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Physical Activity and Maternal and Neonatal Outcomes in Obese Pregnant Women

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by
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This dissertation is dedicated to my parents, Betty and Alan Friedman.

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Abstract

Physical Activity and Maternal and Neonatal Outcomes in Obese Pregnant Women

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Maternal obesity and physical inactivity during pregnancy are independently associated with unfavorable maternal and neonatal metabolic outcomes. Previous research in non-gravid adults suggests physical activity provides protection from many chronic diseases irrespective of body weight. The primary purposes of this dissertation were to determine the impact of obesity on maternal metabolic health (lipid metabolism, inflammation, insulin resistance) and neonatal metabolic health (adiposity, inflammation, insulin resistance), and to determine if adverse maternal and neonatal metabolic health is improved in obese pregnant women who are physically active during pregnancy compared to sedentary obese women. The secondary purpose of this dissertation was to examine the relationships between maternal and neonatal metabolic health.

Three groups of pregnant women were compared between 32 and 37 weeks gestation (N=50). Groups consisted of: 1) lean women, 2) obese sedentary women, and 3) obese physically active women. Body composition (skinfold anthropometry), physical fitness levels (submaximal cycle

test), and physical activity levels (accelerometry) were assessed. Maternal plasma markers of insulin resistance (Homeostatic Model Assessment-Insulin Resistance (HOMA-IR)) and systemic inflammation (C – reactive protein) were measured at rest. Lipid oxidation rate and lipolysis were measured at baseline, during a 30-minute bout of low-intensity exercise, and during a 1-hour recovery period. Cord blood was collected at parturition to measure neonatal plasma insulin resistance, inflammation, and free fatty acid concentration. Neonatal body composition was measured 24-48 hours postpartum via skinfold anthropometry and air displacement plethysmography.

In Chapter 2, maternal and neonatal outcomes were compared between lean and obese pregnant women. Obese pregnant women had higher maternal inflammation, insulin resistance, and lipid oxidation rates. Maternal lipid oxidation rate and inflammation were positively correlated. Maternal inflammation was positively correlated to insulin resistance and blood pressure. Therefore, lipid metabolism may be contributing to inflammation and subsequent insulin resistance and hypertension in obese pregnant women.

In Chapter 3, maternal and neonatal outcomes were compared between obese sedentary and obese physically active pregnant women. Physically active obese women had lower systemic inflammation compared to sedentary obese women; thus, regular physical activity may improve inflammation in obese pregnant women.

In Chapter 4, the relationships between maternal and neonatal metabolic outcomes were examined. There were no correlations between maternal and neonatal metabolic outcomes across

all women in the study. Several relationships between maternal and neonatal outcomes were found when comparing lean or obese women separately, which suggests that the mechanisms linking maternal and neonatal metabolic health are complex and potentially BMI-dependent.

In Chapter 5, the relationship between intensity of physical activity and maternal inflammation was examined. Low-intensity physical activities had the strongest negative correlation to systemic inflammation. Data from Chapter 5 also suggest that small daily increases in low-intensity physical activities may be enough of a stimulus to elicit clinically meaningful reductions in inflammation. Thus, pregnant women should be encouraged to participate in low-intensity physical activities in order to reduce their systemic inflammation and improve their long-term health.

Overall, results from this dissertation project suggest that obesity during pregnancy has unfavorable implications for maternal metabolic health. However, a physically active lifestyle might mitigate these alterations, particularly maternal systemic inflammation. Pregnant women of all body weights should be encouraged to participate in daily physical activity, even low-intensity activity, in order to improve their health and the future health of their offspring.

Chapter 1: Background and Significance

1.1 Maternal Obesity during Pregnancy- The Public Health Problem

The obesity epidemic is an international public health crisis. Concurrent with record rates of obesity in the general population is the increasing prevalence of maternal obesity. Maternal obesity has increased 60% in the past two decades[1] with nearly 1 in 3 women now entering pregnancy obese[2]. Maternal obesity increases the risk for a wide-array of maternal complications including miscarriages[3,4], preterm delivery[4,5], excessive gestational weight gain[6,7], gestational diabetes[8,9], preeclampsia and other hypertensive disorders[5,10], prolonged labor[11], delivery complications[12-14], and medically-necessary caesarean deliveries[5,15]. In addition, maternal obesity contributes to a considerable number of short and long-term neonatal outcomes including undetected anomalies or malformations from reduced sensitivity on ultrasounds[16-18], acute trauma during delivery[13,14], attention-deficit and emotional disorders[19], neural tube defects[20,21], excess adiposity[1,22], and insulin resistance[23,24].

In addition to the serious health implications, obesity during pregnancy has a profound effect on health care costs as the average total costs (practitioner visits, medications, inpatient and outpatient visits) are estimated to be nearly 40% higher in obese women when compared to lean women[25]. The increasing number of women entering pregnancy obese is a significant health problem; thus, understanding the mechanisms by which obesity induces unfavorable outcomes as well as establishing potential therapeutic targets is critical in order to improve health problems and reduce costs associated with obesity during pregnancy.

1.1.1 Fetal Origins Hypothesis

Maternal obesity causes many physiologic changes that increase the risk of poor maternal health during pregnancy and postpartum. Unfavorable physiologic changes may also play a crucial role in adverse fetal programming leading to unfavorable neonatal outcomes with long-term health implications[1,13,26,27]. One of the primary hypotheses attempting to explain the connection between maternal and neonatal physiology is the "fetal origins hypothesis"[28,29]. The "fetal origins hypothesis" suggests that the intrauterine metabolic environment plays a crucial role in neonatal "programming"; thus, the origins of adult health diseases such as obesity, cardiovascular disease, and diabetes may be predetermined by *in utero* exposures[28,29]. Understanding the implications of obesity on maternal health, the intrauterine metabolic environment, and neonatal health is important as obesity may be the most common health risk for the developing fetus[1]. It is critical that metabolic alterations in obese pregnant women are identified in order to develop interventions that modulate the factors contributing to adverse pregnancy outcomes. In particular, maternal lipid metabolism, insulin resistance, and inflammation may be altered in obese pregnant women, and these factors may play a role in the "programming" of neonatal adiposity, insulin resistance, and inflammation.

1.1.2 Lipid Metabolism

Lipid metabolism in non-gravid obese adults

Two aspects of lipid metabolism are lipid oxidation rate and lipolysis, which are closely related to each other during resting and exercise conditions[30]. Lipid oxidation rate is the rate at which lipids are oxidized as a source of energy, while lipolysis is the breakdown of adipose tissue stores (hydrolysis of triglycerides into glycerol and free fatty acids which are then released into

the bloodstream). In obesity, lipid metabolism is altered which may contribute to unfavorable metabolic perturbations such as insulin resistance, oxidative stress, and inflammation[31,32].

Previous data on lipid oxidative capacity in obesity are equivocal; some studies demonstrate higher lipid oxidation rate in obesity[33,34], while others show lipid oxidation rate is down-regulated[35-37]. For example, Berggren et al. found lower fatty acid oxidation rate in obese individuals and attributed it to a defect in the ability to properly oxidize lipids in skeletal muscle[37]. On the contrary, Goodpastor et al. determined that during acute low-intensity exercise (i.e. when lipid metabolism is stimulated), lipid oxidation rates appear to be higher in obese men compared to lean men[33]. Altered rates of lipid oxidation are important as these rates are intricately related to inflammation and glucose metabolism. In non-gravid individuals, lipid oxidation signals pathways that initiate an inflammatory response[31,38], demonstrating a connection between lipid metabolism, inflammation and cardiovascular disease risk. Similarly, increased lipolysis may lead to higher plasma free fatty acid concentration and glucose intolerance in non-gravid adults[39]; thus providing a connection between lipid metabolism and insulin resistance.

Increased lipid oxidation may also lead to excess generation of reactive oxygen species, which are known byproducts of lipid oxidation[32]. Bell et al. concluded that incomplete fatty acid oxidation is increased and complete fatty acid oxidation is decreased in muscle cells from obese individuals when compared to muscle cells from lean individuals[40]. Thus, incomplete, partial oxidation is increased in obesity, but complete, efficient oxidation is decreased[40]. This finding may explain the equivocal findings on lipid oxidation rates in obesity.

The potential impact of incomplete maternal lipid oxidation on obese pregnant women could be substantial as it will produce excess reactive oxygen species leading to a state of oxidative stress. In non-gravid adults, oxidative stress is associated with inflammation, damage to vascular cell walls, high blood pressure, cardiovascular disease, cancer, neurodegenerative disease, hepatic disease, decreased aerobic capacity, and physiologic aging[41-46]. In addition, oxidative stress is believed to be a deleterious factor leading to insulin resistance, beta-cell dysfunction, impaired glucose tolerance, and ultimately type 2 diabetes mellitus[47-49]. Moreover, non-gravid obese adults exhibit increased systemic oxidative stress when compared to normal-weight individuals[50]. It appears that obesity-induced oxidative stress may be a significant causal factor in the development of many obesity-related disease states in adults, particularly insulin resistance and inflammation[45,48,49,51]. Because reactive oxygen species are a by-product of inefficient lipid metabolism, higher lipid oxidation rates may contribute to a pathological metabolic process that can ultimately lead to inflammation and insulin resistance and contribute to adverse metabolic health in obese populations.

Maternal lipid metabolism in obese women during pregnancy

A careful understanding of maternal lipid metabolism during pregnancy is important as maternal lipids are believed to play a major role in fetal development[52,53]. Lipid oxidation and lipolytic rates are elevated in normal-weight pregnant women compared to non-pregnant women, particularly near then end of gestation as a mechanism to spare glucose for the fetus[52-54]. Maternal lipid oxidation and lipolysis in obese women during pregnancy and their relationships to unfavorable neonatal outcomes have not been previously studied. Thus, the influence of maternal obesity on lipid metabolism during pregnancy is the next logical, and so far unexplored,

step in examining the role of altered maternal substrate metabolism in adverse maternal and neonatal health outcomes in this population. The finding of altered lipid metabolism during pregnancy in obese women may suggest that unfavorable metabolic adaptations contribute to maternal, and perhaps neonatal, inflammation and insulin resistance (as both inflammation and insulin resistance are related to alterations in lipid metabolism in obese non-gravid adults[31,39]).

Animal model data suggest impaired maternal whole-body fatty acid oxidative capacity in obesity may precede development of offspring adiposity and its associated co-morbidities[26]. Understanding maternal lipid metabolism in obese women during pregnancy might uncover important information regarding metabolic dysregulation and how it impacts maternal and neonatal outcomes in this population. In addition, the knowledge gained may inform future interventions as lipid oxidation rate and lipolysis are physiologic mechanisms that could eventually be targeted for potential therapies as both can be impacted by diet and exercise[55,56].

1.1.3 Inflammation

Inflammation in non-gravid obese adults

Acute inflammation is an important and necessary biological response to noxious stimuli[57]. Inflammation stimulates an immune response that can combat acute threats to the body's homeostasis. However, sustained, inappropriate inflammatory responses can have detrimental effects on health[57].

It is well-established that obesity is associated with low-grade, chronic, systemic inflammation[58]. Low-grade systemic inflammation secondary to obesity plays an important role in the pathogenesis of many chronic diseases including metabolic syndrome, cardiovascular disease, diabetes, and hypertension[59]. C-reactive protein (CRP) is the most commonly utilized marker of inflammation, and it has been associated with adiposity, hyperinsulinemia, insulin resistance, low HDL concentrations, and cardiovascular disease[60-62]. CRP has been distinguished as the most important inflammatory biomarker for cardiovascular disease and cardiovascular disease risk[63-65].

Inflammation in obese women during pregnancy

Maternal markers of inflammation, including CRP, are elevated in normal physiologic pregnancy[66]. Therefore, the addition of obesity leads to further elevated levels of systemic inflammation during pregnancy[67]. Indeed, Catalano et al. found that obese pregnant women have higher levels of CRP when compared to normal-weight pregnant women[68]. It is hypothesized that the higher levels of inflammation in obese pregnant women may contribute to the well-established increased risk for the development of metabolic complications during pregnancy such as insulin resistance and hypertension. In fact, Ozgu-Erdinc et al. found that first trimester CRP levels are correlated with later development of gestational diabetes[69], suggesting maternal inflammation may be predictive of maternal insulin resistance later in pregnancy. Similarly, early pregnancy maternal inflammation has also been linked with the development of preeclampsia[70]. Higher maternal inflammation during pregnancy may also contribute to increased maternal risk for future development of metabolic syndrome, insulin resistance, diabetes, hypertension, and cardiovascular disease[71].

Interestingly, maternal inflammatory changes during pregnancy are believed to extend into the placenta, suggesting that the fetus of a woman with excessive inflammation is exposed to an inflammatory environment during development[72]. This exposure might predispose neonates to have a higher risk for the development of metabolic disease in adulthood[73,74]. In support, Catalano et al. found that obese pregnant women had higher maternal and umbilical cord blood concentrations of inflammatory markers (IL-6 and CRP) compared to lean women[68]. This finding also suggests maternal inflammation may directly contribute to neonatal inflammation. Overall, maternal obesity and subsequent inflammation might contribute to poor outcomes in pregnant women and their offspring. The relationships between maternal inflammation and neonatal metabolic outcomes are unclear. It is critical these relationships are established in order to understand perinatal development of metabolic disease and thus, inform future intervention strategies.

1.1.4 Insulin Resistance

Insulin resistance in obese non-gravid adults

Insulin resistance reflects an impairment in the tissues' (e.g. skeletal muscle, liver, adipose tissue) ability to respond to the effects of insulin, which impairs how well the tissue is able to clear glucose.[75] When tissues become insulin resistant, the pancreatic beta-cells secrete higher amounts of insulin until they become deficient[76]. The combination of insulin resistance and beta-cell deficiency leads to dysregulation of blood glucose.

Obesity is the most common risk factor for impaired insulin action[75] and the development of type 2 diabetes[77]. Adipose tissue, which is abundant in obesity, releases a number of factors that contribute to the development of insulin resistance[78]. Insulin resistance has serious consequences as it precedes the development of type 2 diabetes and cardiovascular disease[79].

Insulin resistance in obese women during pregnancy

Physiologic insulin resistance occurs during normal pregnancies as the result of human placental lactogen, elevated lipids, and the release of several cytokines[75]. However, when a pregnant woman is also obese, insulin resistance is elevated further which leads to poor outcomes in pregnant women and their neonates[8,68]. Insulin resistance can develop into a diagnosis of gestational diabetes, which is defined as glucose intolerance with onset or first recognition during pregnancy. Further, obese and morbidly obese pregnant women are 2.6 and 4 times more likely to develop insulin resistance and gestational diabetes, respectively[5,9]. Insulin resistance and gestational diabetes can be very problematic to maintaining a healthy pregnancy[9]. Women who develop gestational diabetes are at an increased risk of adverse perinatal, maternal and neonatal outcomes, recurrence of gestational diabetes in subsequent pregnancies, as well as development of type 2 diabetes postpartum[8,80,81].

Interestingly, insulin resistance in obese pregnant women appears to translate into the offspring. Neonates from overweight and obese women have impaired insulin action compared to neonates of normal-weight women[22]. However, this has not been well-studied in obese pregnant women. The contribution of maternal metabolism, including insulin resistance, to fetal programming and neonatal outcomes in obese pregnant women remains unclear.

1.1.5 Neonatal Adiposity

Maternal obesity increases the risk for giving birth to infants with excess adiposity[1,22,82] as maternal obesity is believed to play a significant role in the development of fetal overgrowth[83]. Obesity in childhood is a strong predictor of adult adiposity, thus, pre-pregnancy maternal obesity may predispose neonates to future obesity[84-86]. In addition to the increased risk for obesity into childhood and adulthood, offspring of obese women are also at elevated risk for future development of obesity-related disease states such as hypertension, insulin resistance, dyslipidemia, and cardiovascular disease[9,82,87]. Neonatal macrosomia itself is an independent risk factor for adult metabolic syndrome[88]; thus, the prevention of macrosomia and excessive neonatal adiposity is vital to combatting the generational cycle of obesity and its associated comorbidities.

1.2 Physical Inactivity during Pregnancy- The Public Health Problem

Pregnant women are less physically active than their non-gravid counterparts[89,90]. Only 23% of pregnant women exercise in accordance with guidelines set by the American Congress of Obstetricians and Gynecologists[91] despite strong evidence supporting the physiological benefits to pregnant women and their neonates[92].

1.2.1 Maternal Benefits of Physical Activity during Pregnancy

Physically active women report fewer pregnancy-related problems such as insomnia, anxiety, nausea, heartburn, leg cramps, round ligament pain, and low back pain[93,94], with the greatest relief seen in those who remain active during the entire nine months of gestation[94]. Exercise also

improves depressive symptoms, mood, and self-image in pregnant women[95,96]. Active pregnant women also demonstrate improved fitness, aerobic capacity, exercise performance, and overall well-being[15,97-99].

Physical activity during pregnancy can also improve the labor and delivery process. Active pregnant women are at decreased risk for abdominal and operative vaginal deliveries[15,100-102]. Similarly, aerobic and non-aerobic exercise training programs are associated with shorter active labors[100,101], although this topic is controversial as some studies have found no difference in duration of labor between active and inactive pregnant women[96,98,103,104]. Physical activity during pregnancy also improves recovery following a vaginal delivery as Price et al. found women who were active during pregnancy were able to return to pre-pregnancy household activities nearly twice as quickly after delivery[15].

Not only can exercise reduce symptoms of pregnancy, incidence of operative delivery, and speed-up recovery time, but physical activity can help limit weight gain during pregnancy. It is well-documented that women who engage in physical activity (e.g. exercise) during pregnancy gain less weight[100,105,106]. Excessive gestational weight gain is the strongest risk factor for maternal postpartum weight retention, and gestational weight gain is related to many adverse maternal and neonatal outcomes[107-113]. Additionally, physically active women have a decreased risk of developing preeclampsia[114-116]. Therefore, the benefits of physical activity during pregnancy are substantial.

Maternal physical activity and maternal lipid metabolism during pregnancy

In non-gravid adults, physical activity increases the efficiency of lipid metabolism[55]. More specifically, endurance exercise training increases the capacity for oxidative metabolism of fatty acids and carbohydrates[117]. In obese non-gravid individuals, skeletal muscle lipid oxidation is deficient, but exercise can correct this impairment[37].

The impact of physical activity during pregnancy on maternal lipid metabolism and its relationship to maternal and neonatal metabolic health has not been studied in normal-weight or obese women. Because lipid metabolism (particularly pathways that target lipid oxidation) has potential to be a future therapeutic target, studies in this area are needed to inform future interventions.

Maternal physical activity and maternal insulin resistance

Previous studies suggest that maternal physical activity during pregnancy can prevent the development of gestational diabetes[118-120]; however, the impact of physical activity on gestational diabetes is controversial as a Cochrane review suggests that evidence supporting the role of physical activity in preventing gestational diabetes is insufficient[121].

In non-gravid adults, physical activity reduces insulin resistance[122], and this effect happens irrespective of weight loss[123] . Similarly, insulin sensitivity is improved in normal-weight pregnant women who are active when compared to their inactive counterparts[124]. Physical activity-associated improvements in insulin resistance might also occur in obese pregnant

women as van Poppel et al. reported lower insulin resistance in active overweight and obese pregnant women compared to inactive overweight and obese women[125]. It is important to consider the implications of physical activity on obese women as they are at the highest risk for insulin resistance, gestational diabetes, and future type 2 diabetes mellitus; thus, physical activity-related improvements in insulin resistance may have the greatest benefit in obese women.

Maternal physical activity and maternal inflammation

In non-gravid adults, systemic inflammation (i.e. plasma CRP) is inversely related to physical activity levels, suggesting that physical activity may reduce inflammation[126]. Further, exercise training lowers systemic inflammation in non-gravid obese women[127]. In pregnancy, physical activity levels before and during pregnancy have been shown to reduce plasma CRP concentrations[128,129]. To our knowledge, the impact of physical activity during pregnancy on maternal and neonatal CRP levels has not been studied in at-risk obese women. One prior study examined the relationship between physical activity during pregnancy and maternal plasma CRP concentration in a combination of overweight and obese women, and they found no association[130]. However, we believe our study design will better allow us to assess the role of physical activity on maternal and neonatal plasma inflammation in exclusively obese pregnant women.

1.2.2 Neonatal Benefits of Physical Activity during Pregnancy

The benefits of maternal physical activity during pregnancy on the fetus begin *in utero*, as regular, weight-bearing maternal exercise is associated with placental growth and volume[131]. Price et al. found that there is less incidence of acute neonatal stress at delivery for neonates of active women[15]. Maternal physical activity during pregnancy also has a positive influence on acute health as active women deliver neonates with higher Apgar scores[102,132,133]. In addition, maternal physical activity may influence neurodevelopment as neonates born to active women have higher neurodevelopmental scores during the first four years of life[134]. Interestingly, animal models have even shown that physical activity during pregnancy mitigates the risk for congenital heart disease in offspring of maternal obesity[135]. With maternal obesity and diabetes being well-established risk factors for congenital heart disease in the offspring[136], the benefits of a physically active lifestyle on neonatal health outcomes among obese pregnant women are substantial. Clearly, the influence of maternal physical activity on neonatal health is widespread and may have a significant influence on the short and long-term health of the offspring.

Maternal physical activity and neonatal adiposity

Regular physical activity during pregnancy in normal-weight women is associated with the delivery of lighter and leaner infants[106,133,137-140]. Women who participate in moderate-to-high intensity exercise throughout their pregnancy have significantly smaller (albeit within a healthy range) babies[106,133,139,140], and are less likely to have large-for-gestation-age neonates[141]. Large-for-gestation-age neonates are at increased risk of shoulder dystocia, brachial plexus injury, skeletal injuries, meconium aspiration, perinatal asphyxia, hypoglycemia, and fetal death[142-147]. Most importantly, neonatal macrosomia (i.e. large-for-gestation-age) is an independent risk

factor for adult metabolic syndrome[88]. Neonates born to active women have lower birth weights, and that nearly 70% of the difference in birth weight between neonates born to active versus inactive women can be attributed to neonatal fat mass[133]. In addition, late-pregnancy energy expenditure is associated with lower neonatal adiposity without reduced neonatal fat-free mass[148]. Further, physical activity during pregnancy appears not only to decrease neonatal weight and fat mass at birth, but it may also produce effects that last in childhood and adulthood. Mattran et al. concluded that higher physical activity levels during pregnancy were associated with lower toddler weights and lower weight-for-height ratios[149]. Similarly, Mourtakos et al. and Clapp et al. suggest physical activity may have an impact on long-term body composition as maternal physical activity was related to offspring body composition at eight and five years of age, respectively[150,151].

The impact of physical activity on neonatal body composition in at-risk obese women is unclear. Hayes et al. found that active obese women delivered neonates with lower abdominal circumferences compared to neonates of inactive obese pregnant women[152]; suggesting physical activity in obese pregnant women may reduce neonatal adiposity. However, abdominal circumference as a means of estimating neonatal body fat percentage is less accurate than air displacement plethysmography[153].

Maternal physical activity and neonatal insulin resistance

The impact of maternal physical activity during pregnancy on neonatal insulin resistance has not been well-studied in normal-weight or obese women. In an animal model by Stanford et al.,

exercise before and during pregnancy improved glucose tolerance and lowered serum insulin concentrations in offspring[154]. Similarly, Carter et al. found that offspring born to dams who exercised during pregnancy had enhanced insulin sensitivity and improved glucose homeostasis as adult rats[155]. This suggests that maternal exercise may have short and long-term impacts on the offspring's neonatal insulin resistance.

Clearly, there is a lack of quality research assessing the impact of physical activity on neonatal metabolic health (e.g. insulin resistance) in humans, particularly in overweight and obese women. Studying offspring of obese women is important to designing future interventions as these women and their offspring may be at the highest risk for future diabetes development. With future obesity and diabetes development being significant public health concerns, exercising throughout pregnancy may play an important role on the long-term metabolic health of the offspring by reducing risk of obesity and diabetes later in life.

Maternal physical activity and neonatal inflammation

Maternal inflammatory changes during pregnancy are believed to extend into the placenta thus exposing the fetus to an inflammatory environment during development[72]. The exposure to inflammation *in utero* might predispose the fetus to the development of metabolic disease in adulthood[73,74]. Indeed, Catalano et al. demonstrated that obese pregnant women had higher levels of inflammatory markers (IL-6 and CRP) in maternal and umbilical cord blood when compared to lean pregnant women[68]. This finding suggests a relationship between maternal and neonatal inflammation. Fortunately, maternal physical activity can reduce maternal inflammation during pregnancy[128,129]; however, it is unknown whether these reductions in maternal inflammation

translate into lower neonatal inflammation. The impact of physical activity on inflammation in obese pregnant women and their neonates is unknown and requires further study.

1.3 Physical Activity during Pregnancy in Obese Women

In summary, pre-pregnancy obesity increases the risk for unfavorable alterations in maternal lipid metabolism, insulin resistance, and inflammation. It also increases the risk for worse neonatal outcomes including excess adiposity, higher insulin resistance, and higher neonatal inflammation. In non-gravid populations, exercise improves lipid metabolism, decreases insulin resistance, and decreases inflammation. In normal-weight pregnancies, exercise also improves maternal lipid metabolism, insulin resistance, and inflammation, as well as neonatal adiposity. However, the role of a physically active lifestyle on metabolic outcomes in obese pregnant women has not been well-studied. Understanding the intrauterine metabolic environment and its impact on short and long-term maternal and neonatal metabolic health in obese pregnant women is critical as obese women are at the highest risk for poor pregnancy outcomes with subsequent long-term implications.

Previous research in non-gravid adults suggests that the presence of multiple comorbidities is more strongly correlated with physical inactivity than overweight/obesity[156,157]. Thus, the overall purpose of this dissertation is to determine if maternal physical activity during pregnancy can mitigate poor maternal and neonatal metabolic outcomes (i.e. maternal lipid metabolism, insulin resistance, inflammation, and neonatal adiposity, insulin resistance, and inflammation) commonly observed in obese women. It is important that these relationships are established in order to develop interventions that modulate metabolic factors contributing to adverse outcomes

in obese pregnant women. Figure 1.1 summarizes Chapter 1 by outlining risks associated with maternal obesity and benefits of maternal physical activity, and their impacts on maternal and neonatal long-term health.

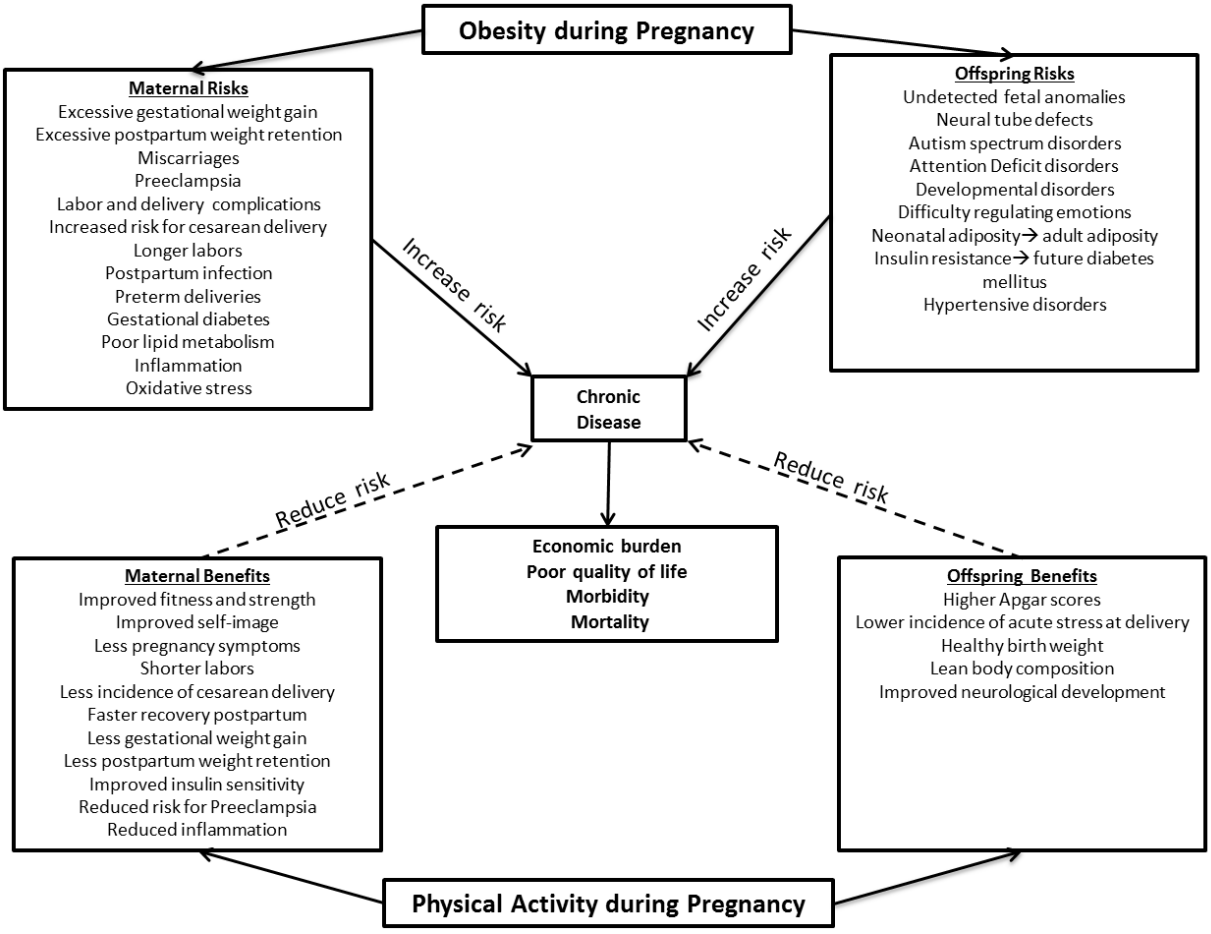

Figure 1.1 Summary of maternal and neonatal risks of obesity and benefits of physical activity

1.4 Purposes and Specific Aims

The **primary purposes** of this dissertation are to (1) compare maternal lipid metabolism, maternal inflammation, maternal insulin resistance, and neonatal metabolic health between lean and obese pregnant women, (2) determine if a physically active lifestyle during pregnancy is associated with improved maternal lipid metabolism, lower maternal inflammation, lower maternal insulin resistance, and better neonatal metabolic health in obese women during late pregnancy, and to (3) determine the relationships between maternal and neonatal metabolic health. Figure 1.2 outlines the aims, groups, and outcome variables for Specific Aims 1-3 in a dissertation project summary. Specific Aim 4 is a secondary analysis and is not shown in Figure 1.2.

Specific Aim 1 (Chapter 2): To compare maternal lipid kinetics during rest and low-intensity exercise, maternal systemic inflammation, and neonatal metabolic outcomes between lean and obese women during late pregnancy.

Hypothesis 1a: Maternal lipid oxidation rate and lipolysis will be higher in obese women compared to lean women during rest and low-intensity exercise.

Hypothesis 1b: Systemic inflammation will be higher in obese women compared to lean women.

Hypothesis 1c: Neonates of obese women will have higher adiposity, higher systemic inflammation, and higher insulin resistance compared to neonates of lean women.

Specific Aim 2 (Chapter 3): To compare maternal lipid kinetics during rest and low-intensity exercise, maternal systemic inflammation, and neonatal metabolic outcomes between physically active and sedentary obese pregnant women during late pregnancy.

Hypothesis 2a: Maternal lipid oxidation rate and lipolysis will be lower in active obese women compared to sedentary obese women.

Hypothesis 2b: Systemic inflammation will be lower in active obese women compared to sedentary obese women.

Hypothesis 2c: Neonates of physically active obese women will have lower adiposity, lower systemic inflammation, and lower insulin resistance than neonates of sedentary obese women.

Specific Aim 3 (Chapter 4): To determine the relationships between maternal lipid kinetics, maternal insulin resistance, and maternal systemic inflammation, and neonatal metabolic outcomes (adiposity, insulin resistance, and inflammation).

Hypothesis 3a: Higher maternal lipid oxidation rate and lipolysis will be associated with higher neonatal adiposity, higher neonatal insulin resistance, and higher neonatal inflammation.

Hypothesis 3b*:* Maternal insulin resistance will be positively correlated with neonatal insulin resistance.

Hypothesis 3c: Maternal systemic inflammation will be positively correlated to neonatal systemic inflammation.

Specific Aim 4 (Chapter 5): To determine the relationship between intensity of physical activity and maternal inflammation.

Hypothesis 4a: Time spent sedentary will be positively correlated with maternal inflammation.

Hypothesis 4b: Moderate intensity physical activity will have the strongest negative correlation to maternal inflammation when compared to light and lifestyle (low-intensity) physical activities.

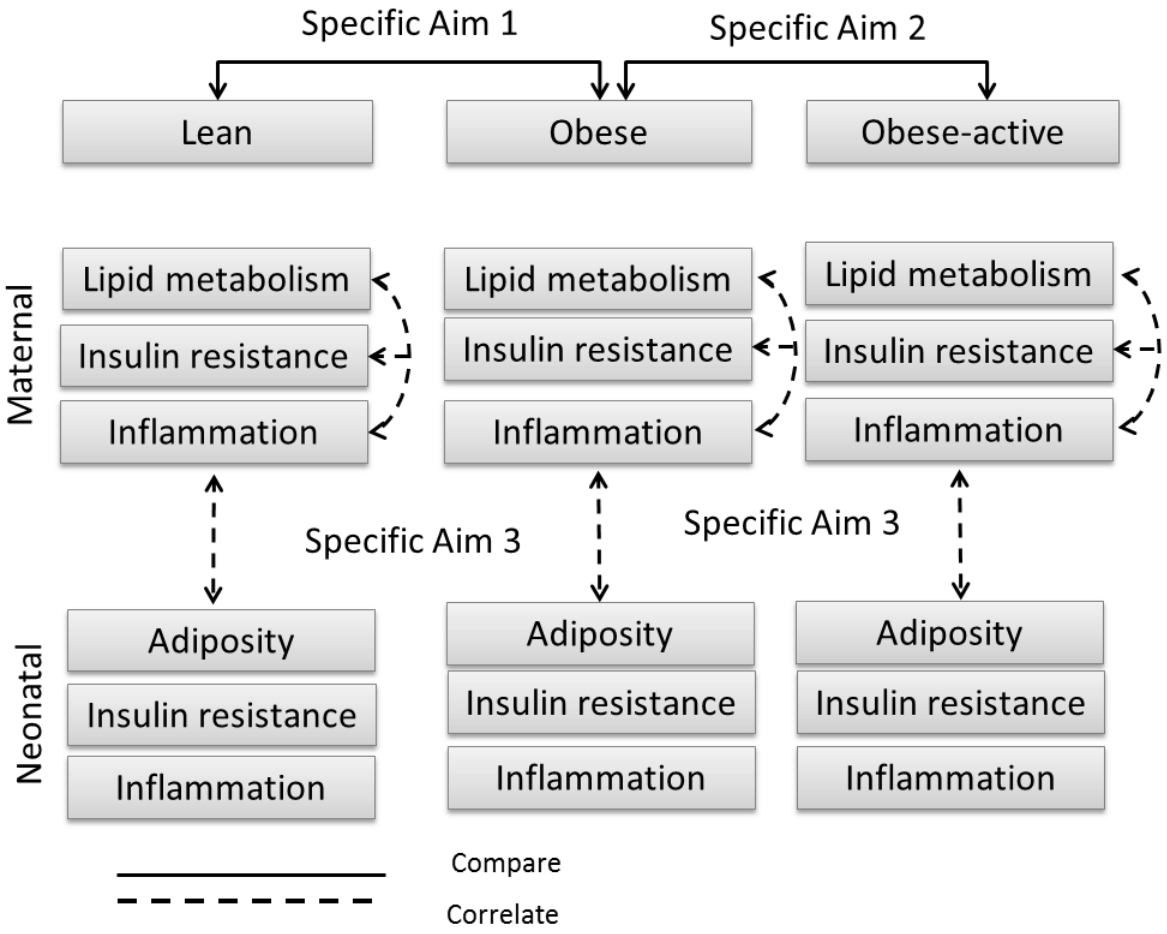

Figure 1.2 Dissertation project summary for Specific Aims 1-3

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Chapter 2: Altered Lipid Metabolism is Associated with Higher Maternal Inflammation in Obese Women during Late Pregnancy

This chapter has been submitted to the *Journal of Obesity Research and Clinical Practice*.

Tinius RA, Cahill AG, Strand ES, Cade WT. Altered lipid metabolism is associated with higher maternal inflammation in obese women during late pregnancy.

2.1 Abstract

Background: Inflammation is elevated in obese pregnant women and is associated with adverse maternal and neonatal outcomes. Maternal lipid metabolism and its relationships with maternal inflammation, insulin resistance and neonatal metabolic health are poorly understood in obese pregnant women.

Methods: 18 lean (age: 26.1 ± 5.0 years, pre-pregnancy BMI: 21.5 ± 1.9 kg/m^2) and 16 obese (age: 25.0 ± 4.8 years, pre-pregnancy BMI: 36.3 ± 4.3 kg/m^2) women participated in this case-control study during the third trimester of pregnancy. Maternal plasma markers of insulin resistance (HOMA-IR) and inflammation (C-reactive protein (CRP)) were measured at rest, and lipid concentration and kinetics (lipid oxidation rate and lipolysis) were measured at rest, during a 30-minute bout of low-intensity (40% VO_{2peak}) exercise, and during a recovery period. Umbilical cord blood was collected for measurement of neonatal plasma insulin resistance, inflammation, and lipid concentration. Neonatal body composition was measured via air displacement plethysmography.

Results: Pregnant obese women had higher plasma CRP (9.1 ± 4.0 mg/L versus 2.3 ± 1.8 mg/L, $p<0.001$) and higher HOMA-IR (3.8 ± 1.9 versus 2.3 ± 1.5, $p=0.009$) compared to pregnant lean women. Obese women had higher lipid oxidation rates during recovery from low-intensity exercise (0.13 ±0.03g/min versus 0.11 ±0.04g/min, $p=0.02$) that was associated with higher maternal CRP ($r=0.55$, $p=0.001$). Maternal CRP was positively associated with maternal HOMA-IR ($r=0.40$, $p<0.02$) and systolic blood pressure ($r=0.40$, $p<0.02$).

Conclusions: Maternal lipid metabolism-associated inflammation may contribute to insulin resistance and higher blood pressure in obese women during pregnancy.

2.2 Introduction

Maternal obesity is a significant public health concern in the United States as one in three women enter pregnancy obese[1]. Maternal obesity is associated with increased risk of maternal complications including excessive gestational weight gain[2,3], gestational diabetes[4], preeclampsia and other hypertensive disorders[5-8], and long-term maternal cardiovascular morbidity[9]. Obesity during pregnancy also contributes to unfavorable offspring metabolic outcomes including excess adiposity[10,11] and insulin resistance[12].

Obese women experience metabolic dysfunction during pregnancy including higher plasma inflammation and lower insulin sensitivity[4,13,14]. Inflammation and insulin resistance are not only interrelated, but also associated with adverse pregnancy outcomes[13,15-19]. Mechanisms contributing to elevated maternal inflammation during pregnancy in obese women are not fully understood. Evidence suggests that non-gravid, obese adults have elevated lipid oxidation rates at rest[20] and during low-to-moderate intensity exercise[21,22], and that excessive lipid oxidation is associated with higher inflammation[23]. Elevated maternal lipid oxidation during pregnancy in obese women might be contributing to increases in maternal inflammation and subsequent alterations in maternal metabolic health. In addition, alterations in maternal lipid metabolism might impact offspring health as maternal obesity contributes to a lipotoxic placental environment that may be associated with increased *in utero* markers of inflammation and altered fetal development[24,25]. However, maternal lipid oxidation and its relationships with maternal and neonatal inflammation and metabolic health have not been previously examined.

The primary purposes of this study were to compare lipid oxidation and lipolysis during late pregnancy between obese and lean women, and to examine the relationships between maternal lipid metabolism, inflammation and insulin resistance. The secondary purpose was to compare neonatal metabolic outcomes (adiposity, inflammation, and insulin resistance) between neonates of lean and obese pregnant women. We hypothesized that obese pregnant women would have higher lipid oxidation rate and lipolysis, and these would be associated with higher maternal inflammation and insulin resistance. We also hypothesized that neonates born to obese women would have higher neonatal adiposity, inflammation, insulin resistance. The knowledge gained from this study may identify lipid metabolism as a mechanism contributing to maternal inflammation and a potential therapeutic target to reduce inflammation and insulin resistance in obese pregnant women.

2.3 Methods

2.3.1 Participants

Thirty-four women participated in the study (lean: n=18, obese: n=16). Women receiving prenatal care at the Women's Health Center and Women's Health Clinic at Barnes Jewish Hospital/Washington University between August 2013 and November 2014 were screened for inclusion. Approximately 350 women were screened, and 50 women who met all criteria with ongoing pregnancies were approached for participation late in their second trimester. Inclusion criteria included women ages 18-44 years, confirmed singleton viable pregnancy with no identified fetal abnormalities (as determined by routine anatomy ultrasound at 18-22 weeks), and pre-pregnancy BMI between 18.0 and 24.9 kg/m^2 for the lean group or pre-pregnancy BMI

between 30 and 45 kg/m^2 for the obese group. Exclusion criteria included: 1) multiple gestation pregnancy, 2) inability to provide voluntary informed consent, 3) self- reported use of illegal drugs (cocaine, methamphetamine, opiates), 4) current smoker who did not consent to cessation, 5) current usage of daily medications by class: corticosteroids, beta-blockers (known to affect lipid metabolism) and anti-psychotics (known to alter insulin resistance and metabolic profiles), 6) diagnosis of gestational diabetes in current pregnancy, history of gestational diabetes, pre-pregnancy diabetes or prior macrosomic (>4500g) infant (each elevate the risk for gestational diabetes in the current pregnancy, or undiagnosed gestational diabetes), 7) history of heart disease, or 8) any other condition that would preclude exercise.

2.3.2 Study Procedures

All study procedures were performed at the Washington University School of Medicine Institute for Clinical and Translational Sciences Clinical Research Unit (CRU). All pregnant women participated in two maternal visits between 32 and 37 weeks gestation. Approval for this study was granted by the Institutional Review Board at Washington University (IRB ID: 201306109, NCT: NCT02039414).

Maternal Visit #1

Body composition was measured using skinfold anthropometry in order to determine maternal percent body fat. Body fat percentage was determined by pressing folds of the skin at seven sites with a caliper (Harpenden Skinfolds Caliper, Baty International, United Kingdom), recording skin thickness, and entering the data into a standardized equation that accounts for age as previously described[26]. Participants also completed the YMCA submaximal cycle test as

previously performed in order to predict cardiorespiratory fitness levels[27]. National Institutes of Health's Dietary History Questionnaire II was completed by each participant to determine potential differences in maternal diet[28]. Previous literature demonstrates that dietary history questionnaires are valid and reproducible among pregnant populations[29].

Maternal Visit 2

Approximately one week after Visit 1, subjects were admitted to the CRU the morning after an overnight fast. The night prior, subjects were instructed to consume a standardized meal consisting of 50% carbohydrates, 30% fats, and 20% protein. Upon admission to the CRU, height, weight, and vital signs were obtained. A catheter (IV) was placed in a hand vein and heated to 55 °C by using a thermostatically controlled box in order to obtain arterialized blood samples as previously described[30]. Participants kept their hand in the box throughout the entire study visit. Participants rested for approximately 30 minutes prior to measuring lipid oxidation rate using indirect calorimetry (True One 2400, Parvomedics, Sandy, UT). Participants laid supine while a canopy was placed over their head for 15 minutes to measure oxygen consumption and carbon dioxide production in order to determine lipid oxidation rate[31]. After the initial indirect calorimetry measurement, a baseline blood collection was obtained. Participants then exercised continuously at approximately 40% of their predicted VO_{2peak} (based on the YMCA submaximal cycle test) for 30 minutes. Lipid metabolism was examined via indirect calorimetry and plasma analysis during and after exercise because low-to-moderate intensity exercise stimulates both adipose tissue breakdown (i.e. lipolysis) and lipid oxidation, and low-to-moderate intensity exercise might mimic their daily activity levels (~3-5 METS, e.g. household chores, caring for other children, walking). Exercise indirect calorimetry was

performed and blood was drawn at the 10, 20, and 30 minute time points of submaximal exercise. After exercise termination, participants returned to supine and blood was drawn 10, 30, and 60 minutes-post cessation of exercise (i.e. recovery). Indirect calorimetry was performed 30 minutes post-exercise for 15 minutes as described for the baseline measurement.

Sample Analyses and Calculations

All samples were immediately placed on ice and plasma was separated by centrifugation within 30 minutes of collection. Plasma samples were stored at -80° C until final analyses were performed. Blood samples for glucose were collected in heparinized tubes and analyzed immediately with an automated glucose analyzer (Yellow Springs Instruments Co, Yellow Springs, OH). Plasma insulin concentration was measured by electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics, Indianapolis, IN). Fasting insulin and glucose levels were used to calculate the homeostatic model assessment-insulin resistance (HOMA-IR)[32]. The HOMA-IR is an index of insulin resistance that reflects fasting glucose concentration measured at the fasting insulin concentration. Inflammation was examined through high-sensitivity C-reactive protein (CRP) and measured by immunoturbidimetric assay (Roach Diagnostics, Indianapolis, IN). Blood samples used to determine plasma free fatty acids were collected in tubes containing EDTA. Plasma free fatty acid concentrations were determined by enzymatic colorimetric assay (Wako Pure Chemical Industries, Osaka, Japan). Lipolysis was calculated by the area under the curve (AUC) for free fatty acids, as previously described[33], from baseline to the end of the study period. A summary score to represent total lipid oxidation rate throughout the study period was determined by calculating the AUC using lipid oxidation rates from baseline, exercise, and recovery time points. Clinical lipid profiles including triglycerides,

total cholesterol, low-density lipoprotein, and high-density lipoprotein were also obtained at the CRU.

Neonatal Measurements

At parturition, neonatal birth weight was obtained. In addition, 44 mL of umbilical vein blood was collected, centrifuged within 30 minutes of parturition, and placed in a -80° C freezer for further analysis. Umbilical cord blood was used to determine neonatal HOMA-IR (insulin and glucose levels), free fatty acid concentration, and CRP.

Within 48 hours of delivery, neonatal anthropometrics were measured in the CRU. Neonatal length (Pediatric Length Board, Ellard Instrumentation LTD, Monroe, WA) and head circumference (Gulick II Tape Measure, model 67020, Country Technology Inc., Gays Mills, WI) were measured. Body composition (fat and lean mass) was measured by skin fold thickness at four different sites (triceps, subscapular, ilium, and thigh, measured by one recorder) and by air displacement plethysmography (Pea Pod, Life Measurement, Inc., Concord, CA). All neonates were full-term (≥37 weeks gestation) at the time of delivery except for one in the obese group. For the neonate born at 36.2 weeks gestation, neonatal anthropometrics were taken when the neonate was 37.1 weeks gestation.

2.3.3 Statistical Analysis

Normality of the distribution for each variable was tested using Kolmogorov-Smirnov tests. Students Independent T-Tests for normally distributed variables and Mann-Whitney U tests for non-normally distributed variables were used to compare metabolic outcomes between lean and

obese groups. Pearson product-moment correlation coefficients for normally distributed variables and Spearman's rank-order correlation coefficient for non-normally distributed variables were used to assess the degree of the relationship between variables. Two-way repeated-measures ANOVAs (group x time) were used with Tukey post hoc analyses when comparing baseline, exercise, and recovery conditions. Study data were collected and managed using REDCap electronic data capture tools hosted at Washington University School of Medicine[34]. All data analyses were conducted using IBM SPSS Statistics, Version 22 (Armonk, New York).

Based on previous data comparing lipid metabolism between lean and obese females at rest and during exercise[22], we estimated an effect size of 1.1. Therefore, with an alpha level of 0.05, 16 women per group were required to power the study at β=0.80.

2.4 Results

2.4.1 Maternal Demographic Characteristics

Obese women had significantly higher pre-pregnancy body mass indexes and body fat percentages than the lean group (Table 2.1). Age, parity, income level, race, and gestation age during study visits were similar between groups (Table 2.1). Obese pregnant women had significantly higher resting energy expenditure compared to lean pregnant women (Table 2.1)

Table 2.1 Maternal demographic and metabolic characteristics in lean and obese pregnant women

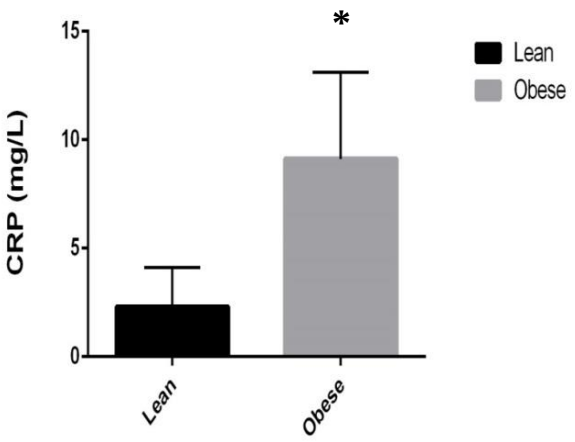
	Lean (n=18) mean±SD	**Obese (n=16) mean±SD**	**t-test p-value**
Age (y)	26.1 ± 5.0	25.0 ± 4.8	0.52
Pre-pregnancy BMI (kg/m^2)*	21.5 ± 1.9	36.3 ± 4.3	<0.001
Body fat (%)*	20.7 ± 4.0	37.7 ± 3.5	<0.001
Gestation age at visit 2 (wks)	35.2 ± 1.0	34.7 ± 1.4	0.25
Gestational weight gain (kg)	14.5 ± 4.5	10.3 ± 9.2	0.12
Heart rate (bpm)	84.1 ± 9.1	90.0 ± 10.8	0.08
Glucose (mg/dL)	76.9 ± 5.5	80.3 ± 7.9	0.24
Insulin (uU/mL)*	12.3 ± 8.2	18.7 ± 8.4	0.01
HOMA-IR*	2.3 ± 1.5	3.8 ± 1.9	0.01
C-reactive protein (mg/L)*	2.3 ± 1.8	9.1 ± 4.0	<0.001
Total cholesterol (mg/dL)	228.4 ± 36.3	207.8 ± 35.9	0.11
HDL (mg/dL)	67.9 ± 18.2	65.1 ± 12.3	0.61
LDL (mg/dL)	130.2 ± 35.0	110.6 ± 34.0	0.24
Triglycerides (mg/dL)	151.8 ± 41.8	160.0 ± 60.9	0.65
Free fatty acids (meq/L)	0.47 ± 0.12	0.49 ± 0.15	0.64
Resting energy expenditure (kcal/day)*	1795 ± 225	2166 ± 291	<0.001
	# of women (%)	**# of women (%)**	**χ^2-test p-value**
Parity			
Nulliparous	11 (61%)	10 (63%)	0.93
Multiparous	7 (39%)	6 (37%)	
Income			
Low income	10 (56%)	8 (56%)	0.75
Mod/high income	8 (44%)	8 (43%)	
Race			
Caucasian	8 (44%)	5 (31%)	0.73
African-American	9 (50%)	10 (63%)	
Other	1 (6%)	1 (6%)	

*p<0.05

2.4.2 Maternal Baseline Metabolic Characteristics

Maternal baseline metabolic characteristics are presented in Table 2.1. Obese pregnant women had significantly higher plasma insulin concentration than lean pregnant women. Obese women had higher HOMA-IR values compared to lean pregnant women. Maternal systolic blood pressure was higher in obese pregnant women compared to lean pregnant women across all time points ($F=3.90$, $p=0.03$). Obese pregnant women had higher plasma CRP concentrations compared to lean pregnant women. CRP, HOMA-IR, and systolic blood pressure data are shown in Figure 2.1.

A.



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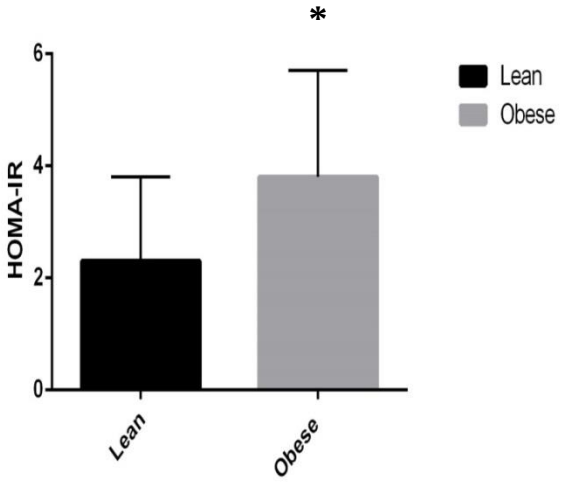


C.

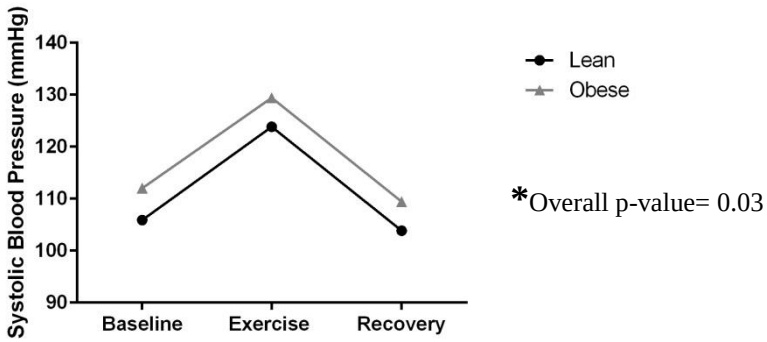


Figure 2.1 Maternal (A) inflammation (B) insulin resistance and (C) systolic blood pressure between lean and obese pregnant women *p<0.05

Dietary composition was similar between groups (Table 2.2). Three women (two lean, one obese) did not complete the dietary survey.

Table 2.2 Average daily dietary composition in lean and obese pregnant women

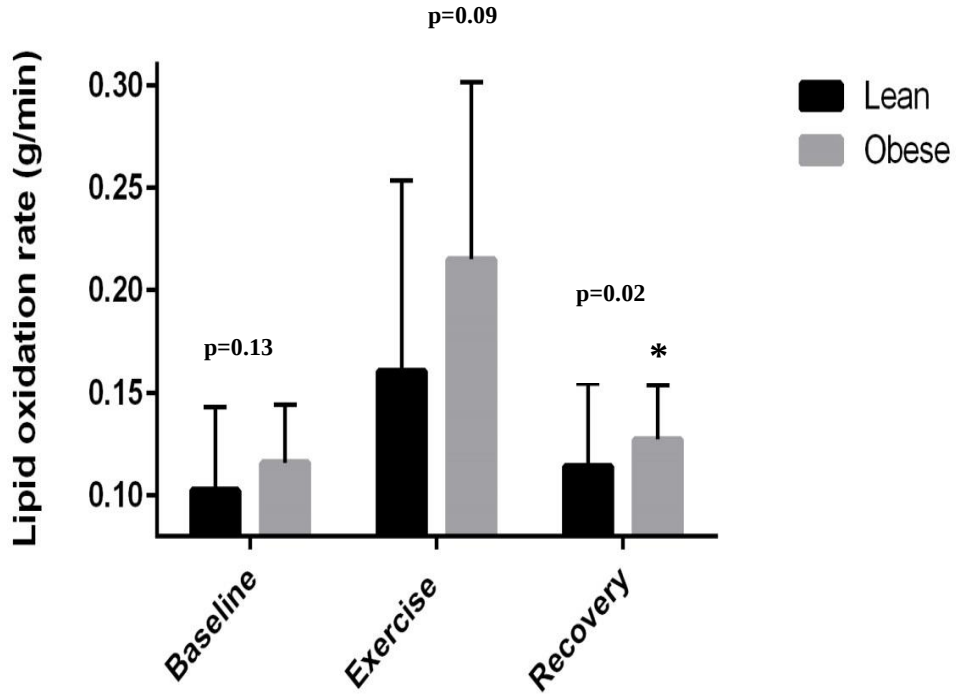
	Lean (n=16) mean ± SD	**Obese (n=15) mean ± SD**	**t-test p-value**
Energy intake (kcal)	2554.7 ± 1574	2058.7 ± 845	0.29
Fat (g)	94.6 ± 50.5	69.1 ± 31.2	0.10
Fat (% of kcal/day)	34.4 ± 5.2	30.6 ± 7.1	0.10
Carbohydrate (g)	353.5 ± 256.2	295.4 ± 137.3	0.44
Carbohydrates (% of kcal/day)	53.1 ± 7.2	56.9 ± 10.6	0.26
Protein (g)	84.1 ± 39.0	73.3 ± 35.9	0.43
Protein (% of kcal/day)	14.3 ± 3.4	14.3 ± 3.5	0.97

*$p<0.05$

2.4.3 Maternal Lipid Metabolism

Pregnant obese women had a higher post-exercise lipid oxidation rate compared to their lean counterparts (recovery: 0.11 ±0.04g/min vs. 0.13 ±0.03g/min, p=0.02) and tended to have higher lipid oxidation rates during baseline and exercise conditions (baseline- lean: 0.10 ± 0.04g/min vs. obese: 0.12 ± 0.03g/min, p=0.13; exercise- lean: 0.16 ±0.09g/min vs. obese: 0.22 ± 0.09g/min, p=0.09) (Figure 2.2A). Total lipid oxidation was significantly higher in the obese group (lean: 11.8 ± 5.4g vs. obese: 15.3±4.4g, p=0.05) (Figure 2.2B). Lipolysis was similar between groups throughout baseline, exercise, and resting conditions.

A.



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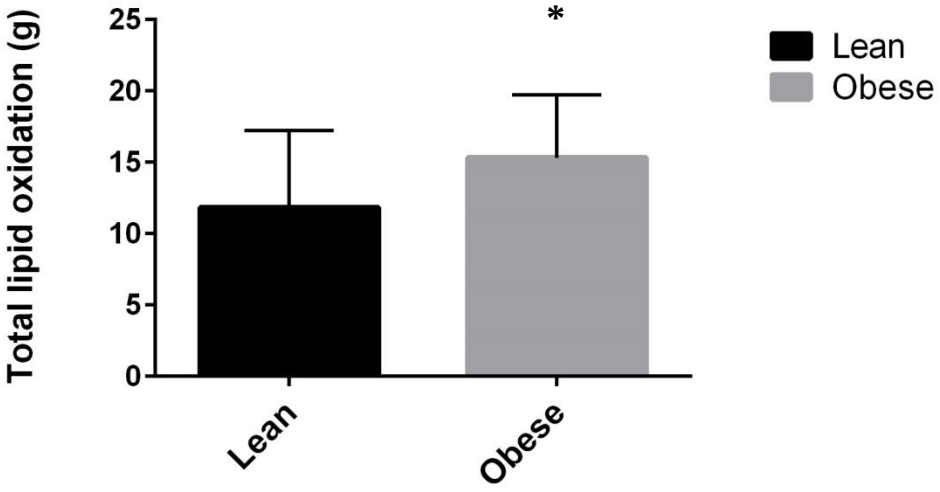
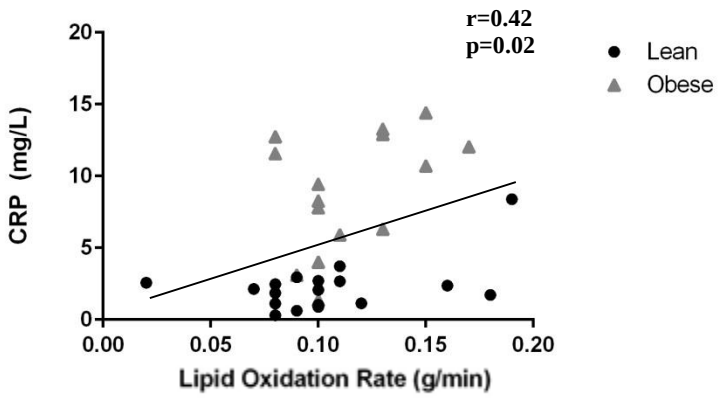


Figure 2.2 (A) Lipid oxidation rates between lean and obese groups at baseline, during exercise, and during recovery from exercise (B) Total lipid oxidation measured by AUC across all timepoints between lean and obese pregnant women *p<0.05

2.4.4 Correlations between Maternal Metabolic Characteristics

CRP was positively associated with lipid oxidation rate at baseline (r=0.42, p=0.02) (Figure 2.3A) and during the 1-hour recovery period (r=.55, p=0.001) (Figure 2.3B). Maternal CRP was also related to total lipid oxidation (r=0.42, p=0.01) (Figure 2.3C).

A.



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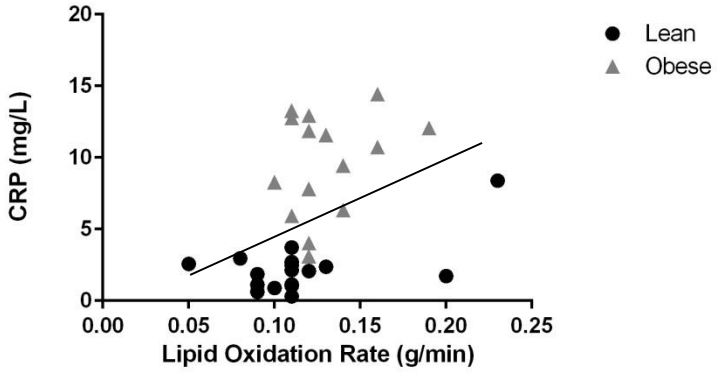

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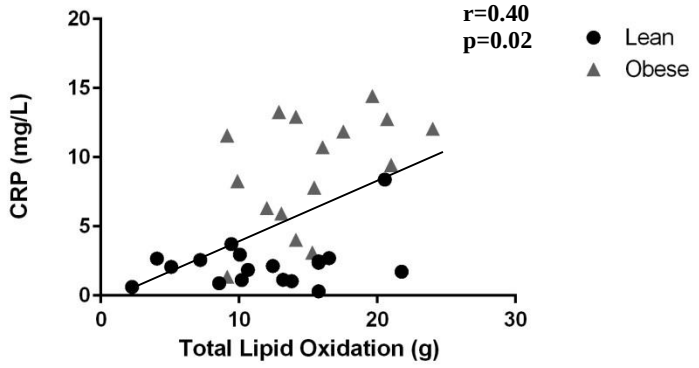
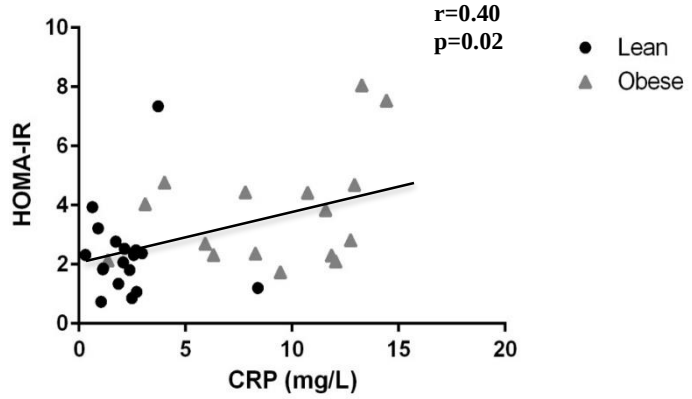


Figure 2.3 Relationships between CRP and (A) baseline lipid oxidation rate, (B) post-exercise recovery lipid oxidation rate, and (C) total lipid oxidation.

Similarly, baseline systolic blood pressure was positively correlated with lipid oxidation rate at baseline (r=0.34, p=0.05) and during the 1-hour recovery period (r=0.39, p=0.02). However, when accounting for CRP, the relationships between lipid oxidation rate and systolic blood pressure did not exist. Maternal CRP was positively correlated with maternal circulating insulin levels (r= 0.44, p=0.01), HOMA-IR (r=0.40, p=0.02) (Figure 2.4A), and systolic blood pressure (r=0.40, p=0.02) (Figure 2.4B). Total lipid oxidation was significantly associated with lipolysis (r=0.57, p=0.001).

A.



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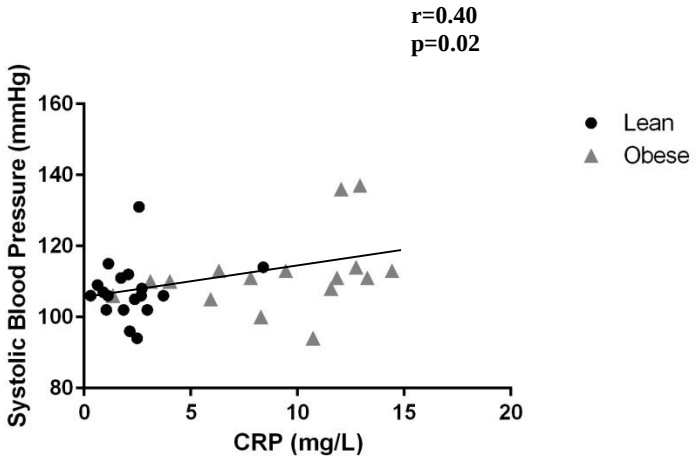

Figure 2.4 Relationships between baseline maternal inflammation and (A) insulin resistance (B) systolic blood pressure

2.4.5 Neonatal Anthropometric and Metabolic Outcomes

Air displacement plethysmography was not performed on three neonates due to time of delivery relative to discharge, but all other neonatal anthropometrics were obtained. Cord blood could not be obtained from one lean and two obese participants. Neonatal anthropometrics and metabolic outcomes were similar between groups (Table 2.3).

Table 2.3 Neonatal outcomes for neonates of lean and obese pregnant women

Neonatal demographic characteristics	**Lean (n=18) mean ± SD**	**Obese (n=16) mean ± SD**	**t-test p-value**
Gestational age at delivery (wks)	39.1 ± 0.9	39.5 ± 1.4	0.33
Birth weight (g)	3221.6 ± 377	3302 ± 447	0.57
Length (cm)	49.5 ± 2.2	49.7 ± 2.2	0.88
Head circumference (cm)	33.7 ± 1.7	34.3 ± 1.2	0.22
Body fat (%)	11.1 ± 4.3	11.1 ± 3.5	0.98
Skinfolds (cm)			
Triceps	4.9 ± 0.8	5.1 ± 1.1	0.61
Subscapular	4.4 ± 0.8	4.4 ± 0.8	0.97
Ilium	4.7 ± 1.3	5.0 ± 1.5	0.56
Thigh	6.4 ± 1.3	6.8 ± 1.8	0.45
	# of women (%)	**# of women (%)**	**χ^2-test p-value**
Mode of delivery			0.27
Vaginal	14 (78%)	9 (56%)	
Cesarean	4 (22%)	7 (44%)	
Gender			0.30
Male	7 (39%)	10 (63%)	
Female	11 (61%)	6 (37%)	
Neonatal cord blood values	**Lean (n=17) mean ± SD**	**Obese (n=14) mean ± SD**	**t-test p-value**
Glucose (mg/dL)	88.4 ± 14.0	80.7 ± 12.8	0.13
Insulin (uU/mL)	7.9 ± 6.2	7.5 ± 4.9	0.98
HOMA-IR	1.7 ± 1.5	1.6 ± 1.2	0.68
Free fatty acids (meq/L)	0.19 ± 0.08	0.16 ±0.06	0.28
C-reactive protein (mg/L)	0.20 ± 0.10	0.24 ± 0.21	0.55

*$p<0.05$

2.5 Discussion

2.5.1 Lipid Metabolism

Our primary novel findings from the study were: 1) maternal lipid oxidation was higher in obese pregnant women compared to lean pregnant women, particularly following an acute bout of low-intensity exercise, 2) maternal lipid oxidation rate was significantly associated with maternal inflammation, and 3) maternal inflammation was related to maternal insulin resistance and systolic blood pressure. These results suggest that elevated maternal lipid oxidation might contribute to increased inflammation and subsequent increases in insulin resistance during late pregnancy in obese women. Importantly, we believe the finding of elevated lipid oxidation rates in obese pregnant women following a mild bout of physical activity is clinically meaningful as this metabolic environment would be representative of women who recently participated in typical daily physical activities (e.g. running errands, cleaning the house, or taking care of another child). Lipid oxidation rate in non-gravid adults is known to increase over resting values during low-to-moderate physical activity and remained effected for 2-3 hours post-exercise[35,36].

In the current study, higher rates of maternal lipid oxidation during rest and post-exercise recovery were associated with greater maternal inflammation. In non-gravid adults, lipid oxidation is intricately related to inflammation and insulin resistance[23,37]. Specifically, lipid oxidation by-products including reactive oxygen species have been shown to signal pathways that initiate an inflammatory response[23,38,39]. The current study also found that higher maternal inflammation was associated with higher insulin resistance, suggesting that maternal inflammation, possibly the result of increased lipid oxidation, might contribute to higher maternal insulin resistance in obese pregnant women. Our results are consistent with Retnakaran

et al. who demonstrated obesity during pregnancy elicits an inflammatory response with possible downstream metabolic sequela including insulin resistance[40]. Similarly, Korkmazer et al. found higher inflammation among insulin resistant pregnant women with and without a classification of gestational diabetes[41]. The clinical implications of this could be substantial as the combination of obesity and insulin resistance during pregnancy can lead to gestational diabetes[13]. Consequently, offspring of obese, insulin-resistant women are at increased risk for preterm delivery and its associated neonatal morbidity, as well as childhood metabolic dysfunction which may potentiate the vicious cycle of obesity and insulin resistance[13]. Taken together, these data suggest a relationship between maternal lipid metabolism, inflammation, and insulin resistance in obese women during pregnancy.

In the current study, lipid profiles were similar between lean and obese pregnant women (triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein). While maternal obesity is associated with increased higher plasma concentrations of triglycerides and low-density lipoproteins early in pregnancy[42], lean and obese women reach similar concentrations during late pregnancy[42]. Our data, measured during late pregnancy, are reflective of the literature demonstrating similar lipid profiles between lean and obese pregnant women. Similarly, maternal and neonatal free fatty acid concentrations in the current study were consistent with Catalano et al. who reported free fatty acid concentrations in obese women and their fetuses were not different than lean pregnant women[42,43].

2.5.2 Inflammation and Blood Pressure

Although not a primary focus of the study, we also found a relationship between maternal inflammation and systolic blood pressure. Maternal systemic inflammation is a known feature of preeclampsia, a pregnancy-specific condition characterized by high blood pressure[44]. In fact, Redman et al. suggest that preeclampsia is ultimately an excessive maternal inflammatory response to pregnancy[45]. High blood pressure disorders during pregnancy, including preeclampsia, are an important cause of morbidity, long-term disability, and even death among pregnant women and their offspring[46]. Inflammation has been identified as a key contributor to pregnancy-specific high blood pressure[45,47] and our findings are consistent with this.

2.5.3 Potential Role of Oxidative Stress

Oxidative stress could provide a link connecting increased maternal lipid oxidation with inflammation and subsequent insulin resistance and hypertension in obese pregnant women. In non-gravid adults, oxidative stress is elevated in obesity[48] and is associated with inflammation, vascular cell wall damage, high blood pressure, cardiovascular disease, poor metabolic function, and insulin resistance[49-57]. It is possible that elevated lipid oxidation rates observed in obese pregnant women result from inefficient and incomplete substrate oxidation; thus, potentially generating partially oxidized substrates (i.e. reactive oxygen species). Bell et al. concluded that incomplete, partial oxidation is increased in obesity[58]. Therefore, increased absolute lipid oxidation in pregnant obese women may be suggestive of increased incomplete oxidation, which may then be contributing to oxidative stress, and ultimately the inflammation and insulin resistance observed in the current study; however, this is speculative and requires further study.

Figure 2.5 depicts proposed pathway for the role of maternal on lipid metabolism and long-term maternal and neonatal outcomes.

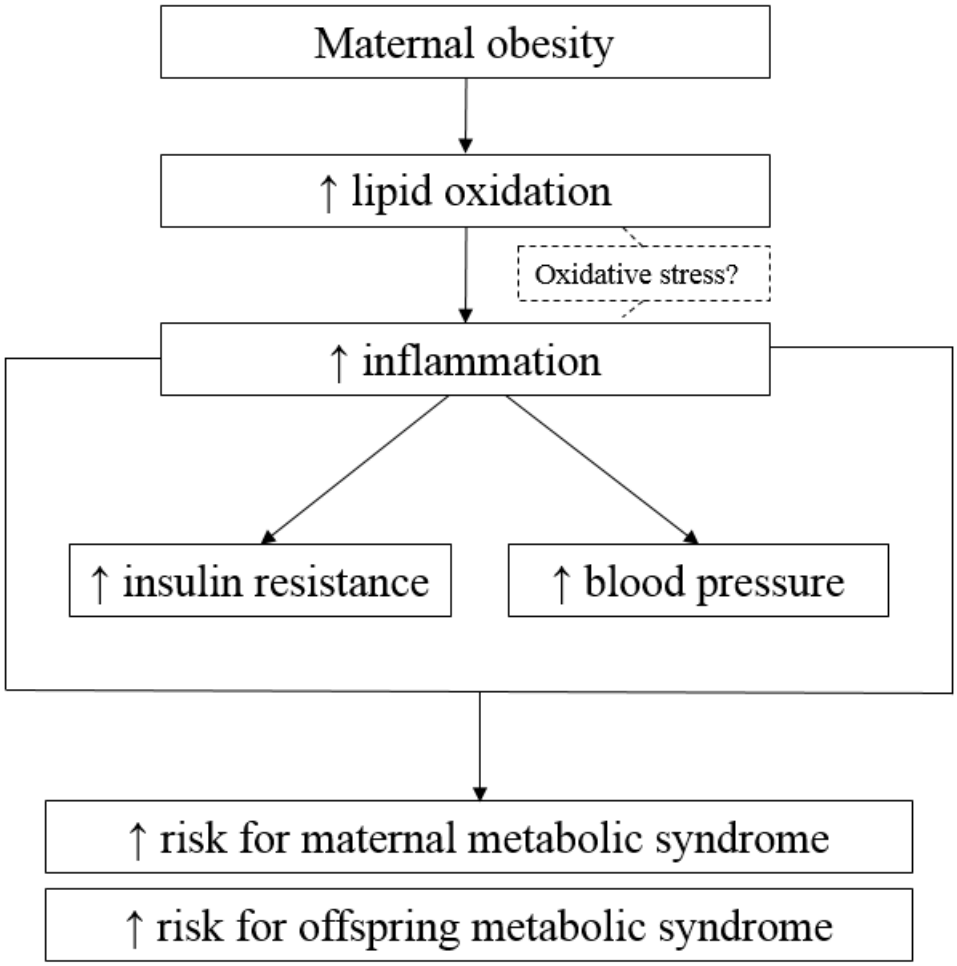

Figure 2.5 Proposed pathway for maternal lipid metabolism and long-term maternal and neonatal outcomes

2.5.4 Neonatal Outcomes

Neonates of pregnant obese woman had similar body composition, insulin resistance, and inflammatory markers as lean pregnant women. These results suggest that despite the metabolic abnormalities associated with obesity during pregnancy, the fetus may be protected from some of these detrimental effects, at least at 24-48 hours post-parturition. However, the "fetal origins hypothesis" suggests the origins of adult health diseases such as obesity, cardiovascular disease, and diabetes may be caused by *in utero* exposures[59,60]. Therefore, it is plausible that metabolic abnormalities may be epigenetically programmed, but are not apparent or measurable until later in life. For example, Liebowitz et al. found that offspring of obese women had higher plasma inflammation at 12 years of age[61]. Offspring of obese women with elevated plasma inflammation during pregnancy may develop inflammation as they age as a result of fetal programming; however, these changes may not be detectable at delivery when umbilical cord blood is obtained. Similarly, Whitaker concluded that obesity during pregnancy more than doubles the risk of obesity in the offspring between ages two and four[62]. Although neonatal body composition in our study was not different between lean and obese pregnant women, it is possible that differences might emerge during the preschool and childhood years. It is also plausible that our sample size was not large enough to detect a difference in birthweight or body composition as it is well-accepted that obese women have larger babies[10,11]. Based on our findings and the current literature, longitudinal studies of offspring born to obese women are needed.

2.5.5 Limitations

A potential limitation of the present study was the observational study design, thus, cause-and-effect relationships could not be determined from these data. Our results should be cautiously interpreted as this pilot study was powered based on our primary outcome of lipid oxidation rate. We are not adequately powered to investigate many other maternal and neonatal outcomes. Bias may have been introduced because measurements and assessments were not blinded (it was obvious who was an obese "case" versus a lean "control"), but uniform data collection procedures were followed for all participants to minimize bias.

2.5.6 Conclusions

We found that obese pregnant women have higher lipid oxidation rates, particularly after an acute bout of low-intensity exercise during late pregnancy. In addition, higher maternal lipid oxidation rate was associated with higher maternal inflammation. Maternal inflammation was related to insulin resistance and systolic blood pressure. We did not find any differences in neonatal metabolic outcomes between lean and obese women. Future studies investigating the role of maternal lipid oxidation-produced oxidative stress and interventions targeting lipid metabolism and inflammation to improve maternal and neonatal health in obese pregnant women are warranted.

2.5.7 Acknowledgements

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Contributions: RAT and WTC researched data, wrote/edited manuscript. EAS and AGC researched data and reviewed/edited the manuscript. The authors declare no conflicts of interest.

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Chapter 3: Maternal Inflammation during Late Pregnancy is Lower in Physically Active Compared to Sedentary Obese Women

This chapter has been submitted to the *Journal of Applied Physiology, Nutrition, and Metabolism*.

Tinius RA, Cahill AG, Strand ES, Cade WT. Maternal inflammation during late pregnancy is lower in physically active compared to sedentary obese women.

3.1 Abstract

Purpose: The primary purpose of this study was to compare maternal plasma inflammation between physically active and sedentary obese women during late pregnancy. The secondary purpose was to examine the relationships between maternal plasma inflammation and lipid metabolism and maternal and neonatal metabolic health in these women.

Methods: A case-control study design was performed in 16 obese-sedentary ((OBS) age: 25.0 ± 4.8 years, body fat: 37.7 ± 3.5%) and 16 obese-active ((OBA) age: 28.9 ± 4.8 years, body fat: 36.6 ± 3.8%) women during the third trimester of pregnancy. Maternal plasma markers of inflammation (C -reactive protein (CRP)) and insulin resistance (Homeostatic Model Assessment-Insulin Resistance (HOMA-IR)) were measured at rest. Plasma lipid concentration and metabolism (lipid oxidation rate and lipolysis) were measured at rest, during a 30-minute bout of low-intensity (40% VO_{2peak}) exercise, and during a resting recovery period using indirect calorimetry. Umbilical cord blood was collected at delivery for measurement of neonatal plasma insulin resistance, inflammation, and lipid concentration. Neonatal body composition was measured 24-48 hours postpartum via air displacement plethysmography.

Results: Maternal plasma CRP concentration was significantly higher in OBS compared to OBA women (9.1 ± 4.0 mg/L versus 6.3 ±2.5mg/L, p=0.02). Maternal plasma CRP concentration was significantly associated with maternal lipolysis (r=0.43, p=0.02), baseline lipid oxidation rate (r=0.39, p=0.03), and baseline plasma free fatty acid concentration (r=0.36, p=0.04).

Conclusions: Maternal physical activity may reduce inflammation during pregnancy in obese women. Lipid metabolism is altered in sedentary obese women and might contribute to elevated systemic inflammation during pregnancy.

3.2 Introduction

Maternal obesity prevalence is at a historic high with nearly one in three women entering pregnancy obese[1]. Pre-pregnancy obesity contributes to maternal inflammation, insulin resistance, and altered lipid metabolism[2,3], as well as neonatal adiposity and insulin resistance; all of which can have serious long-term health implications for women and their offspring[4-7]. In particular, maternal inflammation may play a significant role in the development of maternal insulin resistance and hypertension- two of the most common health issues diagnosed in obese pregnant women[8-10]. Excessive maternal inflammation might also negatively contribute to maternal long-term health as it is predictive of future cardiovascular disease risk in non-gravid adults[11].

Physical inactivity is recognized as an independent risk factor for obesity, insulin resistance, and type 2 diabetes in non-gravid adults[12,13]. The physiological and hormonal changes associated with pregnancy magnify this risk during and after pregnancy by causing an increase in adiposity and insulin resistance[14]. In pregnant women of normal body weight, physical activity reduces inflammation[15,16], as well as increases maternal insulin sensitivity[17]. In obese pregnant women, physical activity may improve maternal insulin sensitivity[18]. In addition, neonates of physically active women have lower adiposity compared to neonates born to inactive women[19,20]. However, the role of a physically active lifestyle on maternal metabolic health, particularly systemic inflammation, in at-risk obese pregnant women and their neonates is poorly understood. To our knowledge, the impact of physical activity on maternal systemic inflammation and lipid metabolism, and neonatal adiposity, insulin resistance, and inflammation has not been studied in obese pregnant women.

The primary purpose of this study was to compare maternal plasma inflammation between physically active and sedentary obese women during pregnancy. The secondary purpose was to examine the relationships between maternal plasma inflammation and lipid metabolism and maternal and neonatal metabolic health in these women. We hypothesized that during late pregnancy, physically active obese women will have lower plasma inflammation, lipid oxidation rate, lipolysis, and insulin resistance compared to sedentary obese women. We also hypothesized that neonates of physically active obese women will have lower adiposity, inflammation, and insulin resistance than neonates of sedentary obese women.

3.3 Methods

3.3.1 Participants

Thirty-two women participated in the study (16 obese-sedentary (OBS), 16 obese-active (OBA)). Four-hundred women receiving prenatal care at the Women's Health Center or Women's Health Clinic at Barnes Jewish Hospital/Washington University between August 2013 and November 2014 were screened for inclusion. Sixty women who met all criteria with ongoing pregnancies were approached for participation late in their second trimester. Inclusion criteria included women ages 18-44 years with a confirmed singleton viable pregnancy and no identified fetal abnormalities (as determined by routine anatomy ultrasound at 18-22 weeks) and pre-pregnancy BMI between 30 and 45 kg/m^2. Physical activity information was gathered from health history questionnaires distributed by the clinic. All women in the active group reported exercising ≥150 minutes per week. Physical activity levels were confirmed via accelerometry. Patients were excluded for any of the following reasons: 1) multiple gestation pregnancy, 2) inability to

provide voluntary informed consent, 3) self-reported use of illegal drugs (cocaine, methamphetamine, opiates), 4) current smoker who did not consent to cessation, 5) current usage of daily medications by class: corticosteroids, beta-blockers, or anti-psychotics (known to alter insulin resistance and metabolic profiles), 6) diagnosis of gestational diabetes in current pregnancy, 7) history of gestational diabetes, pre-pregnancy diabetes or prior macrosomic (>4500g) infant (each elevates the risk for gestational diabetes in the current pregnancy, or undiagnosed gestational diabetes), 8) history of heart disease, or 9) any other condition that would preclude exercise. Approval for this study was granted by the Institutional Review Board at Washington University School of Medicine (IRB ID: 201306109, NCT: NCT02039414). All women gave informed consent prior to participating in the study.

3.3.2 Study Procedures

All study procedures were performed at the Washington University School of Medicine Institute for Clinical and Translational Science's Clinical Research Unit (CRU). All pregnant women participated in two maternal visits between 32 and 37 weeks of gestation.

Maternal Visit 1

Body composition was measured using skinfold anthropometry in order to determine maternal percent body fat. Body fat percentage was determined by pressing folds of the skin at seven sites with a caliper (Harpenden Skinfolds Caliper, Baty International, United Kingdom), recording skin fold thickness, and entering the data into a standardized equation that accounts for age as previously described[21]. Cardiorespiratory fitness levels were assessed using the YMCA submaximal multistage cycle ergometer test as described by Beekley et al. (2004) using a Lode

Corvial Recumbent cycle ergometer (Lode B.V., The Netherlands). Results were used to predict participant's VO_{2peak} via the VO_2-Heart Rate extraction method, which is considered more accurate for pregnant women than the Astrand-Ryhming test[22]. A three-lead electrocardiogram was applied to monitor heart rate during the exercise test. Participants also completed the National Institutes of Health's validated Dietary History Questionnaire II to determine potential differences in diet[23].

Maternal physical activity levels were objectively assessed during the week following visit one using the ActiGraph GT3X+ accelerometer (ActiGraph LLC, Pensacola, FL). The GTX3+ was placed on the non-dominant wrist with non-removable wristbands. The wristbands were cut off by the study team when they returned for their second visit to ensure the accelerometers were worn for the entire data collection period. Data was collected for seven consecutive days at 30 Hz. The accelerometer output was sampled by a 12-bit analog-to-digital converter. The percentage of time spent sedentary as well as the amount of time spent participating in different categories of physical activity ranging from light and lifestyle to moderate were calculated using algorithms corresponding to the following activity counts: sedentary: 0 - 99 counts/min, light: 100 - 759 counts/min, lifestyle: 760 - 1951 counts/min, moderate: 1952-5724 counts/min[24,25].

Maternal Visit 2

Approximately one week after Visit 1, participants were admitted to the CRU the morning after an overnight fast. The night prior, subjects were provided with written instructions from the research unit dietician for consuming a balanced meal with approximately 50% carbohydrate,

30% fat, and 20% protein. Upon admission to the CRU, height, weight, and vital signs were obtained. A catheter (IV) was placed in a hand vein and heated to 55 °C by using a thermostatically controlled box in order to obtain arterialized blood samples as previously described[26]. Participants kept their hand in the box throughout the entire study visit. Participants laid supine for 30 minutes and rested quietly prior to a baseline 15-minute resting indirect calorimetry measurement using the TrueOne Canopy Option and TrueOne Metabolic Cart (TrueOne 2400, Parvomedics, Sandy, UT). Lipid oxidation rate was calculated by measurement of oxygen consumption and carbon dioxide production as previously described[27]. After a baseline blood draw, participants exercised at approximately 40% of their predicted VO_{2peak} (based on the YMCA submaximal cycle test) for 30 minutes. Blood was drawn at 10, 20, and 30 minute time points during submaximal exercise. Indirect calorimetry was also performed at 10, 20, and 30 minutes (for 2 minutes at a time) during submaximal exercise using an exercise mouthpiece and nose clip for calculation of lipid oxidation rate during low-intensity exercise. Lipid metabolism was examined during exercise because exercise upregulates adipose tissue breakdown (lipolysis) and lipid oxidation[28]. In addition, we believe the exercise paradigm mimicked daily activity levels (~3-5 METS, e.g. household chores, caring for other children, walking), thus, representing metabolism during and after normal daily activities. After exercise termination, participants returned to supine and blood was drawn 10, 30, and 60 minutes-post cessation of exercise (i.e. recovery). Resting indirect calorimetry using the canopy option was performed a second time for 15 minutes midway through the post-exercise recovery period. Visit 2 study procedures are shown in Figure 3.1.

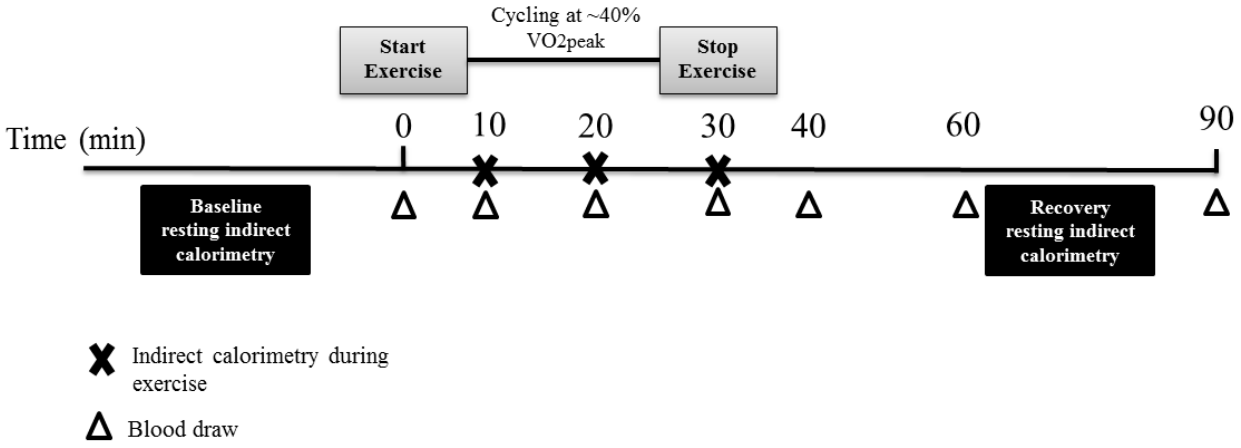


Figure 3.1 Summary of visit 2 procedures

Sample Analyses and Calculations

All blood samples were immediately placed on ice and plasma was separated by centrifugation within 30 minutes of collection. Plasma samples were stored at -80ºC until final analyses were performed. Blood samples for glucose were collected in heparinized tubes and analyzed immediately with an automated glucose analyzer (Yellow Springs Instruments Co, Yellow Springs, OH). Plasma insulin concentration was measured by electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics, Indianapolis, IN). Insulin and glucose levels were used to calculate the homeostatic model assessment-insulin resistance (HOMA-IR)[29,30]. The HOMA-IR is an index of insulin resistance that reflects fasting glucose concentration measured at the fasting insulin concentration. High-sensitivity C-reactive protein (CRP) was measured by immunoturbidimetric assay (Roach Diagnostics, Indianapolis, IN). Blood samples used to determine plasma free fatty acids were collected in tubes containing EDTA. Plasma free fatty acid concentrations were determined by enzymatic colorimetric assay (Wako Pure Chemical Industries, Osaka, Japan). Lipolysis was calculated by the area under the curve for free fatty acids as previously described [31] from free fatty acid concentrations from baseline and throughout the exercise and recovery periods.

Neonatal Measurements

Upon admission to labor and delivery, maternal weight was measured and gestational weight gain was calculated. At parturition, neonatal birth weight was obtained. In addition, 44 mL of umbilical cord blood was collected, centrifuged within 30 minutes of delivery, and stored at

-80ºC until further analyses were performed. Umbilical cord blood was used to determine neonatal HOMA-IR, free fatty acid concentration, and inflammation (CRP).

Within 48 hours of delivery, neonatal anthropometrics were measured in the CRU. Neonatal length (Pediatric Length Board, Ellard Instrumentation LTD, Monroe, WA) and head circumference (Gulick II Tape Measure, model 67020, Country Technology Inc., Gays Mills, WI) were measured. Body composition (fat and lean mass) was measured by skin fold thickness (Harpenden calipers, Baty International, West Sussex, UK) at four different sites (triceps, subscapular, ilium, and thigh) and by air displacement plethysmography (Pea Pod, Life Measurement, Inc., Concord, CA). All neonates were full-term (≥37 weeks) at the time of delivery except for one in the OBS group. For the neonate born at 36.2 weeks gestation, neonatal anthropometrics were taken when the neonate was 37.1 weeks gestation.

3.3.3 Statistical Analysis

The study was primarily powered based on CRP data in sedentary obese pregnant women from Sen et al.[32] who demonstrated that sedentary obese women had CRP levels over twice as high as lean pregnant women (no studies have compared plasma CRP levels between physically active and inactive obese pregnant women). Using a two-tailed alpha of 0.05, 30 total participants (15 per group) were needed to adequately power our study at 0.85 (beta=0.15). One neonate in the OBA group was lost to follow-up, thus, an extra participant was recruited to ensure adequate power.

Normality of the distribution for each variable was tested using Kolmogorov-Smirnov tests. Student's Independent T-Tests for normally distributed variables and Mann-Whitney U tests for non-normally distributed variables were used to compare metabolic outcomes between OBA and OBS women. Two-way repeated-measures ANOVAs (group x time) were used with Tukey post hoc analyses when comparing baseline, exercise, and recovery conditions. Covariates were used to statistically account for differences in age, race, parity, income, and dietary composition between groups when necessary. Pearson product-moment correlation coefficients for normally distributed variables or Spearman's rank-order correlation coefficient for non-normally distributed variables were used to assess the degree of the relationships between variables. Partial correlations were used to adjust for potential confounders. All tests were two-sided with a p-value <0.05 denoting statistical significance. Data were collected and managed using Research Electronic Data Capture (REDCap), hosted at Washington University School of Medicine[33]. All data analyses were conducted using IBM SPSS Statistics, Version 22 (Armonk, New York).

3.4 Results

3.4.1 Maternal Demographic Characteristics

OBA women were older than OBS women, but all other demographics were similar between the groups (Table 3.1).

Table 3.1 Maternal demographic and metabolic characteristics in OBS and OBA pregnant women

	OBS (n=16) mean±SD	**OBA (n=16) mean±SD**	**t-test p-value**
Age (y)*	25.0 ± 4.8	28.9 ± 4.8	0.03
Pre-pregnancy BMI (kg/m^2)	36.3 ± 4.3	34.0 ± 3.7	0.09
Body fat (%)	37.7 ± 3.5	36.6 ± 3.8	0.43
Gestation age at visit 2 (wks)	34.7 ± 1.4	34.4 ± 1.3	0.58
Gestational weight gain (kg)	10.3 ± 9.2	9.5 ± 6.8	0.78
Heart rate (bpm)	90.0 ± 10.8	88.5 ± 12.3	0.73
Systolic blood pressure (mmHg)	112.0 ± 10.9	111.6 ± 8.8	0.96
Diastolic blood pressure (mmHg)	71.5 ± 4.3	69.3 ± 7.9	0.33
Glucose (mg/dL)	80.3 ± 7.9	80.2 ± 8.3	0.96
Insulin (uU/mL)	18.7 ± 8.4	15.6 ± 9.1	0.24
HOMA-IR	3.8 ± 1.9	3.2 ± 2.3	0.24
C-reactive protein (mg/L)*	9.1 ± 4.0	6.3 ± 2.5	0.02
Total cholesterol (mg/dL)	207.8 ± 35.9	212.6 ± 46.1	0.74
HDL (mg/dL)	65.1 ± 12.3	68.5 ± 14.1	0.47
LDL (mg/dL)	110.6 ± 34.0	112.9 ± 39.9	0.87
Triglycerides (mg/dL)	160.0 ± 60.9	155.9 ± 59.9	0.85
Free fatty acids (meq/L)	0.49 ± 0.15	0.45 ± 0.14	0.46
Lipolysis (meq·min/L)*	42.1 ± 11.0	36.9 ± 7.2	0.03
Resting energy expenditure (kcal/day)	2167 ± 291	2099 ± 338	0.55
	# of women (%)	**# of women (%)**	**χ^2-test p-value**
Parity			0.29
Nulliparous	10 (63%)	7 (44%)	
Multiparous	6 (37%)	9 (56%)	
Income			0.28
Low income	8 (50%)	5 (31%)	
Mod/high income	8 (50%)	11 (69%)	
Race			0.34
Caucasian	5 (31%)	9 (56%)	
African-American	10 (63%)	6 (38%)	
Other	1 (6%)	1 (6%)	

*p<0.05

Dietary composition was similar between groups; however, there was a trend for OBA women to consume a higher percentage of dietary fats (Table 3.2). Dietary fats were used as a covariate in subsequent analysis involving lipid metabolism because dietary fat consumption can significantly influence lipid metabolism[34].

Table 3.2 Average daily dietary composition in OBS and OBA pregnant women

	OBS (n=15) mean±SD	**OBA (n=16) mean±SD**	**t-test p-value**
Energy intake (kcal)	2058.7 ± 845	2369.0 ± 1252	0.43
Fat (g)	69.1 ± 31.2	94.0 ± 62.9	0.18
Fat (% of kcal/day)	30.6 ± 7.1	34.9 ± 5.0	0.06
Carbohydrate (g)	295.4 ± 137	295.0 ± 122	0.99
Carbohydrate (% of kcal/day)	56.9 ± 10.6	51.4 ± 7.9	0.11
Protein (g)	73.3 ± 35.9	98.5 ± 68.1	0.21
Protein (% of kcal/day)	14.3 ± 3.5	16.1 ± 3.2	0.15

*$p<0.05$

OBA women spent significantly less time sedentary (72.3 ± 4.3% vs. 58.4 ± 5.9%, $p<0.001$), and more time in light (9.5 ± 1.7% vs. 7.6 ± 0.9%, $p=0.001$), lifestyle (15.2 ± 2.2% vs.10.4 ± 0.8%, $p<0.001$), and moderate (16.9 ± 4.4% vs. 9.7 ± 2.2% vs., $p<0.001$) physical activities than the OBS women (Figure 3.2).

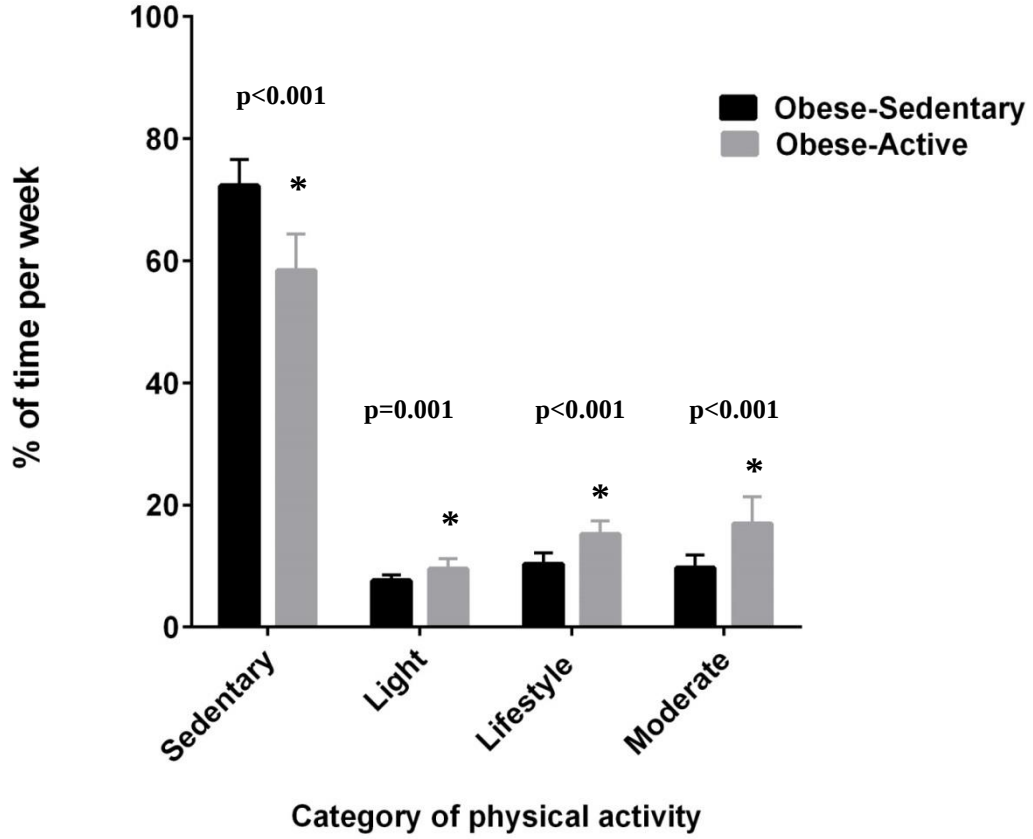


Figure 3.2 Time spent sedentary and participating in light, lifestyle, and moderate physical activities in OBS and OBA pregnant women *p<0.05

OBA women also had higher predicted fitness levels compared to OBS women (33.7± 6.4 ml/kg/min vs. 24.7± 3.5 ml/kg/min, $p<0.001$). All OBA reported their primary mode of activity being walking (n=16), while reported secondary activities were biking (n=1), swimming (n=1), yoga (n=2), weight-lifting (n=2), and using the elliptical machine (n=2).

3.4.2 Maternal Metabolic Characteristics

Maternal plasma CRP concentration was significantly lower in OBA women compared to OBS women (6.3 ± 2.5 mg/L versus 9.1 ± 4.0 mg/L, $p=0.02$) (Figure 3.3). When adjusting for differences in age, race, parity, and income between groups, CRP remained significantly lower in OBA women compared to OBS women ($p=0.002$).

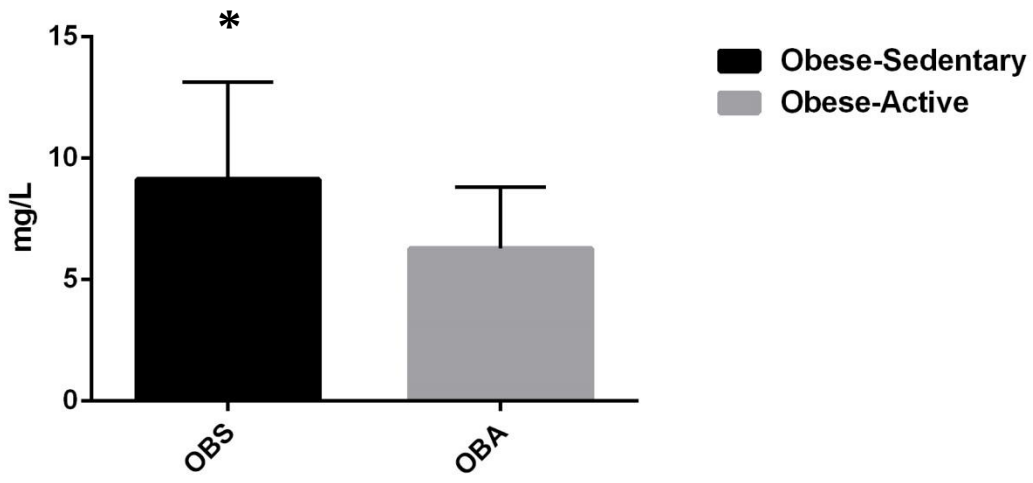
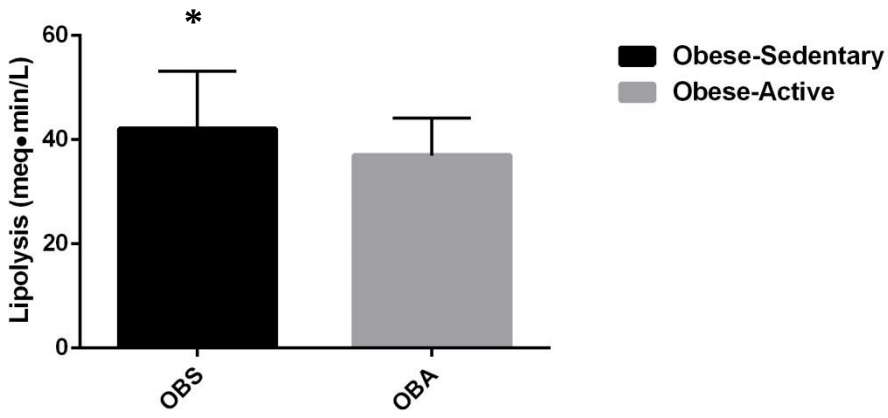


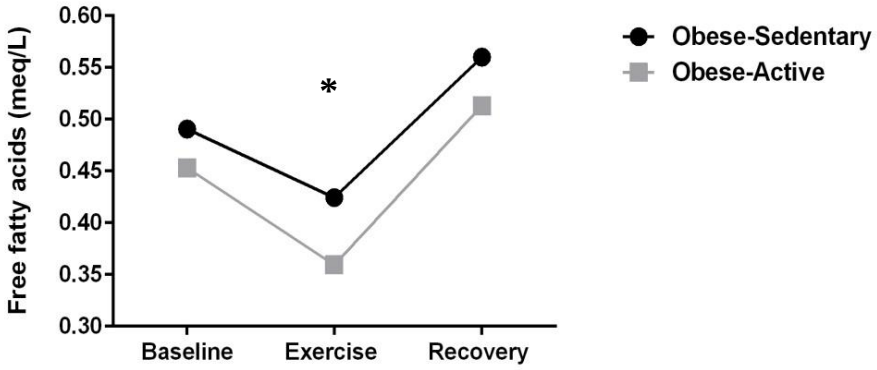
Figure 3.3 Maternal CRP in OBS and OBA pregnant women *p<0.05

OBA women had a lower lipolysis (36.9 ± 7.2 meq·min/L vs. 42.1 ± 11.0 meq·min/L, p=0.03) (Figure 3.4A) and tended to have lower circulating free fatty acids (F= 3.04, p=0.09), particularly during the exercise bout (30 minute time point) (0.36 ± 0.10 meq/L vs. 0.42 ± 0.13 meq/L, p=0.03) (Figure 3.4B) compared to OBS women. There were no differences in lipid oxidation rates between groups (Figure 3.4C).

A.



B.



C.

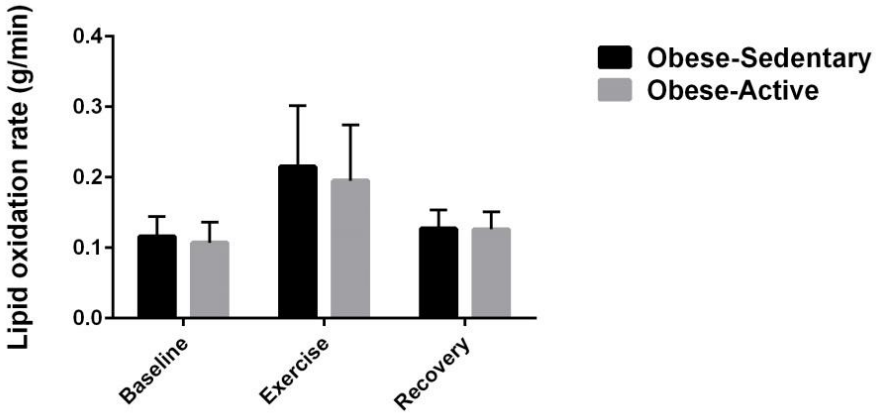


Figure 3.4 (A) Maternal lipolysis (B) Maternal plasma circulating free fatty acids during baseline, exercise, and recovery conditions (C) Maternal lipid oxidation rates in OBS and OBA women during baseline, exercise, and recovery conditions *$p<0.05$

Maternal lipolysis was significantly correlated to maternal plasma CRP concentration (r=0.43, p=0.02). Maternal baseline free fatty concentration was also correlated to maternal plasma CRP concentration (r=0.36, p=0.04). Similarly, maternal baseline lipid oxidation rate and maternal plasma CRP concentration were positively correlated (r=0.39, p=0.03).When the relationship between lipid oxidation and inflammation was analyzed in the OBA and OBS groups individually, lipid oxidation and CRP had a moderate positive correlation in OBS women (r=0.50 p=0.06), but not in OBA women (r= -0.12, p=0.67) (Figure 3.5).

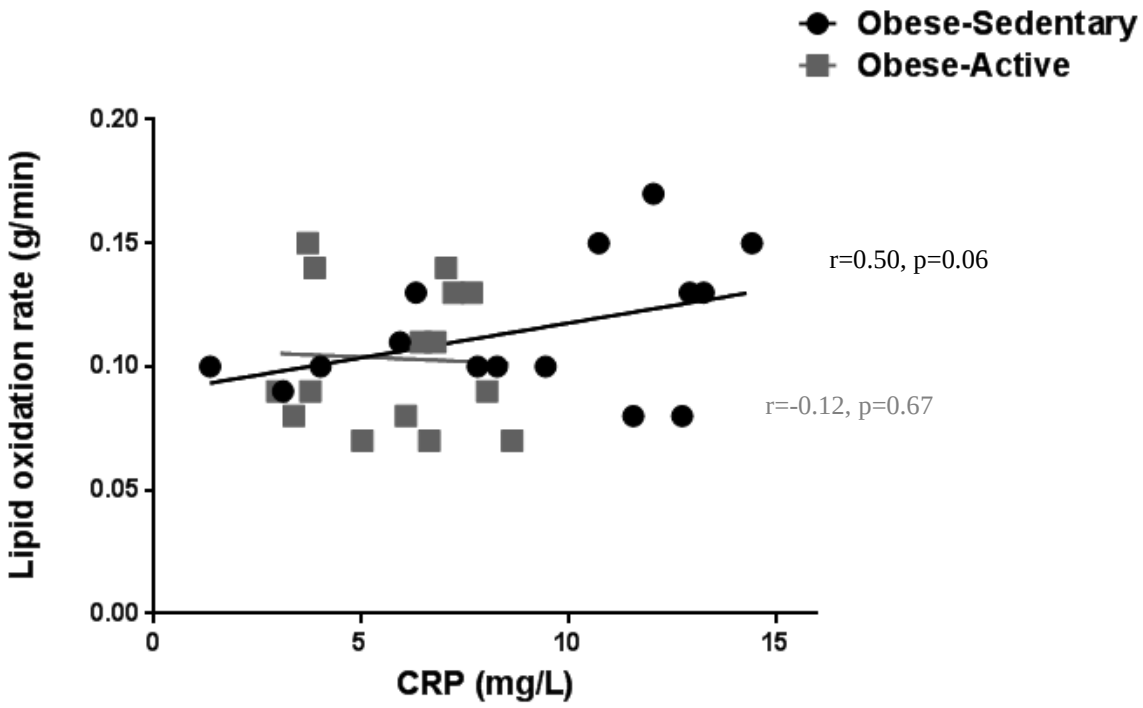


Figure 3.5 Relationship between maternal lipid oxidation rate and maternal CRP in OBS and OBA women

3.4.3 Neonatal Outcomes

Neonatal anthropometrics were not obtained for one neonate who was lost to follow-up. Cord blood could not be obtained for two OBS and four OBA women/neonates. Neonatal anthropometric and metabolic outcomes were similar between groups (Table 3.3).

Table 3.3 Neonatal outcomes for neonates of OBS and OBA pregnant women

Anthropometric outcomes	**OBS (n= 16) mean±SD**	**OBA (n=15) mean±SD**	**t-test p-value**
Gestational age at delivery (wks)	39.5 ± 1.4	39.0 ± 1.3	0.25
Birth weight (g)	3302 ± 447	3299.9 ± 515	0.99
Length (cm)	49.7 ± 2.2	49.6 ± 2.3	0.99
Head circumference (cm)	34.3 ± 1.2	34.3 ± 1.3	0.92
Body fat (%)	11.1 ± 3.5	12.4 ± 2.9	0.29
Skinfolds			
Triceps	5.1 ± 1.1	4.7 ± 0.9	0.32
Subscapular	4.4 ± 0.8	4.3 ± 0.6	0.83
Ilium	5.0 ± 1.5	4.7 ± 1.2	0.54
Thigh	6.8 ± 1.8	6.4 ± 1.3	0.50
	# of neonates (%)	**# of neonates (%)**	**χ^2-test p-value**
Mode of delivery			0.72
Vaginal	9 (56%)	11 (69%)	
Cesarean	7 (44%)	5 (31%)	
Gender			0.72
Male	10 (63%)	9 (56%)	
Female	6 (37%)	7 (44%)	
Cord blood values	**(n=14) mean±SD**	**(n=12) mean±SD**	**t-test p-value**
Glucose (mg/dL)	80.7 ± 12.8	88.3 ± 20.7	0.31
Insulin (uU/mL)	7.5 ± 4.9	9.0 ± 7.2	0.57
HOMA-IR	1.6 ± 1.2	1.9 ± 1.6	0.55
Free fatty acids (meq/L)	0.16 ±0.06	0.16 ± 0.07	0.89
C-reactive protein (mg/L)	0.24 ± 0.21	0.16 ± 0.14	0.32

*$p<0.05$

There were no significant relationships between maternal and neonatal adiposity (r= -0.25, p=0.18), maternal and neonatal inflammation (r=0.19, p=0.37), or maternal and neonatal insulin resistance (r=0.20, p=0.35).

3.5 Discussion

3.5.1 Maternal Outcomes

The primary finding of this study was that during late pregnancy, maternal systemic inflammation, measured by C-reactive protein, was lower in physically active obese women compared to sedentary obese women during late pregnancy. Our finding is consistent with Hawkins et al. who found that physical activity has a protective effect on CRP in normal-weight pregnant women during the second trimester[35]. Our study extends this work by demonstrating the benefits of physical activity on inflammation also apply to obese pregnant women during late pregnancy- a population at high risk for excessive inflammation and its downstream sequela. This finding may be clinically significant as higher inflammation might contribute to the increased acute and chronic risk for the development of metabolic complications (e.g. insulin resistance, gestational diabetes, hypertension, metabolic syndrome, cardiovascular disease)[8,36,37] as well as additional risks for maternal infection, preterm delivery, and severe preeclampsia[9]. Additionally, higher inflammation in sedentary obese women during pregnancy will likely persist into postpartum[38]; thus, potentially contributing to a higher long-term diabetes and cardiovascular disease risk[11].

In non-gravid obese adults, excessive plasma free fatty acids and lipid oxidation are believed to initiate an inflammatory response[39]. In the current study, maternal lipid oxidation and lipolysis were associated with maternal inflammation, suggesting that this relationship between lipid metabolism and inflammation also exists in obese pregnant women. We suspect maternal physical activity during pregnancy might modulate lipid metabolism as obese active women had lower plasma free fatty acid concentrations and lower lipolysis. Our findings are consistent with data in non-gravid adults that suggests endurance exercise training increases efficiency of lipid metabolism including decreasing plasma free fatty acid turnover and oxidation during submaximal exercise[40]. Our study suggests maternal lipid metabolism might be moderately improved in obese women who participate in physical activity, and that this improvement may contribute to lower inflammation. Our finding is clinically important as lipid metabolism may be a modifiable upstream target that contributes to systemic inflammation in obese pregnant women.

Another possible mechanism for physical activity-associated improvements in maternal inflammation in obese pregnant women is through a reduction in oxidative stress. Inflammation is believed to be a manifestation of oxidative stress, which is increased in obese individuals[41]. Studies have demonstrated that exercise training upregulates antioxidant capacity, thus decreasing oxidative stress[42]. In our cohort of obese pregnant women, it is plausible that regular physical activity improved their antioxidant capacity which decreased their oxidative stress, and this lead to a subsequent decrease in inflammation. Further support of this theory is the connection between lipid metabolism and oxidative stress; oxidative stress is a known by-product of inefficient lipid metabolism[43]. Therefore, the lower lipolysis and free fatty acid

concentration found in obese active women may be contributing to a reduction in oxidative stress, and thus, a reduction in systemic inflammation. However, oxidative stress and antioxidant capacity were not measured in the current study; therefore, the role of oxidative stress is speculative.

Upon further examination, we noted that the relationship between lipid oxidation and inflammation was primarily driven by the obese sedentary group. Lipid oxidation and inflammation were positively correlated in obese sedentary women, but this relationship did not exist in the obese active group. This suggests that although lipid oxidation rates were not different between obese sedentary and obese active groups, the positive correlation between lipid oxidation and inflammation in obese sedentary pregnant women may be due to inefficient and incomplete lipid oxidation. Incomplete lipid oxidation may then be contributing to downstream inflammation, which is supported by the increased C-reactive protein in the obese sedentary group. Bell et al. suggests that in obesity, lipid oxidation may be incomplete and inefficient, leading to oxidative stress[39,43]- a known contributor to inflammation[41]. It is well-established that exercise improves efficiency of lipid metabolism as well as reduces oxidative stress[40,44]. Therefore, it is plausible that the reason for the lack-of association between lipid oxidation and inflammation in the obese active group was due to obese active women having improved efficiency of lipid metabolism resulting in complete oxidation of lipids, lower oxidative stress, and lower systemic inflammation. Unfortunately, intermediate by-products of lipid metabolism were not measured, and we did not assess whether or not lipid oxidation was complete; therefore, this is speculative.

3.5.2 Neonatal Outcomes

Contrary to our hypothesis, there were no differences in neonatal metabolic outcomes between obese sedentary and obese active women. Our study suggests that although neonates of obese active women were not leaner, exercise during pregnancy in this at-risk population does not appear to have a harmful impact on neonatal birth weight. These data are clinically important as the neonatal risks associated with obese pregnant women participating in exercise regimes are largely unknown.

Maternal inflammation and insulin resistance were not related to any neonatal metabolic outcomes (adiposity, insulin resistance, or inflammation). These findings suggest that maternal metabolic health may not have an acute impact on neonatal metabolic health. However, metabolic abnormalities may be programmed but not apparent or measurable until later in life[45]. One of the primary ideas behind the "fetal origins hypothesis" is the programming of poor metabolic health can remain latent for many years in the offspring[45,46]. Several studies have noted a long-term impact of maternal obesity or physical activity on neonatal adiposity and inflammation[47,48]. Thus, long-term follow-up of neonates born to obese sedentary and obese active women are needed to truly determine the impact of physical activity on long-term offspring health in obese pregnant women.

3.5.3 Conclusions

We found that inflammation is lower in physically active obese pregnant women when compared to sedentary obese pregnant women. The reduction in inflammation among physically active obese women might be related to improvements in lipid metabolism as measures of lipid

metabolism and inflammation were related. Maternal physical activity did not influence neonatal metabolic outcomes, but long-term follow-up is needed.

3.5.4 Acknowledgements

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Contributions: RAT and WTC researched data, wrote/edited manuscript. EAS and AGC researched data and reviewed/edited the manuscript. The authors declare no conflicts of interest.

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Chapter 4: Relationships between Late Pregnancy Maternal and Neonatal Metabolic Health in Lean and Obese Women

4.1 Abstract

Introduction: The purpose of this study was to examine the relationships between maternal and neonatal metabolic health in lean and obese women during late pregnancy.

Methods: Fifty women participated in this study between 32-37 weeks gestation (N=50). Maternal indices of insulin resistance (homeostatic model assessment-insulin resistance (HOMA-IR)) and inflammation (C - reactive protein (CRP)), as well as lipid concentration and kinetics (lipid oxidation rate and lipolysis) were measured under basal conditions. Umbilical cord blood was collected at parturition for measurement of neonatal plasma insulin resistance, inflammation, and lipid concentration. Neonatal body composition was measured 24-48 hours postpartum via air displacement plethysmography.

Results: There were no significant relationships between maternal and neonatal metabolic outcomes in a cohort of lean and obese pregnant women. In lean pregnant women, maternal pre-pregnancy BMI was positively associated with neonatal body fat percentage ($r=0.65$, $p=0.01$). However, in obese pregnant women, maternal pre-pregnancy BMI trended towards a negative correlation with neonatal body fat percentage ($r=-0.34$, $p=0.06$). In the neonates, there was a trend for neonatal HOMA-IR to be positively correlated with neonatal body fat percentage ($r=0.30$, $p=0.07$).

Conclusions: We found no associations between maternal lipid metabolism, insulin resistance, and inflammation and neonatal adiposity, insulin resistance, and inflammation in a cohort of lean and obese pregnant women.

4.2 Introduction

The "fetal origins hypothesis" suggests that the origins of adult chronic health conditions (e.g. diabetes, cardiovascular disease) are initiated by *in utero* exposures[1]. This hypothesis states that unfavorable metabolic health may "program" the fetus to have metabolic characteristics that can lead to future disease[2-5]. Metabolic characteristics associated with future cardiovascular disease include (but are not limited to) altered lipid profiles, inflammation, insulin resistance, and obesity[6-10]. Alterations in maternal metabolism may play a crucial role in adverse fetal programming, leading to unfavorable maternal and neonatal outcomes with long-term health implications[11-13].

A careful understanding of maternal lipid and glucose metabolism, and their relationships to neonatal health, is critical as both are believed to play an important role in fetal development and maternal and neonatal metabolic health[14-17]. Lipid metabolism may contribute to maternal health during pregnancy as it is intricately connected to inflammation and insulin resistance in non-gravid adults[18,19]. Maternal inflammation and insulin resistance during pregnancy can have serious implications for pregnant women and their neonates[14,20,21]. In addition, maternal lipid and glucose metabolism have been linked with changes in neonatal body composition and birthweight[7,22-24]. Despite the connections between maternal metabolism and neonatal body composition, few studies have examined the direct relationships between maternal metabolism (i.e. lipid metabolism, insulin resistance, and inflammation) and neonatal metabolic outcomes including adiposity, inflammation, and insulin resistance. The purpose of this study was to examine the relationships between late pregnancy maternal and neonatal metabolic health in lean and obese women. We hypothesize that higher maternal lipid oxidation rate and lipolysis will be

associated with higher adiposity, inflammation, and insulin resistance in neonates. We also believe maternal and neonatal inflammation and insulin resistance will be positively correlated. Establishing relationships between maternal and neonatal metabolic characteristics might aid the development of interventions that modulate the maternal metabolic environment that contributes to adverse outcomes and fetal programming during pregnancy.

4.3 Methods

4.3.1 Participants

Fifty (n=50) women participated in the study between 32 and 37 weeks gestation. Of these 50 women, 16 were lean (18.5≤ BMI< 25.0 kg/m^2) and 32 were obese (30kg/m^2≤ BMI <45kg/m^2). Five-hundred women receiving prenatal care at the Women's Health Center at Barnes Jewish Hospital/Washington University were screened for inclusion. Women who met all criteria with ongoing pregnancies were approached for participation late in their second trimester. Inclusion criteria were ages 18-44, confirmed singleton viable pregnancy with no identified fetal abnormalities (as determined by routine standard of care ultrasonography at 18-22 weeks), and pre-pregnancy BMI between 18.5 and 24.9 (lean) or 30 and 45 kg/m^2 (obese). Exclusions included: 1) multiple gestation pregnancy, 2) inability to provide voluntary informed consent, 3) self-reported use of illegal drugs (cocaine, methamphetamine, opiates), 4) current smoker who does not consent to cessation, 5) current usage of daily medications by class: corticosteroids, anti-psychotics (known to alter insulin resistance and metabolic profiles), 6) diagnosis of gestational diabetes in current pregnancy, history of gestational diabetes, pre-pregnancy diabetes or prior macrosomic (>4500g) infant (which elevate the risk for gestational diabetes in the current pregnancy, or undiagnosed gestational diabetes), or 7) history of heart disease.

4.3.2 Study Procedures

All study procedures were performed during two visits at the Washington University School of Medicine Institute for Clinical and Translational Sciences Clinical Research Unit (CRU).

Maternal Visit 1

Maternal body composition was measured using skinfold anthropometry. Body fat percentage was determined by pressing folds of the skin at seven sites with a caliper (Harpenden Skinfolds Caliper, Baty International, United Kingdom), recording the skin fold thickness, and entering the data into a standardized equation that accounts for age as previously described[25]. In order to account for variables that may distort the relationships between maternal and neonatal outcomes, maternal fitness and physical activity levels, and maternal dietary composition were measured. Maternal fitness levels were assessed using the YMCA submaximal cycle test on a recumbent bicycle (Lode Corvial Recumbent, Lode B.V., The Netherlands)[26]. Maternal physical activity levels were objectively assessed for one week using the ActiGraph GT3X+ accelerometer (ActiGraph LLC, Pensacola, FL). The GTX3+ was placed on the non-dominant wrist for seven consecutive days. Data was collected at 30 Hz and the output was sampled by a twelve-bit analog-to-digital-converter. The percentage of time spent sedentary as well as percentage of time spent in different levels of physical activity ranging from light and lifestyle to moderate, were calculated using algorithms and cut points from Freedson et al. in the ActiGraph software[27]. In order to assess dietary composition, participants completed the validated National Institutes of Health's Dietary History Questionnaire II[28]. Previous literature demonstrates that dietary history questionnaires are valid and reproducible among pregnant populations[29].

Maternal Visit 2

Subjects were admitted to the CRU the morning after an overnight fast. The night prior, subjects were provided written instructions for consuming a balanced meal with 50% carbohydrate, 30% fat, and 20% protein. Upon admission to the CRU, height, weight, and vital signs were obtained. A catheter (IV) was placed in a hand vein and heated to 55 °C by using a thermostatically controlled box in order to obtain arterialized blood samples. Participants laid supine for 30 minutes prior to a 15-minute resting indirect calorimetry measurement (TrueOne 2400, Parvomedics, Sandy, UT). Lipid oxidation rate was calculated via oxygen consumption and carbon dioxide production as previously described[30].

Sample Analyses and Calculations

All blood samples were immediately placed on ice and plasma was separated by centrifugation within 30 minutes of collection. Plasma samples were stored at -80ºC until final analyses were performed. Blood samples for glucose were collected in heparinized tubes and analyzed immediately with an automated glucose analyzer (Yellow Springs Instruments Co, Yellow Springs, OH). Plasma insulin concentration was measured by electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics, Indianapolis, IN). Insulin and glucose levels were used to calculate the homeostatic model assessment-insulin resistance (HOMA-IR)[31,32]. The HOMA-IR is an index of insulin resistance that reflects fasting glucose concentration measured at the fasting insulin concentration. High-sensitivity C-reactive protein (CRP) was measured by immunoturbidimetric assay (Roach Diagnostics, Indianapolis, IN). Blood samples used to determine plasma free fatty acids were collected in tubes containing EDTA. Plasma free fatty acid concentrations were determined by enzymatic colorimetric assay (Wako Pure Chemical

Industries, Osaka, Japan). Lipolysis was calculated by the area under the curve for free fatty acids as previously reported[33] from baseline through exercise and the recovery period.

Neonatal Measurements

Upon admission to labor and delivery, maternal weight was measured and gestational weight gain was determined. At parturition, neonatal birth weight was obtained. In addition, 44 mL of umbilical cord blood was collected, centrifuged within 30 minutes of parturition, and placed at -80ºC until further analysis was performed. Umbilical cord blood was used to determine neonatal HOMA-IR (insulin and glucose levels), free fatty acid concentration, and inflammation (CRP).

Within 48 hours of delivery, neonatal anthropometrics were measured in the CRU. Neonatal length (Pediatric Length Board, Ellard Instrumentation LTD, Monroe, WA) and head circumference (Gulick II Tape Measure, model 67020, Country Technology Inc., Gays Mills, WI) were measured. Body composition (fat and lean mass) was measured by skin fold thickness measurement at four different sites (triceps, subscapular, ilium, and thigh) and by air displacement plethysmography (Pea Pod, Life Measurement, Inc., Concord, CA). All anthropometric measurements were taken on full-term neonates (≥37 weeks gestation).

4.3.3 Statistical Analysis

Normality of the distribution was tested for each variable using Kolmogorov-Smirnov tests. Pearson Product-Moment Correlation Coefficients for normally distributed variables and Spearman's Rank-Order Correlation Coefficients for non-normally distributed variables were

used to assess the degree of the relationship between variables. In order to assess the impact of discrete variables such as race, parity, and income on neonatal outcomes and account for potential confounding, Student's Independent T-Tests for normally distributed variables and Mann-Whitney U Tests on non-normally distributed data were performed. Partial correlations were used to adjust for potential confounders. Relationships were also analyzed within each group (lean and obese) in order to determine how pre-pregnancy BMI may impact mechanisms linking maternal and neonatal health. Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at Washington University School of Medicine[34]. All data analyses were conducted using IBM SPSS Statistics, Version 22 (Armonk, New York)

4.4 Results

4.4.1 Maternal Outcomes

Maternal demographic characteristics are shown in Table 4.1.

Table 4.1 Maternal demographic characteristics in lean and obese pregnant women

	All (N=50) mean ± SD	**Lean (n=18) mean ± SD**	**Obese (n=32) mean ± SD**
Age (y)	26.6 ± 5.0	26.1 ± 5.0	26.9 ± 5.1
Pre-pregnancy BMI (kg/m^2)	30.2 ± 7.5	21.5 ± 1.9	35.2 ± 4.1
Body fat (%)	31.2 ± 8.8	20.7 ± 4.0	37.1 ± 3.7
Resting systolic blood pressure (mmHg)	110.2 ± 9.4	107.3 ± 8.1	111.8 ±9.8
Resting diastolic blood pressure (mmHg)	70.0 ± 6.8	69.3 ± 7.6	70.4 ± 6.4
Resting heart rate (bpm)	87.4 ± 10.8	84.1 ± 9.1	89.3 ± 11.4
Gestational weight gain (kg)	11.8 ± 7.1	14.5 ± 4.5	10.3 ± 7.9
	# of women (%)	**# of women (%)**	**# of women (%)**
Race			
African-American	26 (52%)	8 (44%)	14 (44%)
Caucasian	21 (42%)	9 (50%)	16 (50%)
Other	3 (6%)	1 (6%)	2 (6%)
Parity			
Nulliparous	28 (56%)	11 (61%)	17 (53%)
Multiparous	22 (44%)	7 (39%)	15 (47%)
Income			
Low	23 (46%)	10 (56%)	13 (41%)
Moderate-to-high	27 (54%)	8 (44%)	19 (59%)

Demographic characteristics, aside from BMI and body fat percentage, were similar between lean and obese women. Neonatal outcomes were similar between women of difference races, as well as multiparous versus nulliparous women. Neonatal CRP was significantly lower in women with moderate-to-high-income versus women with low-income (moderate-to-high-income: 0.16 ± 0.12 mg/L vs. low-income: 0.26 ± 0.19 mg/L, p=0.02). Maternal dietary composition and physical activity levels were similar between groups and were not related to any neonatal outcomes. Table 4.2 shows maternal dietary composition and physical activity levels.

Table 4.2 Maternal dietary composition and physical activity levels in lean and obese pregnant women

Daily dietary consumption	**All (N=50) mean ± SD**	**Lean (n=18) mean ± SD**	**Obese (n=32) mean ± SD**
Total energy (kcal)	2333 ± 1257	2554 ± 1574	2218 ± 1068
Fat (g)	86.3 ± 50.6	94.6 ± 50.5	81.9 ± 51.0
Fat (% of kcal/day)	33.4 ± 6.0	34.4 ± 5.2	32.8 ± 6.4
Carbohydrates (g)	315.0 ± 181.0	353.5 ± 256.2	295.2 ± 127.4
Carbohydrates (% of kcal/day)	53.7 ± 8.7	53.1 ± 7.2	54.1 ± 9.5
Protein (g)	85.6 ± 50.1	84.1 ± 39.0	86.3 ± 55.5
Protein (% of kcal/day)	14.9 ± 3.4	14.3 ± 3.4	15.3 ± 3.4
Predicted VO_{2peak} (ml/kg/min)	30.0 ± 6.3	30.7 ± 5.7	29.2 ± 6.8
Physical activity (% of time during 1 week)			
Sedentary (%)	64.3 ± 7.5	62.4 ± 4.3	65.4 ± 8.7
Light (%)	9.1 ± 1.7	10.0 ± 1.5	8.5 ± 1.6
Lifestyle (%)	13.2 ± 2.9	14.0 ± 2.2	12.7 ± 3.2
Moderate (%)	13.5 ± 4.3	13.7 ± 2.5	13.3 ± 5.0

4.4.2 Neonatal Outcomes

Cord blood could not be obtained for seven participants. Neonatal anthropometrics were not obtained for one participant. Neonatal demographic characteristics were similar between neonates of lean and obese women. Neonatal data are shown in Table 4.3.

Table 4.3 Neonatal demographic characteristics for lean and obese pregnant women

	All (N=50) mean ± SD	**Lean (n=18) mean ± SD**	**Obese (n=32) mean ± SD**
Gestational age at delivery (wks)	39.2 ± 1.2	39.1 ± 0.9	39.3 ± 1.4
Gestation age during anthropometric measurements (wks)	39.4 ± 1.2	39.6 ± 1.1	39.6 ± 1.3
Birth weight (g)	3272 ± 438	3221 ± 376	3301 ± 472
Body fat (%)	11.5 ± 3.6	11.1 ± 4.3	11.7 ± 3.3
Length (cm)	49.6 ± 2.2	49.5 ± 1.1	49.7 ± 2.2
Head circumference (cm)	34.1 ± 1.4	33.7 ± 1.7	34.3 ± 1.2
	# of neonates (%)	**# of neonates (%)**	**# of neonates (%)**
Gender			
Boy	26 (52%)	7 (39%)	19 (59%)
Girl	24 (48%)	11 (61%)	13 (41%)
Mode of delivery			
Vaginal	34 (68%)	14 (78%)	20 (63%)
Cesarean section	16 (32%)	4 (22%)	12 (37%)

4.4.3 Relationships between Maternal Metabolic Health and Neonatal Outcomes

Maternal and neonatal metabolic outcomes were not related to each other, even when accounting for potential confounders. Table 4.4 shows correlations between primary maternal and neonatal metabolic outcomes. Neonatal glucose concentration was significantly higher in women who delivered vaginally versus a cesarean section (vaginal: 88.6 ± 16.8mg/dL vs. cesarean: 77.7 ± 8.3mg/dL, p=0.008). Therefore, mode of delivery was accounted for when examining relationships between maternal and neonatal glucose, insulin, and HOMA-IR using a partial correlation controlling for mode of delivery.

Table 4.4 Relationships between maternal and neonatal metabolic outcomes

	Maternal **mean ± SD**	**Neonatal** **mean ± SD**	**Correlation between maternal & neonatal (R value, p-value)**
BMI (maternal)/ Ponderal Index (neonatal)	30.2 ± 7.5	2.7 ± 0.3	r= 0.02, p=0.91
Body fat (%)	31.2 ± 8.8	11.5 ± 3.6	r= 0.03, p=0.84
C-reactive protein (mg/L)	5.7 ± 4.0	0.21 ± 0.15	r= 0.12, p=0.46
Free fatty acids (meq/L)	0.47 ± 0.14	0.17 ± 0.07	r= -0.05, p=0.97
Glucose (mg/dL)	79.1 ± 7.3	85.8 ± 15.7	r= 0.21, p=0.19
Insulin (uU/mL)	15.4 ± 8.8	8.0 ± 5.6	r= 0.13, p=0.41
HOMA-IR	3.1 ± 1.9	1.7 ± 1.4	r= 0.09, p=0.59

*$p<0.05$

Maternal lipid metabolism (lipid oxidation rate and lipolysis) was not related to neonatal metabolic outcomes. Relationships between maternal lipid metabolism and neonatal outcomes can be found in Table 4.5.

Table 4.5 Relationships between maternal lipid oxidation rate and lipolysis and neonatal metabolic outcomes

	Maternal lipid oxidation rate (g/min) mean± SD	**Maternal lipolysis (meq·min/L) mean± SD**
Maternal value	0.11 ± 0.03	39.7 ± 10.2
Neonatal outcome	**Correlation between maternal lipid oxidation rate and neonatal outcome (r, p-value)**	**Correlation between maternal lipolysis and neonatal outcome (r, p-value)**
Body fat (%)	r= -0.003, p=0.99	r= -0.23, p=0.13
HOMA-IR	r=0.10, p=0.52	r= 0.04, p=0.83
CRP (mg/L)	r=0.13, p=0.43	r= -0.09, p=0.60
Free fatty acids (meq/L)	r=0.15, p=0.35	r= -0.03, p=0.87

*$p<0.05$

4.4.4 Additional Findings across all BMI Categories

There was a trend for neonatal HOMA-IR to be correlated with neonatal body fat percentage (r=0.30, p=0.07) (Figure 4.1).

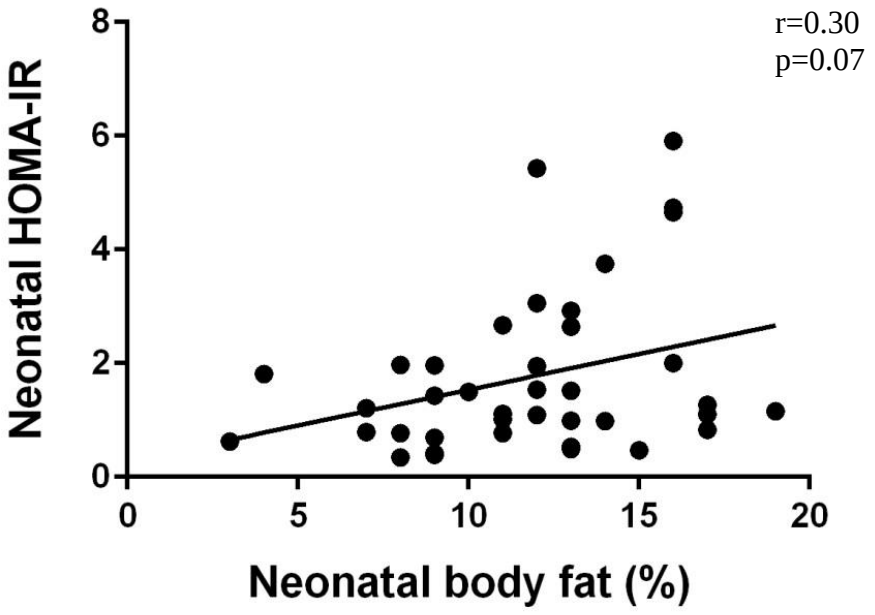

Figure 4.1 Relationship between neonatal body fat percentage and neonatal HOMA-IR

4.4.5 Relationships between Maternal and Neonatal Outcomes separated by BMI

In lean pregnant women, maternal pre-pregnancy BMI was positively associated with neonatal body fat percentage (r=0.65, p=0.008). However, in obese pregnant women, maternal pre-pregnancy BMI trended towards a negative correlation with neonatal body fat percentage (r=-0.34, p=0.058). These trends did not change when accounting for gestational weight gain, which has been shown to influence the relationship between maternal BMI and neonatal birthweight and body composition[35].

In lean pregnant women, maternal lipid oxidation rate was significantly correlated with neonatal insulin (r=0.68, p=0.005) and neonatal HOMA-IR (r=0.64, p=0.006). These relationships did not exist among obese pregnant women.

4.5 Discussion

Contrary to our hypotheses, maternal lipid metabolism, insulin resistance, and inflammation were not related to neonatal adiposity, insulin resistance, and inflammation. Thus, maternal metabolic health does not appear to have an acute impact on neonatal metabolic outcomes. Our negative findings suggest that in a cohort of both lean and obese pregnant women, the fetus appears to be protected acutely from maternal metabolic perturbations experienced during pregnancy. However, the "fetal origins hypothesis" suggests that the origins of adult health diseases such as obesity, cardiovascular disease, and diabetes may be caused by *in utero* exposures, and that the programming of poor metabolic health may remain latent for years[1,36].

Therefore, it is plausible that metabolic abnormalities may be epigenetically programmed, but are not apparent or measurable until later in life. To elaborate, physiologic variations may be present that could turn particular genes on and off and effect how genes are read without a change in the actual DNA sequence; thus, changes may be "programmed" but not actually measurable at birth. Long-term offspring follow-up is needed to draw definitive conclusions.

4.5.1 Maternal and Neonatal Insulin Resistance

The lack-of correlation between maternal and neonatal insulin resistance is inconsistent with previous studies in lean and obese women[21]. Catalano et al. demonstrated a positive correlation between maternal and neonatal insulin resistance in a cohort of lean and obese pregnant women[21]. A possible explanation for the contrary findings could be explained by the labor and delivery process endured by the study participants. Catalano et al. only measured maternal and neonatal HOMA-IR in women who presented to labor and delivery for an elective cesarean delivery; therefore, they did not undergo the process of labor. Most women included in the present study underwent labor prior to either mode of delivery, which may have impacted both maternal and neonatal blood glucose and insulin levels. In addition, we included neonates of vaginal deliveries. It has been shown that neonates whom were delivered vaginally have lower neonatal glucose concentrations[37], which we also found in the present study. Because neonatal HOMA-IR is largely determined by neonatal glucose levels, it is possible altered neonatal glucose concentrations due to mode of delivery contributed to our contradictory findings. In addition, Catalano et al. measured maternal HOMA-IR upon presentation to labor and delivery between 37 and 40 weeks, while we measured maternal HOMA-IR between 32-37 weeks

gestation. Because insulin resistance increases until the end of gestation[8], this may also help explain the inconsistent findings.

4.5.2 Maternal BMI, Body Composition, and Neonatal Body Composition

Contrary to the literature[38], we did not find a significant association between maternal pre-pregnancy BMI or late gestation body fat percentage and neonatal body fat percentage in the combination of lean and obese pregnant women. Interestingly, when examining the relationship between maternal pre-pregnancy BMI and neonatal adiposity in lean women only, we found a strong positive correlation consistent with the literature. However, in exclusively obese women, we noted a moderate negative correlation. Although obesity is traditionally believed to contribute to larger babies[39-41], obesity has been shown to increase the risk for small-for-gestation-age neonates as well[42,43]. It is possible that in our cohort of obese women, the negative relationship could indicate an increased risk for small-for-gestation-age neonates. Similar to macrosomic neonates, small-for-gestation-age neonates are also at-risk for poor long-term health outcomes[40,42,44,45]. The notion that obesity might increase risk for small-for-gestation-age neonates may also help explain the lack-of-association between maternal pre-pregnancy BMI and neonatal adiposity across all groups in our study.

Another possible explanation for not finding a relationship between maternal pre-pregnancy BMI and neonatal body fat percent could be related to the study population. Previous studies by Starling et al. and Hull et al. (two studies that found a positive relationship between maternal BMI and neonatal body fat percent) did include women of a wide range of body mass indexes;

however, only 20% and 12%, respectively, were classified as obese (BMI ≥30kg/m^2)[38,46]. Our cohort consisted of 64% obese pregnant women. These findings indicate a greater need for analyzing the impact on pre-pregnancy BMI on neonatal adiposity in exclusively obese women. Several studies have analyzed the relationships between maternal BMI and neonatal birthweight in obese women[40-42,47,48], but literature on pre-pregnancy BMI and neonatal adiposity, particularly using high-quality measurements such as air displacement plethysmography technology, is limited[38,46,49].

4.5.3 Maternal and Neonatal Inflammation

The current study did not find an association between maternal and neonatal inflammation. Most cytokines do not cross the placenta; thus, our results support the concept that maternal inflammation does not directly transfer across the placenta and lead to fetal inflammation[21,50]. It is possible that neonates may be "programmed" for higher systemic inflammation or other poorer outcomes later in life. In support of this concept, Leibowitz et al. found that offspring of obese women had higher inflammatory markers at 12 years of age[51]. Despite a number of neonatal risks associated with maternal inflammation[52], we do not yet understand the connection, if any, between maternal and neonatal inflammation.

4.5.4 Maternal Lipid Metabolism and Neonatal Outcomes

We found no associations between maternal lipid metabolism and neonatal outcomes including adiposity, insulin resistance, and inflammation. Crume et al. found that maternal circulating lipids during the second half of pregnancy predicted higher neonatal birth weight[23]. In our study,

maternal plasma free fatty acid concentration as well as maternal lipid oxidation and lipolysis did not relate to any neonatal outcomes. However, our data were collected at different time points than Crume et al. making it difficult to compare results considering lipid metabolism changes throughout pregnancy[15].

When lean pregnant women were analyzed independently, maternal lipid oxidation was significantly correlated with neonatal insulin concentration and neonatal HOMA-IR. However, these relationships did not exist among obese pregnant women. These data indicate that mechanisms contributing to neonatal insulin and insulin resistance may differ based on pre-pregnancy BMI status, and that lipid metabolism may play a more significant role in determining neonatal insulin resistance in lean women. The reason for these differences is unclear.

4.5.5 Neonatal Adiposity and Insulin Resistance

In our study, we noted a trend for neonatal HOMA-IR to be positively correlated with neonatal body fat percentage. Similarly, Catalano at el. concluded that neonatal body fat percentage was positively correlated to neonatal insulin resistance[21]. This finding suggests that the known relationship between body fat percentage and insulin resistance in non-gravid adults[53] appears to exist very early in life.

4.5.6 Limitations

The present study results are based strictly on relationships between maternal and neonatal outcomes; therefore, most of our findings, and the interpretations of those findings, are speculative. The lack-of associations between maternal and neonatal outcomes, and the

associations between outcomes in obese and lean women when separated, elucidate the complexity of examining the role of maternal health status during pregnancy and neonatal health at delivery. We also had a small sample size of 50 participants, thus, larger numbers may be needed to detect significant relationships.

4.5.7 Conclusions

We found no associations between maternal lipid metabolism, insulin resistance, and inflammation, and neonatal adiposity, insulin resistance, and inflammation across all participants. Long-term follow up is needed to determine the long-term effects of maternal metabolic health on childhood metabolic health. The relationships between maternal and neonatal metabolic health are complex and might be dependent on maternal BMI status.

4.6 References

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Chapter 5: Low-Intensity Physical Activity is Associated with Maternal Systemic Inflammation during Late Pregnancy

This chapter is in preparation for submission to the Journal of *Applied Physiology, Nutrition, and Metabolism*.

Tinius RA, Cade WT. Low-intensity physical activity is associated with systemic inflammation during late pregnancy.

5.1 Abstract

Background: Excessive maternal inflammation during pregnancy increases the risk for maternal and neonatal metabolic complications; however, maternal physical activity during pregnancy appears to reduce maternal inflammation. The purpose of this study is to examine the relationship between maternal physical activity intensity and maternal inflammation during late pregnancy.

Methods: Maternal physical activity levels (sedentary, light, lifestyle, and moderate), fitness levels, and systemic inflammation (plasma C-reactive protein (CRP) concentration) were measured between 32-37 weeks gestation.

Results: Maternal plasma CRP was negatively associated with time spent in light and lifestyle physical activities (Light: r= -0.40, p= 0.01; Lifestyle: r= -0.31, p= 0.03), but not with time spent in moderate physical activity (r= -0.18, p= 0.21). Higher maternal plasma CRP tended to correlate with more time spent sedentary (r= 0.27, p= 0.06). We also noted that small daily increases in light and lifestyle activities could elicit a clinically meaningful change in inflammation.

Conclusions: Pregnant women should be encouraged to incorporate more low-intensity physical activities into their daily routines in order to decrease systemic maternal inflammation and potentially improve maternal and neonatal pregnancy outcomes.

5.2 Introduction

Low-grade chronic inflammation, often secondary to obesity, plays an important role in the pathogenesis of many chronic diseases including metabolic syndrome, cardiovascular disease, diabetes, and hypertension[1-4]. During pregnancy, maternal systemic inflammation is physiologically elevated[5,6]. Excessive inflammation during pregnancy however increases the risk for metabolic complications such as insulin resistance/gestational diabetes and hypertension/preeclampsia[6-8]. Increased inflammation might also increase risk for future maternal disease including metabolic syndrome, insulin resistance, diabetes, hypertension, and cardiovascular disease[9]. Maternal inflammatory changes during pregnancy appear to extend into the placenta potentially exposing the fetus to an inflammatory environment during development[10], which might contribute to preterm delivery, infection, and the programming of adult metabolic disease[6,11,12].

Light and vigorous maternal physical activities during pregnancy are associated with lower systemic inflammation in the second trimester[13,14]. However, the relationship between physical activity intensity and maternal inflammation in late pregnancy is unclear. The purpose of this study is to examine the relationship between physical activity intensity and inflammation during late pregnancy.

5.3 Methods

5.3.1 Participants

Fifty women pregnant women participated in the present study (N=50).

5.3.2 Study Procedures

Pre-pregnancy body mass index (BMI) was calculated at initiation of prenatal care or via self-report if presentation for care was >10 weeks gestation. All other measures were taken at 32-37 weeks gestation. Body composition was measured using 7-site skinfold anthropometry (Harpenden Skinfolds Caliper, Baty International, United Kingdom)[15]. Fitness levels were assessed using the YMCA submaximal cycle test on a recumbent bicycle (Lode Corvial Recumbant, Lode B.V., The Netherlands). In addition, maternal physical activity levels were assessed for one week one using the ActiGraph GT3X+ accelerometer (ActiGraph LLC, Pensacola, FL). The Actigraph was placed on the non-dominant wrist with non-removable wristbands. ActiGraph data was collected for seven consecutive days at 30 Hertz (compliance for the present study was 100% as all 50 women wore the wristband for all seven days). The percentage of time spent sedentary as well as the amount of time spent participating in different categories of physical activity ranging from light and lifestyle to moderate were calculated using algorithms corresponding to the following activity counts: sedentary: 0 - 99 counts/min, light: 100 - 759 counts/min, lifestyle: 760 - 1951 counts/min, moderate: 1952-5724 counts/min[16]. Inflammation was measured via fasting plasma concentration of high-sensitivity C-reactive protein (CRP). CRP was measured by immunoturbidimetric assay (Roach Diagnostics, Indianapolis, IN).

5.3.3 Statistical Analysis

Maternal CRP was not normally-distributed. Thus, Spearman's rank order correlation coefficients were used to assess the degree of relationships between CRP and all other variables. Additionally, Student's independent t-tests were used to compare time spent in low-intensity activities (light or lifestyle) between those who are at risk for cardiovascular disease based on the established cut-off of a CRP value ≥3.0mg/L (≥3.0mg/L represents higher risk for cardiovascular disease[17]) and those who were not. Logistic regression was also used to determine the amount of physical activity necessary to reduce the odds of having a CRP value ≥3.0mg/L.

5.4 Results

5.4.1 Maternal Demographics and Metabolic Characteristics

Maternal demographic and metabolic data can be found in Table 5.1.

Table 5.1. Maternal demographic and metabolic characteristics

	Mean ± SD
Age (y)	26.6 ± 5.0
Pre-pregnancy BMI (kg/m^2)	30.2 ± 7.5
Body fat (%)	31.2 ± 8.8
Resting systolic blood pressure (mmHg)	110.2 ± 9.4
Resting diastolic blood pressure (mmHg)	70.0 ± 6.8
Resting heart rate (bpm)	87.4 ± 10.8
Gestational weight gain (kg)	11.8 ± 7.1
C-reactive protein (mg/L)	5.7 ± 4.0
Predicted VO_{2peak} (ml/kg/min)	29.7 ± 6.4
	# of women (%)
Race	
African-American	26 (52%)
Caucasian	21 (42%)
Other	3 (6%)
Parity	
Nulliparous	28 (56%)
Multiparous	22 (44%)
Income	
Low	23 (46%)
Moderate-to-high	27 (54%)
Physical activity levels (% /week)	
Sedentary	64.3 ± 7.5
Light	9.1 ± 1.7
Lifestyle	13.1 ± 2.9
Moderate	13.5 ± 4.3

5.4.2 Relationship between CRP and Physical Activity

Maternal plasma CRP concentration was associated with maternal body fat percentage at 32-37 weeks ($r= 0.70$, $p<0.001$). Higher maternal fitness levels were associated with lower maternal plasma inflammation ($r= -0.30$, $p= 0.03$). Higher maternal plasma CRP concentration tended to correlate with more time spent sedentary ($r=0.27$, $p= 0.06$).Maternal plasma CRP was negatively associated with time spent in light and lifestyle physical activities (Light: $r= -0.40$, $p= 0.01$; Lifestyle: $r= -0.31$, $p= 0.03$); CRP was not correlated with time spent in moderate physical activity ($r= -0.18$, $p= 0.21$). Correlations are shown in Figure 5.1.

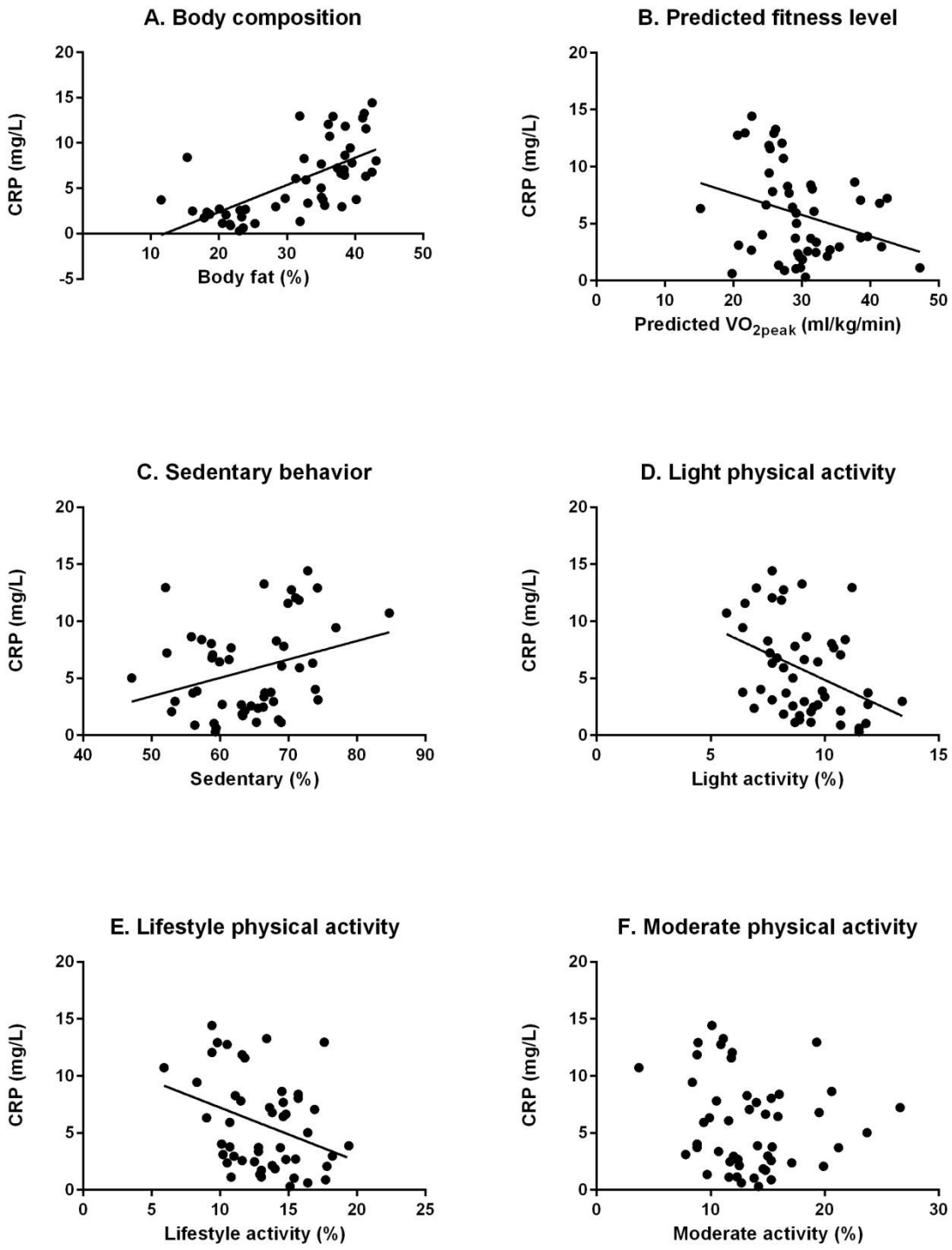

Figure 5.1 Maternal inflammation (CRP) in relation to maternal body composition (A), fitness levels (B), and physical activity levels (C-F).

There were no differences in CRP between the women who only walked and the women who walked in conjunction with other modes of exercise such as biking, swimming, yoga, and weight-lifting (6.2 ± 3.1 mg/L versus 6.3 ± 1.9 mg/L, p= 0.94).

The amount of time spent in low-intensity physical activities (light and lifestyle variables were combined) was 24.4 ± 3.8% (351 minutes) for women with a CRP value <3.0mg/dL versus 21.1 ± 4.4% (304 minutes) for women who had a CRP value ≥3.0mg/L (p=0.02). The odds of having CRP≥3.0mg/L were reduced by 16% for every additional 14 minutes per day of low-intensity physical activity (Exp(B)=0.84, 95% CI [0.72-0.98], p=0.03).

5.5 Discussion

The primary finding of our study is that time spent in light and lifestyle physical activities during late pregnancy is associated with lower maternal systemic inflammation. To our knowledge, this is the first study to show relationships between low-intensity maternal physical activity and maternal systemic inflammation during late pregnancy. Our results are consistent with Hawkins et al. who found that light-intensity physical activity had a negative association with CRP among women in their second trimester[13]. Understanding the relationships between physical activity intensity and inflammation during late pregnancy (i.e. the present study) is imperative as women are most likely to decrease their physical activity levels near the end of gestation[18]. In addition, our study examined these relationships in a cohort of predominantly obese women, which is important because these women are at the highest risk for excessive maternal inflammation and its downstream sequela[19].

In addition to the negative correlation between maternal light and lifestyle physical activity and maternal inflammation, we also found that more time spent sedentary during pregnancy tended to be associated with higher maternal systemic inflammation. Surprisingly, there was not a significant relationship between time spent in moderate physical activity and inflammation. Taken together, our data suggest that low-intensity physical activity may reduce inflammation in pregnant women, and that decreasing time spent sedentary and increasing low-intensity activities may be enough of a stimulus to elicit reductions in maternal systemic inflammation. Our findings also suggest that more intense physical activities such as swimming, cycling, and weight-lifting might not necessarily be superior to walking during pregnancy in terms of lowering maternal inflammation; however more research in necessary to confirm this.

Based on the present study, we believe pregnant women should be encouraged to increase physical activity during day-to-day tasks (e.g. taking the stairs, parking further away, walking instead of driving) in order to reduce systemic inflammation late in pregnancy. Not only do these types of lifestyle changes appear to elicit reductions in maternal systemic inflammation, but they are easier for pregnant women to adopt compared to structured exercise programs. In fact, the most commonly reported barriers to physical activity during pregnancy are lack of time, employment obligations, childcare responsibilities, fatigue, and pregnancy-related discomfort[20-24]. Incorporating less sedentary time and more low-intensity physical activities into daily routines might help women overcome these barriers as these modifications do not involve large time commitments (e.g. does not require a blocked-off period of time to go the gym); they can be performed in the workplace (e.g. take the stairs at work, use a standing desk, take frequent breaks at work to walk); they can be done with children (e.g. playing in the yard with other kids or pets,

going for a slow walk with a stroller or wagon, walking around a store); and they are not overly fatiguing activities that may be uncomfortable during late pregnancy.

Upon further analysis, our data suggest that spending an additional 14 minutes per day during late pregnancy in low-intensity (i.e. light or lifestyle) physical activities may reduce the odds of having a CRP value over the clinical threshold of 3.0mg/L by 16%. In addition, pregnant women with CRP values below this clinical threshold spent on average only 47 additional minutes per day doing light and lifestyle activities than those who were above this clinical value. Therefore, even small daily increases in light and lifestyle activities could elicit a clinically meaningful change in inflammation.

Therefore, it appears that pregnant women should be encouraged to become more physically active in order to improve their inflammation levels, and ultimately their metabolic health and the future health of their neonate.

5.5.1 Conclusions

Maternal inflammation is related to low-intensity physical activities (light, lifestyle) but not moderate intensity activities. Pregnant women should consider incorporating low-intensity physical activities into their daily routines in order to improve markers of inflammation.

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Chapter 6: Conclusions

6.1 Summary and Significance of Key Findings

The primary purposes of this dissertation were to (1) compare maternal lipid metabolism, maternal inflammation, maternal insulin resistance, and neonatal metabolic health between lean and obese pregnant women, (2) determine if a physically active lifestyle during pregnancy was associated with improved maternal lipid metabolism, lower maternal inflammation, lower maternal insulin resistance, and better neonatal metabolic health in obese women during late pregnancy, and to (3) determine the relationships between maternal and neonatal metabolic health.

6.1.1 Chapter 2

The primary purposes of Chapter 2 were to compare lipid oxidation rate and lipolysis during late pregnancy between obese and lean women, and to examine the relationships between maternal lipid metabolism, inflammation and insulin resistance. We found that obese pregnant women have higher mean lipid oxidation rate, particularly following an acute bout of low-intensity exercise. In addition, higher maternal lipid oxidation rate was associated with higher maternal inflammation. This is consistent with data in non-gravid adults that demonstrates lipid oxidation is related to inflammation[1]. The current study also found that higher maternal inflammation was associated with higher insulin resistance, suggesting that maternal inflammation, possibly the result of greater lipid oxidation, might contribute to higher maternal insulin resistance in obese pregnant women. Our results are consistent with Retnakaran et al. who demonstrated that obesity during pregnancy elicits an inflammatory response with possible downstream metabolic sequela including insulin resistance[2].

Although not a primary focus of the study, we also found that higher maternal inflammation was associated with greater resting systolic blood pressure. High blood pressure disorders during pregnancy, including preeclampsia, are an important cause of morbidity, long-term disability, and even death among pregnant women and their offspring[3]. Inflammation has been identified as a key contributor to pregnancy-specific high blood pressure[4,5] and our findings are consistent with this.

Therefore, Chapter 2 demonstrated that obese pregnant women have altered lipid metabolism, and this alteration might contribute to higher maternal inflammation, insulin resistance and blood pressure. Clinically, these findings are important as insulin resistance (gestational diabetes mellitus) and high blood pressure (hypertension or preeclampsia) are two major contributors to perinatal complications in obese pregnant women. Our study suggests that inflammation-related alterations in lipid metabolism might be a contributing factor toward these unfavorable pregnancy outcomes.

6.1.2 Chapter 3

The primary purpose of Chapter 3 was to compare maternal inflammation between physically active and sedentary obese women during pregnancy. We found that inflammation was lower in physically active obese pregnant women when compared to sedentary obese pregnant women. To our knowledge, this is the first study to demonstrate that physical activity might reduce inflammation in obese pregnant women. Chapter 3 also examined the relationships between maternal inflammation and maternal lipid metabolism. Alterations in lipid metabolism (i.e.

higher lipolysis and higher plasma free fatty acid concentration) among obese women might be contributing to inflammation as measures of lipid metabolism and inflammation were related. Interestingly, this relationship appeared to be driven primarily by the obese-sedentary group as lipid oxidation and inflammation were positively correlated in obese-sedentary women, but this relationship did not exist in obese active women. Therefore, it is plausible that the reason for the lack-of association between lipid oxidation and inflammation in the obese active group was due to obese active women having improved efficiency of lipid metabolism resulting in complete oxidation of lipids, lower oxidative stress, and lower systemic inflammation. However, because reactive oxygen species or intermediate by-products of lipid metabolism were not measured, this is speculative. Lastly, neonatal metabolic outcomes were not different between active and sedentary obese pregnant women, but long-term follow-up is needed to examine potential long-term effects of physical activity on offspring health.

6.1.3 Chapter 4

The purpose of Chapter 4 was to examine the relationships between late pregnancy maternal and neonatal health in lean and obese women. We found no associations between maternal lipid metabolism, insulin resistance, and inflammation and neonatal adiposity, insulin resistance, and inflammation across all participants. Although we did not find any significant relationships between maternal and neonatal metabolic health, based on other longitudinal studies, maternal metabolism might have induced epigenetic changes that are not evident until later in the child's life[6]. Long-term follow up is needed to determine potential long-term effects of maternal metabolic health on childhood metabolic health.

Several relationships between maternal and neonatal metabolic health were found when examining obese and lean women independently. This may highlight the complexity of understanding the relationships between maternal and neonatal metabolic health in women of varying body compositions.

6.1.4 Chapter 5

The purpose of Chapter 5 was to examine the relationship between maternal physical activity intensity and maternal inflammation during late pregnancy. Maternal plasma inflammation was negatively associated with time spent in light and lifestyle physical activities, but not with time spent in moderate physical activity. Higher maternal plasma inflammation tended to correlate with more time spent sedentary. Higher maternal fitness level was associated with lower maternal plasma inflammation. Overall, our data suggest that low-intensity physical activity might reduce inflammation in pregnant women, and that decreasing time spent sedentary and increasing low-intensity activities may be enough of a stimulus to elicit reductions in maternal systemic inflammation. Our findings also suggest that more intense physical activities such as swimming, cycling, and weight-lifting might not necessarily be superior to walking during pregnancy in terms of lowering maternal inflammation. Therefore, we believe pregnant women should be encouraged to become more physically active in order to reduce inflammation levels and improve their metabolic health and the future health of their neonate. Low-intensity activities that are enjoyable and can be easily incorporated into their everyday lives should be recommended.

6.1.5 Overall Conclusions

Overall, this dissertation suggests that maternal lipid metabolism, inflammation, and insulin resistance are related to each other and altered in obese pregnant women. However, obese pregnant women that engage in physical activity have lower systemic inflammation which is associated with better maternal metabolic health. Despite the influences of obesity and physical activity on maternal health, none of these metabolic alterations appeared to directly translate into immediate changes in neonatal metabolic outcomes. However, long-term follow-up is needed to better address whether or not obesity and/or physical activity during pregnancy had a long-term impact on the health of the offspring. In addition, we found that low-intensity physical activities may be enough of a stimulus to reduce inflammation in lean and obese women. Taken together, this dissertation suggests that obese pregnant women should engage in physical activity, even low-intensity activities, in order to reduce their inflammation and potentially improve their health and the health of their offspring.

6.2 Limitations

This dissertation project had several potential limitations including: 1) cross-sectional study design, 2) small sample size, and 3) potential bias during data collection. Although it was prospective, this observational, cross-sectional study could only look at the relationships between groups and between outcomes, and thus, could not determine cause and effect. Second, this study was powered based on primary outcomes for each aim, thus, examination of other variables might have been underpowered. Lastly, bias may have been introduced because measurements and assessments were not blinded as women were recruited based on what group they were assigned to (i.e. it was obvious who was lean versus obese-sedentary versus obese-active).

Uniform data collection procedures were followed for all participants to minimize bias and data were objective in nature. In addition, all maternal and neonatal data collections were performed by the same person to minimize interpersonal variability, which can be particularly important with anthropometric measurements such as skinfolds.

6.3 Future Directions

6.3.1 Oxidative Stress

One future direction of the proposed line of research is to examine the role of maternal lipid metabolism-mediated oxidative stress on inflammation, insulin resistance, and neonatal metabolic health. Changes in maternal oxidative stress might provide a link between increased maternal lipid oxidation, inflammation, and subsequent insulin resistance and hypertension in obese pregnant women. In non-gravid adults, oxidative stress is elevated in obesity[7] and is associated with inflammation, vascular cell wall damage, high blood pressure, cardiovascular disease, poor metabolic function, and insulin resistance[8-16]. It is possible that elevated lipid oxidation rates observed in obese pregnant women might be the result of inefficient and incomplete substrate oxidation; thus, resulting in the generation of partially oxidized substrates (i.e. reactive oxygen species). Bell et al. concluded that incomplete, partial oxidation is increased in obesity[17]. Therefore, increased absolute lipid oxidation in pregnant obese women may be suggestive of increased incomplete oxidation, which may then be contributing to oxidative stress, and ultimately the inflammation and insulin resistance observed in the current study; however, this is speculative and requires further study. It is also well-established that exercise improves efficiency of lipid metabolism as well as combats oxidative stress[18,19]; therefore, physical activity

also has the potential to alter the relationships between lipid oxidation, inflammation, and oxidative stress. Figure 2.5 (Chapter 2) depicts the proposed pathway for the role of maternal lipid metabolism in long-term maternal and neonatal outcomes, and the potential role of oxidative stress in this pathway.

6.3.2 The Effects of Physical Activity on Maternal and Neonatal Health

Current guidelines recommend all pregnant women without contraindications participate in 30 minutes or more of moderate-intensity, daily physical activity[20]. Despite the well-established benefits, only 23% of pregnant women engage in the recommended amounts of physical activity[21]. Obese women tend to be less active to begin with, and tend to further reduce their physical activity levels during pregnancy[20]. With maternal obesity rates increasing in concordance with increasing rates of obesity in the general population, studies that demonstrate both the safety and efficacy of physical activity in obese pregnant women are needed. This dissertation project provides the first evidence that maternal physical activity is beneficial in improving maternal inflammation in obese women during late pregnancy. Based on our findings, future studies need to assess the effect of physical activity on lipid metabolism, inflammation, and insulin resistance in randomized controlled clinical trials. In addition, future studies should also follow neonates born to obese and/or physically active women into childhood and adulthood to truly understand the long-term implications of obesity and physical activity on "fetal programming" and offspring health.

6.3.3 Dissemination of Physical Activity during Pregnancy Research

In addition to the studies that demonstrate the safety and efficacy of physical activity in obese pregnant women, we also need dissemination tools in place to allow these messages to translate into the clinic and the community. Obese pregnant women report receiving little or no exercise advice from their health care provider[22,23]. Pregnancy can be considered a "teachable moment", and thus, can be a powerful incentive to establish a healthy lifestyle[24]. With the obesity epidemic being a significant public health concern, research elucidating the causes of childhood and subsequent adult obesity, and the design and implementation of interventions to reduce it, are critical.

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18. Fisher-Wellman K, Bell HK, Bloomer RJ. Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders. *Oxidative medicine and cellular longevity.* Jan-Mar 2009;2(1):43-51.

19. Martin WH, 3rd. Effects of acute and chronic exercise on fat metabolism. *Exercise and sport sciences reviews.* 1996;24:203-231.

20. Seneviratne SN, McCowan LM, Cutfield WS, Derraik JG, Hofman PL. Exercise in pregnancies complicated by obesity: achieving benefits and overcoming barriers. *Am J Obstet Gynecol.* Apr 2015;212(4):442-449.

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22. Weir Z, Bush J, Robson SC, McParlin C, Rankin J, Bell R. Physical activity in pregnancy: a qualitative study of the beliefs of overweight and obese pregnant women. *BMC Pregnancy Childbirth.* 2010;10:18.

23. Stengel MR, Kraschnewski JL, Hwang SW, Kjerulff KH, Chuang CH. "What my doctor didn't tell me": examining health care provider advice to overweight and obese pregnant women on gestational weight gain and physical activity. *Womens Health Issues.* Nov-Dec 2012;22(6):e535-540.

24. Phelan S. Pregnancy: a "teachable moment" for weight control and obesity prevention. *Am J Obstet Gynecol.* Feb 2010;202(2):135 e131-138.

Curriculum Vitae

Rachel Tinius, MS, ACSM-EP-C
August 2015

Contact Information
Movement Science Program
Program of Physical Therapy
Washington University School of Medicine
Campus Box 8502
4444 Forest Park Blvd. Room 1101
St. Louis, MO 63108-2212
E-mail: tiniusr@wusm.wustl.edu

Education

Predoctoral Student *Fall 2011-Fall 2015* *Washington University in St. Louis*

- Movement Science- Program in Physical Therapy
- Translational Physiology Laboratory
- Qualification exams in biomechanics, biocontrol, and bioenergetics: June 2013
- Dissertation proposal: December 2013
- Advisor: Todd Cade, PT, PhD
- Cumulative/Final GPA: 4.0

TL1 Predoctoral Clinical Research Training Program
Fall 2013-Spring 2015 Washington University in St. Louis

- The Clinical Research Training Center at Washington University supports a select group of trainees, as they embark on careers as outstanding patient-oriented researchers, by teaching them how to:
  - Design and conduct clinical research
  - Analyze data
  - Consider relevant ethical and legal issues
  - Write manuscripts and grants
  - Develop and present scientific posters
  - Compete for research funding
- Advisors: Jay Piccirillo, MD, FACS; Jeffrey Peipert, MD, PhD; Susan Stark, PhD, OTR/L

M.S. Clinical Investigation Fall 2013-Fall 2015 Washington University in St. Louis

- Advisor: Jay Piccirillo, MD, FACS
- Cumulative/Final GPA: 4.0

M.S. Exercise Science Spring 2010-Summer 2011 Western Kentucky University

- Designated Graduate Scholar of the College of Health and Human Services
- Advisor: Scott Lyons, PhD
- Cumulative/Final GPA: 4.0

B.S. Exercise Science Fall 2006-Fall 2009 Western Kentucky University

- Designated Scholar of the College of Health and Human Services
- Advisor: Scott Lyons, PhD
- Cumulative/Final GPA: 4.0

Professional Certifications

ACSM Certified Exercise Physiologist November 2014 National Certification

- Ability to conduct physical fitness assessments, interpret results, develop exercise prescriptions, and apply behavioral and motivational strategies to apparently healthy individuals and individuals with medically controlled diseases and health conditions
- Support clients in adopting and maintaining healthy lifestyle behaviors
- Skill in management, administration, and supervision of fitness programs
- Scored 744/800 on certification exam

Practice in Pedagogy Fall 2011-Spring 2015 Washington University in St. Louis

- Extensive knowledge of effective pedagogy, including evidence-based teaching methods, by participating in advanced-level Teaching Center workshops
- Classroom teaching experience that includes observation and feedback from faculty and students
- Developed and refined a well-informed teaching approach

Work Experience

Washington University- Program in Physical Therapy Fall 2011-Present St. Louis, MO
Translational Physiology Lab
Research Assistant

- Assistance with ongoing research projects (W. Todd Cade, PT, PhD, PI)
- Hyperinsulinemic/euglycemic clamp/isotope infusion studies
- Stable isotope tracer serum processing
- Mass spectrometry
- Indirect calorimetry (resting and exercise)
- Body composition testing (adults, children, and infants)
- Exercise testing and training
- Air displacement plythesmography (Bod Pod and Pea Pod Certified)
- Elisa kit protocols
- Clinical exercise testing for BJH patients (ECG, blood pressures, VO_2 measurement)

Teaching Assistant

- Principles in Human Physiology (Spring 2013)
    - Lectures:
        - *Adipose Tissue as an Endocrine Organ*
        - *Pregnancy Physiology, Common Pregnancy Complications, Pregnancy and Exercise*
- Women's Health Unit- Doctorate of Physical Therapy Program (Spring 2013, Spring 2014, & Spring 2015)
    - Lectures:
        - *Pregnancy Physiology and Exercise in Pregnancy*
- Teaching Center Annual Teaching Symposium (August 13, 2014)
    - Discussion leader for first time teaching assistants on discipline-specific topics

Maryville University- Biology Program Spring 2015 St. Louis, MO
Adjunct Professor

- Anatomy and Physiology (Spring 2015)

WKU Department of Kinesiology Recreation and Sport Fall 2010-Summer 2011 Bowling Green, KY
Graduate Assistant

- Class instructor : PE 101- Jogging & PE 100- Lifetime Fitness & Wellness
- Teaching Assistant: Exercise Physiology Lab
- Conducted Research in the Exercise Physiology Lab- Skills acquired listed below
    - Indirect Calorimetry
    - Flow Cytometry
    - Skinfold Calipers
    - Force plate-motion analysis software

Vetta Sports Fall 2010- Spring 2015 O'Fallon, IL
Soccer Coach

- Coordinate and conduct practice sessions for ages 3-10
- Coach games

WKU Athletic Training Room Spring 2009-Winter 2009 Bowling Green, KY
Internship Position

- Assistance with WKU athletes' treatment and injury rehabilitation
- Experience with electrical muscle stimulation and therapeutic ultrasound
- Experience with many different injuries and rehabilitation protocols

WKU Department of Physics Fall 2009 Bowling Green, KY
Biophysics Lab Assistant

- Instructed Lab (PHYS 223)
- Helped students understand material and grade papers (Instructor: Dr. Van der Meer)

Centerfield Park Spring 2004- Fall 2011 O'Fallon, IL

- Summer job- work concessions and handle finances

Publications

Friedman R A , Navalta J W , Fedor E A , Kell H B , Lyons T S , Arnett S W , Schafer M A . Repeated high-intensity Wingate cycle bouts influence markers of lymphocyte migration but not apoptosis . Appl Physiol Nutr Metab 2012, 37(2): 241246.

Friedman, R.A. "Investigation of C-Reactive Protein and Leptin as Biomarkers of Obesity with Potential Clinical Utility" (2011). *Masters Theses & Specialist Projects.* Paper 1091. http://digitalcommons.wku.edu/theses/1091

Abstracts

Friedman, R.A., S. Hunt, A. Maunsell, and J.W. Navalta. Repeated High-Intensity Anaerobic Bouts Influence Lymphocyte Migration but not Apoptosis. International Journal of Exercise Science, 5(1): S55-S57, 2010.

Hunt, S., Friedman, R. A. Maunsell, S. Bhamare, J.W. Navalta, and S. Lyons. Excess Post-exercise Oxygen Consumption Duration is significantly Greater in the Morning Compared to the Afternoon. International Journal of Exercise Science, 5(1): S44-S46, 2010.

Professional Presentations

R.A. Tinius, A.G. Cahill, W.T.Cade. Physical Activity and Maternal and Neonatal Metabolic Outcomes in Obese Pregnant Women (Platform Presentation). American College of Sports Medicine National Conference. San Diego, CA, May 2015.

R.A. Tinius, A.G. Cahill, W.T.Cade. Physical Activity and Maternal and Neonatal Metabolic Outcomes in Obese Pregnant Women. ACTS Translational Science 2015 Conference. Washington, DC, April 2015.

R.A. Tinius, A.G. Cahill, W.T.Cade. The Role of Physical Activity on Maternal and Neonatal Outcomes in Obese Pregnant Women. Diabetes Day Symposium. St, Louis, MO, October 2014.

R.A. Tinius, A.G. Cahill, W.T.Cade. The Role of Physical Activity on Maternal and Neonatal Outcomes in Obese Pregnant Women. Institute of Clinical and Translational Sciences 9th Annual Symposium. St. Louis, MO, October 2014.

R.A. Tinius, A.G. Cahill, W.T.Cade. The Influence of Maternal Physical Activity on Labor and Delivery Outcomes in Obese Pregnant Women. Institute of Clinical and Translational Sciences 9th Annual Symposium. St. Louis, MO, October 2014.

R.A. Tinius, A.G. Cahill, W.T.Cade. Effects of Maternal Obesity on Lipid Metabolism, Oxidative Stress and Neonatal Outcomes. ACTS Translational Science 2014 Conference. Washington, DC, April 2014.

R.A. Tinius, A.G. Cahill, W.T.Cade. Effects of Maternal Obesity on Lipid Metabolism, Oxidative Stress and Neonatal Outcomes. 19th Annual Graduate Research Symposium. St. Louis, MO, February 2014.

R.A. Tinius, A.G. Cahill , W.T. Cade. "Increased maternal lipid oxidation in obese pregnancy is associated with higher infant birth weight." William H. Danforth Scientific Symposium Celebrating the March of Dimes. St. Louis, MO, November 2013.

R.A. Tinius, A.G. Cahill , W.T. Cade. "Increased maternal lipid oxidation in obese pregnancy is associated with higher infant birth weight." Institute of Clinical and Translational Sciences 8th Annual Symposium. St. Louis, MO, October 2013.

R.A. Friedman, S.W. Lyons, J.W. Navalta, M. Schafer, S. Arnett. Biomarkers of Obesity with Potential Clinical Utility. American College of Sports Medicine's National Conference. San Francisco, CA, May 2012.

R.A. Friedman, S. Hunt, A. Maunsell, and J.W. Navalta. Repeated High-Intensity Anaerobic Bouts Influence Lymphocyte Migration but not Apoptosis. International e-Conference on Kinesiology and IntegratedPhysiology, http://kinesiology.econferenceintl.com, 2010.

S. Hunt, R.A. Friedman, A. Maunsell, S. Bhamare , J.W. Navalta, and S. Lyons. Excess Post-exercise Oxygen Consumption Duration is Significantly Greater in the Morning Compared to the Afternoon. International e-Conference on Kinesiology and Integrated Physiology, http://kinesiology.econferenceintl.com, 2010.

SELECTED ACCOMPLISHMENTS

Academics

- WKU Graduate Scholar of the College of Health and Human Services-2011
- WKU Graduate Student-Athlete of the Year- 2011
- WKU Exercise Science Female Outstanding Graduate Student of the Year- 2010-2011
- WKU Scholar of the College of Health and Human Services- 2009
- WKU Student-Athlete of the Year- 2009
- WKU Exercise Science Female Outstanding Student of the Year- 2008-2009
- Designated a President's Scholar at WKU in 2008-2009
- Department of Foreign Language Outstanding Achievement Award-2006
- WKU College of Health and Human Services Freshman Academic Achievement Award-2006

Athletics

Soccer (WKU)

- Nominated as WKU's Representative for NCAA Woman of the Year- 2010
- Lowe's Senior CLASS Leadership award candidate (one of thirty nationwide)- 2009
- NSCAA/Adidas Scholar-Athlete All-Region Team- 2008 & 2009
- ESPN the Magazine Academic All-American- 2008 & 2009
- ESPN the Magazine Academic All-District- 2007
- NSCAA All-Region Team- 2009
- Voted by peers as Team Captain- 2009
- WKU Coach's Award for Outstanding Commitment- 2009
- Preseason All-Sunbelt Conference- 2009
- First Team All-Sunbelt Conference- 2008 & 2009
- Great Lakes All-Region Team- 2008 & 2009
- Second Team All-Sunbelt Conference- 2007

Track and Field (WKU)

- All-Sunbelt Conference in the 3000m Steeplechase-2010

Post-College Running

- 1st place female Corvette half-marathon- October 2011
- 1st place female in the Total Fitness mini-marathon - March 2012
- 1st place female in the Bowling Green 26.2 Marathon- November 2012
- 3rd place female in the Missouri Cowbell Marathon - October 2013
- Placed 1,501/12,308 female runners- Boston Marathon- April 2015