The Influence of Prenatal Exercise Mode on Maternal Cortisol Levels during Pregnancy

By

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ABSTRACT

Background: Cortisol is essential for regulating metabolism, immune function, and stress response. During pregnancy, cortisol levels naturally rise to support fetal growth and development. However, elevated maternal cortisol levels are linked to adverse pregnancy outcomes, such as preeclampsia and preterm birth. While exercise is known to normalize cortisol levels in non-pregnant women, its impact during pregnancy remains unexplored. This study aims to examine the influence of different modes of exercise during pregnancy on maternal cortisol levels. **Methods:** This study consisted of women with singleton pregnancies, who were randomly assigned to one of four groups: aerobic (AE), resistance (RE), combination exercise (CE), or an attention control group (CON). Blood samples were collected at 16- and 36-weeks' gestation to measure cortisol levels. Participant exercise sessions were supervised and tracked. **Results:** In all evaluated exercise types (AE, RE, and CE) the participants had normal levels of cortisol, normal birth outcomes, with no adverse pregnancy outcomes, and thus were deemed safe for pregnant women. The study observed different change patterns in

cortisol levels from early to late pregnancy among exercising groups. At 16 weeks, all the maternal cortisol levels had a small effect size, which suggested the group values were similar. At 36 weeks, the AE and CE groups had a small effect size, which showed no difference between those values; but in the RE group, there was a large effect size, which showed a difference. The change scores of the maternal blood cortisol levels showed CON, RE, and CE groups increased from early to late pregnancy, however AE decreased from early to late pregnancy. **Discussion:** This study enhances the understanding of how exercise during pregnancy affects cortisol levels. While the RE group compared to the CON group showed a moderately strong increase at 36 weeks, this can be due to the large effect size. It provides insights into safe and beneficial exercise regimens, aiming to inform healthcare professionals and expecting mothers about the role of exercise in managing cortisol levels and promoting healthier pregnancy outcomes.

The Influence of Prenatal Exercise Mode on Maternal Cortisol Levels during Pregnancy

A Thesis

Presented to the Faculty of the Department of Kinesiology

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In Partial Fulfillment of the Requirements for the Degree

Masters of Science in Kinesiology

By

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INTRODUCTION

Worldwide, approximately 3-5% of pregnancies are complicated by preeclampsia (Vakil et al., 2022), and another 11.1% are born preterm (Obst et al., 2022). Furthermore, preeclampsia and preterm birth are associated with high maternal cortisol (Nepomashcy et al. 2006). However, elevated cortisol during pregnancy is associated with fetal growth restriction and infant developmental delays (Reis et al., 1999; Davis & Sandman, 2006).

Katsu & Baker, (2021) found in the non-gravid (non-pregnant population) state, cortisol plays a crucial role in regulating protein and carbohydrate metabolism. Cortisol also affects various physiological processes such as glucose utilization, blood pressure maintenance, immune function, and the body's anti-inflammatory response, its primary role seems to lie in influencing peripheral glucose utilization. Katsu & Baker, (2021) also discovered that cortisol can increase blood sugar through gluconeogenesis, and it is essential for the functions of the central nervous system as well as the digestive, hematopoietic, renal, and reproductive systems. Allen & Sharma, (2024) also noted that corticotropin-releasing hormone (CRH) originated from the hypothalamus, which promotes the anterior pituitary to release Adrenocorticotropic hormone (ACTH) and, in turn, triggers the adrenal cortex to release cortisol and androgens. Elevated cortisol levels establish a negative feedback loop, reducing CRH secretion from the hypothalamus. Therefore, it is important to understand how levels of cortisol change throughout pregnancy.

Once the duration of pregnancy increases, serum cortisol levels increase from the first to the third trimester of pregnancy (Osayande & Ekpruke, 2017). During pregnancy, maternal cortisol levels increase two to four times that of non-gravid levels to meet both maternal and fetal physiological needs (Wolpert, 2016). This increase in cortisol levels is important, due to its

vital role in fetal growth and development (Wolpert, 2016). When referring to the actions in the fetus, the steroid hormones aid in the development of the respiratory system, brain and cardiovascular system. These steroid hormones are also associated with several other fetal tissues such as central nervous system, kidney, hindgut, testis, bile ducts, and lungs through midgestation (Cole et al., 2019). Valsamakis et al., (2020) observed that placental CRH, along with cortisol and other hormones (like aldosterone, estradiol, and testosterone are produced from cholesterol within specialized endocrine cells found in the adrenal gland, ovary, and testis (Cole et al., 2019) that cross to the placenta, may lead to a slowdown in fetal growth, decreased birth weight, and the onset of preterm labor in fetuses experiencing prenatal stress. Valsamakis et al., (2020) also revealed that elevated placental CRH has been proposed as a potential predictor for preterm, term, or post-term birth.

Nevertheless, exercise during pregnancy has been shown to be beneficial for both maternal and child health. Exercise helps normalize cortisol levels in non-gravid and gravid (pregnant population) women (Budnik-Przybylska et al., 2020). Cortisol is often perceived as playing a counterproductive role in exercise, potentially hindering adaptation to exercise training, which is due to its catabolic effect on protein turnover. However, cortisol has catabolic actions within the human body, these actions can be beneficial and productive in response to the stress of exercise and the exercise training process. Cortisol is also required to regulate energy metabolism, thus an increase in cortisol levels with an increase in exercise is expected (HACKNEY & WALZ, 2013).

The majority of the literature is on non-gravid women and how exercise such as aerobic, resistance (strength training), and a combination (aerobic and resistance training), affects their cortisol levels. Regarding aerobic exercise, one study in a non-gravid population found that participating in high-intensity exercise for bouts of <15 minutes had an increase in cortisol post-exercise (Consitt et al., 2002). In another study in a gravid population found that after 40 minutes

of walking had a mean increase of 105 nmol•l^{-1} immediately post-exercise (McMURRAY et al., 1996). For resistance training, one study identified that resistance exercise of high intensity can elicit short-term cortisol decreases in non-gravid women. For hypertrophy it decreased from 17.29 ± 1.20 to 10.17 ± 1.27 μg/dl, strength went from 17.96 ± 2.51 to 10.62 ± 1.22 μg/dl, and power-type training went from 17.95 ± 2.13 to 9.67 ±1.31 μg/dl (TaheriChadorneshin et al., 2021). Lastly, in combination exercises, one study also found that non-gravid women had high levels of urinary cortisol at rest compared to those who solely participated in strength or endurance training. Before the participants began they started with a mean of 45.32 nmol•$24h^{-1}$ and increased after 12 weeks with a mean of 83.13 nmol•$24h^{-1}$ (Bell et al., 2000). There is scant research focused on the influence of different exercise modes during pregnancy on maternal cortisol levels.

As stated by the previous literature it is known that cortisol will increase naturally throughout pregnancy, and in acute adaptations, resistance training decreases cortisol levels, and combination training increases cortisol levels in non-gravid populations. In the gravid populations, it is known from previous literature that aerobic training increases cortisol levels. What is unknown is whether exercise influences cortisol levels and what the chronic response is to exercise and cortisol levels.

Therefore, the purpose of this research is to determine if exercise mode influences cortisol levels during pregnancy. It is hypothesized that any type of prenatal exercise mode will result in lower levels of cortisol in late pregnancy, with smaller change scores from 16 to 36 weeks compared to non-exercise controls at the same time point.

Definitions:

- Gravid: women who are pregnant
- Non-gravid: women who are not pregnant.
- Cortisol: is a glucocorticoid produced from the adrenal cortex (Bleker et al., 2017); it has many functions in the human body, such as mediating the stress response, regulating metabolism, inflammatory response, and immune function (Thau et al.,2024)
- Gravida: the number of pregnancies a woman has had
- Parity: the number of times a woman has given birth to a live or non-viable baby, regardless of gestation

Inclusions:

To be eligible for the study all gravid participants had to enroll prior to 16 weeks' gestation and be cleared by their physicians to participate in physical activity. Participants were accepted regardless of their physical activity levels (current or past). Additionally, all participants had to be between the ages of 18 and 40, with a pregnancy body mass index (BMI) of 18.5-39.9. They also cannot be currently using alcohol, tobacco, recreational drugs, or medications for mental health disorders, or have mental health disorders. They also can not currently meet any of the contradictions to exercise in pregnancy as outlined by the American College of Obstetricians and Gynecologists and the American College of Sports Medicine (ACSM) guidelines.

Exclusions:

Participants were excluded if they have chronic conditions such as HIV, AIDs, diabetes, hypertension, or other cardiovascular disease. If the participants are using tobacco, alcohol, or other recreational drugs, and if they are using medications that affect fetal development.

Participants were also excluded if they did not have reliable transportation, email and phone. This study is also limited to English-speaking women as well.

LITERATURE REVIEW

The search engines that were used to conduct the literature review were PubMed and Google Scholar. The information that was gathered is from the years 1996-2024.

Elevated cortisol levels during pregnancy are associated with adverse pregnancy outcomes (i.e., miscarriages, pre-eclampsia, fetal growth restriction, pre-term birth). Due to this correlation, it is imperative to determine how to control maternal cortisol (Davis & Sandman, 2006; Nepomnaschy et al., 2006; Reis et al., 1999). Exercise has been used in the non-gravid and gravid populations to help normalize cortisol levels (Budnik-Przybylska et al., 2020); thus, the purpose of this study is to determine what mode of exercise helps control cortisol levels throughout pregnancy. This chapter will provide an overview of cortisol, how exercise mode influences cortisol levels, and how cortisol changes during pregnancy. The topics discussed throughout this chapter will provide an overview of the current state of literature and our current gaps in knowledge.

An overview of Cortisol

Cortisol is synthesized within the zona fasciculata layer of the adrenal cortex. ATCH, originating from the anterior pituitary, serves to enhance low-density lipoprotein (LDL) receptors and elevates the activity of cholesterol desmolase. This enzyme is crucial as it catalyzes the conversion of cholesterol to pregnenolone, marking the pivotal step in cortisol synthesis (Thau et al., 2024). Cortisol also helps mediate the stress response regulating metabolism, the inflammatory response, and the immune function. When referring the glucose, cortisol enhances the supply of blood glucose to the brain and exerts its effects on the liver, muscle, adipose tissue, and pancreas as well (Thau et al., 2024). Cortisol is produced from the adrenal cortex and also

has a catabolic effect that includes the breakdown of cellular proteins. Cortisol also plays a role in insulin resistance, due to cortisol decreasing glucose entry into the insulin-sensitive tissues. Within normal pregnancy, the resting levels of cortisol are elevated, especially in the third trimester (Osayande & Ekpruke, 2017).

Cortisol additionally shows an important role in fetal development. It allows the facilitating of the development of vital organs during the latter stages of gestation, including the respiratory system, kidneys, gastrointestinal tract, and brain (Cole et al., 2019). In the final weeks of pregnancy, there is a rise of the glucocorticoids which are required to prepare the fetus for life after birth. However, Valsamakis et al., (2020) recognized that elevated levels of glucocorticoids in the fetus resulting from prolonged fetal cortisol production are linked to intrauterine growth restriction. When the infants are preterm, they miss the window of glucocorticoid stimulation. With this, they are born with many maturation abnormalities that require immediate treatment postnatally in the neonatal intensive care unit (NICU) (Cole et al., 2019) It is also worth noting that mothers who are at risk of delivering preterm are administered glucocorticoids to expedite fetal lung maturation and prevent respiratory diseases (Busada & Cidlowski, 2017). These medications mimic the natural hormone cortisol, which plays a crucial role in fetal development.

This information will help assist with the current research question that is trying to be answered. We know that cortisol is an inflammatory process along with pregnancy, however, what we do not know is if different exercise modes affect the cortisol levels of gravid women.

Cortisol levels versus mode of exercise

Much of the existing literature on cortisol levels and exercise modes addresses the effects of exercise on cortisol in non-gravid populations. Below is an exhaustive overview of the existing research that assesses the effects of exercise on cortisol levels in non-gravid populations, along with limited research on gravid populations.

Aerobic exercise and cortisol

Research on the impact of aerobic exercise on cortisol levels is limited focusing on both non-gravid and gravid populations. When compared to female athletes and sedentary controls, Consitt et al., (2002) did a cross-sectional research study conducted by Tegelman, which discovered that elite female athletes exhibited higher cortisol levels in comparison to those of sedentary individuals. In non-gravid women, Consitt et al., (2002) investigated acute effects where the serum cortisol levels increased after exercise cessation in women with <15-minute bouts of high-intensity exercise. The type of sample that was given was unclear along with how many weeks the intervention was. Consitt et al., (2012) also found that in endurance training, cortisol levels peak due to both exercise intensity and duration. Since this study involved non-pregnant women, further research is needed to determine how these findings might apply to pregnant women engaging in aerobic exercise. Although, based on the information provided by the study, these affects would still apply to gravid women. In the gravid population, McMURRAY et al., (1996) conducted a study with 10 women with a singleton pregnancy ranging from 22-28 weeks. Within this study, the participants partook in either a 40-minute aerobic dance program or 40-minute treadmill, walking at a moderate intensity for three weeks. Nonfasted venous blood samples were taken at rest, post-exercise, and 20 minutes after recovery. While McMURRAY et al., (1996) were looking at the acute effects, they concluded that the serum cortisol levels were not affected by the dance program, but there was an elevation with treadmill walking. While the study by McMURRAY et al., (1996) showed an increase in cortisol more research is needed to fully understand the relationship between aerobic exercise and cortisol in gravid women.

Resistance exercise versus cortisol

A study conducted by TaheriChadorneshin et al., (2021) focused on resistance-trained women, primarily bodybuilders, who were non-gravid. TaheriChadoreshin et al., (2021) gathered fasted venous samples immediately post-exercise, and it was also a three-week intervention at

a moderate intensity, and this study looked into acute effects. TaheriChadoreshin et al., (2021) found that any resistance exercise types showed a significant decrease in serum cortisol levels. Additionally, the authors noted that the serum cortisol levels decreased remarkably after participating in hypertrophy training, which involved performing at 70% of a one-rep maximum. TaheriChadoreshin (2021) also detected that their results showed hypertrophy (70% of 1RM) and strength (90% of 1RM), which decreased cortisol levels in bodybuilding women right after exercise. Interestingly, two articles contradicted each other in response to high intensity or high-volume protocols. Consitt et al., (2012) found that resistance exercise at a high intensity can show short-term increases of cortisol levels in women. Kraemer & Ratamess, (2012) provided a counterclaim, that metabolically demanding protocols that involve high total work (i.e., high volume, moderate to high intensity, and short rest periods) have triggered the most significant acute cortisol response, with minimal changes observed during traditional strength/power training. It is worth noting that all the studies mentioned above investigated acute adaptations instead of chronic adaptations. This would support the ongoing research aimed to determine whether high-volume or high-intensity exercise protocols are beneficial or detrimental to cortisol levels, helping to clarify the current conflicting findings on this issue.

Combination exercise versus cortisol

There are a few articles that evaluated the influence of combination exercise on cortisol levels. One article by Consitt et al., (2012) discussed that non-gravid women had developed an increase in urinary cortisol at rest compared to those who strictly participated in strength or endurance training. Consitt et al., (2012) measured the cortisol levels post-exercise, 24 hours after no training for 24-48 hours. The participants were not fasted and exercised for 16 weeks at a moderate intensity. Along with this, it was also discussed that the strength gained during the combination training was also correlated with elevated urinary cortisol levels in the final 8 weeks of training. With the mixture of resistance and aerobic exercises, the thought is if these non-gravid women's cortisol levels increase while participating in combination training, the gravid

women would have the same reaction. Since the gravid population already undergoes heightened cortisol levels through pregnancy if it would change. It is important to note that this study examined acute adaptations rather than chronic responses. Further research in this mode of exercise would be very beneficial to get a better understanding of whether the cortisol levels would elevate more throughout exercising compared to their normal levels, be maintained, or decrease.

How is cortisol affected while being pregnant

In a healthy pregnancy, cortisol is associated with a regulated inflammatory process (Pinheiro et al., 2015). Guardino (2016) recognized that women's cortisol levels typically rise two to four times during a normal pregnancy. With this, cortisol levels also allow for creating a suitable environment for pregnancy, while dysregulation could lead to adverse obstetric outcomes (such as preterm birth and preeclampsia) (Bleker et al., 2017). The relationship between cortisol and pregnancy can help determine many topics. Jones et al., (2006) discussed that cortisol levels can be beneficial when examining links between adverse pregnancy outcomes such as 'preterm delivery, and poor fetal growth', along with 'psychosocial stressors, individual appraisal of stressors and conditions in pregnancy such as infection, elevated maternal corticotropin-releasing hormone levels.' Additionally, more research is needed to understand the influence exercise has on cortisol levels during pregnancy and its role in attenuating adverse pregnancy-related outcomes.

Summary

Elevated cortisol levels during pregnancy are associated with adverse outcomes such as miscarriages, pre-eclampsia, fetal growth restriction, and pre-term birth. To alleviate these risks, understanding how to regulate maternal cortisol levels is important. Since exercise has been studied in non-gravid individuals, its impact on gravid individuals needs to be investigated further. Since limited literature exists on how different exercises affect cortisol levels, this is a

major knowledge gap. Further research is needed to clarify this relationship and its implications for maternal and fetal health.

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METHODS

Participants

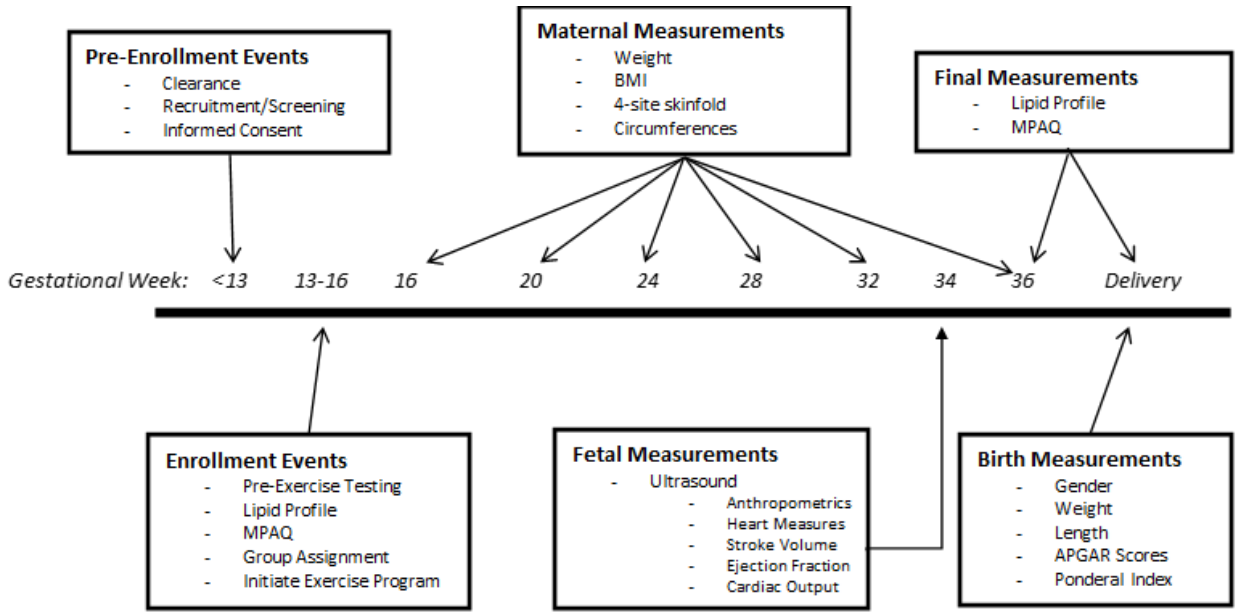
Within the study, women with a singleton pregnancy who had received clearance from their physician to participate in exercise were recruited. These women could have been previously active or sedentary and were between the ages of 18 and 40 years old. Participants had a BMI (body mass index) ranging from 18.5-39.9 and a gestational age of 13-16 weeks. They were required not to be using alcohol, tobacco, recreational drugs, or medications for mental health disorders. Women with pre-existing type 1 or type 2 diabetes mellitus, hypertension, other cardiovascular disease, or other diseases that can affect fetal development, such as HIV, AIDS, or lupus, were excluded from the study. Any participant diagnosed with gestational diabetes (GDM) during the study remained in the study, but their results were analyzed separately from other participants

Study Procedure

Having described the participant characteristics, the focus now will be on the detailed procedure. Figure 1 shows the study procedure layout. Before 16 week's gestation, participants were recruited through brochures, physician's office promotions at local OB/GYN clinics in Eastern North Carolina, as well as email announcements sent to faculty and staff. Informed written consents were acquired from each participant before enrollment. Upon enrollment and prior to or at 16 weeks' gestation, they also needed physician clearance, screening, informed consent, testing visits, and questionnaires were completed. All study protocols were also IRB-approved and East Carolina University Institutional Review Board-approved.

Figure 1

Study Protocol



Once the participants completed the informed consent and were enrolled into the study, the next step is to complete Enrollment Events. During the visit, we obtained a fasted venous sample collected between 6:00-8:00 am, before exercise. This sample was prepared and stored for later analysis of cortisol. Participants were also asked to perform a modified Balke treadmill test to determine their target heart rate range. Once that was completed, on a separate day they would complete a 1 repetition maximum testing, where they were shown proper form and how to complete certain exercises by study staff. After completion, participants were then randomized into four separate groups: aerobic exercise (AE), resistance exercise (RE), combination exercise (CE), and control (CON). The randomization was conducted using a random sequence generator (GraphPad, San Diego, CA) by the principal investigator, and allocation was concealed prior to the group assignment.

Once randomized, participants were asked to attend three days of 50-minute supervised exercise sessions Monday- Friday from 7:00 am – 7:00 pm, with an additional day on Saturday from 6:00 – 11:00 am at the facilities. Resting heart rate and blood pressure were taken at the

start of the session and at the end of each session. Throughout the sessions, heart rate was monitored to ensure that the participants were within their target heart rate range, along with monitoring their RPE (rate of perceived exertion) using the Borg's scale, which helps ensure that they are staying at the moderate-intensity range.

At 36 weeks' gestation, another fasted venous blood sample was collected between 6:00-8:00 am, prior to exercise, and again was prepared and stored for later analysis of cortisol.

Multiplex Kits- Immunoassay Procedure

In order to run cortisol levels, Multiplex- Immunoassay was used. For sample preparation, 250 µL of serum was placed into a microfuge tube, followed by the addition of 375 µL of acetonitrile. The mixture was vortexed for five seconds and then incubated at room temperature for 10 minutes. After another five-second vortex, the sample was centrifuged at 17,000 xg for five minutes. Subsequently, 500 µL of the supernatant was carefully transferred into a 96-well collecting plate. The samples were dried using a speed vacuum at the highest setting and then covered and stored at ≤ -20 °C overnight. Before further processing, the samples were brought back to room temperature, reconstituted, and diluted.

Reagents were brought to room temperature (20-25 °C) before starting the assay. Once at room temperature, 200 µL of assay buffer was decanted from the plate. After shaking, 50 µL of each standard or control was added to the respective wells. Also, 50 µL of assay buffer in the sample wells, and 50 µL of assay buffer in the background, standards, and control wells. For serum or plasma samples, an assay buffer was used. The appropriate control culture medium was used as the matrix solution. Next, 50 µL of the diluted extracted sample was added to the appropriate wells. Then 25 µL of primary antibodies were added to each well, the plate was sealed with foil, and incubated at room temperature for two hours without aspirating. After incubation, the mixing bottle was vortexed, and 25 µL of primary antibodies were added to each well, the plate was sealed with foil, and incubated at room temperature for two hours without

aspirating. After incubation, the mixing bottle was vortexed, and 25 µL of mixed beads were added to each well. The plate was sealed with a plate sealer, wrapped in foil, and placed on a shaker overnight (16-18 hours) at 2-8°C.

Following overnight incubation, sample well contents were removed and washed with a wash buffer. Then, 50 µL of detection antibodies were added to each well and incubated for one hour at room temperature without aspirating. After the incubation, 50 µL of streptavidin-phycoerythrin were added per well and incubated for 30 minutes at room temperature. The well contents were removed and washed three times with 200 µL of wash buffer. Finally, 150 µL of sheath fluid plus or drive fluid plus were added per well, and the plate was resuspended on a shaker plate. The plates were then read on a Luminex 200 using xMAP technology (100 µL, 50 beads per bead set) and xPONENT software for analysis.

Statistical Analysis

For sample size justification, the goal was to recruit 48 participants, allowing for 12 per exercise group. This number included a 10% attrition rate to account for potential dropouts. Statistically, 44 participants were required, equating to 11 per exercise group. Ultimately, 30 participants were enrolled: 11 in the control group, 7 in the aerobic group, 5 in the resistance group, and 7 in the combination group. Although 44 participants were needed to achieve sufficient power, the study was underpowered with only 30 participants. Participant's data was also excluded if they were not compliant throughout the study. Compliance was assessed at needing to maintain 80% attendance or higher starting with their first week of enrollment through delivery).

All statistical analyses were run using SPSS software. Analysis of Variance (ANOVA) was used to test differences between each exercise group and maternal characteristics such as pre-pregnancy BM, age, race, gestation length, gravida, parity, and attendance. This statistical test works by analyzing the levels of variance within more than two groups through samples

taken from each other. Some of the assumptions are normality, homogeneity of variance, and independence (*What Is ANOVA (Analysis Of Variance) Testing?*, n.d.). Since using ANOVA, the comparison of multiple groups to the maternal characteristics were able to reduce any error when looking at the comparisons. ANOVA also showed a better understanding of the differences in the maternal characteristics between the different exercise groups.

Chi-square was used to analyze group differences when the dependent variable was measured at a nominal level. Some of the assumptions were that data in the cells should be frequencies or counts, levels or categories of the variables should be mutually exclusive, study groups must be independent. This means that a different test must be used if the two groups are related and there are two variables, both are measure categories, usually at the nominal level (McHugh, 2013). Chi-square analysis was used to test the between-group differences in maternal race. It also showed a better understanding of diversity within the study.

Descriptive statistics can quantify and describe the basic characteristics of a given data set. Some ideas that you can do with descriptive statistics are you can define subject characteristics, measure data trends, compare events, populations, or phenomena, and validate existing conditions (Descriptive Statistics in Research, n.d.). This analysis was used to show an initial overview of the data and to help identify any general trends and patterns.

T-test analyses are used to test differences between the means of two groups, or the difference between one group's mean and standard value (T-Test Theory for Surveys, n.d.). Some common assumptions regard scale measurement, random sampling, normality of data distribution, adequacy of sample size, and equality of variance in standard deviation (What Assumptions Are Made when Conduction a T-Test? n.d.). A T-test was used to compare the means of two groups to determine if there was a statistically significant difference between our control group and exercise groups. An Independent T-test was used for the comparison of cortisol levels between a group of individuals who performed regular exercise and a group who

did not exercise at all. Cohen's D is used to represent the magnitude of the differences between two or more groups. The calculation of an effect size estimate was used to represent the difference between the two populations (Piasta & Justice, 2010). The assumptions are independent observations, normality, and homogeneity (Cohen's D-effect size for T-Tests, n.d.). Cohen's D was used since the sample size was underpowered. Using Cohen's D helped provide the measure of the magnitude of the effect and showed the practical implications of the findings. The absolute value was taken of each, it was used to look at the effect size of the pre-cortisol, post-cortisol, and change score of the cortisol levels.

Regression analyses were run using JMP statistical software. Regression analysis is used for analyzing different factors that can influence an objective. It can also further help understand how different variables impact each other and what the predicted outcomes are (Regression Analysis, n.d.). Some assumptions suggest there is a linear relationship between the dependent and independent variables, and that the latter aren't highly correlated in their own right (Regression Analysis, n.d.). Regressions were run to examine the relationship between the dependent variables (maternal characteristics) and the independent variables (exercise groups), this helped control for confounding variables.

Samples with coefficients of variation (CVs) >10% were removed from the analysis to limit sample variability. Removing samples with a high CV allows more consistency, which enhances the ability to detect true differences in relationships in the analysis. The limitation of CVs ensures that the values that were obtained from each well are the true values.

RESULTS

This chapter presents the results of the study and highlights the significant findings and their implications. The study cohort consisted of 30 pregnant women, all with low-risk singleton pregnancies and cleared to engage in exercise by an obstetric provider. All participants had a healthy pregnancy free from complications, such as GDM or hypertensive disorders of pregnancy. On average, participants were approximately 30.57 ± 4.94 years old with an average body mass index (BMI) of 29.24 ± 4.38 kg/m², indicating an overweight status, with a gravida of 2.03 (1,4), with full-term delivery at 39.66 ± .80. On average, the attendance was .90 ± .08, participants were excluded from statistical analysis if they were not deemed complaint (80% attendance or higher).

There were no group differences in maternal descriptors (pre-pregnancy BMI, age, race, gestation length, gravida, and attendance compliance), except for parity (Table 1).

Table 1

Maternal Characteristics Across Exercise Types

Table 1: Maternal Characteristics Across Exercise Type.

	Control (n=11)	*Aerobic (n=7)*	*Resistance (n=5)*	*Combination (n=7)*	*p-value*
Pre-Pregnancy BMI (kg/m^2)	29.74±-4.02	29.68±-5.45	28.82±-6.21	28.29±-2.91	.91
Age (years)	30.18±-4.60	30.00±-4.97	33.80±-4.44	29.42±-5.83	.46
Race ‡ (n) %					.25
Asian	(0) 0%	(1) 14%	(0) 0%	(1) 14%	
Black	(3) 27%	(0) 0%	(0) 0%	(0) 0%	
White	(8) 73%	(6) 86%	(5) 100%	(6) 86%	
Gestation Length (wks.)	39.68±-.79	39.74±-1.04	39.22±-.48	39.84±-.79	.61
Gravida †	2 (1,4)	1 (1,4)	2 (1,3)	2 (1,3)	.37
Parity †	1 (0,3)	0 (0,1)	1 (0,2)	0 (0,1)	.047
Attendance Compliance	--	.92 ± .07	.86 ± .06	.92 ± .10	.31

Table 1:BMI = Body Mass Index; Data are presented as mean ⍰ SD; ‡ chi-square analysis presented as (sample number) percentages; † Mann-Whitney U tests performed due to non-normal distribution, and data presented as median (minimum, maximum).

Table 2

Change scores between 16- and 36- week cortisol levels

	Control (n=11)	*Aerobic (n=7)*	*Resistance (n=5)*	*Combination (n=7)*	*p-value*
16-wk cortisol (pg/ml)	59588.71±-33490.76	72391.0±-21964.91	66603.00±-25460.29	72537.00±-17959.37	0.15
36-wk cortisol (pg/ml)	80065.50±-64722.81	75136.99±-74971.64	149308.33±58593.99	72767.49±-64682.57	0.09
Change score	18820.83± 66923.67	-37382.36± 55917.22	54426.00±12869.34	-1567.52 ±67821.67	.43

Maternal cortisol levels at 16-week gestation are similar between the groups (Table 2, Figure 1). Although there is a trend toward the significance of cortisol levels at 36 weeks, this is not significant (Table 2, Figure 2). Although there are no between-group differences in the change score of cortisol, there is a pattern for decreased levels with AE, increased with RE, and a sum of no change with CE (Table 2, Figure 3). It is also important to note that the change score, results differ due to the inclusion of specific time points at 16 and 36 weeks for participants; additionally, some participants did complete both measures at both 16 and 36 weeks.

Figure 2

16-week maternal blood cortisol levels with the mean and standard error

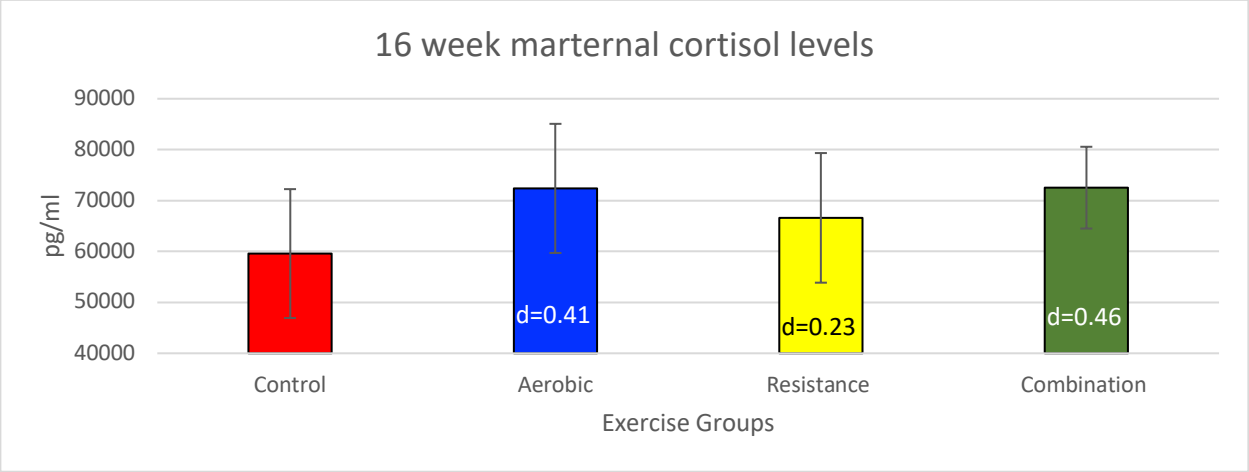


Figure 2 shows the 16-week maternal blood cortisol levels with a p-value of .15. Within this figure you can see that control is represented as red, aerobic as blue, resistance as yellow, and combination as green. The effect size (Cohen's d) was compared between the control group and aerobic, resistance, and combination groups at 16 weeks. For the effect size, the standard guidelines were used: 0.2 equates to a small effect size, 0.5 equates to a medium effect size, and 0.8 equates to a large effect size (Leppink et al., 2016). Since the effect sizes were all small, this suggests group values are similar among these values. The effect size between control and aerobic groups were .41, control and resistance groups were .23, and control and combination groups were .46.

Figure 3

36-week maternal blood cortisol levels with the mean and standard error

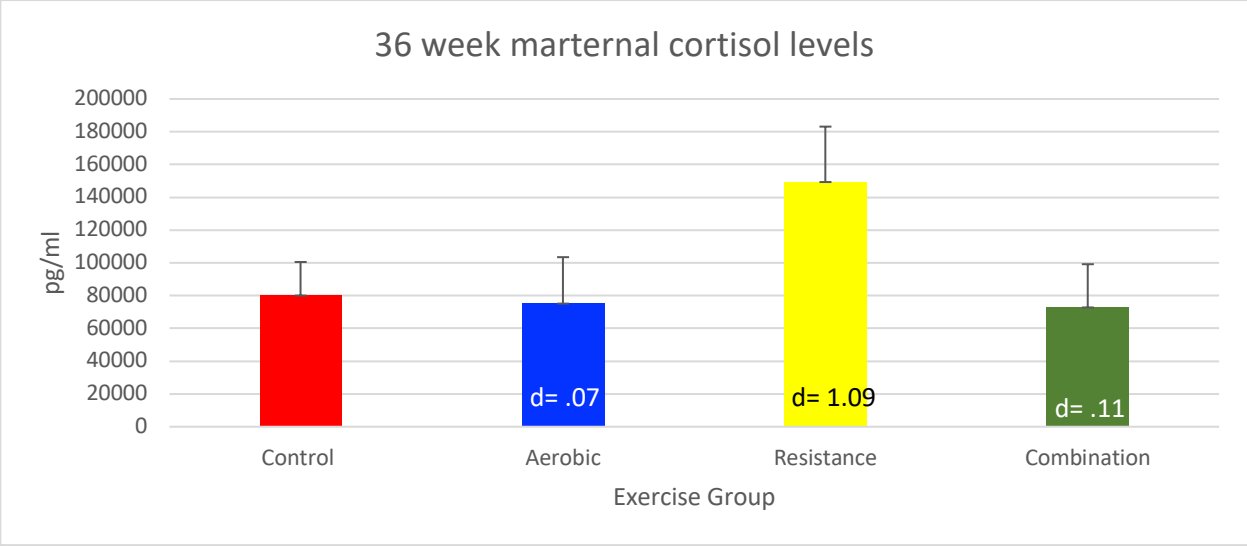


Figure 3 shows the 36-week maternal blood cortisol levels with a p-value of .09 (which is trending towards significance). Within this figure, control is represented as red, aerobic as blue, resistance as yellow, and combination as green. The effect size (Cohen's d) was compared between the control group and aerobic, resistance, and combination groups at 36 weeks. Since aerobic and combination had a small effect size, we can say there is no difference between those values. However, resistance is different compared to the control with a large effect size.

The effect size of control and aerobic groups were .07, control and resistance groups were 1.09, and control and combination groups were .11.

Figure 4

Change Scores of maternal blood cortisol levels with the mean and standard error

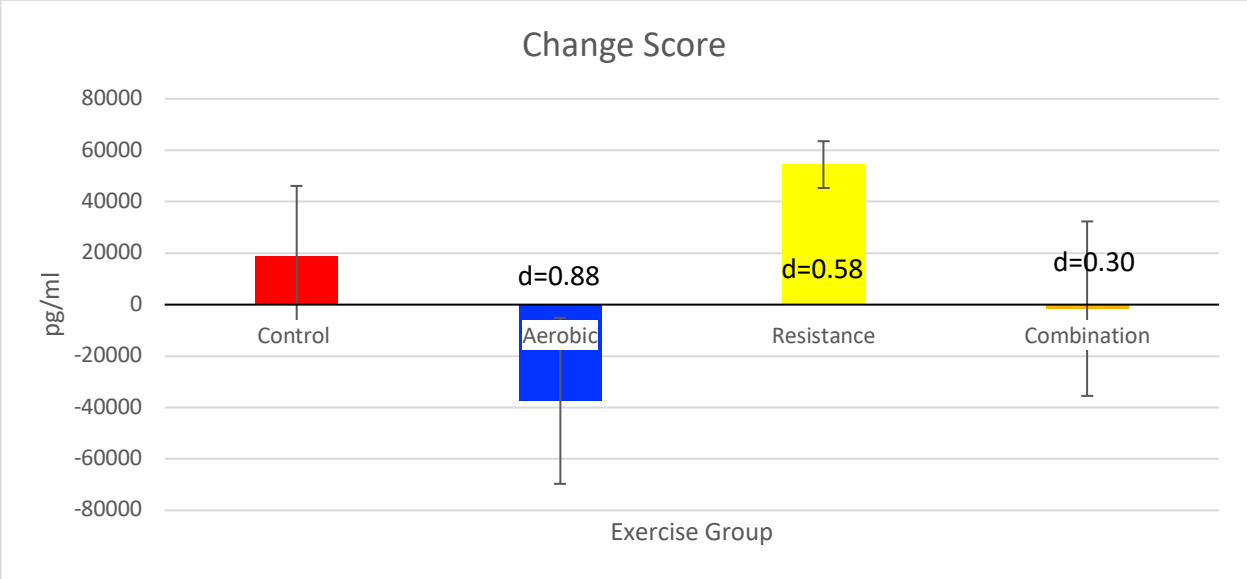


Figure 4 shows the change score of maternal blood cortisol levels with a p-value of .43. Within this figure again, control is represented as red, aerobic as blue, resistance as yellow, and combination as green. The control increased from early to late pregnancy, aerobic decreased from early to late pregnancy, resistance increased from early to late pregnancy, and the combination was the summation of aerobic and resistance. The effect size of control compared to aerobic was .88, control compared to resistance was .58, and control compared to the combination was .30.

Regression analysis

There were no significant models to predict maternal cortisol levels. The dependent variable in this study was cortisol and the independent variable in this study was the exercise group. Although not significant, there is a trend toward significance in predicting 36-week maternal cortisol. This trend suggests that increased gravida, pre-pregnancy BMI, and the CON

group might be related to increased late-pregnancy maternal cortisol (Table 3). Similarly, although not significant, there is also a trend toward significance in predicting maternal cortisol change from early to late pregnancy. This trend suggests that increased pre-pregnancy BMI and no exercise might be related to greater increases in maternal cortisol across pregnancy (Table 3). When using JMP for the regression, it excluded resistance training.

Table 3

36-week cortisol: Regression and Change Score Regression

Table 3: 36-week cortisol: Regression

Outcomes	Estimate	Std. Error	P-value	Lower 95%	Upper 95%
36-week cortisol		**Adjusted R^2= 0.19**	**F= 2.20**	**p= 0.09**	
Gravida	24017.36	11981.49	0.06	-975.599	49010.32
Pre-Pregnancy BMI	5372.29	2879.31	0.08	-633.85	11378.43
Exercise Group					
Aerobic	-6757.14	21529.54	0.76	-51666.98	38152.70
Combination	-7409.56	22466.68	0.75	-54274.23	39455.12
Control	-26929.12	20118.2	0.19	-68894.95	15036.71
Change Score: Regression					
Cortisol Change Score		**Adjusted R^2= 0.27**	**F= 2.32**	**p= 0.13**	
Pre-Pregnancy BMI	6705.62	2944.38	0.05	145.13	13266.10
Exercise Group					
Aerobic	-55669.96	26552.81	0.06	-114833.3	3493.39
Combination	-2191.41	24127.48	0.93	-55950.78	51567.96
Control	7630.01	2944.38	0.72	145.13	13266.10

Table 3: BMI = Body Mass Index; Gravida, Pre-Pregnancy BMI, and exercise group were relevant for 36-week regression; Pre-Pregnancy BMI and exercise group were relevant for change score

DISCUSSION

This study aimed to determine the influence of exercise mode on maternal cortisol levels during pregnancy. The hypothesis is that any type of prenatal exercise mode will result in lower levels of cortisol in late pregnancy, with smaller change scores from 16 to 36 weeks compared to non-exercise controls at the same time point. The main findings from this study are that cortisol levels in CON (control), RE (resistance exercise), and CE (combination exercise) all increased from early to late pregnancy, whereas AE (aerobic exercise) decreased from early to late pregnancy, and the change in cortisol across time (AE decreased, RE increased and CE was the summation of these exercise modes). In addition to the main findings, it was shown that all exercise types are safe for women to participate.

The first main finding is cortisol levels in CON, RE, and CE all increased from early to late pregnancy, whereas AE decreased from early to late pregnancy. Similar to the current findings, a study with 25 healthy non-gravid and gravid women between the ages of 21-35, and the women were divided into five groups, which was similar to the study being done since they had a control group and were comparing them across the other groups which represented different trimesters (Osayande & Ekpruke, 2017). The results of this study indicated that serum cortisol levels showed a progressive increase from the first to the third trimester, with the highest levels in the third trimester of pregnancy (Osayande & Ekpruke, 2017). In another study Scott et al., (1990), it was noted that when looking at saliva, cortisol levels in the late stage of pregnancy were notably higher at all times of the day when compared to non-gravid women. In the second trimester, the mean levels of cortisol were also higher to some degree when compared to the non-gravid women. It is also worth noting Osayande & Ekpruke, (2017) and Scott et al., (1990), identified that the participants were not exercising and showed there were an increase in cortisol levels from the first trimester to the third trimester. These results agree with the current findings within the control group which found there is an increase in

cortisol levels from the first trimester to the third trimester. It is important to acknowledge that Osayande & Ekpruke, (2017) used microgram per deciliter whereas our units are picograms per milliliter, for example, 20μg/dl equals 200,000 pg/ml in the current study.

An additional key result is the change in cortisol across time. As we studied the changes in cortisol across time certain trends indicated that aerobic exercise levels decreased, resistance exercise levels increased, and combination exercise levels had a summation effect of aerobic and resistance training. Importantly, aerobic alone or in combination trended towards decreased cortisol levels. In a study done by Bessinger et al., (2002) found 13 healthy pregnant women were in their first or early second trimester, with the same inclusion criteria that we had, except that the women were required to have been exercising aerobically for a minimum of three months ranging between two to three times a week for at least 30 minutes. Their results stated that the exercise (30-minute treadmill walk at 65% of the participants predicted maximal heart rate at the same time of day) resulted in increased cortisol concentration after exercise. Bessinger et al., (2002) also found that the increase in cortisol levels may be associated with either a rise in transcortin, a decline in the sensitivity of the hypothalamic-pituitary-adrenal axis, or both. It is also noteworthy that later studies found plasma cortisol levels decreased during moderate-intensity exercise in water but increased after a 40-minute treadmill walk. Although not significant, resistance exercise levels also showed an increase. Mulligan et al., (1996) recruited ten healthy non-gravid women experienced in resistance training, though not competitive lifters, and measured serum cortisol concentrations pre-exercise, immediately post-exercise, and at 15- and 30-minutes post-exercise. Mulligan et al., (1996) primary findings indicated that serum cortisol levels significantly increased at all post-exercise time points (immediately and at 15 minutes post-exercise). This differs from the current study, which collects maternal samples at 16 and 36 weeks. This is important to note due to Mulligan et al., (1996) looking at the acute effect of exercise rather than the chronic effect of exercise at 16 and

36 weeks. It is also important because they both had the same results of resistance exercise levels increased.

Lastly, the current study found that combination exercise showed a summation of resistance and aerobic training. A study done by Eklund et al., (2016) involved 29 non-gravid women aged 18-40 who completed a 24-week training intervention. The study examined the effects of extended training on acute exercise responses, comparing endurance training followed by strength training (E+S) to strength training followed by endurance training (S+E). Unlike Eklund's study, our current study allows participants to choose whether to complete endurance training or aerobic training first. In Eklund et al., (2016) study, participants were divided into two groups, with blood samples collected at weeks 0 and 24, both pre-exercise, mid-exercise, post-exercise, and at 24- and 48 hours post-exercise. The results showed that cortisol levels remained statistically unchanged throughout the training period for both groups.

In addition to the main findings, it was recognized that all exercise types are safe for women to participate in while pregnant, based on pregnancy outcomes. Adverse pregnancy outcomes such as preterm birth and preeclampsia are associated with high maternal cortisol levels. Exercise helps normalize cortisol levels in both non-gravid and gravid women. In another article, Gascoigne et al., (2023) reported that multiple studies have shown that individuals who are physically active throughout pregnancy tend to have a lower risk of preterm birth. McMURRAY et al., (1996) conducted a study with women who were at 22-28 weeks of a singleton pregnancy within this study. They only discussed the acute adaptations, not mentioning gestational length; however, all the women went to term and delivered healthy babies. Since McMURRAY et al., (1996) looked at the acute adaptations and their values increased, our results reported a chronic adaptation, which decreased. That shows that all types of exercise are safe for the gravid population. In Osayande & Ekpruke, (2017) looking at the values they received, our results were lower which also showed a positive trend that exercise is

safe for pregnant women. Within the study, Osayande & Ekpruke, (2017) used micrograms per deciliter when compared to this study which used picograms per milliliter. For example, 35 μg/dl would be 350,000 pg/ml within this study, per the results that were given our values were under 350,000 pg/ml.

The strengths of this study are that we monitored their exercise, progress, heart rate, and blood pressure throughout the time that they would come in and work out. We also were able to place the participants in a randomized control trial, along with the recommended levels of exercise for pregnancy. We additionally collected samples at the 16 and 36-week mark. The first limitation of this study was the sample size. Another limitation was the study is only conducted in Eastern North Carolina, and giving birth at ECU Health Hospital in Greenville, NC. We also did not measure maternal stress nor diet, which can influence cortisol levels. The last limitation would be that when conducting change score, some of the participants did not have certain time points at 16 or 36 weeks, or some participants had both 16- and 36-week time points.

Currently, there is little research on the area of pregnancy and exercise modality regarding pregnancy outcomes. Thus, the results of the study offer novel and important insights into how different modes of exercise can positively impact cortisol levels during pregnancy and consequently positively influence pregnancy outcomes. Some of the takeaways are that there needs to be more literature reviewing how cortisol levels are affected throughout pregnancy while exercising, especially over time. This is an important takeaway since there is little research on how different types of modes of exercise can affect cortisol levels along with pregnancy outcomes. These findings can also help inform healthcare professionals and expecting mothers about the role of exercise in managing cortisol levels and promoting healthier pregnancy outcomes.

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APPENDIX A: Informed Consent

East Carolina University

Maternal Informed Consent to Participate in Research

Information to consider before taking part in research that has no more than minimal risk.

Title of Research Study: ENHANCED by **Mom**

UMCIRB #: 12-002524

Principal Investigator: Dr. Linda May, MS, PhD

Institution/Department or Division: Foundational Sciences and Research

Address: 1851 MacGregor Downs Rd, MS 701, Greenville, NC 27834

Telephone #: 252-737-7072

Researchers at East Carolina University (ECU) study problems in society, health, environment, behavior and the human condition. Our goal is to try to find ways to improve the lives of you and others. To do this, we need the help of volunteers who are willing to take part in research.

Why is this research being done?

The purpose of this research is to help improve the health of mothers and their children. The decision to take part in this research is yours to make. By doing this research, we hope to learn the effects of certain activities during pregnancy on your health as well as the health and growth of your child. Components of your health and your baby's health in which we are interested include heart health, body composition, motor skill development, and effects of health at a cellular level.

Why am I being invited to take part in this research?

You are being invited to take part in this research because you are a healthy pregnant mother between the ages of 18 and 40 and have obtained permission from your obstetric provider to participate in certain activities. If you volunteer to take part in this research, you will be one of about 600 women to do so.

Are there reasons I should not take part in this research?

If you do not believe that you can continue to participate in the activities described below 3-4 times per week until the end of your pregnancy, then you should not participate in this study.

What other choices do I have if I do not take part in this research?

You can choose not to participate. If you would like to engage in activities during your pregnancy to improve your health, then you can do this on your own at a local fitness or yoga facility and/or with a personal trainer instead of participating in this research study.

Where is the research going to take place and how long will it last?

The research procedures will be conducted at the following locations:

- Human Performance Laboratory in the Ward Sports Medicine Building (Initial Testing)
- Fitness Instruction, Testing, and Training (FITT) Building – Training Location
- Heart Institute at ECU (ECHI) – Training Location
- Activity Promotion Laboratory in Minges Coliseum – Training Location
- ECU Ob/Gyn clinic (Brody Outpatient)
- Vidant Medical Center (Collection of placenta, and umbilical cord, and cord blood samples)

During the length of the study, you will need to go to ECU Ob/Gyn clinic once, for an ultrasound and fetal ECG and an approved ECU training location 3-4 times a week until you deliver. The study will last the duration of your pregnancy term and, with your permission, we would like to follow your baby's growth up to 12 months old. The total amount of time you will be asked to volunteer for this study is approximately 72 hours over the next 6 months.

What will I be asked to do?

Every participant who agrees to take part in this study will be required to do the following:

- Between 13-16 weeks, you will:
    - Have blood drawn, via venipuncture and finger stick, (a few tablespoons) to determine the level of fats, glucose, and lactate in your blood.

    - Complete a supervised treadmill test to determine your safe activity level during pregnancy
    - Complete a quick max strength test following your treadmill test
    - Complete nutrition and activity questionnaires to determine the foods that you normally eat as well as the activities that you usually do.
- Every 4 weeks (16, 20, 24, 28, 32, and 34/36 wks) we will take the following measures: heart rate, blood pressure, weight, height, BMI, body fat with calipers, and body measures (arm, leg, waist), and ask you to complete questionnaires to determine your health progress related to your assigned activity.
- Between 34-36 weeks pregnant, we will have a
    - Research health professional do an ultrasound of your baby to record his/her body size and heart measures to determine his/her growth.
    - Research health professional perform a fetal ECG to determine how healthy your baby's heart is in relation to your activity.
    - Have blood drawn, via venipuncture and finger stick, (a few tablespoons) to determine the level of fats, glucose, and lactate in your blood
- At the time of delivery, with your permission, a staff member of Vidant Medical Center and our study staff will collect a sample of your umbilical cord, placenta, and the membrane surrounding the placenta.
    - We will test for specific nutrient levels (essential fatty acids and B vitamins). In the future we may test for other compounds such as, hormones.
    - We will share coded samples with our research collaborators for additional testing. The additional testing may include testing for other nutrient levels, compounds, structure analysis of the tissues, etc.
    - We will store your coded specimens in our locked laboratory for the purpose of continuing research with new and improved technology.
    - Your coded specimens may be shared with researchers outside of ECU however no identifying information will be shared.

☐ I *grant* Dr. May and her research team permission to take a sample of my umbilical cord, placenta, and membrane surrounding the placenta for research analysis.

☐ I *do not grant* Dr. May and her research team permission to take a sample of my umbilical cord, placenta, and membrane surrounding the placenta for research analysis.

Participant Signature: _____ Date: _____

- Once your baby is born we will

- record the following measures from your and your baby's medical record (your glucose levels, length of labor and delivery, baby's heart rate, birth weight, birth length, etc.) to determine how your activity is related to these measures.
- Ask you to complete a nutrition & activity questionnaires at one, six and twelve months after your baby is born.

If you agree to take part in this study you will be randomly assigned (like the flip of a coin) to one of four groups:

- Intervention Group #1 – Aerobic Training such as walking on the treadmill, biking, rowing, and other aerobic activities

- Intervention Group #2 – Resistance Training such as toning with resistance bands, light weights, and weight machines.

- Intervention Group #3 – Combination Training will use activities from aerobic and resistance training such as walking on the treadmill and toning with resistance bands, light weights or weight machines.

- Intervention Group #4 – Mindfulness Training will involve light stretching, breathing exercises, walking, meditation, and stress management instruction.

After you are randomly assigned to an activity group, you will come to our facility for 50 minutes, 3 times per week to participate in your supervised sessions with our trained staff. Your heart rate will be monitored during these sessions with a strap that fits around your chest and we will ask you to describe your perceived exertion. Additionally, we will take your blood pressure before and after each session for safety purposes.

Every participant who agrees to take part in this study may **CHOOSE** to do the following:

- At 16 and 20 weeks for 30 minute session (as individual or in groups), we will provide specific nutrition education/counseling for pregnancy.

What possible harms or discomforts might I experience if I take part in the research?

There are possible risks (the chance of harm) when taking part in this research. During the blood draws, you may feel minor discomfort, from the needle stick, but this will be temporary. You may experience light-headedness during the blood draw, as well as during the activities. If you experience this, then please tell the study staff present with you and you will be seated immediately until the feeling disappears. You may experience discomforts related to the activity to which you are assigned such as, but not limited to, muscle soreness. You may experience minor discomfort during the body fat measure as this involves 3 pinches on the skin; this feeling will be minor and temporary.

What happens if I am injured because I took part in this study?

If you are injured as a result of taking part in this study and need medical treatment, please inform study staff or your injury and contact your doctor. The Principal Investigator will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance, you would be responsible for any costs. If you feel this injury was a result of medical error, you have legal rights to receive payment for this injury even though you are participating in a study.

What are the possible benefits I may experience from taking part in this research?

Other people who have participated in this type of research have experienced a variety of changes, such as: improved mood, self-esteem, and energy levels. By participating in this research study, you may also experience the benefits of improved cardiovascular health. For some, there may be no benefit at all by participating in this study.

Will I be paid for taking part in this research?

We will not be able to pay you for the time you volunteer while being in this study; however, you will receive coupons or small gifts from local businesses once a month during your participation in this study.

What will it cost me to take part in this research?

It will not cost you any money to be part of the research. Dr. May's research funds will pay the costs of all procedures.

Who will know that I took part in this research and learn personal information about me?

To do this research, ECU and the people and organizations listed below may know that you took part in this research and may see information about you that is normally kept private. With your permission, these people may use your private information to do this research:

- Any agency of the federal, state, or local government that regulates human research. This includes the Department of Health and Human Services (DHHS), the North Carolina Department of Health, and the Office for Human Research Protections
- The University & Medical Center Institutional Review Board (UMCIRB) and its staff, who have responsibility for overseeing your welfare during this research, and other ECU staff who oversee this research.
- People designated by Vidant Medical Center and Vidant Health

How will you keep the information you collect about me secure? How long will you keep it?

Once data is entered into the database, then it will be coded with a 3 digit number. Only Dr. May will hold a separate database with the code key and identifiers. The code key with identifiers will be kept in a password-protected file on Pirate drive, an ECU approved and protected electronic storage server. The research data will be kept in REDCap, a password protected database. Upon publication only aggregated de-identified data will be published. Data taken during the study will be kept locked in our database indefinitely, for the purpose of future data analysis and dissemination of study findings. You may contact Vidant's Privacy office at 1-888-777-2617 with any question or concerns for how they will help to keep your information secure.

Will I receive anything for the use of my private identifiable information or identifiable biospecimens?

If the research conducted on your private identifiable information or identifiable biospecimens leads to a commercially valuable product, you will not be eligible for any of the profits either because it will be impossible to identify the information or biospecimen that led to the product or because you are transferring ownership of that sample.

Will my identifiable biospecimen be used for whole genome sequencing?

Whole genome sequencing is the process of determining the complete DNA sequence of an individual at a single time. However, further analysis must usually be performed to provide any biological or medical meaning of this sequence. For this research, whole genome sequencing will not occur.

What if I decide I do not want to continue in this research?

If you decide you no longer want to be in this research after it has already started, you may stop at any time. You will not be penalized or criticized for stopping. You will not lose any benefits that you should normally receive. A written or verbal statement must be given to Dr. May or her research coordinator via sodmenhanced@ecu.edu.

Who should I contact if I have questions?

The people conducting this study will be available to answer any questions concerning this research, now or in the future. If you have questions, then you may contact the Principal Investigator at 252-737-7072 (voicemail can be received 24 hours) or her research coordinator via sodmenhanced@ecu.edu. You may also contact Vidant Risk Management at (252) 847-3310.

If you have questions about your rights as someone taking part in research, you may call the University & Medical Center Institutional Review Board (UMCIRB) at phone number 252-744-2914 (days, 8:00 am-5:00 pm). If you would like to report a complaint or concern about this research study, you may call the Director of the UMCIRB, at 252-744-1971.

Is there anything else I should know?

We would also like to continue to follow you and your child after delivery to determine long-term health benefits. We will be asking for your consent to collect birth records about your baby through the use of another consent document. You may be contacted in the future by the study team for further measures of you and your child.

Most people outside the research team will not see your name on your research record. This includes people who try to get your information using a court order.

Identifiers might be removed from the identifiable private information or identifiable biospecimens and, after such removal, the information or biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you or your Legally Authorized Representative (LAR). However, there still may be a chance that someone could figure out the information is about you

I have decided I want to take part in this research. What should I do now?

The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- I know that I can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

_____

Participant's Name (PRINT) **Signature** **Date**

Person Obtaining Informed Consent: I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above and answered all of the person's questions about the research.

Person Obtaining Consent (PRINT) **Signature** **Date**

APPENDIX B: IRB Approval

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

Closure Notification

From: Biomedical IRB

To: Linda May

CC: Lindsey Rossa

Date: 11/9/2023

Re: FR00002503
2023 Final Report for UMCIRB 12-002524
ENHANCED by Mom

I am pleased to inform you that your request to close this study has been approved on 11/03/2023.

It is your responsibility to ensure that you retain all research related documents, including the consent form(s), if applicable, for a period of no less than three years. If you have any questions or need for any reason to re-open this research study, please contact the UMCIRB office prior to implementing any research actions.

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