The Effect of Exercise Mode During Pregnancy on Maternal, Placental, and Cord Inflammatory Markers

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ABSTRACT

Excessive inflammation during pregnancy is associated with adverse pregnancy outcomes. Specifically, increased pro-inflammatory tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1 beta (IL-1ß), interleukin-8 (IL-8), and c-reactive protein (CRP) have been associated with gestational diabetes, preeclampsia, intrauterine growth restriction, and preterm birth. Similarly, pathologies of the maternal-fetal interface, such as recurrent spontaneous abortion, are associated with high pro-inflammatory biomarkers. This also increases fetal exposure to high inflammation *in utero*, contributing to the increased risk of cardiometabolic and mental conditions later in life. In non-gravid adults, exercise is an effective method for reducing pro-inflammatory and increasing anti-inflammatory biomarkers, differing with exercise mode. Currently, no research has directly compared the effect of exercise modes during pregnancy on maternal, placental, and fetal inflammatory biomarkers.

The purpose of this dissertation was to determine the effect of maternal aerobic, resistance, or combination (aerobic + resistance) exercise during pregnancy on maternal, placental, and cord inflammation. Samples from healthy pregnant women with singleton pregnancies that participated in a randomized control trial exercise intervention were obtained. The exercise intervention consisted of 150-minutes of moderate-intensity aerobic, resistance, or combination exercise per week from ≤16-wks gestation until delivery. First, we investigated the

effect of exercise mode on inflammation in maternal plasma at enrollment (≤16-wks) and 36-wks gestation. Second, we examined the concentration of inflammatory biomarkers in placental tissue. Third, cord blood was analyzed for inflammatory biomarkers. All studies utilized Luminex xMAP technology to quantify biomarker concentration. The second and third studies also identified protein and pathway differences with exercise using a label-free proteomics.

Maternal, placental, and cord blood inflammatory biomarkers were similar between groups. However, these data revealed that other exercise metrics predicted inflammatory biomarker concentration. Specifically, increased weekly exercise duration predicted lower IL-6 and IL-8 and increased weekly exercise frequency predicted higher TNF-α in late pregnancy maternal plasma. Increased weekly exercise intensity predicted lower placental fibrinogen. Lastly, total exercise volume, exercise mode, and pre-pregnancy BMI predicted cord blood IL-6 and exercise mode and pre-pregnancy BMI predicted cord blood cortisol.

Label-free proteomics revealed significantly different proteome landscapes in aerobic, resistance, and combination exercisers placental tissue and cord blood compared to controls. We identified multiple downregulated proteins in placentas of exercisers that related to inflammation and immunity. Similarly, there was a downregulation in cord blood pathways that related to innate and adaptive immunity, possibly indicating reduced downstream inflammatory biomarkers.

Altogether the studies within this dissertation did not support our central hypothesis that exercise mode would differentially alter inflammatory biomarkers during pregnancy. However, our studies highlight the importance of monitoring all exercise metrics (e.g., frequency, intensity, time, type), as each related to changes in maternal, placental, and fetal inflammation. We were also able to identify other inflammatory proteins and pathways in placental tissue and cord blood, providing targets for future projects. Collectively, the studies within this dissertation support the safety of exercise during pregnancy, further emphasizing the possible benefits, regardless of exercise mode. The projects in this dissertation extend the knowledge on how antenatal exercise impacts inflammation during pregnancy, thus impacting maternal and infant health.

The Effect of Exercise Mode During Pregnancy on Maternal, Placental, and Cord Inflammatory Markers

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

AE,	Aerobic Exercise
APOs,	Adverse Pregnancy Outcomes
BIPOC,	Black, Indigenous, People of Color
BMI,	Body Mass Index
CE,	Combination Exercise
CRP,	C-Reactive Protein
FDR,	False discovery Rate
GWG,	Gestational Weight Gain
GDM,	Gestational Diabetes
g-HTN,	Gestational Hypertension
HR,	Heart Rate
IL-6,	Interleukin-6
IL-1ß,	Interleukin-1 Beta
IL-8,	Interleukin-8
IL-10,	Interleukin-10
IUGR,	Intrauterine Growth Restriction
LC-MS/MS,	Liquid Chromatography Tandem Mass Spectrometry
LFQ-MBR,	Label-Free Quantification Matching Between Runs
MET,	Metabolic Equivalent
MET*MIN,	Metabolic Equivalent per minute
MET/HOURS/WEEK	Metabolic Equivalent per hours per week
PE,	Pre-eclampsia
PSMs,	Peptide spectrum matches
RCT,	Randomized Controlled Trial
RPE,	Rating of Perceived Exertion

ROS,	Reactive Oxygen Species
RE,	Resistance Exercise
Th1,	T-Helper Type 1
Th2,	T-Helper Type 2
Th17,	T-Helper Type 17
Treg,	T Regulatory Cells
TNF-α,	Tumor Necrosis Factor Alpha
THR,	Target Heart Rate
VO2peak,	Peak Volume Oxygen Consumption
1RM,	One Rep Max

CHAPTER 1

Introduction and Literature Review

The purpose of this literature review is to synthesize the current state of scientific evidence regarding the effects of exercise during pregnancy on maternal, placental, and cord blood inflammatory biomarkers. In this review, we examine the effect of excessive inflammation on pregnancy health and outcomes. Furthermore, we delve into the existing literature surrounding how exercise during pregnancy may impact maternal, placenta, and cord inflammation during pregnancy.

Recent reports indicate that 29% of US women have obesity (BMI > 30.0 kg/m^2) prior to pregnancy.[1] Obesity or excessive gestational weight gain (GWG) can result in excessive inflammation during gestation.[2,3] Further, prolonged excessive inflammation is known to increase the risk of adverse pregnancy outcomes (APOs), such as intrauterine growth restriction or preterm birth.[4–6] Additional pregnancy conditions, such as gestational diabetes (GDM)[7–9] or preeclampsia (PE)[10], are also associated with high levels of inflammatory biomarkers. The placenta, as a mediator between the maternal and fetal environments during pregnancy, supports optimal conditions for proper growth and development. It is suggested that excessive inflammation in the maternal environment impacts placental expression [11,12] of inflammatory biomarkers and, thus, can alter the intrauterine environment.[13–17] This inflammatory environment *in utero*, a critical period when tissues and organs are created,[18] thus programs the fetal towards disease and increased risk of developing cardiometabolic diseases later in life.[14–17,19,20] Conversely, exercise during pregnancy elicits numerous health benefits for pregnant women (i.e., decreased risk of APOs, controlled GWG) and their offspring (i.e., decreased adiposity, improved cardiometabolic health).[21–30] Recent evidence suggests that aerobic (AE) or combination (CE, aerobic + resistance) exercise during pregnancy may help regulate and decrease inflammation.[31–35] Interestingly, in non-gravid individuals, exercise modes appear to differentially impact inflammation.[36–38] Yet, no research has directly compared the effect of exercise modes

during pregnancy on inflammation across the maternal, placental, and fetal compartments.

I. Role of Elevated Inflammation on Pregnancy

For female reproductive physiology, in general, controlled inflammation is important to regulate processes associated with ovulation, menstruation, placentation, and parturition.[39,40] Thus during pregnancy excessive inflammation can be harmful to placental function, which affects fetal growth and development.[4,12,41,42] The inflammatory response is characterized by the upregulation of cytokines which can be mediated through T-cell activation from T-helper (Th) and T-regulatory (Treg) cells. Th type 1 (Th1), type 2 (Th2), type 17 (Th17), and Treg cells are a part of adaptive immunity and work in balance during pregnancy to assist in tolerating the fetus and to initiate parturition.[42,43] Th1 and Th17 cells can increase pro-inflammatory biomarkers (i.e., IL-6, IL-1β, TNF-α) as well as macrophages and adipocyte activity, further stimulating the release of pro-inflammatory cytokines and proteins such as c-reactive protein (CRP).[44,45] However, Th2 and Treg works to balance the pro-inflammatory biomarkers by stimulating the release of anti-inflammatory cytokines, such as IL-10.[4,42] Specific to pregnancy, the timing and balance of these pro- and anti-inflammatory cytokines has a critical role in maternal, placental, and fetal health and development.[46] Pathologies of the maternal-fetal interface, such as recurrent spontaneous abortion, placental abruption, and PE is associated with high proinflammatory cytokines and decreased anti-inflammatory cytokines.[42] Therefore, the balance of pro- and anti-inflammation is critical for preventing fetal rejection and promoting full-term delivery.[40]

While controlled inflammation is critical to a healthy pregnancy, other factors (e.g., pre-pregnancy obesity,[47] excessive gestational weight gain[2]) and/or maternal exposures (e.g., high psychological stress,[48] poor diet[49]) can result in increased pro-inflammation. Increased pro-inflammatory cytokine production is linked with adverse pregnancy related conditions and diseases thus compromising maternal and fetal health.[4] Specifically, pro-inflammatory cytokines TNF-α, IL-6, IL-1ß, and CRP have been associated with APOs, such as gestational insulin

resistance and GDM,[7–9] PE,[50] intrauterine growth restriction, [4] preterm birth.[5,6] Considering the communication between the maternal environment and developing fetus during pregnancy, it is feasible that this elevated inflammation during pregnancy may also impact the placenta and fetal environments as well. Due to the role TNF-α, IL-6, IL-1ß, and CRP in pregnancy and APOs, we will primarily focus on these specific biomarkers for the subsequent sections of this review.

III. Role of Elevated Inflammation on the Placenta

The placenta supports a successful pregnancy by producing hormones, enzymes, and cytokines while simultaneously protecting the fetus from infection and excessive amounts of pro-inflammatory molecules.[51,52] However, an elevated pro-inflammatory maternal environment is associated with changes in infant health outcomes and could be due to changes in the placental inflammatory biomarkers.[15,53] However, the mechanism by which the fetus is exposed to these biomarkers has not been fully elucidated. Animal research has demonstrated that pro-inflammatory IL-6, TNF-α, and IL-1ß, as well as anti-inflammatory IL-10, are upregulated in placenta tissue following maternal immune activation via poly(I:C) injection.[54] Additionally, this study also reported upregulated IL-6 and TNF-α mRNA in placenta tissue that was exposed to maternal immune activation.[54] Further research finds increased macrophage activation and IL-1ß, IL-6, IL-10, and TNF-α gene expression in placenta tissue of obese mice in late pregnancy; similarly, there is increased mRNA expression of TNF-α, IL-6, IL-8, and IL-18 in placental cotyledonary tissue in obese sheep at mid-gestation.[55,56] Lastly, research in women with APOs that have increased inflammation (e.g., GDM, PE) also show increased placental expression of TNF-α, IL-6, and IL-1ß.[11,12] Collectively, it is clear placental functioning is altered as a result of increased maternal inflammation during pregnancy. As a result, this could lead to more pro-inflammatory biomarkers entering the fetal environment and, thus, impact fetal health and development.

III. Role of Elevated Inflammation on Fetus and Infant Health

Increased maternal inflammation appears to also alter fetal exposure to inflammatory biomarkers, as evidenced by increased concentrations in cord blood.[34,57] Thus, excessive maternal inflammation can lead to changes in inflammatory exposures within the fetal compartment. There is an association between maternal inflammation during pregnancy and umbilical cord blood CRP and IL-6.[34,57] Additionally, newborns of women with increased TNF-α gene expression also have higher TNF-α gene expression and lower IL-6 gene expression in cord blood.[58] These data support the concept that maternal inflammation alters fetal exposure to inflammation *in utero*. The elevated maternal inflammation, as seen in GDM or PE, altering cord blood inflammation *in utero* could be a contributing factor to the increased risk for infants who later develop cardiometabolic and/or mental conditions.[17,20,59] Animal research has found maternal inflammation and hyperoxia during pregnancy linked with cardiac dysfunction as well as changes to lung structure and function.[16,17] In humans, increases in maternal mean pro-inflammatory ratio during pregnancy resulted in a linear decrease in leukocyte telomere length, which impacts long-term physiological risk factors as well as disorders later in life.[15] A recent literature review also concluded that levels of inflammation across gestation is linked to offspring neurodevelopmental outcomes such as brain structural and functional changes.[14] Therefore, there is a clear relationship between maternal inflammation and overall offspring developmental changes. Furthermore, maternal inflammation during pregnancy can lead to changes in the infant inflammation after delivery. After controlling for BMI, environmental exposures, and genetic risk factors, increased maternal CRP during pregnancy is associated with increased 6-month old infant blood CRP.[13] Collectively, scientific evidence supports that elevated maternal inflammation leads to increased inflammatory exposure *in utero* and altered postnatal infant inflammation. Maternal inflammation influence on infant inflammatory biomarkers is likely mediated through changes to placental expression of inflammatory markers, due to its intermediary role between maternal and fetal environments.[13,15,41,51,54,60–63] Increased maternal inflammation is associated

with changes in placental, fetal, and infant inflammation as well as linked with adverse health outcomes for all compartments (Figure 1.1). Thus, it is critical to determine the most effective methods for regulating inflammation during pregnancy, to improve maternal and infant health.

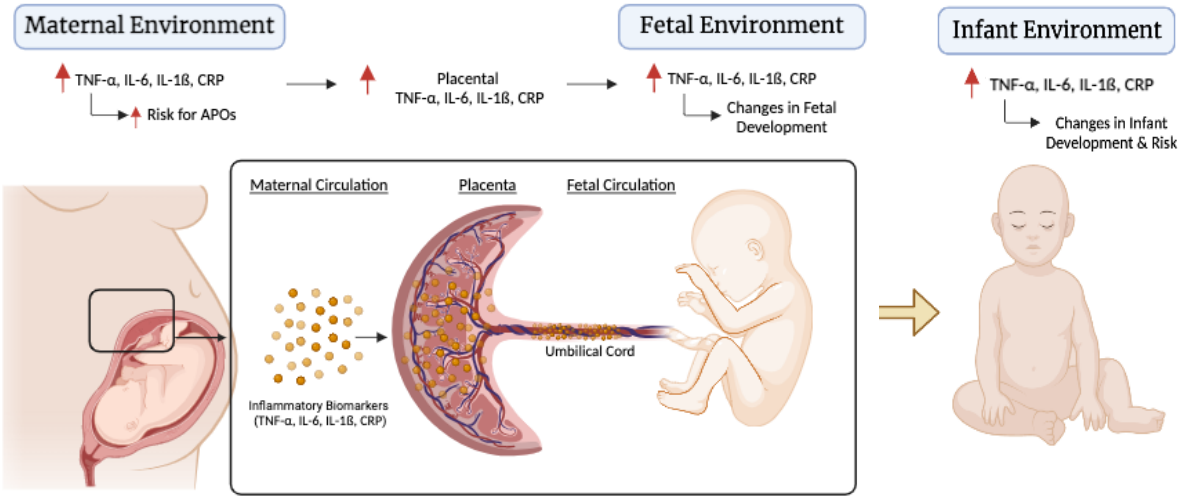

Figure 1.1. Summary of the relationship between elevated maternal inflammation during pregnancy and its influence on placental, fetal, and infant inflammation exposure *in utero* and after delivery. TNF-α = tumor necrosis factor alpha, IL-6 = interleukin 6, IL-1ß = interleukin 1 beta, CRP = c-reactive protein. Created with BioRender.com

IV. Exercise Impact on Pregnancy & Inflammation

Exercise during pregnancy elicits numerous positive effects on maternal, placental, and infant outcomes. Specifically, women who exercise during their pregnancy have reduced risk for APOs, better controlled gestational weight gain, and improved cardiovascular measures. [21–25] Placental tissue of women who regularly exercise during pregnancy have enhanced nutrient and gas exchange, are more efficient, and have lower reactive oxygen species (ROS). [64,65] Infants born to women who exercised during pregnancy have lower risk of macrosomia, lower neonatal and infant adiposity, and improved cardiometabolic health.[26,27,29,30,66,67] In non-pregnant populations, exercise decreases pro-inflammatory biomarkers (IL-1ß, IL-6, TNF-α), thus helping to support healthy inflammation.[36–38,68] However, its suggested that the magnitude of change in inflammatory biomarkers as a result of exercising, differs depending on exercise mode.

Research found that non-gravid adults with type 2 diabetes who participate in aerobic exercise (AE) or combination exercise (aerobic + resistance, CE) experience decreases in CRP from pre- to post-intervention compared to the exercise-counseling and control groups.[37] However, those in the CE group had greater decreases in CRP compared to the AE. Regression analyses found that exercise mode and volume were independent predictors of changes to CRP. Importantly, this study reported no differences in leisure time physical activity volume, which included the exercise sessions, between the AE and CE intervention groups. In addition, the CE group significantly decreased pro-inflammatory IL-1ß, TNF-α, and IL-6 while experiencing increases to anti-inflammatory IL-10 and IL-4 from baseline.[37] The change in IL-1ß, IL-6, IL-10, and IL-4 were all altered to a greater extent relative to the AE group. Taken together, the findings described suggest that exercise mode was largely driving these differences.[37] Another literature review summarized differences in the concentration of inflammatory biomarkers following AE, resistance exercise (RE), or CE in diabetics.[36] In short, this review reported that CE, RE, and AE all decrease CRP, IL-6, and IL-1ß, but CE decreased these biomarkers to a greater extent compared to AE or RE alone.[36] Studies in older adults found that AE significantly reduced CRP, IL-6, and TNF-α from pre- to post-intervention, whereas RE group only decreased TNF-α.[38] Lastly, a review in RE alone in healthy adults concluded that RE can induce significant decreases to CRP, but remains inconclusive of the effect on TNF-α and IL-6. [69] Collectively, these data support that regular exercise can be an effective tool at decreasing pro-inflammatory biomarkers and increasing anti-inflammatory biomarkers in a non-gravid population and differ with exercise modes.

In pregnancy, cross-sectional research on the effect of physical activity (PA) during pregnancy on maternal inflammation is conflicting.[32–34,70,71] However, considering the different maternal demographics and methodologies for assessing PA across studies, it is difficult to draw conclusions. Supervised exercise interventions using a randomized control trial (RCT) study-design may better indicate unbiased, causal relationships between antenatal exercise effect on

inflammation. Data from a supervised AE intervention shows lower TNF-α in late pregnancy maternal serum relative to intermittently active controls.[31] CRP also trended lower in women with AE relative to those who did not, with significant reductions in CRP for those who maintained or increased their exercise throughout pregnancy.[72] Similarly, pregnant women who regularly achieved 11,000 steps/day have lower CRP in late pregnancy compared to the control group.[35] Supervised CE during pregnancy also decreases TNF-α with a reduced increase to IL-1ß in maternal serum compared to non-exercising controls.[73] Taken together, it appears that AE or CE can aid in lowering excessive inflammation during pregnancy in maternal blood. To our knowledge, the effect of RE exercise on maternal inflammation has not been explored. Similarly, more research is needed to establish whether the extent of inflammation decrease differs depending on exercise mode in maternal blood.

In cord blood, CRP has shown to be slightly, but insignificantly, lower in pregnant women with obesity who did 150-minutes per week of physical activity compared to inactive controls.[32] In mice, research found that swimming regularly before and during pregnancy led to decreased IL-6 in male offspring compared to no exercise.[74] Therefore, data suggest that AE may reduce cord blood biomarkers, however research in human participants is needed. Acosta-Manzano et al. found that supervised CE exercise during pregnancy lowers cord blood IL-6 and TNF-α relative to non-exercising controls.[73] There has been no supervised AE or RE interventions examining the effect these exercise modes have on cord blood inflammation.

To summarize the current literature, exercise during pregnancy can be used as a method for decreasing inflammatory biomarkers. However, although exercise during pregnancy is beneficial for improving placental structure and function, there is no research to date examining the effect of exercise mode (AE, RE, or CE) on placental inflammatory biomarker concentration. The effect RE alone during pregnancy has on maternal, placental, or cord blood inflammation is also unexplored. Considering the effect excessive inflammation has on maternal, placental, and

infant health outcomes, it is imperative to determine the most effective method for reducing inflammation during pregnancy.

VI. Conclusion

In conclusion, research supports that AE or CE can regulate maternal inflammation during pregnancy and that CE specifically appears to also help reduce inflammatory biomarkers in cord blood. However, there is no research examining how: 1. AE impacts placenta or cord blood inflammation; 2. RE impacts inflammation in maternal blood, placenta, or cord blood; and 3. CE impacts placental inflammatory biomarkers. Given the observed effects of different exercise modes to control inflammation in non-pregnant populations, it is probable that this effect also occurs for pregnant women. However, maternal exercise research in this area remains scarce; therefore, more research directly comparing the influence of exercise modes during pregnancy on inflammation is needed. Understanding if a specific exercise mode is most effective at reducing inflammation during pregnancy will provide critical information on specific exercise recommendations for women and clinicians. Further, understanding the influence exercise mode has on the communication between mom, placenta, and fetus, can ensure we are improving maternal and infant health during pregnancy and long-term.

CHAPTER 2

Effect of Exercise Mode during Pregnancy on Maternal Inflammatory Biomarkers

ABSTRACT

Excessive inflammation during pregnancy is associated with adverse pregnancy outcomes (APOs) such as gestational diabetes, preterm birth, and depressive symptoms. Exercise during pregnancy is an effective tool for reducing pro-inflammatory biomarkers. However, it is unclear how exercise modes differentially impact maternal inflammation in late pregnancy. The aim of the present study was to compare the influence of a supervised aerobic (AE), resistance (RE), or combination (AE + RE; CE) exercise intervention from early (≤16-wks) pregnancy through delivery. A total of 56 women with healthy singleton pregnancies were randomized to either 150-minutes of supervised moderate-intensity AE (n=14), RE (n=14), CE (n=15) or control (CON; n=13). Maternal plasma was collected at enrollment (≤16-wks) and at 36-wks gestation. Plasma tumor necrosis factor alpha (TNF-α); interleukins (IL) -6, -8, -1ß, -10; c-reactive protein (CRP); and cortisol were analyzed using Luminex xMAP technology. On average, participants were similar in all demographics (e.g., age, pre-pregnancy BMI, gestational weight gain). Exercise volume (MET*min) was significantly higher for all exercisers relative to CON. All inflammatory biomarkers were similar between groups ($p>0.05$). Linear regressions found that weekly exercise duration predicted late pregnancy IL-6 and IL-8 ($p=0.02$ and 0.04, respectively) while weekly exercise frequency predicted TNF-α ($p=0.003$). In conclusion, while all exercise modes had similar inflammatory biomarker concentrations in late pregnancy, exercise duration and frequency predicted some of the late pregnancy inflammatory biomarker concentrations, providing insights for future exploration. Overall, our findings support the safety of exercise during pregnancy, indicating no negative impact on maternal biomarkers during pregnancy, supporting the growing body of evidence that exercise of any kind reported in the current study is safe during pregnancy.

INTRODUCTION:

Excessive pro-inflammatory cytokine production is linked with adverse pregnancy outcomes (APOs), potentially compromising maternal and fetal health.[4] Specifically, excessive inflammatory cytokines such as IL-6, IL-1ß, CRP, and TNF-α have been linked to pregnancy complications such as gestational diabetes (GDM),[7–9] intrauterine growth restriction (IUGR),[4] depressive symptoms,[75] and preterm birth (PTB).[5,6] For female reproductive physiology, in general, controlled inflammation is important to regulate processes associated with ovulation, menstruation, placentation, and parturition.[42,76,77] Thus, during pregnancy, uncontrolled inflammation can be harmful during placentation, which affects fetal growth and development, as well as parturition.[42,76,77]

Exercise is an effective tool for reducing pro-inflammatory biomarkers (e.g., TNF-α, IL-1ß, IL-6, CRP) in pregnant and non-pregnant populations. Interestingly, in non-gravid adults exercise mode has differential effects on inflammatory biomarkers. In non-gravid older adults, aerobic exercise (AE) reduces blood TNF-α, CRP, and IL-6, whereas resistance exercise (RE) decreased TNF-α only from pre- to post-intervention. [38] A review on the effect of RE on inflammatory biomarkers in non-gravid adults reported that RE significantly reduced CRP, but not IL-6 or TNF-α.[69] Similarly, a review assessing exercise mode in type 2 diabetics reported reduced inflammatory biomarkers with AE or resistance exercise (RE) alone, but greater decreases in biomarkers with combination (CE, aerobic + resistance).[36] TNF-α is lower in pregnant women who participate in aerobic exercise (AE) relative to physically active controls. [31] Similarly, pregnant women who do combination exercise (aerobic + resistance; CE) have significantly lower late pregnancy TNF-α in maternal blood along with lower IL-8 and TNF-α in postnatal colostrum.[73,78] Although not significant, pregnant women who do CE also tended to have lower late pregnancy IL-6 and a reduced increase in IL-1ß in maternal blood relative to controls.[73] Collectively, these results indicate that the mode of exercise may not be equally effective at reducing pro-inflammatory biomarkers. Yet, to our knowledge, there is currently no research

directly comparing the influence of exercise modes during pregnancy on inflammatory biomarkers in maternal blood.

Considering the relationship between excessive inflammation with maternal and infant health outcomes during pregnancy, it is essential to determine the best method for reducing excessive inflammation in the maternal environment. Therefore, the goal of the current study was to examine the effect of exercise modes on inflammatory biomarkers in maternal plasma in late pregnancy. We hypothesized that all exercisers would have reduced TNF-α, IL-6, IL-1ß, and CRP in late pregnancy relative to CON, with CE exercisers having the lowest concentrations.

METHODS:

Study Design and Participants. This is a secondary analysis, pooling samples of women recruited from three randomized controlled trials (RCTs), to examine the effect of exercise mode on maternal inflammatory markers. Each of the RCTs recruited low-risk, healthy women with singleton pregnancies; participants were eligible if they met the following criteria: 18-40 years old, pre-pregnancy body mass index (BMI) 18.5 to 39.9 kg/m^2, $\leq$ 16-weeks (16-wks) gestation, and were cleared to exercise by their obstetric provider. Women were excluded if they did not meet these criteria or if they reported having a medical condition known to alter fetal development (e.g., HIV/Aids, cancer, diabetes, untreated hypertension), or use of any of tobacco products, alcohol, recreational drugs, or medications (e.g., oral hypertensive, insulin). Written informed consent was obtained upon enrollment. Those who enrolled were randomized to either 150-minutes of moderate exercise (aerobic, resistance, or combined aerobic + resistance) or control. All protocols were approved by East Carolina University (ECU) Institutional Review Board and registered in clinicaltrials.gov (NCT03517293 and NCT04805502). All procedures were conducted at ECU.

Pre-intervention Testing & Randomization. After enrollment, participants completed a Modified Balke treadmill test, as previously validated, to determine baseline cardiorespiratory fitness and

calculate individualized target heart rate (THR) ranges for moderate intensity exercise sessions. [79] Peak oxygen consumption (VO_{2peak}) was estimated by measuring oxygen consumption and carbon dioxide production via indirect calorimetry (Parvo Medics, TrueOne2400, Sandy UT) while maternal heart rate (HR) was continuously monitored (Polar FS2C HR monitor). Women recruited between March 2020 and October 2021 had THR zones estimated from their pre-pregnancy physical activity levels and age to minimize exposure during the COVID-19 pandemic. All THR zones corresponded to maternal HR at 60-80% VO_{2peak}, reflecting moderate intensity. Additionally, women performed a 1-repetition max (1RM) test to establish baseline strength, which was used to program resistance exercises. Participants were randomized via computer sequencing (GraphPad, Boston, USA) to either aerobic (AE), resistance (RE), combination (aerobic + resistance; CE) exercise, or a stretching/breathing attention control group (CON).

Exercise Intervention. All exercise participants (AE, RE, CE) were supervised by trained exercise instructors in ECU facilities following a standard protocol.[80] Briefly, all exercisers were asked to complete 150-minutes of moderate-intensity activity each week from enrollment until delivery (~24-wks). Each exercise session consisted of a 5-minute warm-up, 50-minutes of activity based on their randomized group, and a 5-minute cool-down. To capture exercise intensity, maternal HR and the Borg scale of perceived exertion (RPE 6-20) were monitored throughout each session. Participants were monitored to ensure they remained within their THR and at a RPE of 12-14 to reflect moderate intensity throughout the session.

The AE group completed moderate intensity training on treadmills, ellipticals, cycle ergometers, rowers, and/or stair stepping equipment, at the participant's discretion. The RE group completed sessions of 2-4 sets aiming for 12 repetitions of each exercise targeting major muscle groups at 60% 1RM. These activities were performed using machines (Cybex; i.e., leg extension, leg curl, shoulder/chest press), dumbbells (i.e., bicep curls, shoulder raises), resistance bands, exercise balls, benches, and/or mats. CE exercisers spent half their exercise sessions completing

AE activities and 25-minutes doing RE activities. These exercises were performed on the same equipment as outlined for each respective activity. To enhance study retention, those randomized to CON were invited to participate in weekly sessions of breathing/stretching. CON participants performed a 5-min warm-up followed by 50-min of stretching activities led by study team member. HR monitors were worn throughout these sessions to ensure maternal HR remained below moderate intensity (<50% VO_{2peak}).

Exercise Metrics. To determine the dose of exercise completed by participants, exercise metrics (volume, intensity, duration, frequency) were totaled each week. Exercise volume (MET*min), as well as weekly duration and frequency were calculated following every session and totaled for the week. At delivery, the total number of exercise sessions and training weeks were totaled for each participant. To examine the averages, weekly duration, intensity (METs), and frequency were totaled and divided by the number of weeks training across pregnancy. To examine the total exercise dose of the intervention, total exercise volume (MET*min) was calculated across the pregnancy. Participant attendance was calculated using the total number of sessions completed each week divided by the number expected (3/week). For this analysis, attendance threshold of 75% was set for all exercisers (AE, RE, CE), as this has previously shown to successfully impact maternal inflammatory biomarkers.[73]

Maternal Measures & Outcomes. Maternal age, race, ethnicity, height, and pre-pregnancy weight were collected via screening questionnaire at the beginning of enrollment. Black or African American, Asian, Native Hawaiian or other Pacific Islander, Native American or Alaskan Native, and Latinx participants were grouped as Black, Indigenous, People of Color (BIPOC). Pre-pregnancy BMI was calculated using self-reported height and weight.

Medications taken during the pregnancy, medical conditions, and incidence of adverse pregnancy condition (e.g., gestational diabetes, intrauterine growth restriction, or preterm birth)

were collected at delivery via electronic health record. Women who were diagnosed with an adverse pregnancy condition were excluded in the current analysis, as these have been associated with increased inflammation.[4,8,9] Gestational weight gain (GWG) was calculated by subtracting the self-reported pre-pregnancy weight obtained in the screening questionnaire from the delivery weight reported in the medical record.

Maternal Blood Collection & Processing: One purple top vacutainer containing EDTA of whole blood was collected from antecubital vein at pre-intervention (≤16-wks) and 36-weeks (36-wks) gestation following an overnight fast (≥8 hours). Upon collection, whole blood was aliquoted into 2, 2 mL microcentrifuge tubes and centrifuged for 15 min, 13 rpm, at 4ºC. Maternal plasma was then separated from red blood cells into 2, 2 mL microcentrifuge tubes. All samples were stored at -80ºC until analysis**.**

Quantification of Maternal Inflammatory Biomarkers: Plasma samples were used to determine the concentration of cortisol, CRP, TNF-α, IL-6, IL-8, IL-1ß, and IL-10 using MILLIPLEX MAP kits. Standards, controls, and samples were prepared using manufacturer's instructions. Each plate was read on a Luminex 200 using xMAP technology and concentrations of each analyte was determined using xPONENT software. To assess cortisol, the Human Circadian/Stress Magnetic Bead Panel kit was used. To assess CRP the Human Cardiovascular Disease (CVD) Magnetic Bead Panel 3 (Acute Phase) kit was used. To assess TNF-α, IL-6, and IL-8 the Human Adipokine Magnetic Bead Panel (Millipore Sigma, Burlington, MA, USA) was used. Finally, the Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Sigma, Burlington, MA, USA) was used to examine IL-1ß and IL-10. Each sample was run in duplicate to determine the concentration of cortisol, CRP, TNF-α, IL-6, IL-8, IL-1ß, IL-10, and then averaged. Due to limited sample availability, all data obtained within the limit of detection for each kit was used. Mean percent difference was calculated for AE, RE, and CE relative to CON. A subset of

participants with data at both 16-wks and 36-wks were taken from each group and used to calculate percent change scores.

Statistical Analyses. The primary outcome of the present research analyses was the quantification of TNF-α. Power analysis using preliminary data indicated a total of 48 participants, or 12 per group was needed to reach significance of $p<0.05$ with 80% power. Outliers were first identified using boxplots and extreme outliers were removed. The remaining potential outliers were checked and removed if there was justification for doing so (i.e., issue during processing affecting sample reliability, medical condition influencing biomarker, etc.). Considering research in this area is limited, potential outliers were thoroughly validated, as these could represent inherent variability in the data. Data were first checked for normality. One-way ANOVAs were used to examine between group differences for all normally distributed data. Kruskal-Wallis non-parametric tests were used for data that were non-normally distributed. Both ANOVAs and Kruskal-Wallis testing utilized a Bonferroni correction factor during post-hoc testing. The *a priori* alpha level for statistical significance was set at $p<0.05$ for all analyses. Analysis of covariance (ANCOVAs) were used to evaluate covariates. To meet the assumption of normality, non-normally distributed biomarkers were $\log_{10}$ transformed before running ANCOVAs and regression analyses. Testing for between group differences and ANCOVAs were conducted using IBM SPSS Statistics for Mac, version 27 (IBM Corp., Armonk, NY, USA). Finally, regression analyses were performed to determine significant predictors of the outcome variables using JMP Statistics for Mac, version 17 (JMP Statistical Discovery, Cary, NC, USA).

RESULTS:

Study population. Maternal blood was analyzed from 92 participants. Of these, 22 were removed due to development of adverse pregnancy outcome (GDM, gestational hypertension, IUGR) or other condition known to affect inflammation (e.g., gestational thrombocytopenia, Sjogren's

syndrome, etc.) by the time of 36wk blood collection; 6 were removed due substance or medication known to alter inflammation (e.g., selective serotonin reuptake inhibitors); and 8 were removed due to low attendance (<75%; 3 AE, 4 RE, 4 CE). Due to small sample sizes, data from 3 participants with attendance of 61%, 67%, and 74% from AE, RE, and CE respectively, were kept in the dataset. These participants had healthy pregnancies, no medical conditions, and took no medications. To ensure the addition of these participants would not skew our outcomes, these data were checked to ensure all were similar to the data of others within their respective groups. Thus, we had a total of 56 samples for analysis (13 CON, 14 AE, 14 RE, 15 CE). In general, groups were similar for all maternal descriptors. As expected, all exercise groups had greater total exercise volume and attendance relative to attention controls (Table 2.1).

Inflammatory biomarker concentration. Pre-intervention 16-wk maternal plasma concentrations of cortisol, CRP, TNF-α, IL-6, IL-8, and IL-1ß was similar except IL-10 which was lower in CE and AE relative to controls (Table 2.2). At 36-wks gestation, all biomarkers were similar between groups (Table 2.2). Similarly, all percent change scores for each biomarker were similar between groups ($p>0.05$; Figure 2.1).

All groups increased cortisol from baseline (Figure 2.1). Cortisol difference relative to CON for each group were -1% for AE, 14% for RE, and -8.8% for CE (Figure 2.2). TNF-α increased for all groups by late pregnancy (Figure 2.1), with AE being 15%, RE being 29%, and CE being 52% higher than CON (Figure 2.2). The percent change at 36-wks for IL-1ß was 210%, 51%, 41%, and 188% for CON, AE, RE, and CE, respectively (Figure 2.1). Though all groups increased from baseline, IL-1ß at 36-wks remained lower for RE and CE groups relative to CON (Figure 2.2).

Although significance was not reached ($p=0.16$) for IL-8, there are distinct patterns of lower levels in AE by 66%, RE by 72%, and CE by 52% relative to CON (Figure 2.2). Similarly, despite no significant between group differences ($p=0.16$) for IL-10 (Figure 2.1), there are distinct patterns of lower levels in AE by 74%, RE by 60%, and CE by 39% relative to CON (Figure 2.2).

ANCOVAs and Regressions. Due to non-normal distribution, all biomarkers were log transformed to meet the normality assumption for ANCOVA and regression testing. To test for between group differences while adjusting for pre-intervention biomarker concentration, pre-pregnancy BMI, GWG, and race (Model 1), there were no differences between the groups for IL-6, IL-8, IL-10, IL-1ß, CRP, and cortisol ($p>0.05$, data not shown). Using Model 1, the corrected model for 36-wk TNF-α was significant ($F=5.90$, $n^2 = 0.76$, $p=0.003$) with enrollment TNF-α being significant ($p=0.00$; Table 2.3). Since the current study was interested in the exercise effect, we ran a second ANCOVA model testing between group differences while adjusting for enrollment biomarker concentration, exercise duration, weekly average intensity, and frequency (Model 2). Using Model 2, there were no differences between groups for IL-6, IL-8, IL-10, IL-1ß, CRP, and cortisol ($P>0.05$, data not shown). Model 2 was significant for 36-week TNF-α ($F=7.72$, $n^2=0.85$, $p=0.003$). From this model, weekly exercise frequency and enrollment TNF-α concentration were significant ($p=0.03$ and 0.00, respectively; Table 2.3).

Linear regressions established that 36-wk IL-6 and IL-8 concentrations were predicted by pre-intervention concentration and weekly exercise duration ($p<0.05$; Table 2.4). TNF-α concentration at 36-wks was also predicted by pre-intervention concentration and weekly exercise frequency ($p=0.003$; Table 2.4). All other models were not significant (data not shown).

DISCUSSION:

The goal of the current study was to examine the effect of exercise modes on inflammatory biomarkers in maternal plasma in late pregnancy. We hypothesized that all exercisers would have reduced TNF-α, IL-6, IL-1ß, and CRP relative to CON in late pregnancy, with CE exercisers having the lowest concentrations. Contrary to our hypothesis, we found similar concentrations between groups for all biomarkers at 36-wks. Interestingly, we found that all exercise types are associated with at least a 20% increase of TNF-α but had reduced IL-8 and IL-10 relative to CON. Lastly, we

found weekly exercise duration predictive of IL6 and IL-8 while weekly frequency was predictive of TNF-α.

We found that all exercise types are associated with at least a 20% increase in TNF-α, with CON participants only increasing ~7%. Considering maternal blood was collected later in the 3rd trimester, we anticipated increases in pro-inflammatory biomarkers. This is due to an increasing pro-inflammatory environment during the final trimester of pregnancy as women get closer to delivery. [42,77,81] Interestingly, all exercisers tended to have higher TNF-α relative to CON at 36-wks gestation, which was unexpected. This is in contrast to another study that noted women who did AE before and throughout gestation had lower TNF-α compared to active controls.[31] However, it is important to note that this study collected maternal blood 2-4 hours postprandially. Meal composition can alter plasma TNF-α and, to the best of our knowledge, was not controlled thus making it challenging to draw comparisons.[31,82] Pregnant women who participate in CE also have lower TNF-α in late pregnancy and reduced increase to IL-1ß.[73] However, CE in this intervention had 2 sessions/week of RE with 1 session/week of AE, compared to our study which split every CE session equally between RE+AE.[73] Among exercisers, trends for TNF-α in our AE and RE groups showed that AE had the lowest TNF-α, followed by RE. Notably, higher concentrations of maternal TNF-α are associated with adverse pregnancy outcomes yet preeclamptic pregnancies and poor perinatal outcomes are associated with lower umbilical cord TNF-α.[7,8,75,83] Regardless, TNF-α in maternal blood for all groups are similar to that or slightly lower than previously reported.[31,73] These findings suggest that TNF-α may respond to either AE or RE better than CE, but regardless, exercise of any mode investigated in the current study, does not negatively impact overall maternal inflammation or pregnancy health. Lastly, in agreement with previous studies, we did not see differences in maternal IL-8 between groups.[73,84] However, we did find all exercise modes *trended* towards lower pro-inflammatory IL-8 and anti-inflammatory IL-10 in late pregnancy relative to CON. Higher IL-8, IL-6, TNF-α and IL-10 are associated with maternal depressive symptoms and pregnancies threatened of preterm labor.[75,85] Our findings

suggest that, exercise of any type as investigated in the current study could result in lower concentrations of these biomarkers, thus resulting in improved outcomes.

Aside from exercise mode, we found greater weekly exercise duration was predictive of IL-6 and IL-8 respectively. Previously, while research in exercising middle-aged men and women found no significant differences in IL-8, women did have increased exercise duration and typically lower concentrations of IL-8, relative to men.[86] We also found that exercise duration was predictive of IL-6, which did not match that found in the previous research.[86] However, the aforementioned study collected their blood samples shortly post-exercise from healthy adults and only observed IL-6 across 4 days of activity. Our study collected blood from pregnant women following an overnight fast (~≥12 hours post exercise) and ~24-wks of exercise training. IL-6 can be either pro- or anti-inflammatory.[87] However, basal IL-6 concentrations are a better representation of general pro-inflammatory status.[87] Thus, the results of the present study could better represent the effect of training on resting IL-6 concentrations in pregnancy and suggest an reduced inflammatory state.

We found that weekly exercise frequency was predictive of higher TNF-α levels. Heavy exercise is thought to increase inflammatory biomarkers (i.e., TNF-α) due to increases in cellular damage that occurs as a response to the activity itself, then return towards resting concentrations by 24 hours post-exercise.[88,89] Although our exercise intervention was moderate-intensity, it is possible that more frequent exercise sessions could have resulted in less time for TNF-α to "recover" post-session. Similarly, while we collected blood following an overnight fast with ~≥12 hours post-exercise, we did not however control for the time of the last exercise session between participants. Therefore, the relationship between exercise frequency and increased TNF-α could result be a result of reduced recovery time between sessions before fasting blood was collected. However, the relationship between exercise frequency and TNF-α needs to be further defined.

This study has strengths that warrant mention. First, the current analysis is the first to directly compare the effect of different exercise modes on inflammatory biomarkers in pregnancy.

Second, we utilized a randomized control trial with a supervised exercise intervention design, which is the strongest evidence for causality. Finally, the methods used for analyzing inflammatory biomarker concentration is a sensitive and precise technique and has been utilized by other studies, allowing for comparisons. While our study has many strengths it is also not without its limitations. First, we had a small sample size, which may be why there is a lack of statistical difference. In addition, due to the small sample size we were not able to control for exercise attendance, maternal stress, or infant sex, which all has the capability to influence our results.[90,91] More research is needed with a more robust sample size to fully elucidate whether a specific type of exercise has a greater impact on maternal TNF-α concentration in late pregnancy.

In conclusion, this study provides novel insights into the effect of exercise mode, duration, and frequency on maternal inflammation during pregnancy. While we found that all exercise modes had similar inflammatory biomarker concentrations in late pregnancy, weekly exercise duration and frequency predicted some of the inflammatory biomarkers of interest, which may provide insights for future exploration. Additionally, all exercisers experienced decreases in IL-8 and IL-10 with increases in TNF-α relative to CON but differed in magnitude depending on mode. Our results indicate that exercise metrics (e.g., mode, frequency, duration) during pregnancy may differentially impact inflammatory biomarkers and should be explored further. Overall, we found no negative impact of exercise on maternal biomarkers during pregnancy, suggesting that the exercise modes investigated in the current study are safe for women during pregnancy.

Tables and Figures

Table 2.1. Maternal and Delivery Descriptors for Maternal Inflammatory Biomarker Analysis

	CON n= 13	AE n= 14	RE n= 14	CE n= 15	p-value
BIPOC[#]	23.1%	21.4%	7.1%	6.7%	0.44
Age (yrs)	30.77 ± 4.71	30.71 ± 3.65	32.21 ± 5.66	30.07 ± 5.55	0.70
Pre-pregnancy BMI (kg/m^2)[$]	28.78 ± 3.56	27.39 ± 5.17	24.99 ± 4.08	27.74 ± 5.30	0.08
Exercise Attendance (%)	63.62 ± 35.05	89.74 ± 10.07**	87.37 ± 9.08*	88.60 ± 9.61**	**0.002****
Total Exercise Volume (MET*min)	6015.04 ± 3296.43	12265.13 ± 3118.75**	12199.73 ± 2494.16**	10725.45 ± 5468.99*	**<0.001*****
GWG (kg)	14.39 ± 4.76	15.76 ± 4.82	15.27 ± 4.82	17.03 ± 6.14	0.60
Gestation Age (wks)	39.83 ± 0.92	39.96 ± 1.01	39.95 ± 0.78	39.95 ± 1.15	0.99
Infant Sex (%F) [#]	30.8%	50.0%	50.0%	20.0%	0.25

All are Mean ± SD. BIPOC = Black or Indigenous People of Color; BMI = body mass index; MET = metabolic equivalents. GWG = gestational weight gain. [$]- Kruskal Wallis test used due to non-normal unconditional distributions. Bonferroni correction. [#]- Chi Square test for between group differences due to categorical data. * = p<0.05, ** = p<0.01, *** = p<0.001 vs CON

Table 2.2. Maternal plasma concentrations of cortisol, CRP, TNF-α, IL-6, -8, -1ß, and -10 at enrollment (≤ 16-wks') and 36-wks gestation

	Enrollment (≤16-wk)					36-wks' Gestation				
	CON n= 8	**AE** n= 9	**RE** n= 9	**CE** n= 9	**p-value**	**CON** n= 8	**AE** n= 10	**RE** n= 9	**CE** n= 8	**p-value**
Cortisol[$] (ng/mL)	62.14 ± 30.32	54.51 ± 29.48	58.60 ± 32.08	72.44 ± 44.01	0.74	90.63 ± 45.71	89.71 ± 57.46	103.77 ± 95.34	82.66 ± 52.33	0.84
CRP[$] (ng/mL)	0.77 ± 0.35	0.74 ± 0.68	0.67 ± 0.46	0.62 ± 0.44	0.84	1.10 ± 0.98	1.36 ± 1.48	0.77 ± 0.71	1.93 ± 1.81	0.44
TNF-α[$]	4.56 ± 3.76	2.32 ± 1.60	2.51 ± 1.02	3.30 ± 2.16	0.48	2.75 ± 1.17	3.16 ± 1.49	3.55 ± 2.42	4.18 ± 2.85	0.85
IL-6[$]	16.35 ± 17.75	48.73 ± 41.95	15.58 ± 21.70	26.42 ± 34.96	0.70	19.92 ± 14.45	45.01 ± 46.49	9.37 ± 12.88	10.77 ± 6.14	0.54
IL-8[$]	8.25 ± 5.76	5.47 ± 0.74	2.32 ± 1.24	5.48 ± 4.86	0.10	9.48 ± 5.86	3.20 ± 2.34	2.68 ± 1.57	4.57 ± 2.77	0.16
IL-1ß	33.64 ± 28.96	35.99 ± 27.96	33.15 ± 25.97	25.36 ± 11.69	0.96	60.50 ± 22.39	62.13 ± 52.95	42.65 ± 55.47	43.19 ± 34.62	0.89
IL-10^	11.33 ± 0.86	**6.18 ± 2.32*^**	9.12 ± 2.63	**4.56 ± 2.10**^**	**0.001****	18.11 ± 13.28	4.62 ± 2.93	7.25 ± 4.28	11.01 ± 7.31	0.16

IL = interleukin, TNF-α = tumor necrosis factor alpha; CRP = c-reactive protein. All biomarkers are measured by picograms per milligram unless otherwise noted. [$]- Kruskal-Wallis test used for both enrollment and 36-wk timepoints due to non-normal distributions. ^-Kruskal-Wallis test used for 36-wk timepoint only due to non-normal distribution. Bonferroni correction. *p<0.05, **p<0.001 vs CON. ^p<0.05 vs RE

Table 2.3. ANCOVA Models for 36 wk Maternal Plasma Biomarkers.

			F	Partial Eta Squared (n^2)	p-value
TNF-α (Model 1)	**R^2=0.76**	**p=0.003**			
Enrollment TNF-α			30.67	0.70	0.00
TNF-α (Model 2)	**R^2=0.86**	**p=0.003**			
Enrollment TNF-α			44.13	0.83	0.00
Weekly Exercise Frequency			6.36	0.41	0.03

Corrected model 1: Enrollment (≤16wks) TNF-α, pre-pregnancy BMI, gestational weight gain, race
Corrected Model 2: Enrollment (≤16wks) TNF-α, weekly exercise duration, weekly average intensity, weekly exercise frequency

Table 2.4. Regression Models for 36 wk Maternal Plasma Biomarkers.

			Estimate	STD Error	t Ratio	Lower 95%	Upper 95%	p-value
IL-6 (Model 1)	**R^2 = 0.90**	**p = 0.02**						
Enrollment [IL-6]			0.90	0.23	3.97	0.32	1.47	0.01
Weekly Exercise Duration			-0.02	0.01	-2.97	-0.04	-0.003	0.03
IL-8 (Model 2)	**R^2 = 0.64**	**p = 0.04**						
Enrollment [IL-8]			0.72	0.23	3.16	0.21	1.23	0.01
Weekly Exercise Duration			-0.01	0.01	-2.30	-0.02	-0.0004	0.04
TNF-α (Model 3)	**R^2 = 0.85**	**p = 0.003**						
Enrollment [TNF-α]			1.07	0.16	6.64	0.71	1.44	0.00009
Weekly Exercise Frequency			0.50	0.20	2.52	0.05	0.96	0.03

Model 1: Enrollment IL-6 concentration, weekly exercise duration, exercise group, weekly exercise intensity (METs)
Model 2: Enrollment IL-8 concentration, weekly exercise duration, exercise group
Model 3: Enrollment TNF-α concentration, exercise session frequency, weekly exercise intensity (METs), weekly exercise duration, exercise group
*p<0.05, **p<0.01, ***p<0.001

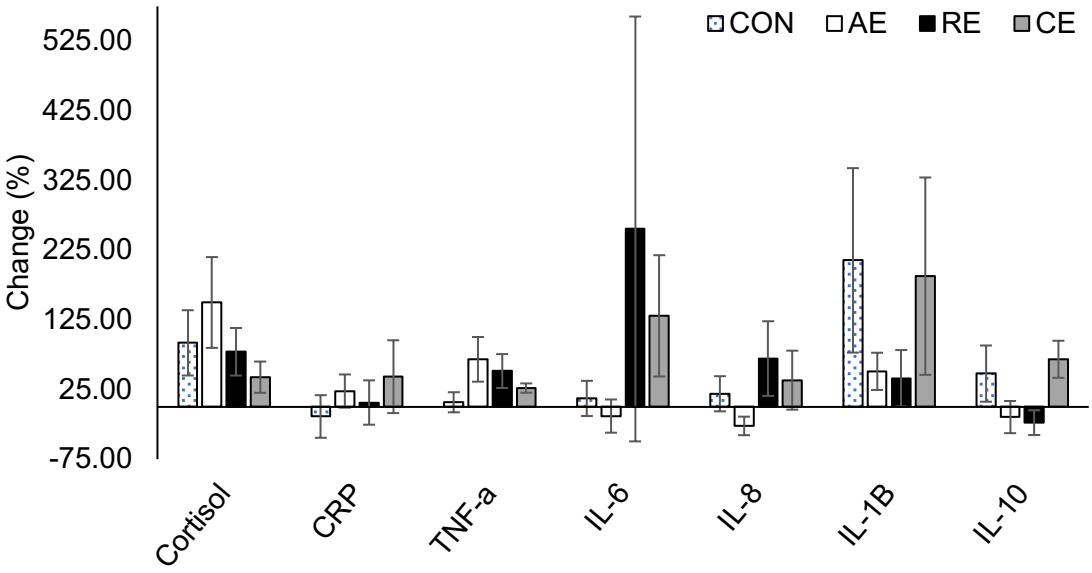

Figure 2.1. Percent change from Enrollment (≤16-wks) to 36-wk Maternal Plasma biomarkers. c-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukins (IL)-6, -8, -1ß, and -10. Values are shown as Mean +/- SEM. CON = control, AE = aerobic exercisers, RE = resistance exercisers, CE = combination (aerobic + resistance) exercisers. p>0.05 for all biomarkers between groups.

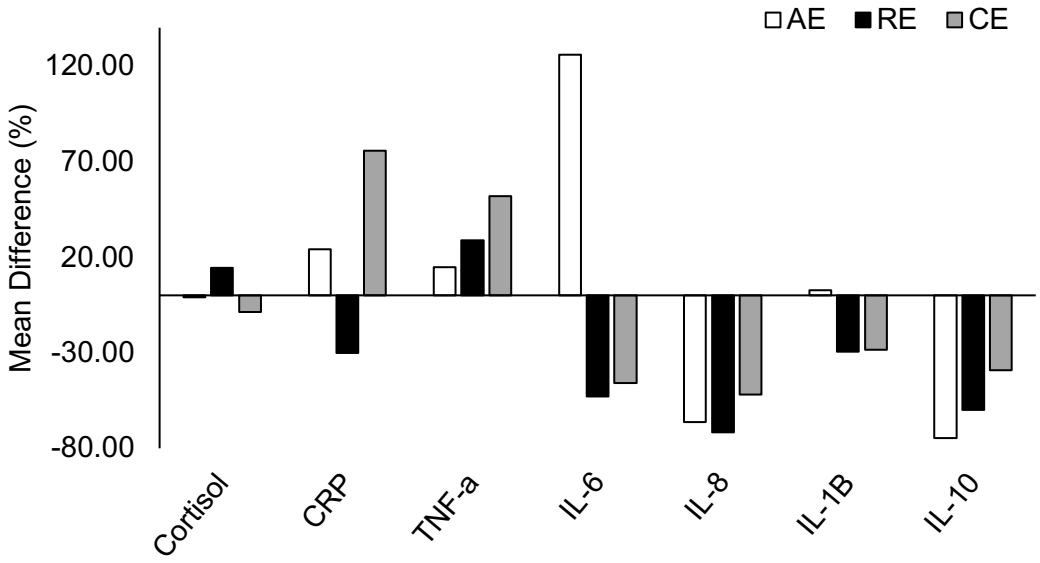

Figure 2.2. Mean percent difference in maternal plasma 36-wk biomarkers from controls. c-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukins (IL)-6, -8, -1ß, and -10. AE = aerobic, RE = resistance, CE = combination (aerobic + resistance) exercise.

CHAPTER 3

Effect of Exercise Mode during Pregnancy on Placental Inflammatory Biomarkers

ABSTRACT

Excessive inflammation in the maternal environment alters placental function and expression of inflammatory biomarkers. Pathologies at the maternal-fetal interface, such as spontaneous abortion, are also have higher pro-inflammatory biomarkers and impact the fetal development. Exercise during pregnancy appears to reduce maternal and infant inflammatory biomarkers which, in part, may be due to improvements to the placental tissue. In non-gravid individuals, exercise mode differentially reduces inflammatory biomarkers. However, it is unclear how exercise modes during pregnancy directly impact placental inflammation. Therefore, the aim of this project was to compare the influence of aerobic (AE), resistance (RE), or combination (AE+RE; CE) exercise during pregnancy on inflammatory biomarkers in placental tissue. A total of 76 women with no adverse pregnancy outcomes were randomized to 150-minutes of supervised moderate intensity AE (n=18), RE (n=17), CE (n=21) or control (CON; n=20). Placenta tissue was collected at delivery and analyzed for tumor necrosis factor alpha (TNF-α); interleukins (IL) -6, -8, -1ß; c-reactive protein (CRP); fibrinogen; and cortisol using Luminex xMAP technology. A label-free proteomics analysis was completed using LC-MS/MS. Database search and protein filters were done with biological replicates for each group (n=10/group). On average, participants similar in age, weight gain, gestation age, and infant sex ($p>0.05$). Pre-pregnancy BMI was lower in RE relative to CON ($p<0.05$). Exercise attendance and total exercise volume were higher for exercisers compared to CON ($p>0.05$). Targeted inflammatory biomarkers in placental tissue were similar between groups ($p>0.05$). After correcting for race, gestational weight gain, pre-pregnancy BMI, infant sex, delivery mode, and group differences, the corrected model for IL-1ß was significant ($R^2=0.42$, $p = 0.04$) for maternal race ($p=0.02$). Linear regressions found exercise intensity to predicted lower placental fibrinogen ($p=0.049$). Placental proteome landscapes for AE, RE, and CE differed from CON ($p<0.001$) and exercisers had a decreased abundance of proteins

that related to the humoral and innate immunity. These findings could be reflective of reduced pathogen exposure during pregnancy, resulting in lower immune proteins, and thus reducing inflammation downstream. Collectively, our findings support that any exercise mode investigated in the current study is safe during pregnancy and may benefit placental inflammation.

INTRODUCTION

The first and third trimesters of pregnancy are characterized by increased pro-inflammation to support proper implantation, placentation, and parturition.[92] However, excessive inflammation during pregnancy is associated with adverse pregnancy outcomes (APOs), like gestational diabetes (GDM), preeclampsia (PE), and intrauterine growth restriction (IUGR).[4,7–10] Pathologies of the maternal-fetal interface, such as endometriosis, recurrent spontaneous abortion, and placental abruption, are also associated with high pro-inflammatory and decreased anti-inflammatory biomarkers.[42,92] Exercise is effective at reducing the amount of pro-inflammatory biomarkers (e.g., IL-1ß, IL-6, TNF-α, CRP) in non-pregnant populations[36,38,68] and, interestingly, may differ depending on exercise mode. Older adults participating in aerobic exercise (AE) have reduced blood IL-6, CRP, and TNF-α, whereas those participating in strength and flexibility exercise only decrease TNF-α from pre- to post-intervention.[38] Similarly, a recent review of exercise mode on inflammation in diabetics reports that AE alone and resistance exercise (RE) alone each reduce pro-inflammatory biomarkers (e.g., CRP, IL-6, IL-1ß) while combined aerobic + resistance exercise (CE) has greater reductions for most pro-inflammatory biomarkers compared to AE and RE alone.[36]

During pregnancy, exercise is safe and beneficial for decreasing APO risk,[21–23] enhancing placental development and perfusion,[64,93,94] and reducing inflammation.[31,32,35,72,73] Specifically, supervised AE lowers TNF-α in maternal serum relative to intermittently physically active controls.[31] Similarly, pregnant women that achieved 11,000 steps/day have lower CRP in late pregnancy compared to those who did less steps and less than controls.[35] CE during pregnancy

decreases TNF-α in maternal serum compared to controls, but not IL-1ß, IL-6, or IL-8.[73] However, there is no research directly comparing AE, RE, and CE on placental inflammatory biomarkers.

Considering the critical role the placenta has between maternal and fetal environments during pregnancy and how it can be impacted in pathologies of the maternal-fetal interface, it is critical to understand how exercise of different modes impact inflammatory biomarkers in placental tissue. Thus, the current study examined the effect of exercise mode on placental inflammatory biomarkers. We hypothesized that the placenta tissue of exercisers (AE, RE, and CE) would have lower concentrations TNF-α, IL-6, IL-1ß, and CRP relative to CON and that, amongst the exercisers, CE would have the lowest concentrations.

METHODS

Study Design and Participants. This analysis pooled samples of women who were recruited from three randomized controlled trials (RCTs) to examine the effect of exercise mode on inflammatory markers in placental tissue. The RCTs each recruited women with singleton-pregnancies that were low-risk and randomized them to either 150-minutes of moderate-intensity exercise (aerobic, resistance, or combined aerobic + resistance) or control. Women were included in the studies if they met the following criteria: pre-pregnancy body mass index (BMI) $\geq$18.5 kg/m^2, aged 18-40, and $\leq$ 16-weeks' gestation. All participants were required to received clearance to participate in exercise during pregnancy by their obstetric provider. Women were excluded if: 1. they did not meet these criteria or 2. if they reported having a medical condition that alters fetal development (e.g., HIV/Aids, diabetes, untreated hypertension, etc.) or the use of tobacco products, alcohol, drugs, or medications (e.g., oral hypertensive, insulin). Upon enrollment, written informed consent was obtained. All protocols were approved by East Carolina University (ECU) Institutional Review Board, registered in clinicaltrials.gov (NCT03517293 and NCT04805502), and were conducted at ECU.

Pre-intervention Testing & Randomization. Prior to randomization, participants were asked to complete a Modified Balke treadmill test, to calculate their specific target heart rate (THR) range that corresponded to moderate intensity, as previously validated. [79] Throughout the test, maternal heart rate (HR) was monitored (Polar FS2C HR monitor) and oxygen consumption and carbon dioxide production were collected via indirect calorimetry (Parvo Medics, TrueOne2400, Sandy UT) to estimate peak oxygen consumption (VO_{2peak}). Between March 2020 and October 2021 THR zones were estimated from the participant's pre-pregnancy physical activity levels and age to minimize exposure during the COVID-19 pandemic. All THR zones reflected moderate intensity, corresponding with maternal HR at 60-80% VO_{2peak}. Participants were also asked to perform a 1-repetition max (1RM) test before randomization to establish baseline strength, which was used to program resistance exercises. After pre-intervention testing was complete, women were randomized to either aerobic (AE) exercise, resistance (RE), combination (aerobic + resistance; CE) exercise, or a stretching/breathing attention control group (CON) via computer sequencing (GraphPad, Boston, USA).

Exercise Intervention. All exercise sessions were overseen by trained instructors in ECU facilities following standard protocols.[80] Exercisers were asked to do 150-minutes of moderate-intensity activity each week until delivery. Exercise sessions each consisted of a 5-minute warm-up, 50-minutes of AE, RE, or CE activities based on their randomized group, and a 5-minute cool-down. Exercise intensity was monitored throughout the sessions via maternal HR and the Borg scale of perceived exertion (RPE 6-20). Participants were asked to be within their THR and at an RPE of 12-14 to reflect moderate intensity throughout the 50-minute exercise bout.

The AE group self-selected available aerobic equipment such as treadmills, ellipticals, cycle ergometers, rowers, and/or stair stepping equipment for their exercise sessions. The RE group completed programmed workouts consisting of 2-4 sets of exercises targeting major muscle groups at 60% 1RM aiming for 12 repetitions of each exercise. These exercise sessions utilized

machines (Cybex), dumbbells, resistance bands, exercise balls, benches, and/or mats. Women in the CE group were asked to spend half their exercise sessions completing AE activities and the other half of the session doing programmed RE activities. The AE and RE activities performed by the CE group were the same as outlined for each respective activity. Those randomized to CON were invited to participate in weekly sessions of breathing/stretching to enhance participant retention. CON participants performed 50-min of stretching activities led by study team member following a 5-min warm-up. Maternal HR was monitored throughout the stretching activity to ensure HR remained below moderate intensity (<50% VO_{2peak}).

Exercise Metrics. Exercise metrics were collected each week and throughout the study to compare exercise dose and included volume, intensity, duration, and frequency. Exercise session volume (MET*min), duration, and frequency were calculated following every session and totaled for the week. Weekly exercise duration, intensity (METs), and frequency were totaled and divided by the number of weeks training across pregnancy. Exercise volume (MET*min) was also calculated across the pregnancy to examine the exercise dose of the intervention. Participant attendance was calculated using the total number of sessions attended each week divided by the number expected (i.e., 3 per week). An attendance threshold of 75% was set for all exercisers (AE, RE, CE), as this successfully impacts maternal and cord inflammatory biomarkers.[73]

Maternal & Delivery Measures. Maternal descriptors (age, race, ethnicity, height, and pre-pregnancy weight) were obtained via questionnaire at enrollment. Self-reported height and weight were then used to calculate pre-pregnancy BMI. For analysis, Black or African American, Asian, Native Hawaiian or other Pacific Islander, Native American or Alaskan Native, and Latinx participants were grouped as Black, Indigenous, People of Color (BIPOC).

Infant sex, delivery mode, medical conditions, incidence of adverse pregnancy condition (e.g., gestational diabetes, intrauterine growth restriction, or preterm birth), and medications were

collected at delivery via electronic health record. Using delivery weight reported in the medical record, gestational weight gain (GWG) was calculated by subtracting the self-reported pre-pregnancy weight from the delivery weight.

Placenta Tissue Processing. Upon delivery the whole placenta, umbilical cord, and tissue surrounding the placenta were collected. At time of collection, approximately 3x3 cm sections of the placental tissue were collected from the villous, chorionic, and basal layers in a peripheral and central location, as determined by the umbilical cord insertion. Following collection, each sample was placed in a 2 mL microcentrifuge tube, flash frozen using liquid nitrogen, then stored at -80°C until tissue homogenization.

Placenta Tissue Homogenate Preparation. Samples from each layer of the peripheral and central placental disk were broken into ~33mg pieces using a mortar pestle in liquid nitrogen, weighed using a standard scale, and combined for homogenization to represent the entire placenta (~200mg total). Samples were placed on ice and combined with 1mL lysis buffer (Merck, Darmstadt, Germany) containing protease inhibitor cocktail (cOmplete Tablets Mini, EDTA-free protease inhibitor cocktail tablets, Roche, Switzerland). Samples were homogenized using a stand homogenizer (Ultra-Turrax, IKA) then centrifuged for 15 minutes at 4°C, 10,000xg. To minimize freeze/thaw cycles, the homogenate was separated into 3 aliquots (~200 uL each) and stored at -80°C until processing.

Placental Protein Quantification. One placental homogenate aliquot was used to measure total protein content using Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford IL, USA) following multiplex assays. Total protein concentration was controlled by dividing the total biomarker concentration by total protein content in each homogenate.

Quantification of Placental Inflammatory Biomarkers. Two aliquots of placental homogenates were used to quantify the concentration of cortisol, CRP, fibrinogen, IL-6, IL-8, IL-1ß, and TNF-α using MILLIPLEX MAP kits. MILLIPLEX Map kit standards, controls, and samples were prepared using manufacturer's instructions. Sample plates were read on a Luminex 200 using xMAP technology. Analyte concentrations were determined using xPONENT software. Cortisol was assessed using the Human Circadian/Stress Magnetic Bead Panel kit. To examine CRP and fibrinogen, the Human Cardiovascular Disease (CVD) Magnetic Bead Panel 3 (Acute Phase) kit was used. Finally, for all the cytokines, TNF-α, IL-1ß, IL-6, and IL-8, the Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Sigma, Burlington, MA, USA) kit was used. Samples were run in duplicate to determine the concentration of cortisol, CRP, fibrinogen, IL-6, IL-8, IL-1ß, TNF-α, and averaged. Samples with average concentrations and a coefficient of variation (CV) <20% were used in the current analysis to limit variability. Mean percent difference was calculated for AE, RE, and CE relative to CON.

Label-Free Proteomics Analysis.

Preparation of mass spectrometry grade peptides from placenta tissue. Total protein concentration was adjusted following BCA protein quantification to 2 ug/uL with a modified lysis buffer, where the protease inhibitor was removed. Proteins were precipitated from 50 uL of sample (~100 ug protein) using ice cold methanol (3:1 v/v) at -20°C and pelleted by centrifugation. The pellets were washed 2 times with ice cold methanol and air dried before further use. Mass spectrometry grade peptides were prepared using PreOmics iST kits (PreOmics GmbH). Precipitated proteins were resuspended in the provided lysis/denaturing buffer, denatured, and alkylated at 95°C for 10 min in a 1.5 mL microcentrifuge tube. Digest reagent was added after cooling to room temperature, and proteins were digested while shaking (600 rpm) for 3 hours at 37°C. Samples were loaded onto the provided microcolumns for purification. Peptides were eluted

using elution buffer via centrifugation, dried under a N2 stream, then resuspended in loading buffer (98:2 water: acetonitrile + 0.1% formic acid) at a concentration of 0.25 mg/mL.

LC-MS/MS for Label-Free Proteomics. Peptides were analyzed by nanoLC-MS/MS using an UltiMate 3000 RSLCnano system (ThermoFisher) coupled to a Q Exactive Plus Hybrid Orbitrap mass Spectrometer (ThermoFisher) via nanoeslectrospray ionization. Peptides were separated using an effective linear gradient of 4-35% acetonitrile (0.1% formic acid) over 135 min. For data dependent acquisition in positive mode, MS spectra were acquired in positive mode. MS1 were performed at a resolution of 70,000 with an AGC target of $2x10^5$ ions and a maximum injection time of 100 ms. MS2 spectra were collected on the top 20 most abundant precursor ions with a charge >1 using an isolation window of 1.5 m/z and fixed first mass of 140 m/z. The normalized collision energy for MS2 scans was 30. MS2 spectra were acquired at 17,500 resolutions with a maximum injection time of 60 ms, an AGC target of $1x10^5$ and a dynamic exclusion of 30 sec.

Database Search and Protein Filtering. FragPipe (v 19.1) [95,96] was used for raw data analysis with default search parameters for open and Label-Free Quantification Matching Between Runs (LFQ-MBR) workflows. Placenta tissue was searched individually with biological replicates (n=10) for each exercise group identified. An initial open search against the canonical + isoforms Uniprot *Homo sapiens* reference proteome (UP000005640, accessed 11/2023) was used to identify potential post-translational modifications for inclusion in the LFQ-MBR workflow. Precursor m/z tolerance was set to -150 to 500 Da and fragment tolerance was ± 20 ppm with 3 missed cleavages for Tryp and Lys-C allowed. Peptide spectrum matches (PSMs) were validated using PeptideProphet and results were filtered at the ion, peptide, and protein level with a 1% false discovery rate (FDR). Based on these initial searches, the following variable modifications were included in the LFQ-MBR analysis: oxidation (+15.5995 Da on Met), deamidation (+0.98401

Da on Gln and Asn), and fixed modification carbamidomethyl (+57.025 Da on Cys). For LFQ-MBR analysis, data were search against the canonical Uniprot *Homo sapiens* reference proteome (UP000005640, accessed 11/2023 and 1/2024). Precursor ion m/z tolerance was ± 20 ppm with 3 missed cleavages for Trypsin/LysC allowed. The search results were filtered by a 1% FDR at the ion, peptide, and protein-level. PSMs were validated using Percolator and label free quantification was carried out using IonQuant [97] Match between runs FDR rate at the ion level was set to 10% for the top 300 runs. Proteins with >95% probability of ID, >2 unique peptides, and in more than 80% of a sample group (*i.e.* 7/10 injections) were considered high confidence IDs and retained for analysis. Intensities were $\log_2$ transformed, normalized to median intensities of the sample group. Relative abundances for low sampling proteins were determined via normal distribution in Perseus.[98]

Statistical Analyses. Power analysis using preliminary data of the primary outcome variable, TNF-α, found that a total of 48 participants (12/group) was needed to reach significance of $p<0.05$ with 80% power. Women with an adverse pregnancy condition (e.g., GDM, IUGR, etc.) were excluded since there is a link between these conditions and increased pregnancy inflammation.[4,8,9] First, all extreme outliers, identified using boxplots, were removed. Potential outliers were removed if there was justification for doing so (i.e., issue during processing affecting sample reliability, medical condition influencing biomarker, etc.). Since research exploring the effect of pregnancy exercise on placenta tissue inflammation is largely unexplored, potential outliers were thoroughly checked since these could be indicative of inherent variability. Following outlier removal, data were checked for normality then one-way ANOVAs were used to examine between group differences. Data that was non-normally distributed were run using Kruskal-Wallis non-parametric tests. Post-hoc testing for both ANOVAs and Kruskal-Wallis utilized a Bonferroni correction factor. Statistical significance was set at $p<0.05$ for all analyses. Analysis of covariance (ANCOVAs) were used to evaluate covariates such as pre-pregnancy BMI, race, GWG, infant

sex, and delivery mode. Testing for between group differences and ANCOVAs were conducted using IBM SPSS Statistics for Mac, version 27 (IBM Corp., Armonk, NY, USA). Finally, regression analyses were performed to determine significant predictors of the outcome variables using JMP Statistics for Mac, version 17 (JMP Statistical Discovery, Cary, NC, USA).

RESULTS:

Quantification of Placental Inflammatory Biomarkers

Study population. Placental tissue was analyzed from 95 participants. In the analysis to quantify the biomarkers of interest (cortisol, CRP, fibrinogen, IL-6, IL-8, IL-1ß, TNF-α), 8 were removed due to an adverse pregnancy outcome (i.e., GDM, IUGR) or other condition known to affect inflammation (e.g., Sjogren's syndrome, thrombocytopenia, etc.); 3 were removed due to medication known to alter inflammation (e.g., selective serotonin reuptake inhibitors); and 8 were removed due to low attendance (<75%; 3 AE, 4 RE, 1 CE). This left a total of 76 samples for analysis (20 CON, 18 AE, 17 RE, 21 CE). In general, groups were similar for maternal descriptors, except the resistance group had a lower pre-pregnancy BMI (Table 3.1). As expected, all exercise groups had greater total exercise volume and attendance (Table 3.1).

Inflammatory biomarker concentration. One additional outlier for cortisol was removed due to discovery of a medical condition that is known to impact that specific biomarker. [99] After review, all other potential outliers were included in the final dataset for analysis, as there was no justification for removal and may be the result of inherent variability. After datapoint removal for outliers, CV >20%, and datapoints that were determined to be outside of each kits limit of detection, sample size for each biomarker varied. Placental concentrations of cortisol, CRP, fibrinogen, IL-6, IL-8, IL-1ß, and TNF-α are similar between groups (Table 3.2).

Using CON group biomarker concentration as the reference, average biomarker concentration percent difference was calculated for each biomarker in the AE, RE, and CE groups

(Figure 3.1). Relative to the CON, AE had lower average concentrations for all biomarkers; RE had lower average concentrations for cortisol and fibrinogen, while CRP, IL-6, IL-8, IL-1ß, and TNF-α had higher averages; and CE had lower average concentrations of cortisol, fibrinogen, IL-6, and IL-8 with increased average concentration of CRP, IL-1ß, and TNF-α (Figure 3.1).

ANCOVAs and Regressions. Due to non-normal distribution, all biomarkers were $\log_{10}$ transformed to meet the normality assumption for ANCOVA and regression testing. All biomarkers were run in ANCOVA analysis to adjust for potential covariates. After adjusting for pre-pregnancy BMI, maternal race, gestational weight gain, infant sex, and delivery mode, there were no between group differences for cortisol, CRP, fibrinogen, IL-6, IL-8, or TNF-α ($p>0.05$; data not shown). The corrected model was significant for IL-1ß ($F = 1.66$, $p = 0.04$, $n^2 = 0.64$) with race being the only significant covariate ($p=0.02$, Table 3.3). After adjusting for weekly exercise session frequency, duration, and average weekly intensity (METs), there were no between group differences for cortisol, CRP, fibrinogen, IL-6, IL-8, IL-1ß, and TNF-α ($p>0.05$, data not shown).

Linear regression established that average weekly exercise intensity (METs) predicted fibrinogen concentration (Table 3.4). All other models were not significant (data not shown).

***Label-Free Proteomics*.**

Study Population. In the global proteomics analysis, 40 samples (10/group) were randomly selected from the original 95 homogenates for analysis to represent the 4 groups. In general, the groups were similar in all maternal and infant descriptors (Table 3.5). As expected, total exercise volume (MET*min) was higher in each of the exercising groups, but did not differ between AE, RE, and CE (Table 3.5).

Between-group Protein Abundances. There are 787 proteins with differential abundances between AE (471), RE (212), or CE (104) compared to CON (Figure 3.2). Firstly, the AE placental

proteome demonstrated 471 proteins ($p<0.001$) with differential abundances compared to CON placentas (Figure 3.2). After Log_2 fold change parameters were set to >1.2 or <-0.8, the AE placental proteome had 77 proteins with differential abundances (73 lower, 4 higher) compared to CON placentas. Of the 77 proteins, 14 (13 lower, 1 higher in abundance) in the AE group were identified to potentially have a role in inflammation and immunity: macrophage mannose receptor 1 (MRC1), dematin (DMTN), immunoglobulin kappa variable 2-28 (IGKV2-28), adrenodoxin (FDX1), complement C4-B (C4B), major vault protein (MVP), galectin-3-binding protein (LGALS3BP), immunoglobulin kappa variable 1D-13 (IGKV1D-13), SH3KBP1-binding protein 1 (SH3KBP1), tyrosine-protein kinase Lyn (LYN), kinectin (KTN1), low affinity immunoglobulin gamma Fc region receptor II-b (FCGR2B), transforming growth factor beta-1-induced transcript 1 protein (TGFB1I1), and macrophage migration inhibitory factor (MIF). Pathway analysis resulted in no significant findings (data not shown).

The RE placental proteome had 212 proteins with differential abundances ($p<0.0001$) relative to the CON placental proteome (Figure 3.2). After the Log_2 fold change parameters, 54 proteins (44 higher, 10 lower) were different between RE and CON placentas. Of the 44 differential proteins between RE and CON placentas, 9 proteins (8 lower, 1 higher) were found to be related to inflammation or immune function (Figure 3.2): C4b-binding protein alpha chain (C4BPA), dematin (DMTN), complement factor H-related protein 1 (CFHR1), probable non-functional immunoglobulin kappa variable 3-7 (IGKV3-7), immunoglobulin heavy constant alpha 1 (IGHA1), immunoglobulin kappa variable 1-6 (IGKV1-6) and 1D-33 (IGKV1D-33), complement C1s subcomponent (C1S), and growth factor receptor-bound protein 2 (GRB2). Pathway analysis resulted in no significant findings (data not shown).

CE placentas had 104 proteins with differential abundances ($p<0.0001$) compared to the CON placentas (Figure 3.2). Log_2 fold change identified 30 proteins with differential abundances between CE and Con placentas. Of these 30 proteins, 18 were lower and 12 proteins were in higher abundance in CE placentas relative to CON placentas. Of these 30 proteins, 8 proteins all

lower in abundance, are related to inflammation or immunity (Figure 3.2): sterol-4-alpha-carboxulate 3-dehydrogenase (NSDHL), immunoglobulin lambda variable 3-9 (IGLV3-9), C4b-binding protein alpha chain (C4BPA), probable non-functional immunoglobulin kappa variable 3-7 (IGKV3-7), DNAJ homolog subfamily c-member 13 (DNAJC13), pregnancy specific beta-1-glycoprotein 5 (PSG5), immunoglobulin heavy constant alpha 1 (IGHA1), and SH3KBP1-binding protein 1 (SH3KBP1). Pathway analysis resulted in no significant findings (data not shown).

Across exercise groups, 1 protein, eukaryotic translation initiation factor 3 subunit K (EIF3K), was in higher abundance in all 3 groups relative to CON placentas. Collectively, the 29 proteins (13 AE, 8 RE, 8 CE) lower in abundance in the placentas of the exercisers that related to inflammation or immunity generally related to adaptive immunity, antigen recognition, and pathways associated with the release of inflammatory molecules (e.g., AMPK, EGFR, JAK-STAT). Whereas the 2 proteins (1 AE, 1 RE) found higher in abundance in placentas of exercisers were related to innate immune response and the MAPK/ERK pathway.

DISCUSSION:

The goal of the current study was to examine the effect of exercise mode on placental inflammatory biomarkers. We hypothesized that the placenta tissue of exercisers (AE, RE, and CE) would have reduced TNF-α, IL-6, IL-1ß, and CRP relative to CON group, with the CE exercisers having the lowest concentrations. Contrary to our hypothesis, we found similar levels of inflammatory biomarkers between groups. However, we found interesting trends specific to exercise group, that exercise intensity predicts placental fibrinogen, and that placentas from exercisers had different proteome profiles compared to the CON.

The current study observed similar levels of cortisol, CRP, fibrinogen, IL-6, IL-8, IL-1ß, and TNF-α between the groups. Previous research shows that exercise positively impacts placental development and reduces systemic pro-inflammatory biomarkers.[32,64,93,94] Ramírez-Vélez and colleagues report placental tissues of CE have increased nitric oxide and endothelial nitric

oxide.[64] Endothelial nitric oxide has an anti-inflammatory effect under normal physiological conditions but pro-inflammatory under pathological conditions.[100] Considering the healthy population in the present study, our findings were unexpected. Maternal BMI has been positively correlated with maternal and fetal plasma TNF-α as well as increased placental IL-1ß and pro-inflammatory pathways (e.g., p38-MAPK, STAT3).[101] Specifically, maternal obesity has been reported to result in higher concentrations of maternal plasma CRP and IL-6 and placental ILs -1, -6, -8, and TNF-α.[102–104] Delivery mode also affects inflammation. Spontaneous vaginal deliveries have greater IL-6, IL-8 and TNF-α concentrations in amniotic fluid compared to elective cesarean section (CS).[105] Placental mRNA expression of IL-1ß and IL-8 is higher, and TNF-α is lower in placentas of women who delivered vaginally vs CS.[106] We included pre-pregnancy BMI and delivery mode in our ANCOVA and regression models to control for differences between groups and did not see an effect. However, due to small sample sizes, we could not stratify by maternal BMI or delivery mode to see if these sub-groups had different outcomes. To our knowledge, we are the first to examine the effect of exercise on specific inflammatory biomarkers within healthy, placental tissue. Current research exploring this topic examines inflammation in maternal, cord, and infant blood and is mostly done in clinical or at-risk populations (e.g., pregnancy disorders, women with obesity, etc.) who tend to display higher inflammation. Thus, it is possible that the relationship between exercise and inflammation may be a result of changes occurring within the maternal environment, thus further influencing changes to development and function of the placental tissue. However, the placenta is known to have an influence over immunological and inflammation control during pregnancy [107] therefore more research is needed to further elucidate the mechanisms by which exercise impacts pregnancy inflammation.

Although not significant, percent changes in the average biomarker concentration for the exercise groups relative to the CON are worth noting. Cortisol and fibrinogen were the only biomarkers that, regardless of modality, decreased for all exercisers relative to the CON. Overall, placentas of AE exercisers had decreased concentrations for all biomarkers compared to CON.

RE placentas tended to have lower cortisol and fibrinogen relative to CON, with all other biomarkers being higher. Lastly, it appeared that placental tissue from CE was roughly split, with 4 biomarkers (cortisol, fibrinogen, IL-6, IL-8) tending to be lower and 3 (CRP, IL-1ß, TNF-α) tending to be higher. Taken together, it could mean that aerobic activity may be important for driving decreases in placental pro-inflammatory biomarkers. There currently are no established ranges for biomarker concentrations in placental tissue nor reductions resulting clinical significance reported. Therefore, more research in this area is needed to elucidate whether these changes result in clinically meaningful changes to pregnancy and delivery health.

We found that exercise intensity predicts placenta fibrinogen concentration. Specifically, as average weekly METs increased, fibrinogen concentration in placental tissue decreased. Previous research shows that high-intensity exercise results in greater reductions of fibrinogen in men aged 42-69 years.[108,109] Similarly, young obese men that do continuous endurance training or circuit resistance training at a moderate-intensity have similar reductions in plasma fibrinogen.[110] In post-menopausal women, those who are physically active have lower plasma fibrinogen compared to those who are sedentary.[111] Therefore, exercise intensity, regardless of mode, may be a more appropriate exercise metric to monitor when assessing fibrinogen.

Our global proteomics analysis found that the placenta proteome landscapes differed for each of the exercise groups compared to CON. In all, the placentas of exercisers had 161 proteins with differential abundances (135 lower, 26 higher) compared to CON. Of those, 31 total proteins in exerciser's placentas had functions related to inflammation. In general, the proteins that were lower in abundance (29 total; 13 AE, 8 RE, 8 CE) across the exercise groups related to adaptive immunity, antigen recognition, and pathways associated with the release of inflammatory molecules (e.g., AMPK, EGFR, JAK-STAT). While the remaining 2 proteins (1 AE, 1 RE) found higher in abundance in placentas of exercisers were related to innate immune response and the MAPK/ERK pathway. Previous research has reported enrichment of inflammation and immune response pathways in placental tissue of obese women.[101,112,113] Immune responses to infection

during pregnancy are due to a combination of signals initiating from both the maternal and fetal-placental unit during pregnancy.[107] Despite our finding of no significant changes to specific pathways among exercisers, the decreases in protein abundance related to immunity could be reflective of decreased pathogen exposure during pregnancy. However, the present study did not test for this and thus, more research is needed. Regardless, we did find that eukaryotic translation initiation factor 3 subunit K (EIF3K), which is involved in the initiation of protein synthesis, was higher in abundance for AE, RE, and CE groups relative to CON. Thus, it is evident there are changes to protein synthesis and pathways related to inflammation occurring in the placentas of exercisers compared to control participants. More research is needed to identify which specific pathways are altered as a result of antenatal exercise and how the resulting changes to exercise effect maternal and infant pregnancy health.

The current analysis is the first to assess the effect of prenatal exercise on placental inflammatory biomarkers. The study is strengthened by the randomized controlled trial design, which is the strongest evidence for causality. We also utilized standard methods for assessment and kits that are valid for these biomarkers. Furthermore, we used sensitive and precise technique for proteomics (LCMSMS). In addition to these strengths, we have limitations to our methods. We had a small sample size and thus did not meet power for biomarker analysis and may be why there is a lack of statistical differences. Although we have a small sample size, we have a diverse population for generalizability of results. Additionally due to the small sample size, we were not able to stratify by pre-pregnancy BMI or delivery mode, which are known to influence inflammatory markers.[102–104,106] Since we evaluated cortisol and inflammation, it is important to note that we did not control for stress levels during pregnancy, which could have influenced results. Lastly, we found that maternal race significantly predicted placental IL-1ß. However, due to the small sample size we were unable to stratify by racial groups, and thus is a limitation of the current study. Future research is needed to further elucidate the relationship between maternal race and placental inflammatory biomarkers.

In conclusion, this study provides novel insights on the effect of exercise mode on placental inflammation. While we found that all exercise modes have similar inflammatory biomarker concentrations in placental tissue, exercise intensity predicted placental fibrinogen. Additionally, proteome landscapes differed between exercisers vs control participants, highlighting several proteins in each exercise group with decreased abundance that may reflect a reduced immune and inflammatory state. More research is needed to better elucidate the relationship of exercise during pregnancy has on placental inflammation. Overall, our findings support that exercise during pregnancy is safe and does not appear to negatively impact placental inflammation.

Tables and Figures

Table 3.1. Maternal and Infant Descriptors for Placenta Inflammatory Biomarker Analysis.

	CON (n =20)	AE (n = 18)	RE (n= 17)	CE (n= 21)	p-value
BIPOC (%)[+]	40.00	22.22	17.65	9.52	0.12
Age (yrs)	29.00 ± 4.92	29.33 ± 4.37	30.82 ± 4.94	31.00 ± 4.69	0.45
Pre-pregnancy BMI (kg/m^2)[$]	27.64 ± 4.54	24.89 ± 5.41	23.61 ± 3.88**	26.75 ± 4.61	**0.01***
Exercise Attendance (%)	62.44 ± 34.36	92.61 ± 5.22***	91.97 ± 5.03***	88.85 ± 7.60***	**<0.001*****
Total Exercise Volume (MET*min)	5251.92 ± 3402.60	13965.62 ± 2898.06***	14079.74 ± 1729.35***	11311.39 ± 4778.46***	**<0.001*****
GWG (kg)	14.04 ± 4.49	16.65 ± 3.90	14.50 ± 3.99	15.62 ± 4.25	0.24
Gestational Age (wks)	39.5 ± 1.06	40.14 ± 0.86	40.18 ± 0.81	39.74 ± 0.99	0.28
Infant Sex (%F)[+]	15.00%	38.89%	47.06%	47.62%	0.11

All are Mean ± SD. BIPOC = Black, Indigenous, People of Color; BMI = body mass index; MET = metabolic equivalents; GWG = gestational weight gain. [$] - Kruskal Wallis test used due to non-normal unconditional distributions. Bonferroni correction. [+]- Chi-Square test run for between group differences due to categorical data* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ vs CON

Table 3.2. Placenta concentrations of cortisol, CRP, IL-6, IL-8, IL-1B, TNF-a, and fibrinogen.

	CON (n=10)	AE (n=9)	RE (n=8)	CE (n=11)	p-value
Cortisol (ng/mg)	3.33 ± 2.83	2.91 ± 2.77	3.04 ± 3.09	2.65 ± 1.40	0.95
CRP	65.86 ± 58.89	53.59 ± 43.79	85.36 ± 97.34	73.75 ± 83.25	0.99
Fibrinogen	34.95 ± 24.37	22.15 ± 10.62	20.12 ± 12.85	31.48 ±19.22	0.43
IL-6	18.51 ± 7.06	18.38 ± 11.34	26.94 ± 15.84	14.63 ± 5.13	0.36
IL-8	153.61 ± 106.29	80.39 ± 52.91	195.80 ± 160.23	149.49 ± 160.14	0.24
IL-1ß	1.87 ± 2.08	0.57 ± 0.21	3.72 ± 4.07	2.06 ± 1.73	0.29
TNF-α	4.71 ± 2.02	3.35 ± 0.77	6.04 ± 3.65	5.22 ± 2.25	0.29

All biomarkers are measured by picograms per milligram weight of placental tissue protein unless otherwise indicated.
CRP = c-reactive protein; IL = interleukin, TNF-a = tumor necrosis factor alpha.

Table 3.3. ANCOVA Model for Placenta Biomarkers.

		F	Partial Eta Squared (n^2)	p-value
IL-1ß (Model 1)	**R^2=0.42**	**p=0.04**		
Race		7.41	0.36	0.02

Corrected Model 1: Race, gestational weight gain, pre-pregnancy BMI, infant sex, delivery mode

Table 3.4. Regression Model for Placenta Fibrinogen

	Estimate	STD Error	t Ratio	Lower 95%	Upper 95%	p-value
Fibrinogen (Model 1) R^2 = 0.20	**p = 0.049***					
Exercise Intensity	-0.12	0.04	-2.84	-0.77	0.47	**0.007****

Model 1: Exercise intensity (weekly average METs), attendance, exercise frequency (per week), exercise mode
*=p<0.05, **=p<0.01

Table 3.5. Maternal and Infant Descriptors for Placental Proteomics Analysis.

	CON (n =10)	AE (n = 10)	RE (n= 10)	CE (n= 10)	p-value
BIPOC (%)	10.00	30.00	30.00	20.00	0.67
Maternal Age (yrs)	31.10 ± 3.90	30.70 ± 4.76	32.10 ± 5.59	28.60 ± 3.53	0.38
Pre-pregnancy BMI (kg/m^2)$^{\$}$	28.88 ± 4.61	27.66 ± 5.81	24.51 ± 4.28	27.77 ± 5.61	0.14
Exercise Attendance (%)$^{\$}$	75.55 ± 24.77	93.81 ± 4.79	88.96 ± 9.33	92.56 ± 7.74	0.21
Total Exercise Volume (MET*min)	5983.88 ± 3070.31	14922.31 ± 2778.47***	12881.98 ± 1878.03***	11866.86 ± 4130.29**	**<0.001**
GWG (kg)	12.93 ± 4.58	15.94 ± 3.81	14.03 ± 4.21	15.59 ± 3.51	0.33
Infant Sex (%F)	50.00%	50.00%	50.00%	60.00%	0.96

Note: All are Mean ± SD. BIPOC = Black, Indigenous, People of Color; BMI = body mass index; MET = metabolic equivalents. $^{\$}$ - Kruskal Wallis test used due to non-normal unconditional distributions. Bonferroni correction * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ vs CON

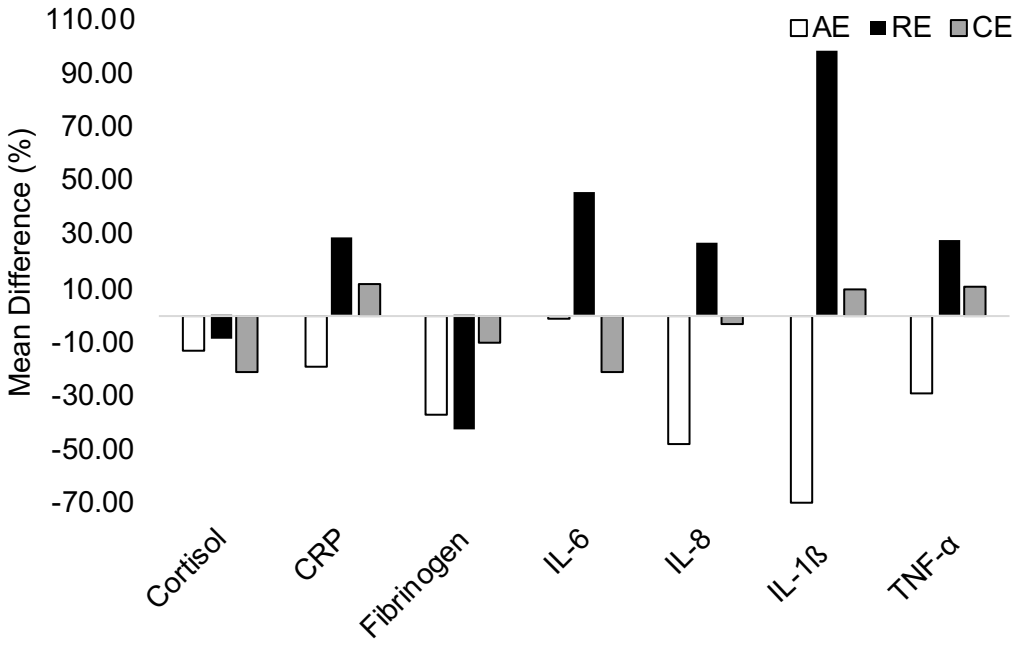

Figure 3.1. Mean percent differences for placental cortisol, c-reactive protein (CRP), fibrinogen, interleukins (IL)-6, -8, -1ß, and tumor necrosis factor alpha (TNF-a). Attention control group (CON) set as reference group for calculations. AE = aerobic exercisers, RE = resistance exercisers, CE = combination (aerobic + resistance) exercisers.

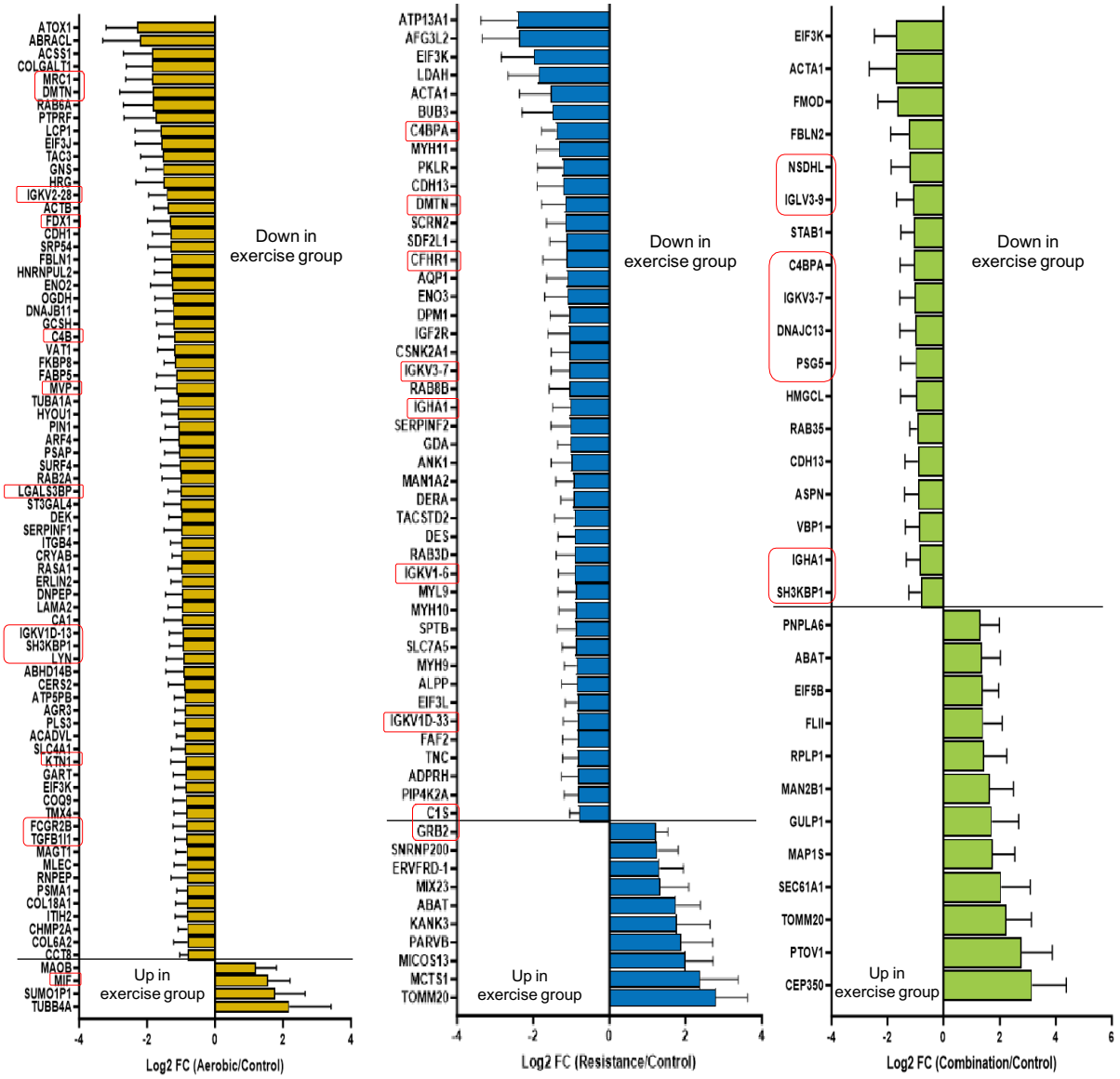

Figure 3.2. Proteins with differential abundances in aerobic vs control (yellow), resistance vs. control (blue), and combination vs. control (green) groups. Boxed proteins are associated with inflammation or immunity.

CHAPTER 4

Effect of Exercise Mode during Pregnancy on Umbilical Cord Inflammatory Biomarkers

ABSTRACT:

Excessive inflammation *in utero* alters fetal development and increases risk for cardiometabolic and mental conditions later in life. Infants born to women who exercise during pregnancy have lower adiposity and improved cardiometabolic health. Similarly, 150-minutes of activity and combined aerobic + resistance exercise alters cord blood biomarkers of inflammation. In non-pregnant individuals, exercise mode differentially reduces biomarkers of inflammation. However, it is unclear how exercise mode during pregnancy differentially impacts inflammation in cord blood. Therefore, the aim of this project was to compare the influence of aerobic (AE), resistance (RE), or combination (AE + RE; CE) exercise intervention during pregnancy on inflammatory markers in cord blood. A total of 48 pregnant women with no adverse pregnancy outcomes were randomized to 150-minutes of supervised moderate intensity AE (n=9), RE (n=15), CE (n=11), or control (CON; n=13). Cord plasma was collected at delivery and analyzed for tumor necrosis factor alpha (TNF-α); interleukins (IL) -6, -8, -1ß, -10; c-reactive protein (CRP); and cortisol using Luminex xMAP technology. Global proteomics analysis was completed using LC-MS/MS label-free approach. Database search and protein filters were done with biological replicates for each group (n=8-10/group). On average, participants were similar in age, gestational weight gain, gestational age at delivery, and infant sex ($p>0.05$). Pre-pregnancy BMI was lower in RE relative to CON ($p<0.05$). Attendance and total exercise volume (MET*min) were higher for all exercisers compared to CON ($p<0.05$). The targeted inflammatory biomarkers in cord blood at delivery were similar between groups ($p>0.05$). Linear regressions found that total exercise volume, exercise mode, and pre-pregnancy BMI predicted IL-6 ($R^2=0.80$, $p=0.04$), while exercise mode and pre-pregnancy BMI predicted cortisol ($R^2=0.68$, $p=0.02$). Proteome landscapes for AE, RE, and CE differed from CON ($p<0.001$). Pathways in exercisers were downregulated related to inflammation and immunity. Our findings suggest that exercise mode or volume alter inflammatory markers in

cord blood. Overall, we found no negative impact of exercise during pregnancy on cord blood inflammatory biomarkers, thus supporting the safety of the exercise modes investigated in the current study.

INTRODUCTION:

Excessive inflammation during pregnancy is associated with adverse pregnancy outcomes (APOs) such as intrauterine growth restriction (IUGR), recurrent spontaneous abortion, or gestational diabetes (GDM).[4,7–10,42] Excessive inflammation in the maternal environment is linked with inflammation in the fetal environment, as evidenced by altered inflammatory biomarker concentration and gene expression in cord blood.[57,58] Specifically, women with pre-eclampsia have increased c-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) concentrations in maternal blood with increased CRP and slightly, but not significantly, increased IL-6 in cord blood.[57] Levels of inflammation across gestation is associated with offspring neurodevelopmental outcomes, such as brain structure and functional changes.[14] Research suggests that an increased maternal pro-inflammatory ratio during pregnancy decreases leukocyte telomere length, impacting physiological factors later in life. [15] Taken together, the increase in maternal inflammation during pregnancy alters infant inflammation exposure *in utero*, potentially contributing to an increased risk for cardiometabolic and/or mental conditions later in life. [17,20,59]

Exercise reduces pro-inflammatory biomarkers (e.g., IL-1ß, IL-6, TNF-α, CRP) in non-pregnant populations[36,38,68] and, interestingly, differs depending on exercise mode. From pre- to post-intervention, aerobic exercise (AE) in older adults decreases blood IL-6, CRP, and TNF-α, whereas strength and flexibility exercise only decrease TNF-α.[38] Similarly, AE and resistance exercise (RE) reduces pro-inflammatory CRP, IL-6, and IL-1ß in diabetics, but combined aerobic + resistance exercise (CE) results in greater decreases in pro-inflammatory biomarkers.[36] In pregnancy, physically active women with obesity have slight, but nonsignificant, reductions in

umbilical cord blood CRP relative to their inactive counterparts.[32] CE during pregnancy leads to lower cord IL-6 and TNF-α but higher IL-1ß, relative to controls.[73] The effect of supervised exercise modes (AE, RE, CE) during pregnancy on cord blood inflammatory markers has not be defined. Considering excessive inflammation can negatively impact infant health outcomes at delivery and later in life, it's crucial to establish the best method for controlling the amount of inflammation in the fetal environment during pregnancy. Therefore, the current study aimed to examine the effect of exercise modes on inflammatory biomarkers in umbilical cord plasma at delivery. We hypothesized that exercisers would have lower cord plasma TNF-α, IL-6, IL-1ß, and CRP compared to CON, with CE exercisers having the lowest.

METHODS

Study Design and Participants. The current study is a secondary analysis that included women recruited from three separate randomized controlled trials (RCTs). This analysis was done to examine the effect of exercise mode on markers of inflammation in cord blood. Each RCTs recruited women with singleton pregnancies that were low-risk and healthy, then randomized them to either 150-minutes of moderate exercise (aerobic, resistance, or combined aerobic + resistance) or control. Women were eligible for study participation if they were: 1. aged 18-40, 2. $\leq$ 16-weeks' gestation, 3. pregnant with a single baby, and 4. had a pre-pregnancy body mass index (BMI) $\geq$18.5 kg/m^2. All women were required to provide clearance from their obstetric provider, indicating it was safe to participate in exercise during their pregnancy. Women who did not meet these criteria or reported having a medical condition known to alter fetal development (e.g., HIV/Aids, cancer, diabetes, untreated hypertension), use of any of tobacco products, alcohol, recreational drugs, or medications (e.g., oral hypertensive, insulin) were excluded. Following eligibility screening, written informed consent was obtained. Protocols were approved by East Carolina University (ECU) Institutional Review Board, performed at ECU, and registered in clinicaltrials.gov (NCT03517293 and NCT04805502).

Pre-intervention Testing & Randomization. Following study enrollment, all women completed a validated Modified Balke treadmill test to determine target heart rate (THR) ranges for moderate intensity. [79] Oxygen consumption and carbon dioxide production were captured via indirect calorimetry (Parvo Medics, TrueOne2400, Sandy UT) and used to estimate peak oxygen consumption (VO_{2peak}) while maternal heart rate (HR) was monitored simultaneously (Polar FS2C HR monitor). To minimize exposure during the COVID-19 pandemic, treadmill testing was suspended between March 2020 and October 2021. Participants enrolled during this time were given THR zones estimated based on their pre-pregnancy physical activity levels and age. All THR zones were used during each exercise session to ensure moderate intensity and corresponded with maternal HR at 60-80% VO_{2peak}. Women enrolled also performed a 1-repetition max (1RM) test to establish baseline muscular strength. This was used to program the level of resistance or weight for resistance exercises. Following pre-intervention testing, participants were then randomized via computer sequencing (GraphPad, Boston, USA) to either aerobic (AE) exercise, resistance (RE), combination (aerobic + resistance; CE) exercise, or a stretching/breathing attention control group (CON).

Exercise Intervention. All exercise participants (AE, RE, CE) were supervised by trained exercise instructors in ECU facilities following a standard protocol from enrollment through delivery (~24 weeks).[80] Exercisers (AE, RE, CE) were asked to meet 150-minutes (3 times per week, 50 min sessions) of moderate-intensity activity each week until delivery. Exercise sessions consisted of a 5-minute warm-up, 50-minutes of activity based on their randomized group, followed by a 5-minute cool-down. Maternal HR and perceived exertion of 12-14, via the Borg RPE 6-20 scale, were monitored throughout each session to ensure moderate intensity was being met.

Participants randomized to AE were asked to complete moderate intensity trainings utilizing treadmills, ellipticals, cycle ergometers, rowers, and/or stair stepping equipment, at the

participant's discretion. RE sessions were pre-programmed and consisted of exercises that targeted major muscle groups. Women were asked to complete each exercise at 60% 1RM for 2-4 sets aiming for 12 repetitions. These exercisers utilized a variety of equipment including: resistance bands, benches, dumbbells, machines (Cybex), exercise balls, and/or mats. CE exercisers spent equal time (~25-min) doing AE and RE activities during each exercise session. These exercises were performed similarly to each respective activity as outlined. CON participants were invited to participate in weekly sessions of breathing/stretching to enhance study retention. Each CON session included a 5-min warm-up and 50-min of stretching. HR was collected throughout these sessions to ensure maternal HR did not reach moderate intensity (<50% VO_{2peak}).

Exercise Metrics. Each week, exercise frequency, intensity (MET*min), duration, and volume were totaled to examine the exercise dose of the intervention. Upon delivery, exercise session frequency and weeks spent training were totaled and used to calculate participant attendance. Attendance was calculated by dividing the number of sessions attended each week by 3. An attendance threshold of 75% for AE, RE, or CE participants was required in the current analysis, as this has previously shown to successfully impact cord inflammatory biomarkers.[73] To examine average weekly exercise dose across the pregnancy, exercise frequency, duration, and intensity (METs) were totaled and divided by the number of training weeks completed at delivery. Total exercise dose of the intervention was explored by examining the total exercise volume (MET*min) across the pregnancy.

Maternal & Delivery Measures. Maternal age, race, ethnicity, were collected via questionnaire at enrollment. Maternal height and pre-pregnancy weight were also collected via self-report and used to calculate pre-pregnancy BMI. Women who identified as Black or African American, Asian,

Native Hawaiian or other Pacific Islander, Native American or Alaskan Native, and Latinx participants were grouped as Black, Indigenous, People of Color (BIPOC) for analysis.

Infant sex, delivery mode, and incidence of adverse pregnancy condition (i.e. GDM, IUGR, gestational hypertension (g-HTN), preeclampsia (PE), preterm birth (PTB)), medications taken during the pregnancy, and medical conditions were collected at delivery via electronic health record. Women who were diagnosed with an adverse pregnancy condition were excluded in the current analysis, as these have been associated with increased inflammation.[4,8,9] Gestational weight gain (GWG) was calculated by subtracting pre-pregnancy weight from the medical record reported delivery weight.

Umbilical Cord Blood Processing: Upon delivery, one purple top vacutainer containing EDTA of mixed arterial and venous cord blood was collected. Delivery samples were stored at 4ºC until study staff could collect. Upon collection, whole blood was aliquoted into 2, 2mL microcentrifuge tubes and centrifuged for 15-min, 13 rpm, at 4ºC. Cord plasma was then separated from cord red blood cells into 2, 2mL microcentrifuge tubes. All samples were stored at -80ºC until analysis**.**

Quantification of Cord Blood Inflammatory Biomarkers: Cord plasma samples were used to examine the concentration of cortisol, CRP, IL-6, IL-8, IL-1ß, TNF-α, and IL-10 using MILLIPLEX MAP kits. The standards, controls, and samples for each kit were prepared using manufacturer's instructions. All sample plates were read on a Luminex 200 using xMAP technology and analyte concentrations were determined using xPONENT software. The Human Circadian/Stress Magnetic Bead Panel kit was used to assess cortisol. The Human Cardiovascular Disease (CVD) Magnetic Bead Panel 3 (Acute Phase) kit was used to examine CRP. The Human Adipokine Magnetic Bead Panel (Millipore Sigma, Burlington, MA, USA) was used to assess IL-6, IL-8, IL-1ß, and TNF-α. Finally, the Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Sigma, Burlington, MA, USA) was used to examine IL-10. All samples were run in duplicate to determine

the concentration of cortisol, CRP, IL-6, IL-8, IL-1ß, TNF-α, and IL-10 then averaged. All data obtained within the limit of detection for each kit was used due to small sample availability.

Label-Free Proteomics

Preparation of mass spectrometry grade peptides from cord plasma: Undepleted proteins from plasma samples (5 µL) were subsequently precipitated using ice-cold methanol (3:1 v/v) at -20° C and pelleted by centrifugation. The pellets were washed with ice cold methanol (x2) and allowed to air dry before further use. PreOmics iST kits (PreOmics GmbH) were used to prepare mass spectrometry grade peptides, as such precipitated proteins were resuspended in the provided lysis/denaturing buffer and denatured and alkylated at 95°C for 10 min in a 1.5 mL microcentrifuge tube. After cooling to room temperature, digest reagent was added, and proteins were digested for 3 hours at 37°C while shaking (600 rpm). Samples were then loaded onto the provided microcolumns for purification. Peptides were eluted via centrifugation using the provided elution buffer, dried to completeness under a N2 stream, and resuspended in loading buffer (98:2 water: acetonitrile + 0.1% formic acid) at a concentration of 0.25 mg/mL.

***LC-MS/MS for Label-Free Proteomics*.** Peptides were analyzed by nanoLC-MS/MS using an UltiMate 3000 RSLCnano system (ThermoFisher) coupled to a Q Exactive Plus Hybrid Orbitrap mass Spectrometer (ThermoFisher) via nanoeslectrospray ionization. Peptides were separated using an effective linear gradient of 4-35% acetonitrile (0.1% formic acid) over 135 min. MS spectra were acquired in positive mode for data dependent acquisition in positive mode. MS1 were performed at a resolution of 70,000 with an AGC target of $2x10^5$ ions and a maximum injection time of 100 ms. MS2 spectra were collected on the top 20 most abundant precursor ions with a charge >1 using an isolation window of 1.5 m/z and fixed first mass of 140 m/z. The normalized collision energy for MS2 scans was 30. MS2 spectra were acquired at 17,500

resolutions with a maximum injection time of 60 ms, a dynamic exclusion of 30 sec, and an AGC target of $1x10^5$.

***Database Search and Protein Filtering*.** FragPipe (v 19.1) [95,96] was used for raw data analysis with default search parameters for open and Label-Free Quantification Matching Between Runs (LFQ-MBR) workflows. Cord plasma was searched with biological replicates (CON = 8, RE= 9, AE & CE = 10) for each group identified. An initial open search against the canonical + isoforms Uniprot *Homo sapiens* reference proteome (UP000005640, accessed 11/2023) was used to identify potential post-translational modifications for inclusion in the LFQ-MBR workflow. Precursor m/z tolerance was set to -150 to 500 Da and fragment tolerance was ± 20 ppm with 3 missed cleavages for Tryp and Lys-C allowed. Peptide spectrum matches (PSMs) were validated using PeptideProphet and results were filtered at the ion, peptide, and protein level with a 1% false discovery rate (FDR). Based on these initial searches, the following variable modifications were included in the LFQ-MBR analysis: oxidation (+15.5995 Da on Met), deamidation (+0.98401 Da on Gln and Asn), and fixed modification carbamidomethyl (+57.025 Da on Cys). For LFQ-MBR analysis, data were search against the canonical Uniprot *Homo sapiens* reference proteome (UP000005640, accessed 11/2023 and 1/2024). Precursor ion m/z tolerance was ± 20 ppm with 3 missed cleavages for Trypsin/LysC allowed. The search results were filtered by a 1% FDR at the ion, peptide, and protein-level. PSMs were validated using Percolator and label free quantification was carried out using IonQuant [97] Match between runs FDR rate at the ion level was set to 10% for the top 300 runs. Proteins with >95% probability of ID, >2 unique peptides, and in more than 80% of a sample group (*i.e.* 7/10 injections) were considered high confidence IDs and retained for analysis. Intensities were $\log_2$ transformed, normalized to median intensities of the sample group. Relative abundances for low sampling proteins were determined via normal distribution in Perseus.[98]

Statistical Analyses. Using preliminary data for TNF-α, our primary outcome variable, a power analysis to reach significance of $p<0.05$ with 80% power needed a total of 48 participants, or 12 per group. Outliers were first identified and removed using boxplots. Any potential outliers identified during outlier assessment were removed if there was justification, such as a reported issue during processing that may affect sample reliability, to do so. All potential outliers were thoroughly explored before removing, as it could be representative of inherent variability of the sample. Data were then assessed for normality. Statistical significance was set at $p<0.05$ for all analyses. Normally distributed data underwent one-way ANOVA testing to examine between group differences and used a Bonferroni correction factor during post-hoc testing. Non-normally distributed data were run using a Kruskal-Wallis non-parametric tests and utilized a Bonferroni correction factor during post-hoc testing. Covariates such as pre-pregnancy BMI, maternal race, GWG, infant sex, and exercise metrics were assessed using Analysis of covariance (ANCOVAs). To meet the assumption of normality, non-normally distributed biomarkers were $\log_{10}$ transformed before running ANCOVAs and regression analyses. ANCOVAs and between group differences testing was conducted using IBM SPSS Statistics for Mac, version 27 (IBM Corp., Armonk, NY, USA). Regression analyses were performed to determine significant predictors of the outcome variables using JMP Statistics for Mac, version 17 (JMP Statistical Discovery, Cary, NC, USA).

RESULTS:

Quantification of Cord Inflammatory Biomarkers

Study population. Cord plasma was analyzed from 82 participants. In the analysis to quantify the biomarkers of interest (cortisol, CRP, IL-6, IL-8, IL-1ß, TNF-α, IL-10), 17 were removed due to development of adverse pregnancy outcome such as gestational diabetes (i.e., GDM, g-HTN, PE, IUGR, PTB) or other condition known to affect inflammation (e.g., beta thalassemia, rheumatoid arthritis, smoking during pregnancy); 5 were removed due to medication known to alter inflammation (e.g., selective serotonin reuptake inhibitors); and 12 were removed due to low

attendance (<75%; 3 AE, 2 RE, 6 CE) or participating in outside exercise not related to their group allocation (1 CON). This left a total of 48 samples for analysis (13 CON, 9 AE, 15 RE, 11 CE). In general, groups were similar for all maternal descriptors, except the RE group had lower pre-pregnancy BMI relative to CON (Table 4.1). As expected, all exercise groups had greater total exercise volume and attendance than CON participants (Table 4.1).

Inflammatory biomarker concentration. After datapoint removal for being outside limit of detection and extreme outliers, sample size for each biomarker varied. Cord plasma concentrations of cortisol, CRP, IL-6, IL-8, IL-1ß, TNF-α, and IL-10 were similar between the groups (Table 4.2).

ANCOVAs and Regressions. After adjusting for pre-pregnancy BMI, maternal race, gestational weight gain, and infant sex, there were no between group differences for cortisol, CRP, IL-6, IL-8, IL-1ß, TNF-α, or IL-10 ($p>0.05$, data not shown). After adjusting for weekly exercise frequency, duration, and intensity, no significant differences between groups were detected for any inflammatory markers ($p>0.05$, data not shown).

Linear regressions established that IL-6 was predicted by total exercise volume, exercise mode, and pre-pregnancy BMI, while cortisol was predicted by pre-pregnancy BMI and exercise mode (Table 4.3). All other models were not significant ($p>0.05$, data not shown).

Label-Free Proteomics.

Study Population. In the global proteomics analysis, 40 samples (10/group) were analyzed. After processing, 2 samples were removed due to medication known to alter inflammation (e.g., selective serotonin reuptake inhibitors) and 1 for doing physical activities outside of her group allocation (CON). This left a total of 37 samples (8 CON, 10 AE, 9 RE, 10 CE) for analysis. In

general, the groups were similar in all maternal and infant descriptors (Table 4.4). As expected, total exercise volume was higher in each of the exercising groups relative to CON (Table 4.4).

Between-group Protein Abundances. Compared to CON, the proteome landscapes for AE, RE, and CE groups differed ($p<0.001$). When pooling all exercise groups together, a total of 61 proteins were found to be lower in abundance in cord plasma of exercisers relative to control (Figure 4.1). Pathway analysis, using proteins that changed in all exercising groups, identified 5 pathways with the greatest decreases relative to control (Figure 4.2). These pathways were: cellular oxidant detoxification, hydrogen peroxide catabolic process, cellular response to toxic substance, reactive oxygen species (ROS) metabolic process, and carbon dioxide transport.

There are 391 proteins with differential abundances between AE (168), RE (115), or CE (108) compared to CON ($p<0.0001$). Firstly, the cord plasma proteome for AE group had 167 proteins higher in abundance and 1 lower in abundance compared to CON ($p<0.1$). Pathway analysis identified 4 pathways with the greatest decreases in AE vs CON: B-cell mediated immunity, lymphocyte mediated immunity, humoral immune response mediated by circulating immunoglobulin, and acute inflammatory response (Figure 4.3).

The cord plasma for the RE group had 114 proteins with lower abundances and 1 with higher abundance compared to CON ($p<0.0001$). Pathway analysis identified 2 pathways with the greatest decreases, as determined by protein abundance in RE vs CON: cellular oxidant detoxification and hydrogen peroxide catabolic process (Figure 4.4).

Finally, cord plasma for CE group had 106 proteins with lower abundances relative to control with 2 proteins with higher abundance ($p<0.0001$). Pathway analysis identified 3 pathways with the greatest decreases in CE vs CON: cellular detoxification, complement activation, and hydrogen peroxide catabolic process (Figure 4.5).

DISCUSSION:

The goal of the current study was to examine the effect of exercise mode on inflammatory biomarkers in umbilical cord plasma at delivery. We hypothesized that cord plasma of all exercisers would have reduced TNF-α, IL-6, IL-1ß, and CRP compared to CON, with CE exercisers having the lowest concentrations. We found similar levels of inflammatory biomarkers between groups. However, we noted exercise mode and volume predicted pro-inflammatory biomarker concentration in cord plasma while controlling for pre-pregnancy BMI. Finally, we found that the cord plasma of exercisers had different proteome profiles compared to control participants.

Contrary to our hypothesis, the present study observed similar levels of cord plasma cortisol, CRP, IL-6, IL-8, IL-1ß, TNF-α, and IL-10 between groups. Our findings suggest that exercise is safe during pregnancy and has no negative impact on inflammatory biomarkers within cord plasma. However, these data should be interpreted carefully, as we were underpowered for these outcomes. Importantly, values for cord IL-6, TNF-α, CRP, IL-8, and cortisol for all groups were within established reference ranges or similar to that of previous research. [114–116] Our values for pro-inflammatory IL-1ß and anti-inflammatory IL-10 were lower than previously reported, however previous studies did not account for adverse pregnancy outcomes (APOs) except preterm birth.[117] Considering the established association between APOs, like GDM, and altered inflammatory states in maternal and cord blood, we excluded participants with these conditions to minimize confounding the data and thus, may explain why the concentrations for IL-1ß and IL-10 were lower in the present study relative to Mestan et al.[4,6,118,119]

We are the first to examine the effect of a supervised prenatal exercise intervention utilizing AE and RE alone on cord plasma inflammatory biomarkers as well as the first to compare 3 modes of exercise. Previously, physically active pregnant women with obesity have slightly, but not significantly, lower CRP in cord blood relative to inactive pregnant women with obesity.[32] This was in agreement with our findings, as we did not see any differences in CRP among groups. In

partial agreement with our results, Acosta-Manzano et al. reported women who did CE during pregnancy have no differences in cord IL-8 or IL-10 relative to controls. However, unlike our findings, this group did report lower cord IL-6 and TNF-α and higher cord IL-1ß in CE relative to control.[73] Importantly, the control group from Acosta-Manzano et al. were asked to continue their regular daily activity, but had significantly more light-intensity and total physical activity time per day relative to the combination group at 16-wks. In the current study, some, but not all, of the participants in the attention control group (CON) completed regular stretching sessions, during which participants were below recommended pregnancy exercise guidelines by maintaining the intensity below moderate throughout each session. Therefore, the controls in the present study are somewhat similar to those from the from Acosta-Manzano et al. research. Interestingly, the CE intervention by Acosta-Manzano et al. had 2 days/week of resistance based activities with 1 day/week aerobic based activities,[73] whereas the present study spent equal time at every exercise session doing both AE and RE exercises. Although there were no differences between groups, the RE group averages for pro-inflammatory TNF-α and IL-6 in the present study are trending similarly to the previous study, whereas the AE group means were the same or slightly higher than CON. Taken together, the trends from the current study and differences shown in cord blood of CE from previous research could suggest that RE may better elicit decreases in pro-inflammatory biomarkers. However, a more robust sample size is needed before reaching this conclusion. Therefore, more research is needed to further elucidate the extent that exercise mode may differentially impact cord plasma.

We found that total exercise volume, exercise mode, and pre-pregnancy BMI predicted IL-6 in cord blood. IL-6 has both pro- and anti-inflammatory properties, depending on its cell-signaling and intracellular regulation.[87,120–123] Muscle-derived IL-6 leads to predominately anti-inflammatory activity, fat-derived IL-6 has predominately pro-inflammatory activity,[120,123] and resting IL-6 is proposed to better represent a pro-inflammatory status.[87] We also found and exercise mode and pre-pregnancy BMI predicted cord plasma cortisol. Cortisol acts as an anti-

inflammatory and is released in response to inflammation.[124] Specifically, increased total exercise volume, pre-pregnancy BMI, and being in the CE or CON groups predicted lower pro-inflammatory IL-6 and anti-inflammatory cortisol in cord plasma. Research on the influence of pre-pregnancy BMI on cord blood inflammation is contradictory,[125–127] and does not explore the influence of lean vs fat mass on cord blood inflammation. However, in non-gravid adults, increased adiposity is associated with higher systemic inflammation and salivary cortisol.[128,129] Previously, CE was associated with increased lean and decreased fat mass, [130] and the present study found slightly, but not significantly, lower cord concentrations of IL-6 and cortisol in the CE group. We also found that the AE group allocation predicted higher cord pro-inflammatory IL-6 and anti-inflammatory cortisol. However, although nonsignificant, our AE groups also had one of the highest average pre-pregnancy BMI and GWG. Taken together, this could indicate that the influence of pre-pregnancy BMI and exercise group to predict cord blood IL-6 may be related to differences in maternal lean and fat mass. In addition, our AE group on average had the highest percentage of female infants, which is linked with higher cord cortisol,[131] body fat percentage, and lower lean body mass, relative to male infants.[132] Although the specific effect of fetal body composition on cord blood inflammatory biomarkers is not defined, it is possible that this could be impacting our results. The current study did not capture lean or fat-free mass specifically, and therefore we are unable to draw firm conclusions on its impact. We were also unable to stratify by infant sex or delivery mode or control for body composition and pregnancy stress, which may further impact our results.[131,133,134]

The cord plasma proteome of exercisers had 391 proteins with differential abundances (168 AE, 115 RE, 108 CE) compared to CON, with AE having the most changes in pathways directly related to inflammation. AE had the greatest decreases in proteins within the following pathways: B-cell mediated immunity, lymphocyte mediated immunity, humoral immune response mediated by circulating immunoglobulin, and acute inflammatory response. Previous research shows that proteomic patterns in plasma of non-gravid habitual aerobic exercisers has decreases

in biological processes related to stress, inflammatory, and immunity.[135] We also found decreases in pathways related to inflammation in the cord plasma of AE compared to control, and therefore may suggest benefits experienced in the maternal compartment may be translated to infant during gestation. Regardless, our findings reflect changes to inflammatory processes in cord plasma of AE, providing specific targets in for future research.

Cord plasma of RE had the greatest decrease in proteins related to cellular oxidant detoxification and hydrogen peroxide catabolic process pathways. Previous studies have seen proteome profiles change following 15 weeks of RE in plasma of women with and without fibromyalgia that related to regulation of immune system process and stress response.[136] Although the aforementioned pathways in the present study were not directly reflective of inflammation, they are related to processes that reduce or remove toxic superoxide radicals and/or hydrogen peroxide, which are molecules that can stimulate inflammatory responses in cells.[137,138] Therefore, the downregulation of these pathways in RE could relate to altered inflammation in cord plasma compared to CON. Considering the CE group activities utilized ones from both AE and RE, it is not surprising that the cord plasma of CE had the greatest decreases in similar processes, i.e., complement activation, hydrogen peroxide catabolic process, and cellular detoxification pathway, relative to CON. Similarly, when comparing all exercisers vs control, cellular oxidant detoxification, hydrogen catabolic process, cellular response to toxic substances, and ROS metabolic process were among the pathways with the greatest decreases. As previously mentioned, decreased proteins within these pathways could indicate a reduced need to buffer molecules known to stimulate inflammation, and thus represent better inflammatory control. More research targeting these specific pathways is needed to establish the relationship between these pathways that may stimulate inflammation and resulting inflammatory responses. Collectively, the findings from our cord plasma proteomics analysis suggest that exercise during pregnancy has a prominent effect on the circulating proteome in umbilical cord plasma related to inflammation. These data provide insights into specific pathways that are altered in cord plasma

with different exercise modes that should be targeted to better understand the influence of exercise mode on cord plasma inflammation.

This study has strengths that warrant mention. First, the current analysis is the first to compare the effect of prenatal exercise modes on cord plasma inflammatory biomarkers, contributing to the growing body of research supporting regular participation in exercise during pregnancy. Second, we utilized a supervised exercise intervention meeting the recommended level of exercise during pregnancy with a randomized controlled trial design, which is the strongest evidence for causality. Furthermore, the methods utilized in the present study to assess specific and global biomarkers and pathways of inflammation were sensitive and precise techniques. In addition to our strengths, we have limitations to our methods. First, we have a small sample size for biomarker analysis which makes it difficult to generalize, however, this is the first study in this area. Additionally, we did not control for stress levels during pregnancy or delivery mode, which may have influenced the results.

In conclusion, this study provides novel insight into the effect of exercise mode on cord blood inflammation. While we found that all exercise modes had similar inflammatory biomarker concentrations, exercise mode and volume predicted the effect on pro/anti-inflammatory IL-6 and anti-inflammatory cortisol in umbilical cord plasma. Similarly, proteome landscapes differed between the exercisers and control participants, identifying several pathways related to inflammation that were improved in exercisers. More research is needed to further define the impact prenatal exercise has on cord plasma inflammation and whether this could be used as a non-pharmacological method for reducing fetal exposure to excessive inflammation. Our findings indicate no negative effects from the investigated exercise modes on cord blood inflammatory biomarkers, supporting safety of exercise during pregnancy on umbilical cord blood.

Tables and Figures

Table 4.1. Maternal and Infant Descriptors for Cord Blood Inflammatory Biomarker Analysis

	CON n= 13	AE n= 9	RE n=15	CE n= 11	p-value
BIPOC (%)[+]	23.08	33.33	6.67	9.09	0.29
Age (yrs)	30.69 ± 4.80	29.22 ± 3.80	30.47 ± 4.27	30.09 ± 5.59	0.90
Pre-pregnancy BMI (kg/m^2)[$]	28.29 ± 4.16	27.44 ± 6.34	23.13 ± 4.23**	26.67 ± 6.16	**0.01**
Exercise Attendance (%)	63.75 ± 31.28	92.64 ± 5.82**	89.10 ± 6.62**	91.13 ± 10.22**	**<0.001*** **
Total Exercise Volume (MET*min)	5679.40 ± 2964.32	14599.10 ± 3865.83**	13048.86 ± 2692.87***	11620.69 ± 4884.87**	**<0.001*** **
GWG (kg)	13.49 ± 4.82	16.99 ± 3.41	15.64 ± 4.12	14.48 ± 4.53	0.27
Gestational Age (wks)[$]	39.35 ± 1.34	40.00 ± 0.92	40.05 ± 0.87	39.86 ± 0.95	0.54
Infant Sex (%F)[+]	23.08	66.67	33.33	36.36	0.21

All displayed as Mean ± SD. BIPOC = Black or Indigenous People of Color; BMI = body mass index; MET = metabolic equivalents; GWG = gestational weight gain. [$] - Kruskal Wallis test used due to non-normal unconditional distributions. [+]- Chi-Square test run for between group differences due to categorical data. Bonferroni correction * = p<0.05, ** = p<0.01, *** = p<0.001 vs CON

Table 4.2. Cord blood concentrations of cortisol, CRP, IL-6, -8, -1ß, -10, and TNF-α using multiplex analysis

	CON n= 9	AE n= 7	RE n= 7	CE n= 7	p-value
Cortisol (ng/mL)	36.73 ± 16.67	57.89 ± 35.15	63.49 ± 31.32	37.58 ± 36.52	0.27
CRP (ng/mL)$	0.12 ± 0.12	0.15 ± 0.08	0.22 ± 0.18	0.14 ± 0.10	0.50
IL-6	11.60 ± 3.68	14.28 ± 7.31	10.98 ± 3.76	8.76 ± 4.82	0.54
IL-8 $	10.45 ± 8.78	5.99 ± 2.10	8.19 ± 4.93	9.26 ± 8.44	0.90
IL-1ß $	1.84 ± 0.50	4.33 ± 3.08	1.85 ± 1.20	6.64 ± 4.80	0.24
TNF-α	6.21 ± 0.73	6.90 ± 1.60	5.74 ± 1.58	7.59 ± 2.05	0.21
IL-10 $	15.98 ± 9.24	38.41 ± 22.14	34.88 ± 29.69	14.26 ± 10.60	0.35

All biomarkers are measured by pg/mL unless otherwise specified. $- Kruskal-Wallis test used due to non-normal unconditional distributions. Bonferroni correction. IL = interleukin, TNF-α = tumor necrosis factor alpha; CRP = c-reactive protein.

Table 4.3. Regression Models for Cord Blood Interleukin 6 (IL-6) and Cortisol

	Estimate	STD Error	t Ratio	Lower 95%	Upper 95%	p-value
IL-6 (Model 1)	**R^2 = 0.80**	**p = 0.04***				
Total Exercise Volume	-0.001	0.0002	-3.94	-0.001	-0.0003	**0.006****
Exercise Group						**0.02***
AE	12.35	3.28	3.76	4.59	20.11	
CE	-7.62	2.10	-3.63	-12.59	-2.65	
CON	-3.51	1.30	-2.7	-6.58	-0.44	
Pre-pregnancy BMI	-0.46	0.18	-2.5	-0.89	0.02	**0.04***
Cortisol (Model 1)	**R^2 = 0.68**	**p = 0.02***				
Pre-pregnancy BMI	-4175.05	1420.31	-2.94	-7243.45	-1106.65	**0.01***
Exercise Group						**0.02***
AE	52113.11	16473.45	3.16	16524.38	87701.84	
CE	-28933.59	10367.82	-2.79	-51331.90	-6536.27	
CON	-24085.91	14540.06	-1.66	-55497.80	7325.98	

Model 1: Total volume, exercise group, pre-pregnancy BMI, attendance, gestational weight gain (GWG)
BMI = body mass index. *=p<0.05, **=p<0.01, ***p<0.001

Table 4.4. Maternal and Infant Descriptors for Cord Blood Proteomics Analysis

	CON n= 8	AE n= 10	RE n=9	CE n= 10	p-value
BIPOC (%) [+]	12.50	40.00	11.11	10.00	0.26
Age (yrs)	31.75 ± 5.18	29.20 ± 3.58	31.56 ± 5.05	31.00 ± 6.73	0.71
Pre-pregnancy BMI (kg/m^2) [$]	29.20 ± 4.64	26.86 ± 6.25	23.28 ± 4.35	26.66 ± 6.43	0.06
Exercise Attendance (%)[$]	63.63 ± 33.98	90.18 ± 9.52	91.41 ± 5.49	89.29 ± 11.54	0.13
Total Exercise Volume (MET*min)	5465.57 ± 3265.50	13092.21 ± 4365.54*	14232.55 ± 1911.52***	11606.14 ± 5390.85*	**<0.001**
GWG (kg)	13.69 ± 4.71	16.79 ± 3.27	13.42 ± 3.52	15.21 ± 2.17	0.15
Gestational Age (wks)	39.53 ± 1.46	39.93 ± 0.90	39.95 ± 1.05	39.93 ± 0.58	0.82
Infant Sex (%F) [+]	25.00	70.00	22.22	30.00	0.10

All displayed as Mean ± SD. BIPOC = Black or Indigenous People of Color; BMI = body mass index; MET = metabolic equivalents; GWG = gestational weight gain. [$] - Kruskal Wallis test used due to non-normal unconditional distributions. [+]- Chi-Square test run for between group differences. Bonferroni correction * = p<0.05, ** = p<0.01, *** = p<0.001 vs CON.

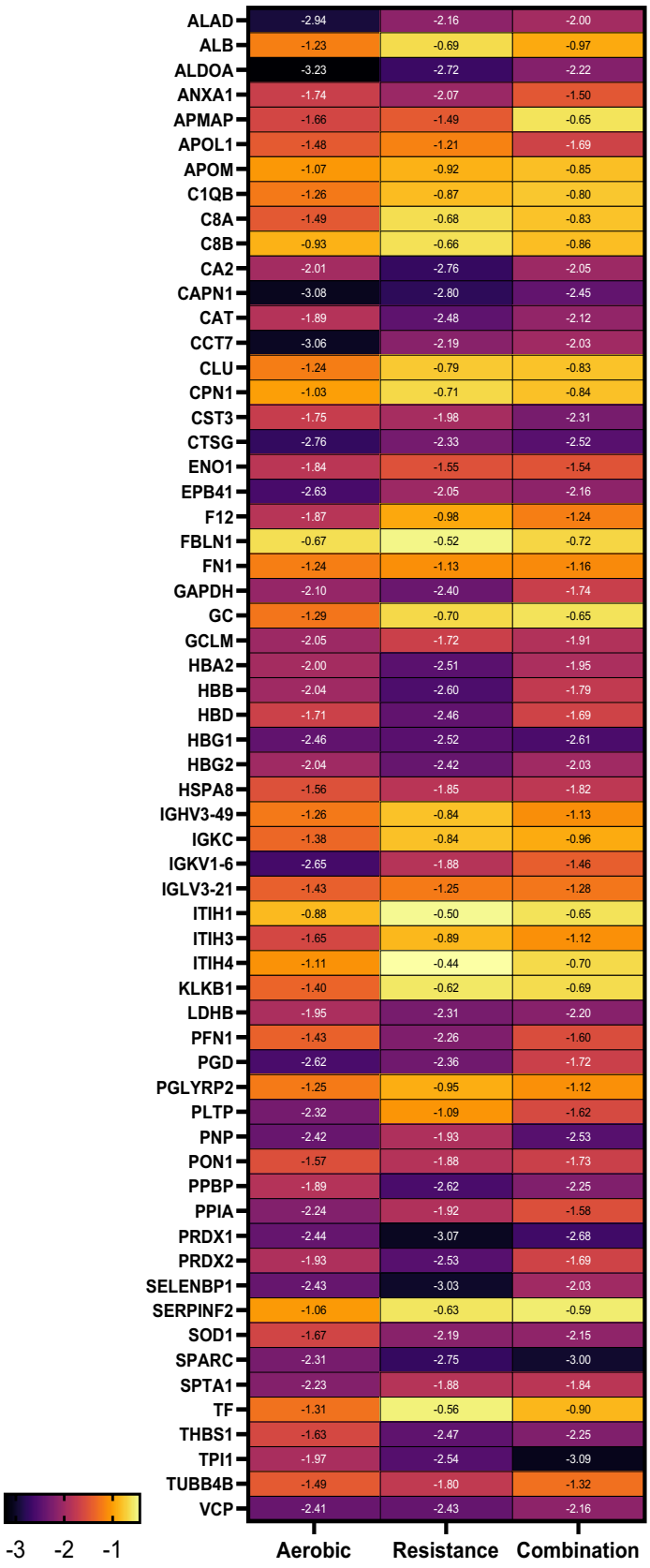

Figure 4.1. Log_2 Protein Abundances in Cord Blood All Exercise (Aerobic + Resistance + Combination) groups vs control.

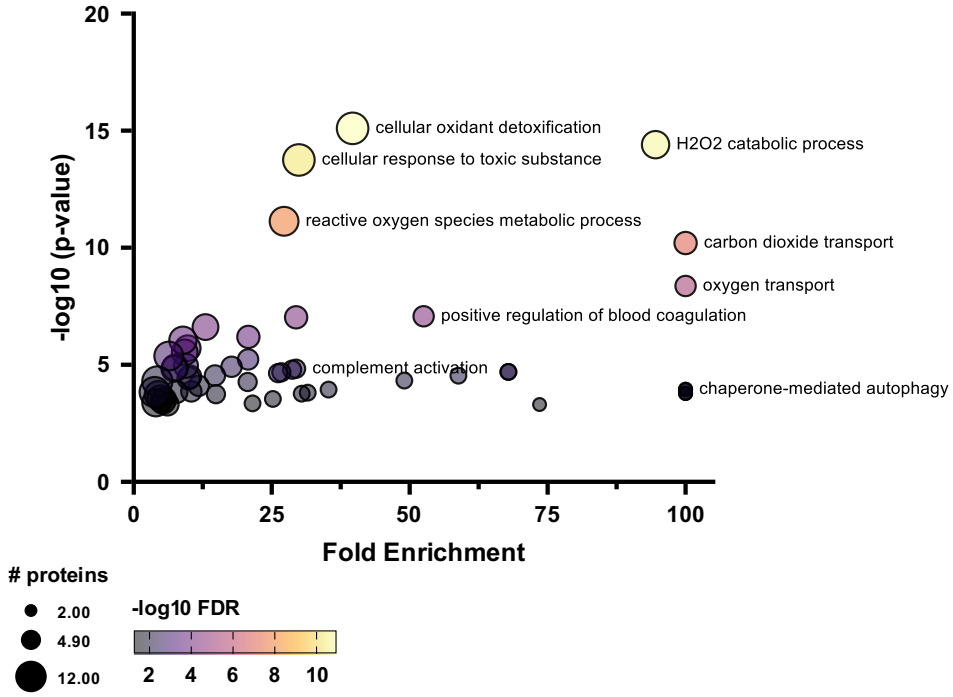

Figure 4.2. Decreased Pathways Identified by Lower Protein Abundance in Cord Blood for All Exercise Groups (Aerobic, Resistance, and Combination exercise) vs Control.

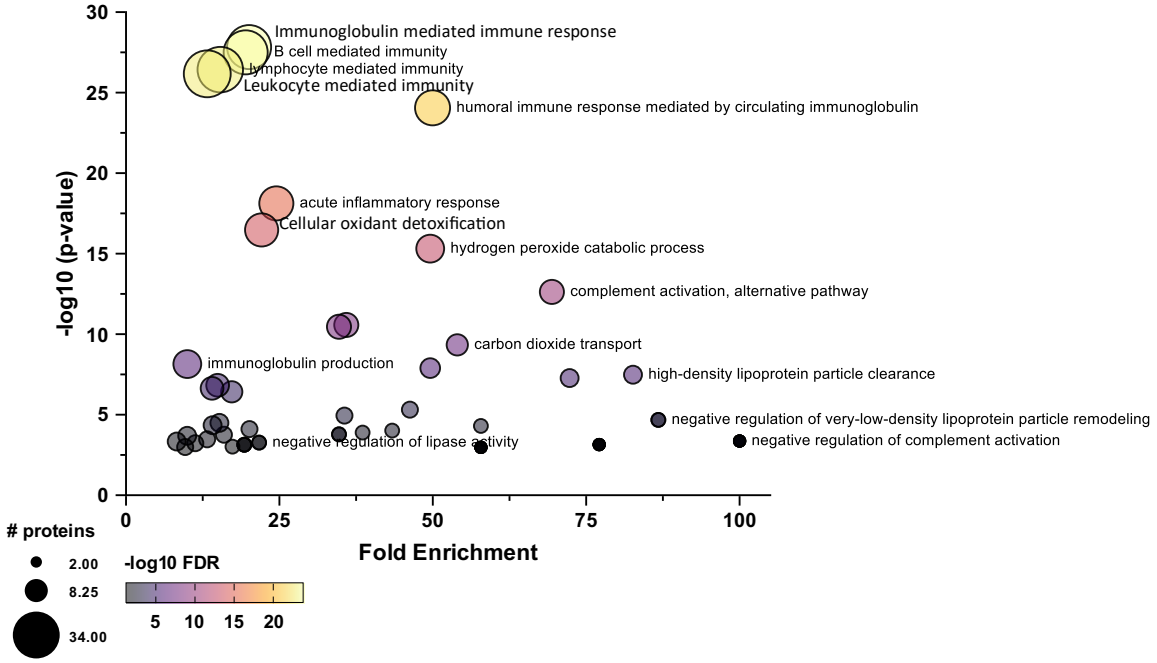

Figure 4.3. Decreased Pathways Identified by Lower Protein Abundance in Cord Blood of Aerobic Exercisers vs Control.

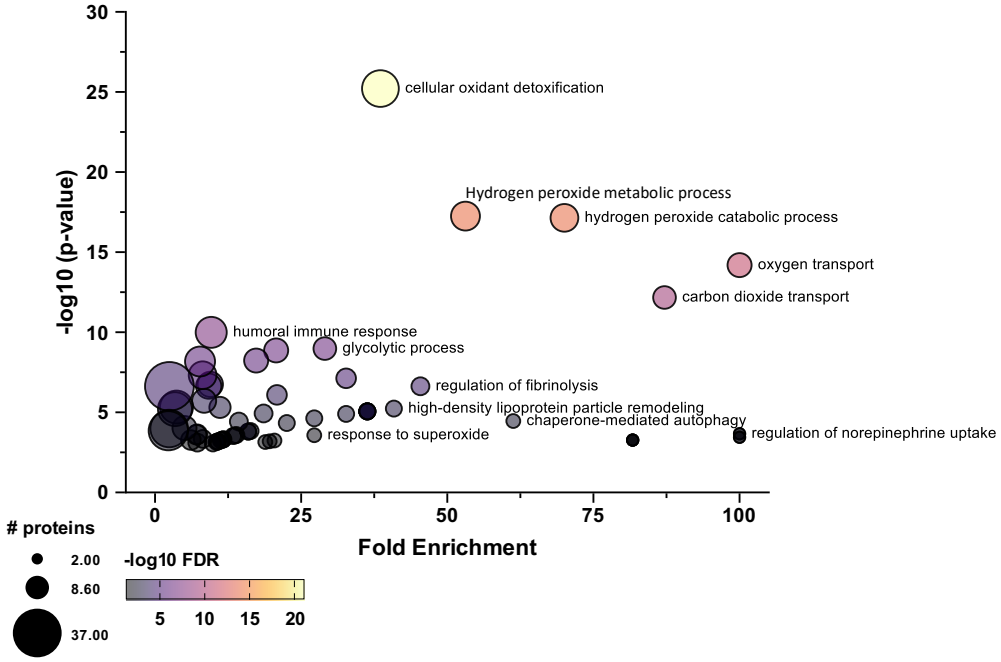

Figure 4.4. Decreased Pathways Identified by Lower Protein Abundance in Cord Blood of Resistance Exercisers vs Control.

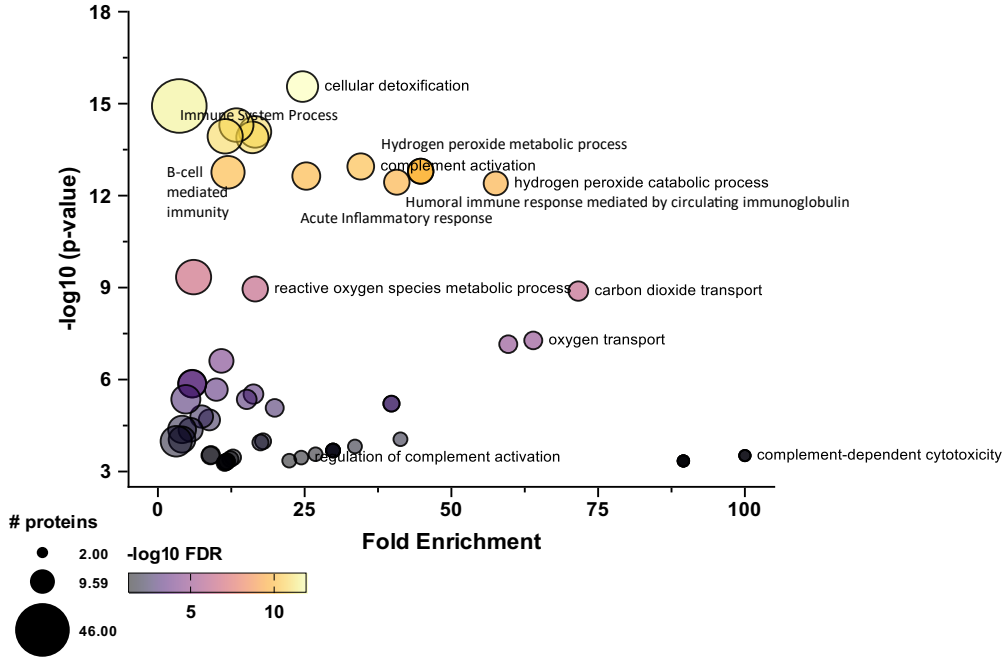

Figure 4.5. Decreased Pathways Identified by Lower Protein Abundance in Cord Blood of Combination Exercisers vs Control.

CHAPTER 5

Summary and Conclusions

Evidence suggests that prolonged, excessive inflammation during pregnancy is linked with adverse pregnancy outcomes (APOs). Specifically, high pro-inflammatory biomarkers such as tumor necrosis factor alpha (TNF-α); interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and c-reactive protein (CRP) and insufficient anti-inflammatory biomarkers such as IL-10 are associated with intrauterine growth restriction, preterm birth, gestational diabetes, or preeclampsia. [4,105,139,5–10,140] The placenta, as the mediator between the maternal and fetal environments during pregnancy, supports optimal conditions for growth and development. Yet, women with APOs linked with excessive inflammation in the maternal environment may also have changes to placental immunoregulation[11,12] and, thus, alter the intrauterine environment.[13–17] An excessive inflammatory fetal environment *in utero*, a critical period for tissue and organ development,[18] thus programs the offspring towards adverse outcomes and potential disease later in life.[14–17,19,20] However, research indicates that exercise during pregnancy may aid in reducing pro-inflammatory biomarkers (e.g. CRP, TNF-α, IL-6).[31,32,73] In non-gravid individuals, exercise mode appears to differentially impact inflammatory biomarker concentrations.[36,38,141]

Despite the suggested differences of exercise modes on inflammation in non-gravid individuals, the impact of exercise modes on inflammation during pregnancy has not been well defined. Therefore, the purpose of this project was to determine the relationship between antenatal exercise mode on pregnancy inflammation utilizing a three-study approach. We examined the influence of antenatal aerobic (AE), resistance (RE), or combination exercise (CE) on maternal inflammatory biomarkers in late pregnancy (Study 1), placental inflammatory biomarkers (Study 2), and cord inflammatory biomarkers at delivery (Study 3). This project also examined the impact of exercise metrics on inflammation (Studies 1-3) and utilized a global proteomics approach to identify additional proteins and pathways associated with inflammation

that were altered (Study 2 and 3). Briefly, across all three studies we found similar concentrations of inflammatory biomarkers between groups for maternal blood, placental tissue, and cord blood. However, our first study found increased exercise duration linked with lower maternal IL-6 and IL-8, while increased exercise frequency was associated with increased TNF-α. Our second project found that increased exercise intensity predicted decreased fibrinogen in placental tissue. Finally, our third study found increased total exercise volume is associated with lower IL-6, while CE is specifically linked with lower IL-6 and cortisol in cord blood. In addition to these findings, our second and third studies found significant proteome differences between exercise groups relative to control. This allowed us to identify several proteins and pathways to target in future research projects to better establish the changes to inflammation that occur with antenatal exercise.

SUMMARY OF KEY FINDINGS

Study 1: Exercise mode and Maternal Inflammation

The first study found similar levels of inflammatory biomarkers in maternal blood between groups, but increased exercise duration was associated with decreased pro-inflammatory markers IL-6 and IL-8, while increased exercise frequency was linked with increased TNF-α. Finding similar late pregnancy maternal inflammatory biomarkers between groups was unexpected. A previous study found AE during pregnancy led to lower TNF-α in late pregnancy relative to physically active controls.[31] However, this study collected maternal blood 2-4 hours postprandially. Meal composition alters plasma TNF-α and, to our knowledge, was not controlled for in the previous research.[31,82] This may explain the discrepancy between our findings and that previously reported. Additionally, maternal pre-pregnancy BMI and infant sex were not reported which may impact maternal inflammation[102,142,143] and therefore difficult to compare populations. Our findings align, in part, with that of previous research in CE during pregnancy. Like the present study, Acosta-Manzano et al. reported that CE during pregnancy does not change IL-6 or IL-1β.

However, this group did find that CE during pregnancy significantly decreases TNF-α in late pregnancy relative to non-exercisers.[73] The CE intervention in previous research had 2 days/week of resistance with 1 day/week of aerobic activities, whereas the present study spent equal time doing RE + AE at every exercise session.[73] Additionally, the first study in this project did have slightly higher pre-pregnancy BMI in our CE group compared to the intervention group in the previous study. Pre-pregnancy BMI can influence maternal inflammatory biomarkers and thus may contribute to why our results differ.[102,143] Although there was no significance in the present study, AE and RE groups had somewhat lower average TNF-α relative to CE. Therefore, these differences could suggest a reduced pro-inflammatory environment with AE or RE training during pregnancy. However, more research is needed that stratifies by pre-pregnancy BMI to better define this relationship.

In the first study we also found that increased weekly exercise duration was associated with lower IL-6 and IL-8 concentrations. Similarly, a previous study found that women had higher exercise duration and trended towards lower IL-8 relative to men.[86] Exercise training is known to decrease basal IL-6. [86,144] Specifically, 4-months of AE training in healthy women decreases basal IL-6 concentration ~60%.[145] Taken together, it is possible that longer exercise sessions over time could result in greater acute decreases in IL-8 and IL-6. This may result in an improved sensitivity to these biomarkers overtime, thus, resulting in lower resting IL-8 and IL-6 with training.

Additionally, we found that exercise frequency was associated with increased TNF-α. An acute bout of intense exercise increases TNF-α, which is suggested to be a result of muscular damage that occurs.[88,89] A recent meta-analysis found that exercise training reduced overall TNF-α concentrations, but those who exercised 3 times per week or less had greater decreases than those that exercised more than 3 times per week.[146] Considering that TNF-α tends to increase in the third trimester[143] and the link between exercise frequency and TNF-α response previously described, it is conceivable that those who exercised more frequently during pregnancy may

experience a heightened TNF-α response. Collectively, these findings support the importance of further research to understand regular maternal changes especially in response to all exercise metrics during (frequency, intensity, duration, volume) related to the influence on maternal inflammatory biomarker concentrations.

Study 2: Exercise mode and Placental Inflammation

The second study had similar inflammatory biomarkers across groups in placental tissue. However, we also found that exercise intensity was associated with lower placenta fibrinogen. Fibrinogen is a protein that is involved in blood clots and can be a marker of inflammation[147,148]; during pregnancy, fibrinogen is elevated in women with gestational diabetes, preeclampsia, and cholestasis.[149–151] Previous research in obese young men found RE training reduces plasma fibrinogen compared to AE training.[110] These researchers suggested this was due to the higher intensity involved in RE relative to AE.[110] Furthermore, another study notes exercise volume (MET/hours/week) and counts per minute collected from accelerometry are both negatively correlated with plasma fibrinogen in healthy adults.[152] Counts per minute is used to determine approximate intensity from accelerometer data[153] and thus, supports that increased exercise intensity results in lower fibrinogen levels in healthy adults. Due to the limited sample size in the current project, we could not stratify by pre-pregnancy BMI or delivery mode, both of which may influence placental inflammatory outcomes. [103,104,106,154] However, ANCOVA analysis did include these covariates and did not change our results. The relationship between exercise intensity and fibrinogen concentration indicates there is an influence of antenatal exercise on placental inflammation. These findings also support the importance of collecting all exercise metrics when performing exercise during pregnancy and may be important for helping to control pregnancy inflammation in the placental.

Study 3: Exercise Mode and Cord Blood Inflammation

The third study examined the effect of exercise mode on cord blood inflammatory markers, to represent inflammation in the fetal environment. This study found similar inflammatory

concentrations in cord blood between groups for all biomarkers. We also found that exercise mode, pre-pregnancy BMI, and exercise volume predicted IL-6 in cord blood. Further, exercise mode and pre-pregnancy BMI predicted cortisol concentration. As mentioned in Study 1 in maternal blood, exercise training decreases resting IL-6.[86,144] Considering the association between maternal and cord blood inflammation and that exercise duration contributes to exercise volume,[13,57] it makes sense that training at a greater volume would result in greater reductions in cord blood IL-6. Regarding exercise group, we found that AE is linked with greater IL-6 and cortisol whereas CE and CON groups were linked to lower IL-6 and cortisol. Interestingly, although there were no significant differences between groups, our AE group had the highest proportion of female offspring whereas CON and CE had the lowest. Female offspring have higher concentrations of cortisol in cord blood and therefore may explain the relationship between AE and increased cortisol concentration.[131] Our findings also indicated that higher pre-pregnancy BMI was predictive of lower IL-6 and cortisol in cord blood. Previous studies show that cortisol and IL-6 are related to body composition. Literature is unclear whether pre-pregnancy BMI influences cord blood biomarkers, as evidenced by contradictory findings.[125–127] However, fat mass specifically is linked with higher salivary cortisol and inversely related to infant IL-6 at 4-months.[129,155] Since BMI and GWG are not specific indicators of fat mass vs. lean mass, it is possible that the results of the present study could reflect differences in maternal body composition. The effect of neonatal body composition *in utero* on cord blood biomarkers such as IL-6 or cortisol has not been defined. However, considering increased adiposity and reduced muscle mass are associated with increased cortisol and IL-6, it is possible the fetal body composition in late pregnancy would also influence these outcomes. Since this study did not capture maternal or neonatal muscle and fat mass, more research is needed to better establish the relationship between maternal and offspring body composition on cord blood inflammatory biomarkers.

Study 2 and 3: Exercise Mode and Proteomics

Lastly, the global proteomics analyses in the second and third studies found significant differences in proteome landscapes of exercisers relative to control participants. The exercise groups had various changes in protein abundances and subsequent pathways that related to inflammation. Although our second study was inconclusive for the pathway analysis, we did see several proteins with decreased abundance in placental tissue that were related to immunoglobulin variable units, C4B complement and binding protein, as well as macrophages. These proteins are involved in the humoral and innate immune responses. [156–159] Our second study also found that all exercise groups had 1 protein, eukaryotic translation initiation factor 3 subunit K (eIF3K), in common with higher abundance. This is a component of the eukaryotic translation initiation factor 3 complex, which is required for the initiation of protein synthesis. [160] Therefore, this suggests that overall protein synthesis appears to be heightened in placental tissue of exercisers, with decreases to proteins related to inflammation. Interestingly, our third study observed that the cord blood of AE group had downregulated pathways directly related to inflammation and immunity. Of those changed, the decreased protein abundance in cord blood of AE group that were of interest were linked to B-cell mediated immunity, lymphocyte mediated immunity, and humoral immune response mediated by circulating immunoglobulin pathways. RE had decreased protein abundance in the pathway related to humoral immune response and CE had reduced protein abundance in complement activation pathway. Taken together, these pathways are all either related to or have an important role in adaptive immunity, which includes T-helper (Th) cells and the downstream inflammatory effects (e.g., TNF-α, IL-6, IL-8, IL-10) that are, in part, targeted by this project.[161] The downregulation of these immune pathways in cord blood of AE, RE, and CE may indicate lower exposure to foreign pathogens for the immune system to fight off. In turn, this would result in lower immune activation and downstream inflammation in the placental and fetal environments. All exercisers also had a significantly lower abundance in proteins within the reactive oxygen species (ROS) metabolic process pathway. This suggests that ROS was reduced in exercisers, thus reducing the inflammatory response, since

ROS is an inflammatory mediator.[162] Interestingly, while the cord blood of AE and RE demonstrated the greatest decreases in specific pathways, the CE group had the greatest decrease in pathways that were similar to both AE and RE and additionally had large decreases in pathways unique to the CE group. This suggests that the cord blood of CE not only has similar benefits of AE and RE individually, but also experiences unique benefits by combining the two exercise modes, thus, supporting an exercise mode effect. Overall, the downregulation in placental proteins and cord blood pathways related to immunity may indicate the placental-fetal interface is in an overall healthier environment for AE, RE, and CE groups. These findings extend the current knowledge, and taken collectively, suggest that exercise during pregnancy results in lower immune activity, and thus inflammation, in placental tissue and cord blood. More research is needed targeting these specific proteins and pathways to better elucidate the influence AE, RE, and CE has on inflammation and immune responses during pregnancy in a larger population.

This project has strengths that warrant mention. First, our project utilized sensitive methods for assessing both specific markers of inflammation and global approach for additional biomarkers and pathways related to inflammation. Second, our overall randomized control trial study design utilizing three separate exercise modes provided the best design for causality. Third, participants of all races and BMIs with low-risk, singleton pregnancies were recruited for the current studies, which helps strengthen the generalizability of our findings. However, this project has limitations. First, we had a small sample size for the studies, however, our sample was diverse to make these new and innovative findings more generalizable. Second, we were not able to control for potential covariates (e.g., pre-pregnancy BMI, stress, delivery mode) which could also have impacted the findings [90,102,104,105] and diminished our ability to detect the effect of exercise. Despite these limitations, these studies are the first for a direct comparison of exercise mode on these and outcomes and thus demonstrate interesting trends for inflammatory biomarkers with exercise during pregnancy. Thus, more research is needed.

CONCLUSION

This dissertation focused on comparing the effect of different exercise modes during pregnancy on inflammation in the maternal, placental, and cord compartments. Considering our findings from each study, this dissertation found that antenatal exercise during pregnancy did not differentially influence inflammatory biomarkers in pregnant women, placentas, or cord blood at delivery. Despite this, our findings did highlight the importance of collecting and monitoring all exercise metrics (e.g., intensity, time, type, frequency, adherence) as each of these are related to changes in inflammatory biomarkers. Additionally, we found differences among proteins and pathways associated with inflammation in placental tissue and cord blood, providing targets for future projects. Collectively these findings are an important contribution to the field, further emphasizing the possible beneficial health impact of exercise during pregnancy, regardless of the exercise modes investigated in the current study. Although our studies did not support our central hypothesis of a differential impact of exercise mode on inflammatory biomarkers during pregnancy, it is important to note we had a small sample, therefore these findings should be interpreted carefully. More research is needed with a larger population of pregnant women to establish whether exercise frequency, intensity, duration, or mode has the greatest impact on inflammation during pregnancy. Altogether, the research studies within this dissertation provide new targets for future research on pregnancy inflammation and extend our knowledge on how antenatal exercise mode impacts inflammatory biomarkers during pregnancy, thus impacting maternal and infant health.

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Appendix A: Institutional Review Board Approval Documents

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
4N-64 Brody Medical Sciences Building- Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office 252-744-2914 · **Fax 252-744-2284** ·
rede.ecu.edu/umcirb/

Notification of Continuing Review Approval: Expedited

From: Biomedical IRB
To: Linda May
CC: Lindsey Rossa
Date: 11/30/2022
Re: CR00009943
UMCIRB 12-002524
ENHANCED by Mom

The continuing review of your expedited study was approved. Approval of the study and any consent form(s) is for the period of 11/29/2022 to 11/28/2023. This research study is eligible for review under expedited categories # 2,5,7. The Chairperson (or designee) deemed this study no more than minimal risk.

As the Principal Investigator you are explicitly responsible for the conduct of all aspects of this study and must adhere to all reporting requirements for the study. Your responsibilities include but are not limited to:

1. Ensuring changes to the approved research (including the UMCIRB approved consent document) are only initiated with UMCIRB review and approval except when necessary to eliminate an apparent immediate hazard to the participant. All changes (e.g. a change in procedure, number of participants, personnel, study locations, new recruitment materials, study instruments, etc.) must be prospectively reviewed and approved by the UMCIRB before they are implemented;

2. Ensuring that only valid versions of the UMCIRB approved, date-stamped informed consent document(s) are used for obtaining informed consent (consent documents with the IRB approval date stamp are found under the Documents tab in the ePIRATE study workspace);

3. Promptly reporting to the UMCIRB all unanticipated problems involving risks to participants and others;

4. Applying for continuing review and receive approval of continuation of the study prior to the study's current expiration date. Application for continuing review should be submitted no less than 30 days prior to the expiration date. Lapses in approval (i.e. study expiration) should be avoided to protect the safety and welfare of enrolled participants and liability to the University; and

5. Submission of a final report when the study meets the UMCIRB criteria for closure. Study approval should not be allowed to expire simply because the study is completed, rather the UMCIRB should be formally notified of study completion via the final report process.

The approval includes the following items:

Document	Description
Aerobic Home Exercise Sheet(0.01)	Additional Items
Circuit Home Exercise Sheet(0.01)	Additional Items
Dietary Assessment(0.01)	Surveys and Questionnaires
ENAHNCED Protocol COVID 19 Addendum (0.01)	Study Protocol or Grant Application
ENHANCED 12-002524-protocol Amendment 40-HIGHLIGHTED 1.26.2020(0.15)	Study Protocol or Grant Application
ENHANCED Protocol 12-002524 Amendment 40-CLEAN-1.26.20(0.22)	Study Protocol or Grant Application
ENHANCED Protocol 12-002524 Amendment 52 - Clean Version 7.6.21_.docx(0.01)	Study Protocol or Grant Application
ENHANCED Protocol 12-002524 Amendment 52 - Highlighted Version 7.6.21_.docx(0.01)	Study Protocol or Grant Application
ENHANCED%20Timeline-2014.pdf(0.01)	Additional Items
Exercise Motivations Inventory(0.01)	Surveys and Questionnaires
Exercise Session Log Sheets(0.01)	Additional Items
International Physical Activity Questionnaire(0.01)	Surveys and Questionnaires
IRB Agreement - ECU-Campbell University 03.30.17(0.01)	Additional Items
Maternal Measurements Form(0.01)	Additional Items
Mindfulness Home Exercise Sheet1.docx(0.01)	Additional Items
Modified Balke Protocol.docx(0.01)	Standardized/Non-Standardized Instruments/Measures
Modified Physical Activity Questionnaire020817(0.05)	Surveys and Questionnaires
Nutrition Powerpoint(0.01)	Additional Items
Psychological Need Satisfaction in Exercise Scale(0.01)	Surveys and Questionnaires
Resistance Home Exercise Sheet(0.01)	Additional Items
Sheffield FFQ.doc(0.02)	Surveys and Questionnaires
Stretching Home Exercise Sheet(0.02)	Additional Items

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.

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Notification of Continuing Review Approval

From:	Biomedical IRB
To:	Linda May
CC:	
	Lindsey Rossa
Date:	7/28/2023
	CR00010154
Re:	UMCIRB 19-001863
	Exercise Modality on Childhood Obesity Risk

I am pleased to inform you that at the convened meeting on 7/26/2023 12:15 PM of the Biomedical IRB, this research study underwent a continuing review and the committee voted to approve the study. Approval of the study and the consent form(s) is for the period of 7/26/2023 to 7/25/2024.

The Biomedical IRB deemed this study Greater than Minimal Risk .

As the Principal Investigator you are explicitly responsible for the conduct of all aspects of this study and must adhere to all reporting requirements for the study. Your responsibilities include but are not limited to:

1. Ensuring changes to the approved research (including the UMCIRB approved consent document) are only initiated with UMCIRB review and approval except when necessary to eliminate an apparent immediate hazard to the participant. All changes (e.g. a change in procedure, number of participants, personnel, study locations, new recruitment materials, study

instruments, etc.) must be prospectively reviewed and approved by the UMCIRB before they are implemented;

2. Ensuring that only valid versions of the UMCIRB approved, date-stamped informed consent document(s) are used for obtaining informed consent (consent documents with the IRB approval date stamp are found under the Documents tab in the ePIRATE study workspace);

3. Promptly reporting to the UMCIRB all unanticipated problems involving risks to participants and others;

4. Applying for continuing review and receive approval of continuation of the study prior to the study's current expiration date. Application for continuing review should be submitted no less than 30 days prior to the expiration date. Lapses in approval (i.e. study expiration) should be avoided to protect the safety and welfare of enrolled participants and liability to the University; and

5. Submission of a final report when the study meets the UMCIRB criteria for closure. Study approval should not be allowed to expire simply because the study is completed, rather the UMCIRB should be formally notified of study completion via the final report process.

The approval includes the following items:

Document	Description
Aerobic Source Document (0.02)	Data Collection Sheet
Circuit Source Document (0.02)	Data Collection Sheet
Control Source Document (0.02)	Data Collection Sheet
Delivery and Birth Record (0.02)	Data Collection Sheet
Dietary assessment-Preg Study.docx(0.01)	Surveys and Questionnaires
Documentation of Informed Consent (0.02)	Data Collection Sheet
ECU OB Support Letter(0.01)	Additional Items
Effect of Exercise Modality during Pregnancy on Childhood Obesity-updated010623(0.05)	Study Protocol or Grant Application
EMCOR-MATERNAL CONSENT-HIPPA-with gift cards-010523(0.14)	Consent Forms

EMCOR-Pediatric Consent-HIPAA-with gift cards(0.15)	Consent Forms
FFQ.docx(0.01)	Surveys and Questionnaires
HIPAA Authorization for Parent Guardian.ActiveMoms.OBOW.4.15.21.doc(0.01)	HIPAA Documentation
HIPAA Authorization Template.ActiveMoms.OBOW.4.15.21.doc(0.02)	HIPAA Documentation
HIPAA-Authorization for Future Research4.15.21.doc(0.02)	HIPAA Documentation
Infant Blood Draw Source Document (0.01)	Data Collection Sheet
Infant Environment Enrichment Questionnaire(0.01)	Surveys and Questionnaires
Infant Feeding Questionnaire(0.01)	Surveys and Questionnaires
Infant Measurements Source Document (0.01)	Data Collection Sheet
Infant Physical Activity and Sleep Questionnaire(0.03)	Surveys and Questionnaires
IPAQ(0.01)	Surveys and Questionnaires
Letter of Support PCHD(0.01)	Additional Items
Maternal Blood Draw Source Document (0.01)	Data Collection Sheet
Maternal Measurements Source Documents(0.02)	Data Collection Sheet
Modifiable Activity Questionnaire (0.01)	Surveys and Questionnaires
OB information form(0.02)	Additional Items
OB Physical Activity Clearance Example Letter(0.01)	Additional Items
Participant Participation Log Document (0.03)	Data Collection Sheet
Promotional Materials(0.01)	Surveys and Questionnaires
Recruitment Agency Recruitment Materials(0.01)	Recruitment Documents/Scripts
recruitment flyer - updated for NIH funding(0.01)	Recruitment Documents/Scripts
Resistance Source Document (0.02)	Data Collection Sheet
Screening & Enrollment Log (0.01)	Data Collection Sheet
Study Recruitment Flyer 1(0.05)	Recruitment Documents/Scripts
Study Recruitment Flyer 2(0.03)	Recruitment Documents/Scripts
Study Timeline for Participants(0.02)	Additional Items
Support Letter Greenville OB(0.01)	Additional Items
TikTok Script from TrialFacts(0.01)	Recruitment Documents/Scripts
Tissue Collection Source Document (0.02)	Data Collection Sheet
Treadmill, 1-RM and Maternal REE testing-updated(0.02)	Data Collection Sheet
updated social media flyer - for NIH funding(0.01)	Recruitment Documents/Scripts

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 following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study:

N. Broskey

The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: None

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

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rede.ecu.edu/umcirb/

Notification of Continuing Review Approval

From:	Biomedical IRB
To:	Linda May
CC:	
	Lindsey Rossa
Date:	9/15/2023
Re:	CR00010229 UMCIRB 20-002367 MAMA Tot - Healthy Weight Parallel Study

I am pleased to inform you that at the convened meeting on 9/13/2023 12:15 PM of the Biomedical IRB, this research study underwent a continuing review and the committee voted to approve the study. Approval of the study and the consent form(s) is for the period of 9/13/2023 to 9/12/2024.

The Biomedical IRB deemed this study Greater than Minimal Risk .

As the Principal Investigator you are explicitly responsible for the conduct of all aspects of this study and must adhere to all reporting requirements for the study. Your responsibilities include but are not limited to:

1. Ensuring changes to the approved research (including the UMCIRB approved consent document) are only initiated with UMCIRB review and approval except when necessary to eliminate an apparent immediate hazard to the participant. All changes (e.g. a change in procedure, number of participants, personnel, study locations, new recruitment materials, study

instruments, etc.) must be prospectively reviewed and approved by the UMCIRB before they are implemented;

2. Ensuring that only valid versions of the UMCIRB approved, date-stamped informed consent document(s) are used for obtaining informed consent (consent documents with the IRB approval date stamp are found under the Documents tab in the ePIRATE study workspace);

3. Promptly reporting to the UMCIRB all unanticipated problems involving risks to participants and others;

4. Applying for continuing review and receive approval of continuation of the study prior to the study's current expiration date. Application for continuing review should be submitted no less than 30 days prior to the expiration date. Lapses in approval (i.e. study expiration) should be avoided to protect the safety and welfare of enrolled participants and liability to the University; and

5. Submission of a final report when the study meets the UMCIRB criteria for closure. Study approval should not be allowed to expire simply because the study is completed, rather the UMCIRB should be formally notified of study completion via the final report process.

The approval includes the following items:

Document	Description
Aerobic Source Document_ActiveMoms-LM.docx(0.02)	Data Collection Sheet
Circuit Data Sheet_ActiveMoms-LM.docx(0.02)	Data Collection Sheet
Delivery and Birth Record_ActiveMoms-LM.docx(0.02)	Data Collection Sheet
Dietary assessment-Preg Study.docx(0.01)	Surveys and Questionnaires
Documentation of INformed Consent(0.01)	Additional Items
Documentation of Informed Consent-LM.docx(0.02)	Data Collection Sheet
FFQ.docx(0.01)	Surveys and Questionnaires
HIPAA Authorization for Future Research(0.01)	HIPAA Documentation
HIPAA-Authorization-Neonate-mamatot.doc(0.03)	HIPAA Documentation
Infant Blood Draw Source Document.docx(0.01)	Data Collection Sheet
Infant Environment Enrichment Questionnaire.docx(0.01)	Surveys and Questionnaires

Infant Feeding Questionnaire.docx(0.01)	Surveys and Questionnaires
Infant Measurements Source Document.docx(0.01)	Data Collection Sheet
Infant Physical Activity and Sleep Questionnaire (1).docx(0.02)	Surveys and Questionnaires
Information for OB providers about MAMA TOT(0.01)	Additional Items
IPAQ.pdf(0.01)	Surveys and Questionnaires
Letter of support-PCHD-Linda May-mamatot.pdf(0.01)	Additional Items
Mama Tot - parallel study-updated.docx(0.05)	Study Protocol or Grant Application
MAMA Tot HIPAA Maternal .doc(0.03)	HIPAA Documentation
MAMA TOT Maternal Consent-HIPPA-Clean-022822.docx(0.05)	Consent Forms
Maternal Blood Draw Source Document.docx(0.01)	Data Collection Sheet
Maternal Measurements _ ActiveMom-LM.docx(0.02)	Data Collection Sheet
MPAQ(0.01)	Surveys and Questionnaires
OB Physical Activity Clearance Example.docx(0.01)	Additional Items
Participant Participation Log Document .docx(0.02)	Data Collection Sheet
Pediatric MAMA TOT W- HIPPA-clean-022822.docx(0.05)	Consent Forms
Resistance Data Sheet_ActiveMoms-LM.docx(0.02)	Data Collection Sheet
Screening & Enrollment Log .docx(0.01)	Data Collection Sheet
Study Flyer .pdf(0.01)	Recruitment Documents/Scripts
Study Flyer 2 .pdf(0.01)	Recruitment Documents/Scripts
Tissue Processing Form-LM.docx(0.02)	Data Collection Sheet
Treadmill, 1-RM and Maternal REE testing(0.01)	Data Collection Sheet
Yoga Data Sheet_ActiveMoms-LM.docx(0.02)	Data Collection Sheet

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 following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study:

None

The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: N. Broskey

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