acceptor O_2 to generate H_2O

Complex V (CV) = fifth enzyme in the electron transport chain, also referred to as ATP synthase. CV is located on the inner mitochondrial membrane

Chapter 1 – Introduction

The incidence of obesity, type 1 diabetes mellitus (T1DM) and type 2 DM (T2DM) are increasing in the United States[12]. In the United States, obesity (OB) impacts almost 40% of women of reproductive age (20 – 39 years)[2]. Maternal obesity (MO) increases risk of pregnancy complications and places a greater risk on the health of the fetus[9]. The placenta is largely responsible for maternal and fetal health. Pregnancy pathologies complicated by pre-existing MO and DM are known to have altered placenta mitochondria [18,20]. We can surmise that a successful and healthy pregnancy is largely dependent on the ability of placental mitochondria to respond to the physiological changes that occur during pregnancy.

Health benefits of exercise have been well established. Lower incidence of post-partum weight retention, excessive gestational weight gain, PE and GDM have been observed with exercise[1,8,22,28]. The placenta undergoes drastic structural and physiological changes during pregnancy, requiring a large amount of ATP. Placental mitochondria are responsible for producing enough ATP via oxidative phosphorylation (OXPHOS) to meet the demands of a developing fetus. The ability of mitochondria to adapt and respond to stimuli makes them attractive targets for interventions to improve pregnancy outcomes. Little research has been done to investigate the influence exercise training has on placental mitochondria OXPHOS capacity.

Purpose

The purpose of this study was to determine the effects of different modes of exercise on placental mitochondria. More specifically, if OXPHOS complex protein expression is altered with different modes of exercise training. We hypothesized any type of exercise during pregnancy

increases placental mitochondria OXPHOS protein expression relative to controls. Based on previous research, we further hypothesized aerobic exercise would have the greatest increase in OXPHOS proteins compared to the resistance and combination exercise groups.

Chapter 2 – Review of Literature

Pregnancy Pathologies

Placental mitochondria dysfunction is the basis for many pregnancy pathologies. Intrauterine growth restriction (IUGR), preeclampsia (PE), and gestational diabetes (GDM) have been linked to increased and decreased mitochondria content[13]. Decreased expression of Mfn2, a protein involved in mitochondria bioenergetics and maintenance, has also been observed in PE placentae[29]. These changes in mitochondrial content and Mfn2 suggest the placenta's diminished capacity to produce ATP, which is primarily done via OXPHOS[9]. Furthermore, diminished ATP production in placental mitochondria may have negative consequences on placenta function and fetal development.

Placenta Programming

Research has found both MO and GDM influence placenta efficiency and fetal outcomes. In animal models, obesity induced with a HFD has been associated with increased fetal weight and risk of stillbirth [27,17]. Increased fetal weight has also been found in human research. One study found infants from obese women with GDM to have increased birthweight, risk of macrosomia, risk of NICU admission, and lower APGAR scores compared to normal weight glucose tolerant women[2]. This research supports the claim that maternal stimuli may program negative fetal outcomes. One must consider the role played by the placenta in propagating the maternal stimuli that induces changes in the fetus.

Role of the Placenta

The placenta joins maternal and fetal physiological systems transporting nutrients, exchanging gas and waste, and regulating hormones[5]. This tissue's ability to meet the dynamic

energy demands of pregnancy is driven by a large amount of mitochondria. Placental mitochondria drive protein synthesis, lipid synthesis, steroid synthesis, the urea cycle, and ATP production[14]. Mitochondrial dysfunction is characterized by an inability to adapt to physiological changes throughout gestation and is the basis for many pregnancy complications[13]. Therefore, it's crucial mitochondria are able to respond to a variety of stimuli in order to fill these different roles.

It's clear that stimuli interact with the placenta to influence the intrauterine environment, which can in turn impact maternal and fetal health. Exercise during pregnancy has been shown to have positive effects on maternal and fetal outcomes[8,21,22]. Therefore, exercise may stimulate placental changes enhancing maternal health and fetal development.

Guidelines for Exercise during Pregnancy

The American College of Sports Medicine (ACSM) and American College of Obstetricians and Gynecologists (ACOG) highlight exercise during pregnancy decreases the incidence of cesarean section, post-partum weight retention, excessive gestational weight gain, PE, and GDM[1,22,24]. For exercise prescription, ACSM and ACOG recommend 150 min/wk of moderate intensity aerobic activity spread throughout the week (30 or more min/day). ACSM also recommends 2-3 or more days of RT depending on experience level, and more than 2-3 days of flexibility exercises. Given the absence of contraindications to exercise, a woman may continue exercise training throughout the entire pregnancy.

As previously mentioned, exercise during pregnancy can decrease risk of developing GDM and PE[1,22] and the basis for many pregnancy pathologies is placenta inefficiency[13]. This suggests the placenta is adapting to exercise to become more efficient. However, it is important

to consider the adaptations that normally occur across gestation.

Placental Mitochondria Plasticity

Drastic physiological changes occur throughout pregnancy requiring mitochondria to adapt and meet energy demands to sustain a growing fetus. Meaning, a higher production of ATP. The electron transport chain (ETC) is a series of 5 enzyme complexes required for OXPHOS and ATP production, therefore, the expression and activity of these complexes are predictors of tissue efficiency. Limited research has been done with placenta tissue OXPHOS activity prior to delivery. In the first trimester, mitochondria content increases and OXPHOS through complex I (CI), complex II (CII), and complex IV (CIV) decreases from 7-10 weeks' and 11 weeks' gestation[14]. An increase in content combined with a decrease in respiration suggests the tissue shifted to become less efficient within the first trimester. This was also found to be true when comparing first trimester and third trimester placenta tissue. The same study found a substantial increase in mitochondria content in third trimester tissue coupled with a decrease in respiration through C I, C II, and CIV compared to first trimester tissue[14].

These findings illustrate that mitochondria respond to a variety of stimuli during different timepoints in gestation. Mitochondrial plasticity found in normal first and third trimester tissue implies plasticity may play a role in the occurrence of pregnancy pathologies.

Placental Mitochondria Related to Gestational Disorders

Many pregnancy pathologies limit placental mitochondria's ability to adapt and respond to stimuli. Pre-existing conditions including MO have also been shown to alter placental mitochondria content[13] suggesting damage to tissue efficiency. Researchers have found placentae from obese women have decreased activity of CI and combined CII/ CIII compared to

normal weight women[12]. Another study found obese women had increased placental mtDNA compared to normal weight controls, suggesting a compensatory mechanism[18].

Placental mitochondrial abnormalities are also present in GDM. Pregnancy complicated with hyperglycemia improves placental mitochondria biogenesis to protect cells from increased oxidative damage[15]. Additionally, an ETC complex analysis found women with GDM treated with medication had decreased expression of CI – CIV[20]. The presented research shows mitochondrial alterations in placenta tissue are different from healthy weight glucose tolerant women. The ability of mitochondria to adapt and respond to stimuli makes them attractive targets for interventions to improve pregnancy outcomes. Exercise as a means of attenuating these pregnancy pathologies has yet to be explored.

Skeletal Muscle Response to Exercise Training

It is well known that skeletal muscle mitochondria positively respond to exercise training. Different modes of exercise have been studied in an effort to establish what type of exercise training produces the most dramatic results in improving mitochondrial function. Porter et al. 2015 examined the impact of chronic RT on skeletal muscle mitochondrial respiratory chain capacity and function in 11 healthy young men through means of a 12-week resistance training exercise intervention. Muscle biopsies taken pre and post intervention were analyzed for respiratory capacity. It was found that only complex I of the ETC had increased protein expression by 11%[23]. Endurance training has been thought to have a greater impact on mitochondrial adaptations response to exercise training compared to RT. A study examined the effect of endurance training on a single leg. Muscle biopsies were taken before and after a 6-week endurance-based knee extensor exercise protocol. Further results from this study showed

a significant increase in CI – CIV activity after the endurance intervention[10]. When comparing the two, the data supports the claim that endurance training has a greater impact on mitochondrial OXPHOS capacity compared to RT. However, the driving forces behind health benefits and signals eliciting mitochondria changes are extensive and still being explored.

Summary

There is ample evidence suggesting exercise during pregnancy decreases risk of poor maternal and fetal outcomes[1,22,27,29]. Limited tissue research has been done to explore the basis for these adaptations to exercise in the pregnant condition. It's been established that resistance training increases CI expression in skeletal muscle[23]. Endurance training has also been extensively studied, and skeletal muscle mitochondria adaptations occur in CI, CII, CIII, and CIV in response to exercise training[10]. Upregulation of these proteins suggests increased OXPHOS capacity and enhanced tissue function. It is important to consider if these adaptations in skeletal muscle are mirrored in the placenta to attenuate the positive maternal and fetal health benefits associated with exercise during pregnancy. A combination of resistance and endurance training has yet to be studied in the context of pregnancy. Additionally, placental mitochondria response to exercise during pregnancy is still being explored.

Chapter 3 – Methods

Participants

The participants in this study were women between 18 and 40 years of age with a low-risk singleton pregnancy, between 13-16 weeks gestation. These women were cleared to exercise by a physician, and did not meet any contraindications to exercise in pregnancy (Table 1), according to ASCM or ACOG guidelines[1,22]. Participants using alcohol, tobacco, recreational drugs, or medications for mental health were excluded from the study. Other exclusion criteria were participants with pre-existing T1DM or T2DM, hypertension, cardiovascular disease, and diseases that can affect fetal development (HIV, AIDS, Lupus). Participants diagnosed with GDM remained enrolled in the study, but were excluded from this analysis.

Absolute Contraindications to Aerobic Exercise During Pregnancy	Relative Contraindications to Aerobic Exercise During Pregnancy
Hemodynamically significant heart disease	Severe Anemia
Restrictive lung disease	Unevaluated maternal cardiac arrhythmia
Incompetent cervix/cerclage	Chronic bronchitis
Multiple gestation at risk for premature labor	Poorly controlled type I diabetes
Persistent second or third trimester bleeding	Extreme morbid obesity
Placenta previa after 26 weeks' gestation	Extreme Underweight (body mass index <12)
Premature labor during the current pregnancy	History of extremely sedentary lifestyle
Ruptured membraned	Intrauterine growth restriction in current pregnancy
Pregnancy induced hypertension	Poorly controlled hypertension/preeclampsia
	Orthopedic limitations
	Poorly controlled seizure disorder
	Poorly controlled thyroid disease
	Heavy smoker

Table 1. Contraindications to aerobic exercise during pregnancy. Adopted from ACSM's guidelines for exercise testing and prescription, 8th ed. Philadelphia (PA): Wolters Kluwer and Lippincott Williams & Wilkins; 2010. P. 185.

Participants received clearance after enrollment; all exercise testing and training started between 13 and 16 weeks gestation (Figure 1). Informed written consent was obtained from each participant prior to enrollment. All protocols were approved by the East Carolina

University Institutional Review Board.

Overview of Procedures

Women were recruited by flyers and promotion at local gyms, pediatric clinics, family medicine practices, and obstetrics/gynecology clinics. Email announcements were also sent to all East Carolina University faculty and staff. After recruitment, physician clearance, informed consent, initial testing, and questionnaires were completed at or prior to 16 weeks' gestation.

Participants were explained each exercise training group and informed they would be randomized into a group for the duration of the study. After enrollment, participants completed an initial testing appointment during which cardiorespiratory fitness testing using the modified Balke submaximal treadmill test was used to establish individual target heart rate (THR) zones. After the treadmill testing, participants completed a one repetition maximum (1RM) test using Cybex machines and various free weights to establish muscular strength and fitness level. All participants were instructed on proper form for exercises performed during the 1RM test.

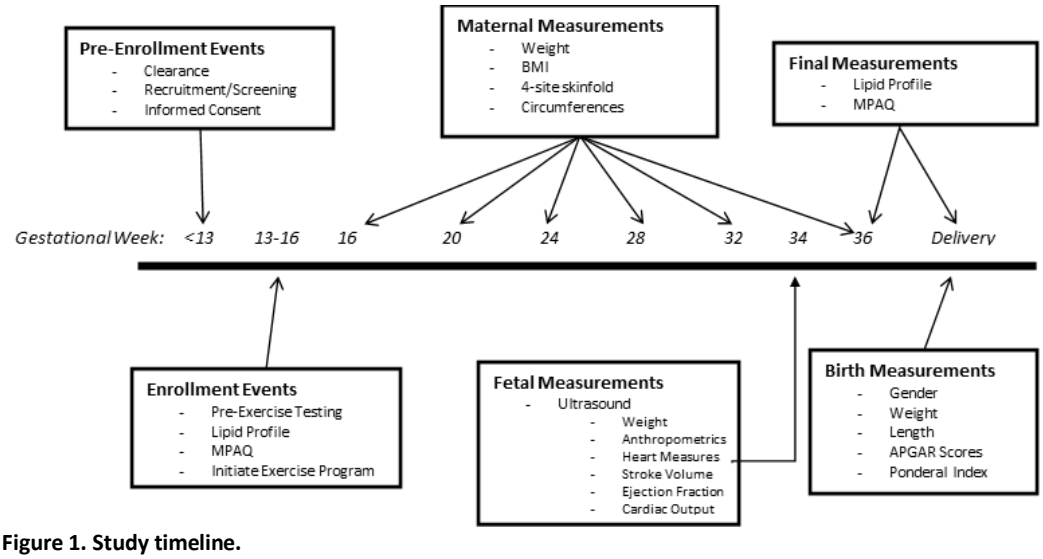

Figure 1. Study timeline.

The intervention started at 13 – 16 weeks' and continued until delivery (Figure 1). Participants were scheduled to attend their supervised exercise program 3 days each week and in total complete 150 minutes of exercise. At home workouts using minimal equipment or body weight exercises were provided to account for scheduling conflicts and social distancing practices associated with COVID-19. Resting heart rate (HR) and blood pressure (BP) were recorded before and after each supervised exercise session. Only HR was recorded pre- and post- exercise if it was done at home. HR was also monitored throughout the entire session and recorded after each exercise bout to ensure participants were staying within their THR zone.

After delivery, placentas were refrigerated until collected and processed. Villous tissue samples were obtained and stored at -80°C until further processing. Participant information including gestational age at time of delivery, infant weight, and infant sex were obtained from the subject's Electronic Health Record (EHR).

Pre-Exercise Testing

Modified Balke Protocol

The modified Balke protocol was used to determine individual THR for exercise prescription and test VO_2 peak comparable to Mottola et al. (2006). ParvoMedics TrueMax 2400 metabolic measurement system was calibrated prior to testing ensuring reliable test data. This included a gas and flow calibrations. Gas calibration was done according to manufacturer instructions on an air tank composed of 16% oxygen and 4% carbon dioxide. Flow rate was calibrated using a 3-L syringe. Prior to starting the test, standing resting BP and HR were taken. HR was measured throughout the entire test via Polar FS2C HR monitor. Additionally, BP, HR, and rating of perceived exertion (RPE) according to Borg's scale (Table 2) were recorded during

the last 30 seconds of each stage with each stage lasting 2 minutes.

At the start of the test, 5 minutes of gas collection at a resting state was followed by a 5-minute warm up at 3.0 mph and 0% grade. Stage one was a continuation of warm-up parameters. As the test progressed, speed was maintained at 3.0 mph while percent grade increased 2% every 2 minutes or with each stage (Table 3). This progression continued until stage 8. The treadmill remained at 12% grade while the speed increased every 2 minutes by 0.2 mph. The test was stopped when participants reached their 85% HR or experienced symptoms outlined by ACSM that require stopping the test[1]. After test completion, a 5-minute cool-down at warm-up parameters was followed by another resting state gas collection for 3-5 minutes.

Borg Scale for Rating of Perceived Exertion (RPE)	
6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Table 2. Borg RPE Scale. Adopted from ACSM's guidelines for exercise testing and prescription. 8th ed. Philadelphia (PA): Wolters Kluwer and Lippincott Williams & Wilkins; 2010.

Modified Balke
Protocol

(VO_2 peak pre-exercise testing)

Stage	Minute	Speed (mph)	Grade (%)	HR (bpm)	BP	RPE
Warm-up	0-5	3.0	0			
1	0-2	3.0	0			
2	2-4	3.0	2			
3	4-6	3.0	4			
4	6-8	3.0	6			
5	8-10	3.0	8			
6	10-12	3.0	10			
7	12-14	3.0	12			
8	14-16	3.2	12			
9	16-18	3.4	12			
10	18-20	3.6	12			

Table 3. Modified Balke protocol for pre-exercise VO_2 peak testing. Adapted from Mottola et al. VO_2 peak prediction and exercise prescription for pregnant women. *Med Sci Sports Exerc*. 2006;38(8):1389-1395.

1 Repetition Maximum Testing (1RM)

During the initial visit, participants performed 1RM testing following the submaximal treadmill test to determine muscular strength and fitness and thus assign appropriate weight loads in their potential exercise prescriptions. Participants were supervised closely to prevent injury and establish good form for the exercises performed. 1RM testing determined the maximum weight the participant could successfully lift while maintaining proper form in 3 or less bouts. Assessing and critiquing form was crucial to accurately determine weight load

maximums for specific muscle groups and eliminate compensatory efforts by other muscle groups to lift more weight. These values were used to establish appropriate starting weights, approximately 60% of maximum load, for the resistance only and combination (resistance plus aerobic) groups.

Modifiable Physical Activity Questionnaire

The Modifiable Physical Activity Questionnaire (MPAQ) was administered at 36 weeks' gestation and one-month after delivery to assess exercise done outside the study protocol. Participants provided information on physical, occupational, and leisure activities. If participants assigned to the control group exceeded 450 METmin/wk of moderate activity, then they were excluded from this study. Women assigned to the resistance group were excluded if they engaged in aerobic activity greater than 450Metmin/wk. Similarly, women assigned to the aerobic group were excluded for engaging in more than 450Metmin/wk of resistance exercise. Women that did not complete the questionnaire at 36 weeks' or 1-month after delivering were also excluded. The MPAQ also collected maternal descriptive measures (maternal age, gravida, parity, weight, and height). Pre-pregnancy BMI was based on self-reported height and weight in the questionnaire. Height and weight was also collected 36 weeks' gestation prior to a supervised training session.

Exercise Protocol

Prior to starting the session, participants were given a Polar FS2C heart rate monitor. All groups began with a 5-minute warm-up using their choice of aerobic equipment including the stationary bike, treadmill, or elliptical. Exercise sessions lasted a minimum of 50 minutes to achieve compliance. Total exercise per week was a minimum of 150 minutes per ACSM and

ACOG guidelines[1,20]. Appropriate intensity was determined using the Borg scale rating of perceived exertion (RPE) in addition to the individual's THR zone throughout the session. The goal perceived exertion was maintaining moderate intensity corresponding to an RPE of 12-14 on the Borg scale.

The aerobic training (AT) group performed aerobic exercise on either a treadmill, elliptical, or recumbent bike for a total of 50 minutes. The trainer leading the session recorded the speed and resistance level shown on the exercise equipment every 10 minutes. Both of which were manipulated by the participant at her discretion to maintain RPE of 12-14 and stay within the THR zone. HR and RPE were recorded every 10 minutes.

The resistance training (RT) group performed resistance exercises also done by the CT group using Cybex machines, free weights, resistance bands, and body weight. Participants did 2-3 sets of 8-15 repetitions of each exercise. Exercise prescriptions for the RT group also had either a full body, lower body, or upper body focus. Each training session had an abdominal core component. Weights were adjusted throughout the intervention to account for an increase in fitness and maintain perceived exertion of moderate intensity.

The combination training (CT) group alternated between AT and RT, completing 5 bouts of each. Aerobic bouts lasted 4.5 minutes on any piece of aerobic equipment mentioned previously. RT bouts consisted of 3 -5 exercises done using Cybex machines, free weights, resistance bands, or body weight. Exercise prescriptions had a full body, lower body, or upper body focus determined by the trainer of the session or per request of the participant. Each session had an abdominal core component as well. Participants completed one set of each exercise in a single RT bout. Each set was 8-15 repetitions. Number of repetitions was

determined based on weight adjustments, discomfort, or breakdown of form. Exercises done using Cybex machines included: leg curl, leg extension, leg press, calf raises, latissimus dorsi pull down, seated row, chest press, shoulder press, and tricep extension. Dumbbells were used to target muscle groups not targeted on Cybex machines in addition to providing participants an alternative way to exercise muscle groups if they experienced discomfort using machines. Weights were adjusted accordingly throughout the intervention to account for increase in fitness and to maintain perceived exertion of moderate intensity. Abdominal core exercises varied based on gestational stage in pregnancy, comfort, and level of fitness.

The control group performed a series of active stretching, breathing, and flexibility exercises. Static stretching was combined with dynamic poses and controlled breathing throughout the session. The series targeted major muscle groups including hamstrings, quadriceps, chest, shoulders, and back. Exercises were also beneficial in alleviating discomfort typically experienced in the later stages of pregnancy. Heart rate remained below each participant's THR zone.

Exercise Adherence

Exercise session attendance was tracked via an electronic record in REDCap[8] and calculated by dividing the number of sessions attended by the total number of possible sessions within the participants' gestational period. Participants were considered "exercise adherent" if their attendance was ≥ 80% of possible exercise sessions. Participants were excluded if they did not meet 80% exercise compliance of 150 min/wk of exercise and attendance three times per week.

Electronic Health Record (EHR)

Gestational Age (weeks) as time of delivery, mode of delivery (vaginal, cesarean), infant weight (kilograms), and infant sex were retrieved from the participant's EHR post-delivery.

Villous Tissue Collection

Placentas were collected and processed within 24 hours of delivery. The umbilical cord was used as a reference point to collect 5 samples of villous tissue located in central and peripheral regions of the placenta. The tissue was blotted on gauze to remove blood clots. Blunt dissection with forceps and scissors allowed the removal and avoidance of collagen and calcium deposits. All samples were immediately placed in liquid nitrogen and stored at -80°C until further processing.

Villous Tissue Processing

Human Placental Tissue Homogenization

All supplies were pre-cooled in liquid nitrogen to prevent tissue thawing. Tissue lysis buffer was prepared using 50mM HEPES, 100mM sodium fluoride, 50mM sodium orthovanadate, 10mM EDTA, 1/200-Protease inhibitor (Sigma, St. Louis, MO), 1/100-Phosphatase (1 and 2) Inhibitor cocktails (Sigma, St. Louis, MO), and 50 μM PUGNAc. Central and peripheral samples of villous tissue were removed from -80°C, placed in liquid nitrogen, and broken into ~25 mg pieces using a mortar and pestle. These pieces were weighed in a precooled homogenization tube to prevent thawing and then immediately placed in liquid nitrogen. Then 500 μL of tissue lysis buffer was added to the sample placed and placed in ice-water after lysis buffer addition. The sample was homogenized using a homogenization probe for 3 rounds of 10 second intervals with 20 seconds between each subsequent round. Following

homogenization, the sample was placed ice prior to the addition of 25 μL of 20% Triton-X and then mixed with a transfer pipette for a final concentration of 1%. Lastly, the sample was pipetted into a microcentrifuge tube.

Protein Extraction

To isolate proteins, 500 μL of tissue homogenate sonicated for 5 seconds on ice. A final concentration of 0.1% SDS was obtained by adding 5.0 u μL of 10% SDS solution, and samples were sonicated for 5 seconds at 20% output control, 2-duty cycle. Samples were then rotated end-over-end in the refrigerator for 120 minutes before being spun 12,000xG. The supernatant was removed, added to a new tube, and prepared for Bicinchoninic acid (BCA) assay by 1:5 dilution of sample with lysis buffer. The aliquot was stored in the refrigerator for short term storage, and -80 °C for long term storage.

Electron Transport Chain Proteins

BCA determined protein concentration per sample, and a spreadsheet provided appropriate water addition to samples to achieve uniform protein concentration. A 9:1 dilution of Laemmli buffer to BME was added to the sample 4:1 dilution. Samples were boiled for 5 minutes using a heat block at 95°. Aliquots had approximately 100μg of protein per 200 μL of volume.

To prepare the gels, they were rinsed with Milli Q water, the comb removed, and the wells rinsed 3-times with run buffer. Gels were put in electrophoresis containers and suspended in run buffer. The ladder, once thawed, was loaded in the first and last lanes. Samples, of 20-30 μg of protein per well, were loaded and optimized as needed. After loading, the power supplier to the electrophoresis container was turned to 100V for 22 minutes or until

proteins migrated halfway down the gel. Voltage was then turned to 200V until proteins migrated to the bottom of the gel. Voltage was removed and turbo transfer was done to transfer the gel to a nitrocellulose membrane. The membrane was put in blocking buffer and rocked for 1 hour. Blocking buffer was replaced with OXPHOS cocktail and rocked in the refrigerator overnight. The membrane was removed and washed 3 times with TBST before adding the secondary antibody and rocked for 1 hour. The membrane was washed again 3 times with TBST, and the last wash was with TBS. After washing, the membrane was suspended with new TBS and imaged using an Oddessy Imager. Images were quantified with BioRad Software, and data was normalized to the loading control.

Statistical Analysis

Participants were excluded if they did not meet 80% exercise compliance of 150 min/wk of exercise and attendance three times per week. Women in the control group participating in more than 450 METmins/wk of moderate activity were also excluded. Women that developed GDM were excluded. Women were stratified by pre-pregnancy BMI and gravida. Healthy weight women had a BMI between 18.5 and 24.9 (kg/m^2). A BMI of 25 (kg/m^2) or greater was classified as OW/OB. Gravida was stratified into G1 and G2+ women. Women pregnant for the first time were classified as G1, while women having two or more pregnancies were classified as G2+. A two-way analysis of variance (ANOVA) was used to compare means of exercisers to control and means of the exercise groups for each ETC complex. Tukey's HSD post-hoc analysis determined which means were significantly different from each other. Statistical analysis was performed on normalized data using SPSS (version 28.0.0, IBM Statistics). Alpha level was set *a prori* at $p < 0.05$ for all statistical tests.

Chapter 4 – Results

We had 45 placentas collected; however, placentas from women diagnosed with GDM were excluded (n=3). Thus, the final placenta tissue samples came from 42 healthy female volunteers with a low-risk singleton pregnancy and physician clearance to exercise. The average participant was 30 years old, pregnant the second time, with healthy weight BMI prior to pregnancy.

Maternal Measurements

Participants were comparable considering age, parity, pre-pregnancy weight, BMI at 36 weeks, weight at 36 weeks, mode of delivery, and GA at time of delivery (Table 4). There was a significant difference in gravida between groups (p=0.03) and a trending difference in pre-pregnancy BMI (p=0.06).

Maternal Characteristics					
	RT (n=9)	AT (n=13)	CT (n=10)	Control (n=10)	P-value
Maternal age (years)	33.2 ± 4.5	30.2 ± 5.6	29.7 ± 2.9	29.3 ± 4.3	0.25
Gravida†	2.0 (1,3)	2.0 (1,5)	1.0 (1,2)	2.0 (1,4)	0.03*
Parity†	0.0 (0,3)	0.0 (0,3)	0.0 (0,1)	0.5 (0,3)	0.40
Pre-Pregnancy BMI (kg/m^2)†	22.2 (19.8,31.6)	23.8 (18.5,42.4)	27.1 (21.0,29.3)	27.9 (22.0,35.7)	0.06
Pre-Pregnancy weight (lbs)†	130 (120,190)	150 (110,271)	162 (115,190)	161.5 (120,235)	0.22
BMI at 36 GA (kg/m^2)†	27.0 (24.2,35.9)	28.8 (24.2,47.2)	28.7 (24.4,34.6)	30.1 (26.1,41.7)	0.43
Weight at 36 GA (lbs)†	162 (144,216)	180 (141,304)	183 (140,235)	189 (141,279)	0.76
Delivery type:					
Vaginal†	88%	92%	60%	70%	0.22
Gestation week	39.8 ± 1.4	39.8 ± 1.2	39.8 ± 0.4	39.6 ± 1.0	0.98

Table 4. Maternal characteristics. Values are reported as mean ± SD. Kruksal-Wallis test for non-parametric data are represented by † and reported median (minimum, maximum). *RT*, resistance training group. *AT*, aerobic training group. *CT*, combination training group. *GA*, gestational age.

Infant Measurements

Although control neonates tended to be heavier (p=0.09), all groups were similar for birth weight (Table 5). Similarly, although there was a larger percentage of female infants in the combination group, groups were similar for percent males and females (Table 5).

Infant Characteristics					
	RT (n=9)	AT (n=13)	CT (n=10)	Control (n=10)	P-value
Birth weight (kg)	3.531 ± 0.360	3.580 ± .318	3.430 ± 0.411	3.862 ± 0.454	0.09
Infant sex:					
Percent male†	67%	85%	40%	80%	0.23
Percent female†	33%	15%	60%	20%	0.23

Table 5. Infant Characteristics. Values are expressed as mean (± SD). Kruksal-Wallis test for non-parametric data are represented by †.

Placenta OXPHOS Expression

There were no significant differences in the expression of OXPHOS proteins between non- exercisers and exercisers (Figure 2).

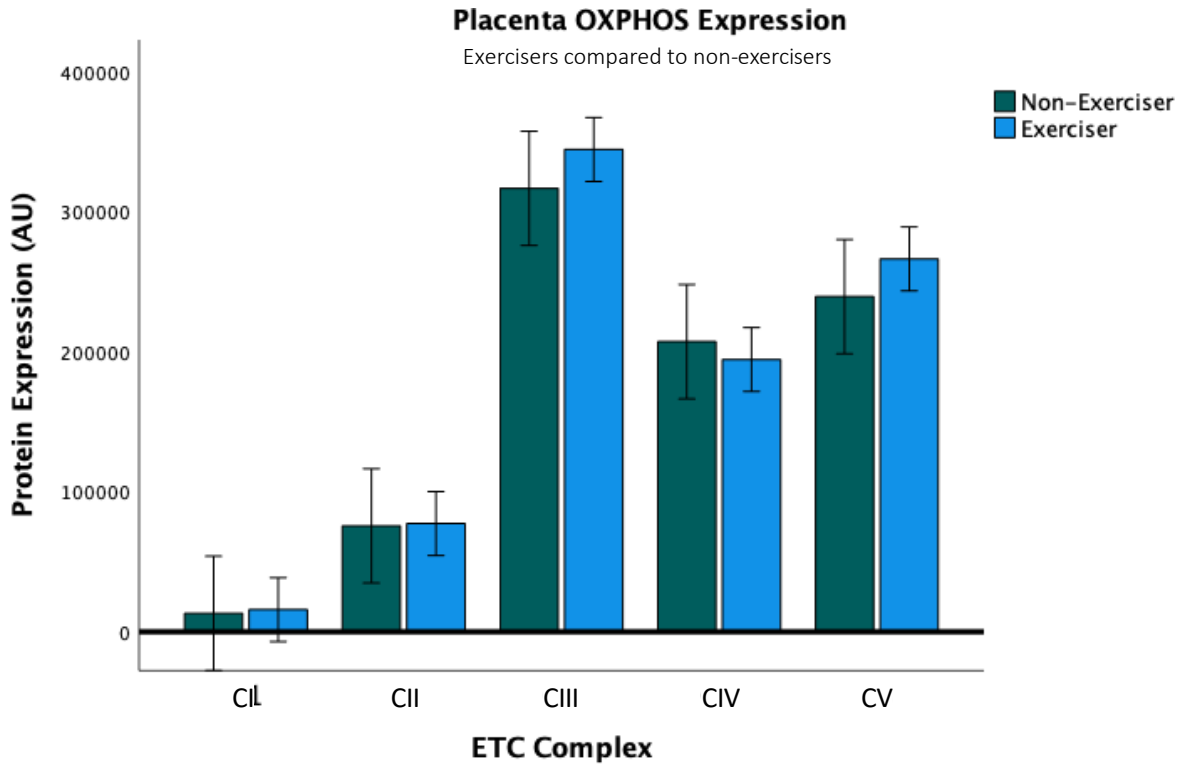

Figure 2. Placenta OXPHOS complexes of exercisers compared to non-exercisers. *OXPHOS*, oxidative phosphorylation. *ETC*, electron transport chain. *CI*, complex I. *CII*, complex II. *CIII*, complex III. *CIV*, complex IV. *CV*, complex V.

When comparing the three exercise groups to control however, AT placentae showed near significant increased CIII expression compared to control (p= 0.06) and significantly higher

compared to RT (p= 0.02) groups (Figure 3). AT also had increased CIV expression compared to CT (p= 0.04). Lastly, CV expression had trends of being greater in AT than control (p=0.09).

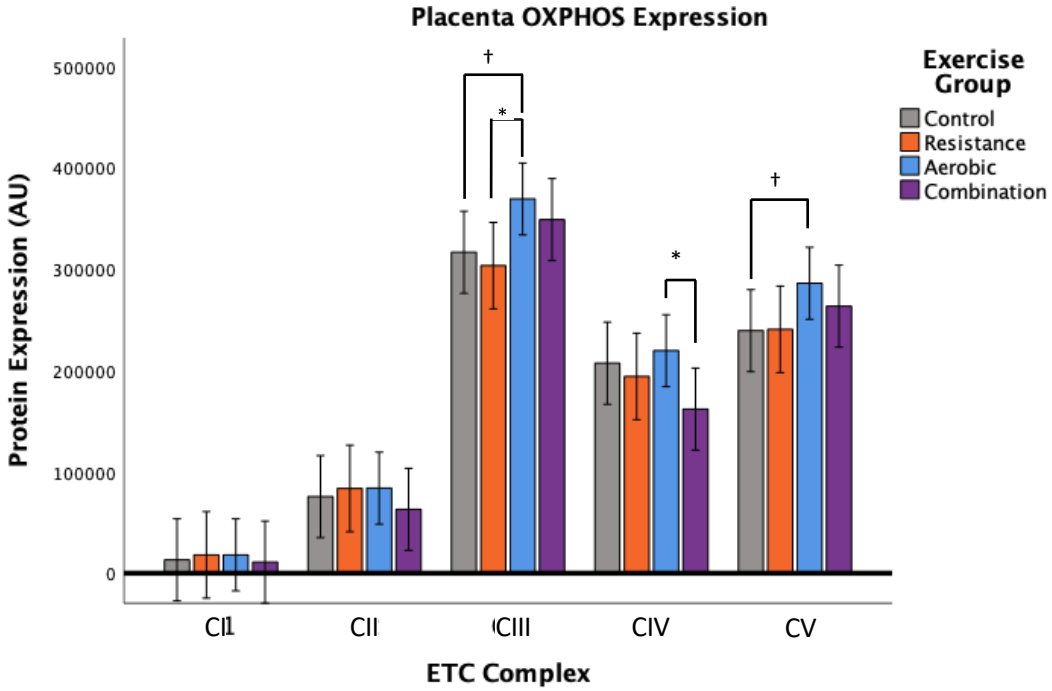

Figure 3. Placenta OXPHOS complexes of all pregnant women for each exercise group. *OXPHOS*, oxidative phosphorylation. *ETC*, electron transport chain. *CI*, complex I. *CII*, complex II. *CIII*, complex III. *CIV*, complex IV. *CV*, complex V. * p<0.05, † p<0.1.

OXPHOS expression stratified by pre-pregnancy BMI

Stratification by BMI showed healthy weight and OW/OB placenta responded differently to exercise. For all women with a healthy pre-pregnancy BMI, placentae from AT group had greater expression of CIII compared to RT (p=0.04) and CT (p=0.01) groups (Figure 4). Additionally, AT showed a trending increase in CV expression compared to RT (p=0.08). CI and CII expression were similar for all exercise groups (Figure 4).

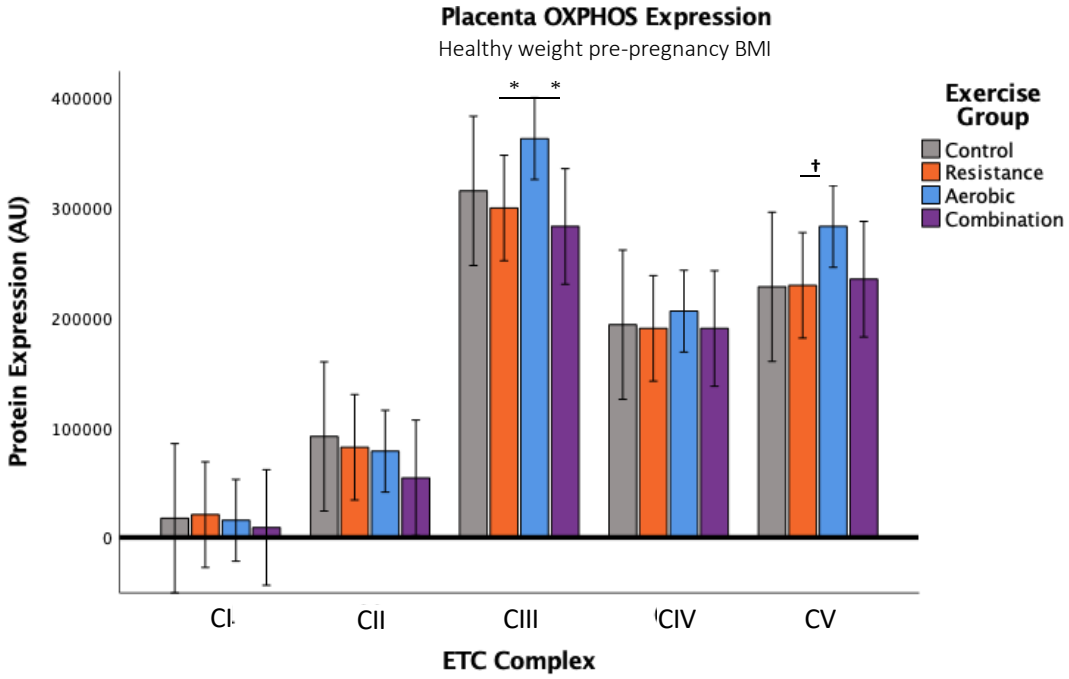

Figure 4. Placenta OXPHOS complex expression for pregnant women with healthy weight pre-pregnancy BMI. *OXPHOS*, oxidative phosphorylation. *ETC*, electron transport chain. *CI*, complex I. *CII*, complex II. *CIII*, complex III. *CIV*, complex IV. *CV*, complex V. * $p<0.05$, † $p<0.1$.

In OW/OB placentae, CIII was greatest in the CT group compared to control (p=0.03) and RT (p=0.06). However, for CIV, AT had greater expression compared to CT (p=0.02). CT placentae also showed a trend toward less CIV expression compared to control (p=0.07). CI and CII expression were similar between groups (Figure 5).

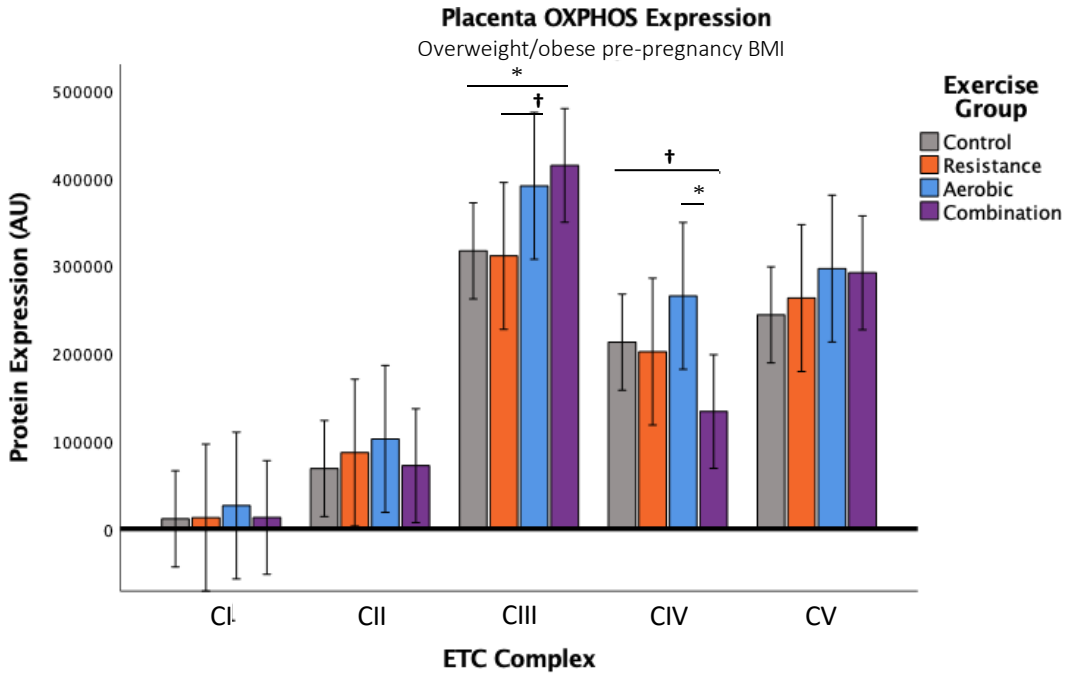

Figure 5. Placenta OXPHOS expression for pregnant women with overweight/obese pre-pregnancy BMI.
***OXPHOS*, oxidative phosphorylation. *ETC*, electron transport chain. *CI*, complex I. *CII*, complex II. *CIII*, complex III. *CIV*, complex IV. *CV*, complex V.**
*** $p<0.05$, $p<0.1$.**

OXPHOS expression stratified by gravida

Stratification of the data by gravida showed there was no difference in placental OXPHOS expression between exercise groups in women that were pregnant for the first time (G1) (Table 6).

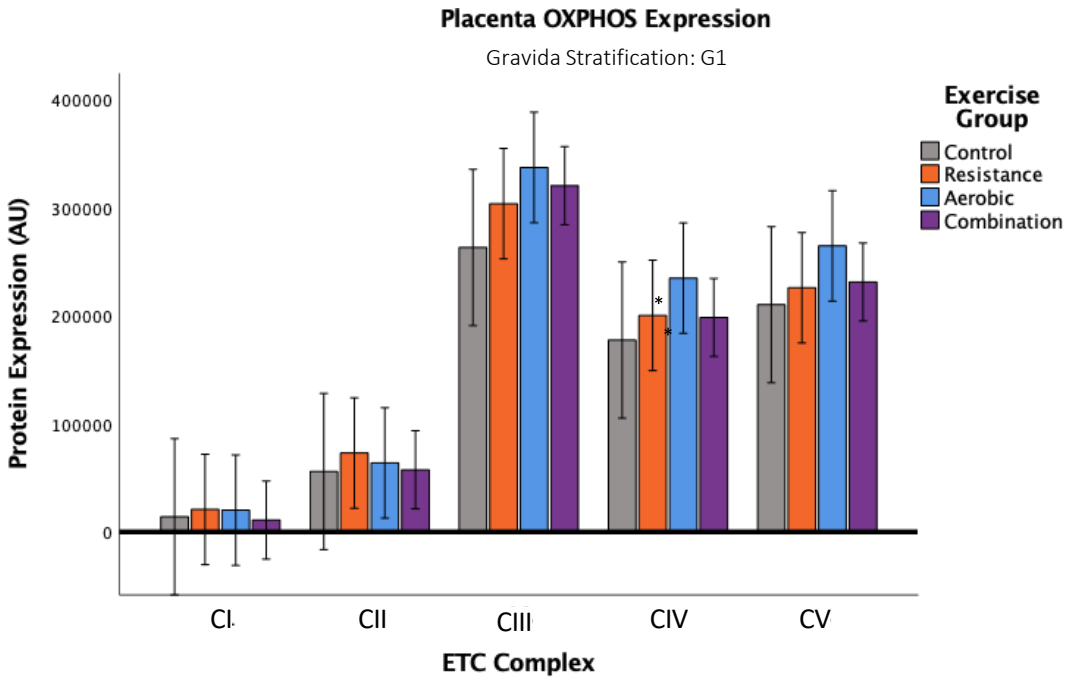

Figure 6. Placenta OXPHOS expression of each exercise group in G1 women. *G1*, first time pregnant women. *OXPHOS*, oxidative phosphorylation. *ETC*, electron transport chain. *CI*, complex I. *CII*, complex II. *CIII*, complex III. *CIV*, complex IV. *CV*, complex V.
*** *p*<0.05, † *p*<0.1.**

However, differences were observed between exercise groups for women having been previously pregnant (Table 7). CT showed greater CIII expression compared to control (p=0.02) and RT (p=0.006). CT had significantly lower CIV expression compared to control (p<0.001), RT (p=0.003), and AT (p<0.001). Lastly, CT showed greater CV expression compared to control (p=0.01) and RT (p= 0.02).

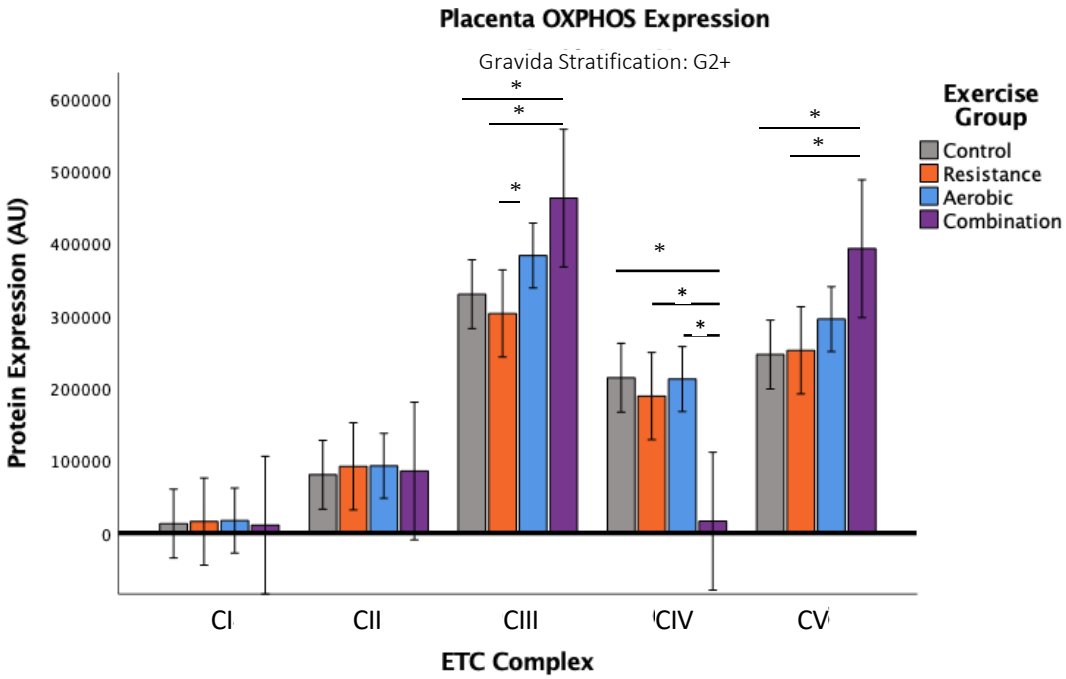

Figure 7. Placenta OXPHOS expression of each exercise group in G2+ women. *G2+,* women having previously been pregnant. *OXPHOS*, oxidative phosphorylation. *ETC*, electron transport chain. *CI*, complex I. *CII*, complex II. *CIII*, complex III. *CIV*, complex IV. *CV*, complex V. * $p<0.05$, † $p<0.1$.

Chapter 5 – Discussion

The purpose of this study was to determine the influence of exercise and exercise mode on placental mitochondria OXPHOS protein expression. Aerobic activity had the greatest change in OXPHOS protein expression. More specifically, placentae from women who participated in AT during pregnancy showed greater expression of complexes III and V compared to controls. After stratifying data by pre-pregnancy BMI, healthy weight women showed greatest change in CIII and CV expression with aerobic exercise. However, placentae of OW/OB women had increased CIII expression and trending decrease in CIV expression with combination training. Stratification by gravida found combination training by G2+ increased CIII and CV expression with lower CIV expression. Currently, there is limited literature on OXPHOS protein expression response to exercise training in placenta villous tissue. This is the first study to examine placental mitochondria adaptations to different types of supervised exercise, at recommended levels, during pregnancy.

In this study, we found aerobic exercise during pregnancy had greater influence on placental mitochondria OXPHOS expression compared to other modes of exercise training. This is consistent with findings in other tissue[10]. Fritzen et al. 2019 found 6 weeks of aerobic training in non-gravid adults improved activity of OXPHOS CI, CII, CIII, and CIV activity in skeletal muscle mitochondria[10]. A 50% increase in citrate synthase (CS), an enzyme reflecting oxidative capacity of mitochondria, activity was also observed. Increased ETC complex activity and oxidative capacity in muscle tissue could be due to greater OXPHOS protein expression, ultimately enhancing the organelle's capacity to produce ATP and improve tissue endurance. Resistance training however has been shown to increase CI expression[23], which was not found

in this study. We found no differences in the expression of CI between exercise groups. Additionally, we found CT did not have a summative influence of RT and AT on OXPHOS expression in placental mitochondria. In fact, CT placentae had lower CIV expression compared to AT. Improvements in CS activity have not been seen in response to RT [23], suggesting no changes in muscle oxidative capacity or tissue efficiency. Based on the current findings and previous literature, it seems AT during pregnancy is the most effective at increasing OXPHOS complex expression compared to control and other exercise groups in this study.

The placental response to different modes exercise may be influenced by pre-pregnancy conditions. OW/OB pre-pregnancy BMI is associated with placenta deficiency [16,17] and placental mitochondria content has been found to be decreased or increased by different studies[13,18]. Obesity is associated with increased inflammation, which contributes to increased oxidative stress during pregnancy. Research has found combined resistance and aerobic training decreases oxidative stress in placenta tissue, suggesting chronic exercise training lowers levels of oxidative stress[25,26]. Similar to the all group data, we found aerobic activity during pregnancy induced greater expression in CIII and CV in healthy weight women. However, in OW/OB women, both AT and CT groups had increased CIII expression with no change in CV expression. Furthermore, in OW/OB women CT placenta had lower CIV expression compared to AT. In this study, placentae from women with OW/OB responded differently than healthy weight women to CT. Being OW/OB alters how the placenta adapts to exercise during pregnancy; it seems that AT or CT throughout pregnancy may be as beneficial to OW/OB women as just AT for healthy weight women. This finding warrants further investigation.

Gravida was also found to influence placenta adaptations to exercise training. Women

experiencing their first pregnancy showed no difference in OXPHOS expression between exercise groups. On the other hand, women who had previously been pregnant responded most to CT. CT induced dramatic changes in CIII, CIV, and CV expression. Interestingly, a similar change of increased CIII and CV with lower CIV is seen in mitochondrial ETC of long-lived dwarf mice tissues (Choksi et al. 2011); this suggests the alteration may help in mitochondrial prolongevity and thus help to maintain healthy placenta function longer. Little is known regarding placenta changes with increasing gravida. One study has noted differing placental vascular development in nulliparous relative to multiparous women[3,4]. This study notes that multipara women have a quicker rise and maintenance of vascular growth factors compared to similar nulliparous counterparts[3]. Thus, it is possible that the influence of exercise throughout pregnancy may help to maintain these higher levels of placental vascular growth factors which can thus stimulate placenta mitochondria. Future research is warranted to determine how the placenta changes and responds to exercise during pregnancy within this population.

Strengths & Limitations

A strength of this study was that women participated in a supervised exercise RCT that met ACSM and ACOG guidelines and achieved a minimum of 80% attendance and exercise compliance. Leisure time physical activity (LTPA) in addition to the study protocol was assessed and used as a determinant for exclusion from this sample. Sample sizes representing each exercise group were considerably large for this type of study. Additionally, this was one of the first studies to evaluate complex V protein expression in placentas. However, we acknowledge this study has limitations. Mode of delivery was not controlled for and may have impacted level of mitochondrial protein expression. Vaginal deliveries have been shown to increase oxidative

stress in placental mitochondria compared to cesarean section, which could impact results[14]. However, our groups were similar regarding the number of vaginal and cesarean deliveries to hopefully minimize this potential effect on the outcomes. Secondly, the time between delivery and tissue collection was variable. Ideally samples would be obtained within a few hours of delivery to minimize physiological changes occurring after the placenta is delivered. Yet, we ensured that all samples were collected within a similar time frame and processed in the same way. This should be controlled for in future research. We did not control for gestation length. Therefore, future research should also control for gestational length since this may impact the placental health overall.

Conclusion

This data demonstrate that any type of exercise is safe since exercise did not negatively alter placenta mitochondria OXPHOS protein expression. Aerobic activity increases expression of OXPHOS complexes in placental mitochondria compared to resistance and combination training as well as controls. Additionally, women with healthy and OW/OB pre-pregnancy BMI have increased placental mitochondria OXPHOS expression with AT and CT. Increases and decreases in CIV expression were found in women previously pregnant participating in CT, and no decrease in expression was found between AT and controls. Therefore, aerobic exercise might be most effective at improving maternal and fetal outcomes and placenta efficiency. The placenta is a mitochondria rich organ facilitating the dynamic energy demands of pregnancy and acts as a mediator between mother and fetus. Mitochondria dysfunction decreases placenta efficiency, and negatively impacts maternal health and fetal development[9], and is the basis for many pregnancy pathologies. Health benefits of exercise during pregnancy include

decreased incidence of PE and GDM[1,22], suggesting exercise may improve placental mitochondria and tissue function.

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Appendix A: Notification of Amendment Approval

From: Biomedical IRB

To: Linda May

CC: Ashland Haire

Date: 8/3/2022

Re: Ame55_UMCIRB 12-002524
UMCIRB 12-002524
ENHANCED by Mom

Your Amendment has been reviewed and approved using expedited review for the period of 8/2/2022 to 12/19/2022. It was the determination of the UMCIRB Chairperson (or designee) that this revision does not impact the overall risk/benefit ratio of the study and is appropriate for the population and procedures proposed.

Please note that any further changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. A continuing or final review must be submitted to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

Document	Description
Changes to Study Team/Personnel	addition of Stancell, Grantham, McLaurin, and Kern

For research studies where a waiver or alteration of HIPAA Authorization has been approved, the IRB states that each of the waiver criteria in 45 CFR 164.512(i)(1)(i)(A) and (2)(i) through (v) have been met. Additionally, the elements of PHI to be collected as described in items 1 and 2 of the Application for Waiver of Authorization have been determined to be the minimal necessary for the specified research.

The Chairperson (or designee) does not have a potential for conflict of interest on this study.

IRB00000705 East Carolina U IRB #1 (Biomedical) IORG0000418
IRB00003781 East Carolina U IRB #2 (Behavioral/SS) IORG0000418