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Offspring and Maternal Health Benefits of Exercise during Pregnancy

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OFFSPRING AND MATERNAL HEALTH BENEFITS OF EXERCISE DURING PREGNANCY

DISSERTATION

A dissertation has been submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Medicine at the University of Kentucky

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ABSTRACT OF DISSERTATION

OFFSPRING AND MATERNAL HEALTH BENEFITS OF EXERCISE DURING PREGNANCY

Maternal lifestyle and nutrient intake during pregnancy can have long-lasting effects on the health of offspring as well as the mother. This dissertation focuses on the impact of maternal exercise during pregnancy on offspring insulin sensitivity and glucose uptake and the maternal effects of exercise during pregnancy.

The first aim of this dissertation was to investigate if exercise prior to and during pregnancy and nursing would improve glucose uptake and insulin sensitivity in mice and rats. In both mice and rats, it was concluded that maternal exercise could enhance whole-body insulin sensitivity and increase glucose uptake into skeletal muscle and adipose tissue in adult offspring compared with offspring from sedentary dams. Maternal exercise also positively influenced male but not female adult offspring body composition; male offspring from exercised dams had significantly decreased fat mass and increased lean mass compared with offspring from sedentary dams.

The second aim of this dissertation was to test whether exercise during pregnancy would improve glucose disposal in mouse dams with diet-induced obesity. Maternal running was effective in reducing fat mass accumulation and glucose intolerance associated with high fat feeding during pregnancy. In high fat diet mice, exercise was also able to improve insulin sensitivity in adipose tissue compared to tissue from sedentary high fat diet mice.

The findings in this dissertation provide new insight into the long-term effects exercise during pregnancy can have on offspring health. Women may be encouraged to start an exercise regimen before and during their pregnancy if they are aware of the life-long benefits it can have for their children. The findings from the second aim present new insight into how exercise can affect pregnancies complicated by maternal obesity and glucose intolerance, and the animal model can be used in the future studies to investigate the offspring effects of maternal exercise during a diabetic pregnancy.

KEYWORDS: Pregnancy, Exercise, Offspring, Glucose, Insulin

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May 16, 2013
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CHAPTER 1

INTRODUCTION

1.1. General Introduction

1.1.1. Developmental Programming. Pregnancy is a critical time for both the mother and developing fetus. Events that occur during pregnancy can have a permanent effect on the health of the offspring. Developmental programming is a theory that the stimuli received from the intrauterine environment can result in long-term changes in an organism that can predispose or protect it from diseases later in life. It is well known that changes in the uterine environment caused by things such as drug use (for example tobacco and alcohol) can cause changes in fetal development that result in physical and mental dysfunction in offspring that last a lifetime. Less studied is how maternal diet and physical activity can affect offspring, particularly offspring metabolic health. Over the past two decades research has emerged showing that maternal diet, whether it is under or over-nutrition can alter offspring insulin sensitivity and increase offspring incidence of type 2 diabetes. This introduction will define and discuss diabetes as well as outline developmental programming of diabetes in humans and animal models. Lastly, this section will introduce the benefits of exercise and the potential use of exercise during pregnancy as an intervention to positively impact offspring health.

1.1.2. Type 2 Diabetes. Type 2 diabetes affects approximately 8 – 9% of the population in the United States and 300 million people are projected to have the disease by 2020 [1]. Consequences of the disease include heart disease, neuropathy, and kidney disease [2]. Type 2 diabetes is characterized by insulin resistance, hyperglycemia, and progressive β cell failure (the pancreatic islet cells responsible for the formation and

secretion of insulin) [3]. In response to glucose stimulation, β cells release insulin which in turn acts primarily on white adipose tissue and skeletal muscle to promote glucose uptake into the cells [4]. My project as described in Chapters 2-4 detected differences in insulin responsiveness in the peripheral tissues so that will be the focus of this introduction. A signaling cascade within the cell is initiated upon insulin binding to its receptor on the cell surface in skeletal muscle and adipose tissue [5]. Insulin binds to the extracellular α subunits of the insulin receptor (IR), causing autophosphorylation of the trans-membrane β subunits. Phosphorylated IR then recruits and phosphorylates a tyrosine residue on insulin receptor substrate 1 (IRS-1). Tyrosine phosphorylated IRS-1 in turn activates phosphatidylinositol 3-kinase (PI3K), which can catalyze the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 activates the 3–phosphoinositide–dependent protein kinase 1 (PDK1). PDK1 can then activate other kinases including Akt and atypical protein kinase C (PKCλ/ζ), leading to the translocation of glucose transporter type 4 (GLUT4) from the cytosol to the membrane for glucose uptake [5, 6].

Obesity is a major risk factor associated with type 2 diabetes. Adipose tissue plays a major role in the development of insulin resistance because it secretes adipokines, a group of cytokines and hormones that can deregulate the activation and activity of proteins involved in the insulin signaling cascade. For example, pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukin 6 (IL-6) released from adipose can lead to serine/threonine phosphorylation of IRS-1, causing inactivation of the protein and decreased translocation of GLUT4 to the cell surface for glucose uptake into the cell [7]. Free fatty acids (FFA) can also work in a similar manner to TNFα,

contributing to insulin resistance. Elevated FFA levels have been shown to produce diacylglycerol (DAG) accumulation in skeletal muscle. This accumulation of DAG can cause serine/threonine phosphorylation of IRS-1, therefore inhibiting insulin stimulated glucose uptake into the cell [8, 9]. On the other hand, adiponectin, a peptide hormone also secreted by adipose tissue (circulating levels are negatively correlated with adiposity) can actually enhance glucose uptake by activating AMP activated protein kinase (AMPK), a protein that stimulates insulin independent glucose uptake in response to a fall in cellular ATP levels [7]. Of particular interest to this project is how levels of these adipokines and peptide hormones are affected by obesity and/or exercise during pregnancy, which will be discussed in subsequent sections.

It is important to mention that both β cell dysfunction and hepatic insulin resistance can contribute to the development of type 2 diabetes. In β cells, deficits in glucose stimulated insulin secretion can occur when the cells are exposed to excess nutrients such as lipids and glucose [10, 11]. This results in an overall decrease in circulating insulin release in response to rising glucose levels, meaning less glucose is taken up into peripheral tissues. Reduction of hepatic insulin sensitivity by factors such as consumption of a high fat diet can further promote a diabetic state [12, 13]. Hepatic insulin stimulation inhibits gluconeogenesis, the process of endogenous glucose production from glycogen stored in the liver [14, 15]. When the liver becomes insulin resistant, endogenous glucose production is not decreased in response to circulating insulin and glucose levels, which contributes to hyperglycemia [16].

1.1.3. Developmental Programming of Diabetes: Human Maternal Malnutrition. Geographical studies conducted in England and Wales were the first to show a link

between a poor intrauterine environment, caused by famine during gestation, to disease in aged offspring [17-19]. In areas where there was maternal malnutrition, David Barker found a correlation between low birth weights, due to poor maternal nutrition, and ischemic heart disease in the adults who had been exposed to famine during their fetal development [17]. A subsequent study looked at individual cases in these areas and found men with the lowest birth weights had approximately a three-fold higher death rate from ischemic heart disease [20]. Other studies in this cohort of offspring also showed links between low birth weight and hypertension and fibrinogen in adulthood [21, 22]. Barker then looked at glucose regulation and type 2 diabetes in these adults and found that lower birth weights were associated with impaired glucose tolerance and type 2 diabetes [23]. Later, Barker and colleague Charles Hales went on to show that adults who had a low birth weight had higher incidence of insulin resistance and type 2 diabetes that was independent of body mass and adiposity [24]. Other groups were also able to demonstrate insulin resistance and type 2 diabetes in later-life related to low birth weight [25-28]. Another well studied population of offspring exposed to maternal malnutrition comes from those who were born during the Dutch Famine between 1944 and 1945. In adulthood, this population showed higher rates of obesity, coronary heart disease, and impaired glucose tolerance and insulin secretion compared to those born before or after the famine period [29-31]. Barker and Hales proposed the “thrifty phenotype” hypothesis to summarize the findings from maternal malnutrition research which states that poor fetal nutrition is detrimental to development and consequently predisposes an individual to type 2 diabetes in adulthood [32]. An individual can be “programmed” during fetal development to have an advantage after birth in an environment that is predicted to have

low nutrient availability, but this programming is disadvantageous when there is sufficient or excess nutrient availability. Since the development of the thrifty phenotype hypothesis, numerous animal models have been used to study the effects of intrauterine growth restriction (IUGR) leading to low birth weights and the effects on offspring insulin sensitivity and glucose regulation.

1.1.4. Developmental Programming of Diabetes: Animal Models of Maternal Malnutrition. Uterine artery ligation and maternal nutrient/protein restriction are two of the most used models to study the effects of IUGR in animals. Uterine artery ligation creates poor nutrient flow to the fetus, a common cause of reduced fetal growth in humans. In rats, ligation results in low birth weight and insulin resistance and glucose intolerance in aged offspring [33]. Offspring also have reduced beta cell mass and higher fasting glucose compared to normal offspring [33, 34]. In rodents, maternal nutrient restriction causes restricted fetal growth and birth weight which is accompanied by impaired beta cell function and glucose tolerance, and hyperinsulinemia in adult life (4 and 12 months of age) compared to offspring from normal fed dams [35, 36]. Other studies using maternal protein restriction in rodents have found similar results. Offspring have decreased beta cell mass as well as reduced insulin content, and in response to glucose stimulation, have decreased insulin secretion compared to offspring from normal fed dams [37-40]. Adult offspring at 15 and 17 months of age also have impaired glucose tolerance [41, 42]. Maternal nutrient restriction has also been studied using sheep. Offspring born to nutrient restricted mothers show decreased insulin sensitivity, increased insulin secretion, along with increased weight gain compared to normal offspring [43].

Using these animal models, researchers have been able to attempt to determine the mechanisms behind the observed insulin resistance and impaired glucose tolerance in offspring exposed to IUGR. Research has shown that rat offspring exposed to IUGR have reduced expression of the p110β subunit of PI3-Kinase, as well as decreased association of the p110β with the p85 subunit in adipose tissue compared to control offspring, which inhibits GLUT4 translocation, decreasing glucose uptake into the cell [40, 44]. In muscle samples from growth restricted offspring, there is decreased expression PCKζ, another important protein in the insulin signaling cascade involved with GLUT4 translocation, compared to expression in control animals [41]. Hepatic insulin resistance has also been observed in IUGR offspring. After insulin stimulation, hepatic glucose production in IUGR is not decreased as in control animals and there is decreased phosphorylation of Akt compared to control animals [45]. These decreases in insulin pathway protein expression and activation could make the IR signaling cascade less sensitive to insulin stimulation and activation.

Other studies have found that IUGR can result in epigenetic changes to genes involved in metabolism and growth. Insulin-like growth factor-1 (IGF-1) is a protein produced mainly by the liver that plays an important role in growth and cellular proliferation by activating the Akt pathway. Circulating levels of this protein are reduced in models of IUGR[46]. Decreased IGF-1 has been linked to epigenetic modifications along the hepatic IGF-1 gene, specifically histone modifications that result in down-regulation of gene transcription [47]. Other epigenetic changes have been associated with the dysfunctional pancreatic β cells found in offspring exposed to IUGR. Pancreatic and duodenal homeobox 1 (Pdx1) is a transcription factor that regulates pancreas growth and

differentiation of β cells [48]. Pdx1 expression is reduced is offspring that experience IUGR compared to normal offspring, predisposing offspring to β cell dysfunction and type 2 diabetes [49]. This reduction in expression is due to histone modifications caused by IUGR that result in silencing of the gene.

In-utero growth restriction affects many tissues and proteins involved with insulin sensitivity and glucose homeostasis. Clearly, poor nutrient supply to the developing fetus has many long-term consequences that can predispose offspring to insulin resistance and diabetes. Interestingly, maternal overnutrition causes many negative effects in the mother herself as well as fetal over-growth that results in similar long-term offspring disease incidence.

1.1.5. Developmental Programming of Diabetes: Maternal Obesity and Gestational Diabetes. Obesity rates are on the rise and almost 30% of women of child-bearing age are considered obese [50]. Obesity during pregnancy can be detrimental to both mother and child. High body mass index and consumption of a high calorie diet are risk factors for the development of gestation diabetes, defined as glucose impairment first recognized during pregnancy [51-53]. Although a natural insulin resistance develops as all pregnancies progress in order to ensure adequate nutrient flow to the fetus, in some women, especially those who are obese, this natural decrease in insulin sensitivity can develop into diabetes [54]. Excess adipose tissues in obese women may play a role in inducing gestational diabetes in obese women by causing an increase in whole body inflammation. Inflammatory cytokine levels, such as TNFα, are positively correlated with adiposity, and can contribute to insulin resistance by inactivating proteins, like IRS-1, in the insulin signaling cascade [8]. Obese pregnant women with gestational diabetes do

indeed have increased circulating levels of these pro-inflammatory cytokines along with down-regulated IRS-1 in skeletal muscle [55-57]. Obesity is also associated with decreased adiponectin levels, a protein that can contribute to increased glucose uptake by promoting AMPK phosphorylation and therefore increase glucose uptake in skeletal muscle [58]. In women with gestational diabetes, adiponectin levels are significantly decreased compared to women experiencing a normal pregnancy and this too may contribute to the impaired glucose tolerance in these women [59]. Finally, increased fat mass is also linked to high levels of circulating triglycerides and free fatty acids which both have insulin desensitizing effects, and these levels are higher in women with gestational diabetes compared to a normal pregnancy [8, 54, 60]. Similar to inflammatory cytokines, these molecules affect proteins in the insulin signaling cascade and can deactivate them [8, 57].

Due to all these changes in protein expression, circulation, and activation, women who develop gestational diabetes are at a substantially higher risk than other women for type 2 diabetes in years after their pregnancies [61]. Therefore, treatment or prevention of gestational diabetes is of high importance. Management of gestational diabetes, however, is also critical as high levels of maternal glucose can be detrimental to the developing fetus. Similar to poor nutrient availability during development, excess nutrient and glucose exposure can also ‘program’ offspring to be predisposed to insulin resistance and diabetes.

Fetal growth increases as a result of high maternal glucose levels [62]. This is due to higher levels of fetal insulin being secreted in response to maternal glucose levels, because although maternal glucose crosses the placenta, maternal insulin does not [62].

Children from gestational diabetic mothers therefore have excess fat mass and are heavier at birth than children from normal women [63]. This increase in body weight and adiposity persists past birth and throughout childhood [63-65]. Even in women with only mild glucose intolerance, as opposed to women with gestational diabetes, infant fat mass and body weight are increased compared to infants of a normal pregnancy [66]. In a well studied population of women with a high incidence of gestational diabetes, the Pima Indian cohort, it has been found that gestational diabetes results in a high risk of type 2 diabetes in offspring compared to those born to nondiabetic women [67, 68]. Of the children who become diabetic in this population, almost all of them are from diabetic mothers [68]. Interestingly, siblings born to the same mothers prior to the development of diabetes or gestational diabetes do not show the same incidence of type 2 diabetes as siblings born to the mothers once diabetes has developed [64].

Animal models of excess calorie intake before and during pregnancy have shown similar results to human studies. However, animal models have allowed researchers follow health outcomes in offspring through adulthood. In rodents, offspring from high calorie fed dams have increased adiposity and elevated blood insulin and glucose levels in adult life between 3 – 6 months of age compared to offspring from normal diet fed dams [69, 70]. One year old offspring from dams fed a high fat diet also exhibit decreased whole body insulin sensitivity (as measure by hyperinsulinemic-euglyemic clamp) when compared with offspring from dams fed a normal diet [71]. These offspring demonstrate β cell dysfunction; they have decreased glucose stimulated insulin release from pancreatic β cells compared to control offspring [71]. Offspring from diet induced obese dams have also been shown to develop frank diabetes by 26 weeks of age [72].

Using animal models, researchers have also been able to identify a number of other negative health outcomes in offspring caused by maternal high calorie feeding; these outcomes include offspring hypertension, fatty streaks on aortas, fatty liver, and hyperphagia [69, 73, 74].

Effective management or prevention of maternal glucose levels and gestational diabetes is extremely important for the health of mother and child. To date, there is evidence that changes to diet and weight loss prior to conception can decrease risk of gestational diabetes. Once gestational diabetes has developed, diet and lifestyle modifications are recommended, and insulin can be used to maintain normal maternal blood glucose levels if needed [75]. Surprisingly, exercise has received very little attention in the treatment and prevention of gestational diabetes [75]. It is widely accepted that exercise has innumerable health benefits, including improving whole body glucose regulation, and may have the same health benefits in pregnant women as in non-pregnant individuals. More recently it has come to light that exercise during pregnancy may also have benefits for offspring. The remainder of this introduction will focus on effects of exercise during normal and diabetic pregnancy, as well as potential benefits of maternal exercise for the next generation.

1.1.6. Exercise during Pregnancy: Maternal Effects in Normal Pregnancies. There are countless health benefits of physical activity, including but not limited to enhanced mood and cognition, weight management, improved cardiovascular health, and decreased risk of disease such as cancer, heart disease, and type 2 diabetes [76-79]. Many physiological adaptations occur in a woman in response to pregnancy as well as exercise, and these can work synergistically to maintain the best health possible for mom and baby.

In pregnancy, the cardiovascular system changes in order to support the developing child. Hormones released by the placenta increase blood vessel elasticity and cause water and sodium retention [80, 81]. Blood volume is thereby increased which in turn can raise cardiac output [80]. Overall cardiac output can be increased by almost 40% compared with the non-pregnant state, allowing for proper blood flow and glucose and oxygen delivery for fetal development [80, 82]. The cardiovascular adaptations that occur in pregnancy are very similar to those that happen in response to exercise training. Regular exercise also results in increased blood volume and cardiac output [83]. Consistent exercise training and pregnancy can have additive effects on cardiovascular structure and function. Women who exercise during pregnancy have up to 15% higher plasma volume compared with sedentary pregnant women [84, 85]. Stroke volume can also be raised as much as 50% in exercising pregnant women compared to sedentary pregnant women because exercise training and pregnancy both increase left ventricular volume [86, 87].

There has been concern that exercise during pregnancy could be harmful for the fetus due to higher maternal body temperature during physical activity bouts [88]. However, greater blood circulation due to exercise and pregnancy also allows the mother to maintain core body temperature since more blood is circulating to the surface/skin, allowing heat to dissipate [85]. Another concern has been the changes in blood flow caused by exercise. During an exercise bout, blood flow is shifted away from digestive and reproductive systems and other organs and redirected towards muscle and skin [83, 89]. This raised fear that exercise would deprive the fetus of blood and therefore oxygen and glucose. In pregnancy, exercise causes a change in blood flow from the fetus to maternal skin and muscle, and at high intensities, this change in flow can be as great as

50% [85]. However, once the exercise bout has ended, there is a rapid return to normal blood flow. Also, women who have trained from the start of their pregnancy actually have increased placental size and improved placental function, meaning that even during physical activity, there is still adequate blood flow to the fetus despite shifts in distribution of flow [90].

There are also several metabolic changes that occur in during pregnancy. Adipose tissue is formed during early pregnancy since it is able to store high amounts of energy. Muscle and fat also become more insulin resistant as pregnancy progresses [91] which causes more maternal fat deposition and decreases maternal glucose uptake and utilization, leaving more glucose for the fetus. Opposite from the pregnancy response, exercise decreases fat mass and increases muscle and bone mass [92]. Exercise also enhances insulin sensitivity in skeletal muscle so that muscle can take up glucose and store it for energy during an exercise bout [93]. Since exercise can counteract the insulin desensitizing effects of pregnancy, it is important that during a healthy pregnancy, a woman eats regularly to maintain glucose levels reaching the fetus [94]. The metabolic effects of exercise training are particularly important in pregnancies complicated by gestational diabetes, which will be discussed in greater detail the next section.

There are many reported maternal benefits of exercising during healthy pregnancy. There are multiple types of exercise including endurance and resistance training. Examples of endurance training are activities such as running, cycling, and swimming while resistance training includes exercises like weight lifting that focus on increasing muscle size and/or strength [95]. Pregnant women who participate in continuous weight-bearing endurance training (such as running or aerobics) are leaner

with decreased fat mass and weight gain compared to sedentary women. Body fat, as measured by skin-fold thickness, was much lower in women who maintained an exercise routine of at least 3 – 5 times a week [94]. They also return to pre-pregnancy weight faster [94]. Women who exercise also have less fluid retention and decreased lower back pain and blood pressure, decreasing the risk of pre-eclampsia (high blood pressure in pregnancy) [96]. Labor time is shorter; in one study 65% of women who remained active throughout pregnancy had a labor of 4 hours or less versus only 31% of control women being in labor for 4 hours or less [94]. Women also complain less of discomfort during pregnancy and labor when exercise was maintained throughout pregnancy. There is up to a 35% decrease in request for pain relievers and 75% decrease in exhaustion during labor compared to sedentary pregnant women [94]. A more subtle benefit of physical activity during pregnancy is improved mood; several studies have reported that, particularly in the third trimester, women who exercise have lower anxiety and are more stable in their moods compared with sedentary women [97, 98].

Clearly, maintaining good physical activity and condition can contribute to healthy pregnancy outcomes. Therefore, the American College of Obstetricians and Gynecologists recommends that women "engage in regular, moderate intensity physical activity to continue to derive the same associated health benefits during their pregnancies as they did prior to pregnancy" [99]. Women who were inactive prior to pregnancy are encouraged to slowly and progressively work up to 30 minutes of exercise [99]. The Society of Obstetricians and Gynecologists of Canada also recommends that all pregnant women without complications should perform some form of endurance and/or strength training to promote a healthy pregnancy [100]. The American College of Sports Medicine

also suggests that healthy women continue a regular exercise regimen throughout pregnancy [101]. All groups advocate safe physical activities such as swimming, jogging, or stationary biking and warn against activities such as contact sports of those that require special balance, for example cycling, that could result in maternal or fetal injury.

1.1.7. Exercise during Pregnancy: Maternal Effects in Diabetic Pregnancies. There are several maternal and offspring risks of gestational diabetes, including increased risk of type 2 diabetes in mother and offspring; therefore physical activity throughout pregnancy could be particularly important if a mother has gestational diabetes. Since exercise has metabolic effects that include enhancing insulin sensitivity, it may be an effective method of preventing or treating gestational diabetes. Given the metabolic impact exercise training can have in humans, it is surprising that there have been relatively few studies looking at the physical activity effects on gestational diabetes, in particular the intensity and frequency of exercise required to improve diabetic outcomes.

As mentioned earlier, studies have shown that exercise can improve insulin sensitivity in muscle [102, 103]. Endurance training has been shown in several studies to increase GLUT4 levels in skeletal muscle, as well as increase levels of muscle glycogen synthase and hexokinase, thereby promoting glucose uptake and phosphorylation in response to insulin stimulation [93, 103-105]. The anti-inflammatory effects of exercise also play a role in improving insulin sensitivity. Research looking at exercise has found that decreases in TNFα with training, which could contribute to insulin sensitizing effects by preventing inactivation of proteins in the insulin signaling cascade [106, 107]. The insulin sensitivity enhancing effects of resistance training has been less studied, but it is thought that it is able to improve overall insulin sensitivity by increasing muscle mass

[108, 109]. Exercise can help regulate blood glucose levels by stimulating insulin independent glucose uptake in skeletal muscle. Muscle contraction stimulates AMPK, which leads to GLUT4 translocation to the cell membrane and glucose uptake into the cell [110-112]. AMPK is a fuel sensor that is stimulated in response to a decrease in the ATP/AMP ratio; therefore when muscles contract and ATP levels decrease and AMP levels increase, AMPK is activated [112, 113].

Exercise is clearly able to affect glucose disposal in several different ways and so it has been studied, although not extensively, in the prevention and management of gestational diabetes. Studies looking at the effects of exercise on risk of developing gestational diabetes have yielded mixed results. Observational studies have found that risk of gestational diabetes can be decreased with various levels of physical activity. Women who reported any level of activity early in their pregnancy showed a 56% decrease in gestational diabetes risk in one prospective study [114]. Dempsey et al also found that, in a separate cohort of women, daily stair-climbing during pregnancy was associated with a 50 – 75% decrease in gestational diabetes risk [115]. This study also showed that intensity and duration of exercise bouts were negatively correlated with incidence of gestational diabetes in the study cohort. Other studies have found that women who reported vigorous exercise before pregnancy, or even low intensity physical activity such as brisk walking, have reduced incidence of gestational diabetes compared to women who were not physically activity [116, 117]. In a meta-analysis, Tobias et al concluded that high levels of exercise before or during early pregnancy decreased gestational diabetes incidence [118]. Using data from the National Maternal and Infant

Health survey, another group found that women who began physical activity at the onset of pregnancy had 57% lower adjusted odds of developing gestational diabetes [119].

Despite these promising findings, other studies have found no clear evidence that physical activity can reduce the risk of gestational diabetes. Han et al pooled data from multiple clinical trials and concluded that there was no evidence that any level of physical activity before or during pregnancy had any effects on development of gestational diabetes[120]. In a recent randomized control trial, pregnant women who were assigned to moderate intensity exercise (combination of endurance, muscle strength, and flexibility training) for 3 days a week were not protected from gestational diabetes development [121]. It is important to note that although there was no effect on the development of gestational diabetes, this exercise regimen did result in fewer incidence of macrosomia in the infants of diabetic mothers compared to those from control diabetic mothers. In another randomized control trial overweight and obese women were put on a moderate intensity aerobic and resistance exercise program consisting of 2, 60 minute sessions a week [122]. Exercise training was unable to reduce fasting glucose levels or improve insulin sensitivity as defined by the homeostatic model assessment index, which estimates insulin sensitivity and β cell function.

Little research has been conducted investigating the effects of exercise on pregnant women who have already developed gestational diabetes; however some yielded positive results. In one, an arm exercise program in which an arm ergometer was used to monitor and maintain heart rates (20 minutes of arm exercise at ~50% maximum capacity; 3 times a week) in women diagnosed with gestational diabetes was able to normalize fasting glucose levels after 6 weeks [123]. In another study, gestational

diabetic women were assigned to diet or diet plus exercise consisting of resistance circuit-type training (i.e. squats, knee extensions, seated row, and lateral pull-down) 3 times a week, and the need for insulin to control blood glucose levels was monitored [124]. In normal weight women, diet and exercise was no different than diet alone at preventing the need for insulin to control blood glucose; however in overweight women, diet plus exercise reduced the need for insulin compared to diet therapy alone. Additionally, another group was able to show that resistance training of moderate intensity with an elastic band 3 times week, was effective in reducing the number of patients with gestational diabetes requiring insulin therapy [125].

Exercise has potential as a treatment or preventative therapy for gestational diabetes, but there is a lack of evidence based studies and animal models that can be used to look at the physiological effects of exercise and the type and intensity of exercise required to improve gestational diabetes outcomes. Animal models will be essential in the study of physical activity effects on gestational diabetes.

1.1.8. Exercise during Pregnancy: Effects on Offspring. There are numerous maternal health benefits of physical activity during healthy and diabetic pregnancies, and more recently, attention has turned to the offspring benefits of maternal exercise. In humans, the offspring effects of maternal exercise can be apparent from birth. In women who exercise before and during pregnancy, rigorous exercise can decrease birth weight, but not to the level associated with IUGR or small for gestational age. This decrease in birth weight is associated with reduced fat mass and no changes in lean mass in the infants [86, 126]. Beginning moderate weight bearing exercise in pregnancy can actually increase birth weight due to increased placental growth and volume; however the

increased birth weight is only due to increased lean mass with no changes in fat mass [127]. Other studies have shown that non-weight bearing, yet fairly intense exercise, such as cycling and swimming had no effects on birth weight [84, 128]. A more recent study, however, found that a non-weight bearing stationary cycling exercise program started mid gestation was effective in reducing birth weight [129]. Others have shown that even weight-bearing exercise, when at reduced intensities compared to what women exercised prior to pregnancy, did not have effects on birth weight [130, 131]. Interestingly, when intense exercise is stopped at some point during pregnancy, because placental growth has already been increased due to the physical activity, infants are born with a higher birth weight than others from mothers who were sedentary throughout the entire pregnancy, and this increase in birth weight is the result of higher levels of fat mass [132]. The type of exercise and timing of exercise during pregnancy can all have different impacts on offspring birth weight, making the amount, type, and timing of exercise during pregnancy important factors to consider in future studies. Currently, it is thought that these differences in birth weight with difference exercise regimens during pregnancy are the result of placental growth changes and oxygen and glucose levels reaching the fetus in response to exercise [90]. Again, it is important to note that although exercise can result in lower birth weights; it does not increase risk of low birth weight (less than 5 pounds 8 ounces) and that infant body length and sizes are not altered by maternal exercise.

In humans, there is still very limited research based information on the long-term outcomes in offspring resulting from maternal exercise during pregnancy. The most extensive research on maternal and offspring effects of exercise during pregnancy has been conducted by clinician James Clapp. When evaluating the behavior of infants 5 days

after birth using the Brazelton Neonatal Behavioral Assessment Scales, he found that offspring from women who exercised throughout pregnancy (an average of 4 times per week and 60% maximum capacity) performed better on orientation and state levels skills than offspring from less active mothers, suggesting that maternal exercise was having an impact on neurological development [133]. In another cohort of women, Clapp looked at the effects of exercise (stair-climbing, aerobics, or running) on offspring morphometric and neuro-developmental outcomes at 1 year of age [134]. Women in the exercise group exercised at least three times a week for 20 minutes per exercise bout, reaching 60 – 90% maximum capacity at their highest intensities. Although at birth the infants from exercise mothers had lower body weight and fat mass compared with those from less active mothers. However, at 1 year, they had comparable body weight and body composition to children from control mothers. There were also no differences in neuro-developmental outcomes as measured by the Bayley Pyschomotor Scales at that age. Using yet another group of exercising women, Clapp measured morphometric and neuro-developmental outcomes in 5 year old children and compared these outcomes to children from less active, control mothers [135]. To be included in the exercise group in this study, women had to exercise (running, aerobics, cross country skiing, or a combination of these) 3 or more times per week for at least 30 minutes a bout and reach at least 55% maximum capacity intensity. As expected, at birth the infants from exercise mothers had lower body weight and reduced fat mass compared with those from control mothers. Interestingly, at 5 years of age, children from exercise mothers continued to weigh less and have reduced fat mass compared with children from control mothers. When neuro-developmental outcomes were measured, Clapp found that although children from both experimental

groups performed similarly in motor and integrative skills tasks, children from exercise mothers performed significantly better in oral language skills and on the Wechsler Scales Intelligence Test compared with children from control mothers. The mechanisms behind the improved neurological outcomes and continued reduction in fat mass in this study are unclear, but this does suggest that exercise during pregnancy can have positive impacts on offspring many years after birth.

Using animal models researchers have been able to start to evaluate the cellular changes associated with the observed effects of maternal exercise in offspring, as well as follow offspring as they age in order to assess the more long-term effects of exercise during pregnancy. In rodent models, many have looked at the impact of maternal exercise on the neurological outcomes in offspring. Lee et al studied the effects of maternal swimming in rats on hippocampal neurogenesis and memory in offspring [136]. Starting at time of conception, pregnant rats in the exercise cohort were forced to swim 10 minutes a day throughout pregnancy. Offspring were tested on postnatal day 21 in a step-down avoidance task for changes in short-term memory function and on postnatal day 29 offspring were euthanized and hippocampal neurogenesis and brain-derived neurotrophic factor (BDNF) mRNA levels were evaluated. Offspring from exercise dams had increased latency to step-down the avoidance task indicating enhanced short-term memory. On postnatal day 29, offspring from exercise dams showed increased expression of BDNF and increased neurogenesis in the hippocampus, the area of the brain associated with learning and memory. Another group also used forced exercise in a rat model of exercise during pregnancy in order to evaluate the effects of maternal exercise on offspring anxiety and neurogenesis in the prefrontal cortex [137]. Dams in the exercise

group were acclimated to treadmill running for one week prior to pregnancy and during pregnancy dams ran at 8 meters/minute for 30 minutes a day, 5 days a week. Offspring from exercise and control dams were tested on postnatal day 26 and at 4 months of age in an open field anxiety test. At both of these age points, offspring expression of BDNF and vascular endothelial growth factor (VEGF) (decreases in both are associated with anxiety levels) was measured. At both ages, offspring from exercise dams performed better in the open field task, indicating lower levels of anxiety compared with offspring from control dams. In the prefrontal cortex, offspring from exercise dams also had increased expression of BDNF and VEGF at 26 days and 4 months of age, indicating increased growth and vascularization in this area of the brain compared to offspring from control dams.

In an identical model of maternal exercise in rats, offspring were tested for changes in spatial memory in response to maternal exercise [138]. At postnatal days 21 and 120 offspring from control and exercise dams were tested for spatial learning and memory using a water maze. Animals were trained to find a platform and then tested for latency to find the platform in a water maze the next day. At both ages, offspring from exercise dams had decreased latency to find the platform in the maze following training compared with offspring from control dams, demonstrating enhanced spatial memory. Offspring from exercise dams also had increased neurogenesis in the hippocampus at both ages compared to those from control dams. Using mice, Park et al used maternal treadmill running during pregnancy (40 minutes/day at 12 meters/minute for the 3 weeks of pregnancy) to look at BDNF in the hippocampus and memory in offspring [139]. On postnatal day 3, offspring from exercise dams had enhanced BDNF expression in the

hippocampus compared to those from control dams, which could functionally improve learning and memory in the offspring. At postnatal day 30, offspring from exercise dams also performed better on a Y-maze task, signifying improved memory compared to offspring from sedentary dams. Finally, Herring et al evaluated the effects voluntary maternal exercise from the start and throughout the duration of pregnancy on offspring Alzheimer outcomes in transgenic mice predisposed for the disease [140]. On postnatal day 150, female offspring from control and exercise dams were evaluated for β-amyloid plaque formation and angiogenesis in the brain. Offspring from exercise dams had significantly decreased β-amyloid plaque burden and increased angiogenesis, as well as an improvement in several other outcomes associated with Alzheimer disease compared with offspring from control dams. All of these studies suggest that at early and later ages, exercise during pregnancy can positively influence offspring brain development and function and potentially protect offspring from age related disease.

Given the effects exercise during pregnancy can have on placental blood flow and glucose and oxygen reaching the fetus, surprisingly few studies have investigated the metabolic outcomes of maternal exercise in offspring. Almost two decades ago, Vanheest and Rodgers assessed offspring metabolic outcomes of maternal treadmill running in diabetic rat dams [141]. Female rats were made diabetic via streptozotocin injection and then divided into control and exercise groups. Exercise rats ran on a treadmill at a pace of 20 meters/minute for 1 hour/day, 5 days a week immediately prior to and during pregnancy. Although there were no changes in maternal glucose regulation as a result of the treadmill training, offspring from exercise dams had significantly lower blood glucose levels during glucose tolerance testing following a glucose challenge at postnatal day 28

compared to those from control diabetic dams. Glucose tolerance in the offspring from exercise diabetic dams was even significantly improved when compared with offspring from control, non-diabetic dams. Despite improvements in glucose disposal, there were no changes in offspring fasting insulin levels as a result of maternal exercise. A more recent study evaluated the ability of maternal exercise to improve offspring metabolic dysfunction programmed by maternal protein nutrient restriction [142]. As described earlier, maternal protein restriction can cause insulin resistance and glucose intolerance in offspring. In this study dams were given a low protein diet which caused hyperglycemia, hypercholesterolemia, and glucose intolerance in offspring by 150 days of age. These parameters were reversed however in offspring from dams that ran on a treadmill for 6 minutes/day, 5 days/week at 65% maximum capacity for 4 weeks prior to pregnancy and throughout pregnancy. The results from this study suggest that exercise during pregnancy can improve glucose regulation in offspring predisposed to glucose intolerance. To date, no studies have been conducted looking at the offspring metabolic effects of maternal exercise in healthy pregnancy.

1.2. Scope of Dissertation

1.2.1. Aims of Dissertation. The main purpose of this project was to determine if voluntary maternal exercise could enhance offspring glucose regulation and insulin sensitivity. The secondary purpose of this project was to evaluate maternal exercise as an intervention for diet induced glucose intolerance during pregnancy.

1.2.2. Rationale. Maternal exercise during pregnancy has been shown to have many effects on offspring outcomes. In humans, offspring exposed to maternal exercise have lower fat mass and body weight at birth [90, 129, 134, 143]. Umbilical cord blood levels of IGF-1 are also decreased with maternal cycling. The long-term effects in human offspring have been less studied [129]. In one cohort of women, 5 year old children from exercising mothers had higher intelligence scores and lower fat mass compared with children from sedentary mothers [135]. This correlates with animal research in which maternal exercise results in increased neurogenesis and improved learning and memory in young offspring [136, 137].

Given that physical activity during pregnancy can affect glucose and oxygen flow to the fetus, surprisingly few people have investigated the metabolic impact this intervention can have on offspring. Using rats, two separate studies have demonstrated that maternal exercise in unhealthy pregnancies (gestational diabetes and maternal nutrient restriction) can improve offspring glucose metabolism compared to offspring from diabetic or nutrient restricted sedentary dams [141, 142]. No studies in humans or animals have looked at the lifelong impact of exercise during a normal pregnancy on offspring insulin sensitivity or glucose disposal.

Determining these long-term effects in offspring will provide insight into the lasting impacts of maternal exercise on offspring. Once the benefits of maternal exercise are realized, physicians may be more likely to encourage women to participate in exercise regimens throughout pregnancy. Women may also be more willing to start a training program if they are educated on the benefits it could have for their children. Exercise during pregnancy is a potential short-term intervention that may decrease susceptibility and incidence of insulin resistance and diabetes in future generations.

Additionally, there has been little investigation into exercise as an intervention for maternal obesity and gestational diabetes. Obesity is a major risk factor for the development of gestational diabetes which is detrimental to the health of both mother and offspring [52]. Gestational diabetes heightens the risk of developing type 2 diabetes after pregnancy and can lead to obesity and diabetes in offspring [68, 144]. Although a few studies have shown that resistance training can decrease the need for insulin to control blood glucose in pregnant women with gestational diabetes [124, 125], there is a general lack of knowledge on the type, timing, and intensity of exercise best used as a treatment in pregnancies complicated by obesity and diabetes. By developing an animal model of maternal obesity that causes maternal glucose intolerance, research can be conducted investigating (1) the impacts of maternal exercise on maternal glucose uptake and insulin sensitivity and (2) eventually study the lasting effects of exercise during an unhealthy pregnancy on offspring. Again, these findings could potentially encourage women to remain or become physically active during their pregnancies and physicians may also be more likely to recommend exercise regimens to their patients at high risk for gestational diabetes.

1.2.3. Hypothesis and Specific Aims. **Hypothesis:** Maternal exercise before and during pregnancy and nursing will increase offspring glucose disposal by enhancing offspring insulin sensitivity in peripheral tissues. Exercise before and during pregnancy will also improve maternal glucose regulation in a mouse model of diet-induced obesity. **Aim1:** Investigate if exercise prior to and during pregnancy and nursing will improve glucose regulation and insulin sensitivity in offspring in mice and rats. **Aim 2:** Test whether exercise during pregnancy will improve glucose regulation in mice dams with diet-induced obesity.

CHAPTER 2

PERINATAL EXERCISE IMPROVES GLUCOSE HOMEOSTASIS IN ADULT OFFSPRING

2.1. Abstract

Emerging research has shown that subtle factors during pregnancy and gestation can influence long-term health in offspring. In an attempt to be pro-active, I set out to explore whether a non-pharmacological intervention, perinatal exercise, might improve offspring health. Female ICR mice were separated into sedentary or exercise cohorts with the exercise cohort having voluntary access to a running wheel prior to mating and during pregnancy and nursing. Offspring were weaned and analyses were performed on the mature offspring that did not have access to running wheels during any portion of their lives. Perinatal exercise caused improved glucose disposal following an oral glucose challenge in both female and male adult offspring ($P < 0.05$ for both). Blood glucose concentrations were reduced to lower values in response to an intraperitoneal insulin tolerance test for both female and male adult offspring of parents with access to running wheels ($P < 0.05$ and $P < 0.01$, respectively). Male offspring from exercised dams showed increased percent lean mass and decreased fat mass percent compared to male offspring from sedentary dams ($P < 0.01$ for both), but these parameters were unchanged in female offspring. These data suggest that short-term maternal voluntary exercise prior to and during healthy pregnancy and nursing can enhance long-term glucose homeostasis in offspring.

2.2 Introduction

In 2007, twenty-three and a half million people in the United States (US) were estimated to have diabetes and this number is increasing [145]. Interestingly, and what is often under-appreciated, is that the metabolic status of an individual is decided not only by their inherited genes, nutritional intake, and physical exercise, but also by maternal nutrition and obesity during pregnancy. In 1992, Barker and Hales put forth the thrifty phenotype hypothesis that suggested that malnourished pregnant mothers produce smaller offspring who have a higher incidence of obesity, diabetes, and heart disease in adulthood. This hypothesis has since been modified to the developmental origins of health and disease (DOHaD) [32, 146, 147].

The DOHaD suggests that the maternal environment and fetal programming lead to a higher incidence of several diseases later in life [148, 149]. A growing number of studies have been designed to provide evidence for the negative impact of DOHaD using mice, rats, and sheep as animal models [40, 69, 150, 151]. Many of these studies are directed at malnutrition through protein restriction or physical stressors that produce similar effects [40, 152-155], but more recent studies are elucidating the metabolic effects of high-fat diet consumption during pregnancy on offspring [69, 155-157].

It has been known since Hippocrates and Galen that physical activity is an important component of a healthy lifestyle [158]. However, knowledge about the contributions of maternal exercise during pregnancy and the long-term consequences on offspring is minimal. In both rats and mice, maternal exercise during pregnancy can improve brain physiology and cognition in the offspring [136, 159, 160]. In humans, five-year-old children born to mothers who exercised regularly during pregnancy had

improved intelligence scores and reduced body mass [135]. Physical activity is already used as a treatment for gestational diabetes in humans, but long-term outcomes in offspring have not been fully investigated [116, 161-163].

Using a mouse model, I set out to explore maternal voluntary exercise as an intervention to improve offspring metabolic health. Ie hypothesized that voluntary exercise prior to and during pregnancy and nursing would benefit offspring metabolic health throughout their adult life. In this report I show that maternal voluntary exercise just prior to and during pregnancy and lactation had a positive impact on glucose regulation and insulin sensitivity in sedentary adult offspring. This is exciting as it indicates that a simple, short-term, non-pharmacological intervention can improve long-term glucose homeostasis in the next generation.

2.3. Material and Methods

2.3.1. Animals and diets. These studies were carried out at the University of Kentucky according to an approved Institutional Animal Care and Use Committee protocol. At 2 months of age, female ICR mice were bred and produced one litter at Taconic prior to shipment to the University of Kentucky at 4 months of age. Females were housed 4 mice per cage for a 2 week acclimation period then separated into 2 groups, sedentary or voluntary exercise and were housed singly for the duration of the study. They were placed in light–controlled boxes (Phenome Technologies) in an environmentally-controlled vivarium between 68 – 72^{o}F with unlimited access to food and water under a controlled photoperiod (14 hour light; 10 hour dark). The dams (and sires) were fed Labdiet® Formulab Diet #5008. Maternal body weight and food intake

were measured once a week throughout the breeding portion of the study. The pregnant dams in the exercise cohort had continual access to running wheels throughout pregnancy until 14 days after giving birth at which point wheels were removed to prevent possible harm to growing pups. Litters were culled to 8 or 9 pups approximately 48 hours after birth. Pups were cross-fostered from other litters from the same group if they did not have at least 8 pups. Pups were weaned on postnatal day 21 onto Teklad Global 18% Protein Rodent Diet #2018 and were housed 4 to 5 mice per cage. The offspring themselves did not have access to running wheels during any portion of the study. Subsequent analyses were performed on the offspring from 20 and 18 different sedentary and exercised nursing dams, respectively.

2.3.2. Exercise. Female ICR mice were housed in cages purchased from Phenome Technologies, Inc. (Lincolnshire, IL). The mice had open access to the running wheels that were mounted within each cage. A mechanical counter was used to record wheel rotations to a desktop computer via ClockLab software (Actimetrics). Sedentary female mice were housed in nearly identical cages that did not contain running wheels. Dams exercised at least 7 days prior to and during pregnancy up through 14 days of lactation. Male mice also had the ability to exercise for the 10 day period while they were in the cages for mating purposes. The exercise was completely voluntary; mice were not forced in any way onto wheels. Offspring did not exercise for any portion of the study.

2.3.3. Oral glucose tolerance test (OGTT). At 31 – 32 (6 h fast), 36 – 37 (3 h fast), and 71 – 72 (3 h fast) weeks of age, offspring of both sexes were fasted and then given an oral gavage of D-(+)-glucose (Sigma Aldrich, St. Louis, MO) at 2 g per kg body weight. Blood glucose was measured in tail vein blood using an Ascensia Breeze 2 meter

(Bayer, Mishawaka, IN) just prior to gavage (time zero) and at 15, 30, 60, and 120 minutes after glucose administration.

2.3.4. Intraperitoneal insulin tolerance test (IPITT). At 33 – 34 (female) and 43 – 44 (male) weeks of age, mice were fasted for 3 hours. Female mice were then given an intraperitoneal injection of porcine insulin (Sigma, I-5523) at 0.75 IU per kg body weight. Male mice were given an intraperitoneal insulin injection at 1.25 IU per kg body weight. Blood glucose was measured at 0, 15, 30, 60, 120, and 180 minutes post-injection from tail vein prick. Female mice that became unresponsive to touch as a result of hypoglycaemia were given a glucose injection (n = 2/20 for sedentary and n = 5/18 for exercise). No further blood glucose readings were taken from the glucose-injected mice but pre-glucose injection values were included in the analysis. No male mice became unresponsive during the procedure. Short fasting times were used for GTTs and ITTs instead of an overnight fast because, in mice, this creates a more physiologically relevant state compared to an overnight fast for evaluating insulin action on glucose uptake [164]. Because mice feed at night, an overnight fast can create a physiological state that is more similar to a 24 hour fast, causing distress to the animal [165]. In an overnight fast, compared to a short fast started in the morning, hepatic glycogen stores are depleted [166], and therefore there can be falsely low blood glucose levels that are not an accurate indicator of subjects' long-term glycemic control, as measured by glycosylated haemoglobin [167].

2.3.5. In vitro soleus muscle glucose uptake. Female offspring born to sedentary and exercise dams were fasted for 3 hours at 37 weeks of age (n = 6 per group). Mouse soleus muscles were then quickly excised immediately after euthanasia. The tissue was

immersed in Krebs-Ringer's bicarbonate buffer (117 mM NaCl, 4.7 mM KCl, 24.6 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $CaCl_2$, 2.5 mM $MgSO_4$) bubbled with 95% O_2 and 5% CO_2. Soleus muscles from one leg were used to measure basal glucose uptake and the contra lateral soleus were used to measure the effects of insulin. All muscles were first incubated with Krebs-Ringer's bicarbonate buffer with 2 mM pyruvate for 30 minutes at 37°C. Soleus muscles were then rinsed and incubated with Krebs-Ringer's buffer containing 1 mM 2-deoxy-D-[1,2-^{3}H]glucose (2D-^{3}HGlucose, 1.5 mCi/mL) and 7 mM D-[^{14}C]mannitol (0.45 mCi/mL) for 10 minutes. Insulin (100 nM) was added to the buffer of the insulin group. Finally, soleus muscles were rinsed with plain Krebs-Ringer's buffer. Tendons were removed and soleus muscles were blotted dry with filter paper and digested with 250 µL of 1 N NaOH at 80°C for 10 minutes. After neutralizing with 250 µL of 1 N HCl, 350 µL of sample was added to scintillation liquid for dual label radioactivity counting. Glucose uptake (per gram tissue weight) was then determined after calculating the intracellular and extracellular space as previously described in Chambers et al [168]. Tissues from male offspring were not tested due to time constraints.

2.3.6. In vitro adipose glucose uptake. A separate cohort of female offspring born to sedentary and exercise dams were fasted for 3 hours at 36 weeks of age (n = 4 per group). Glucose uptake was performed as previously described with some modifications [169]. Briefly, parametrial fat was removed immediately after euthanasia and cut into 5 – 10 mg sized explants. Explants were washed three times in 0.5 mL of Krebs-Ringer buffer (KRH buffer; pH 7.4) supplemented with 1% BSA. Explants were incubated in KRH buffer with 1% BSA at 37°C for 30 min to establish basal conditions. Explants

were then transferred to 24-well plate containing 450 uL of KRH buffer with either 0 or 100 nM insulin and incubated for 15 min at 37°C under 5% CO_2. The assay was initiated by the addition of 50 µL of 4.5 mM 2-deoxyglucose containing 0.5 µCi of ^{14}C-2-deoxyglucose (57.7 mCi/mmol; Perkin Elmer, NEC495A00). After 15 min the assay was terminated by transferring tissue explants to ice-cold KRH buffer supplemented with 1% BSA, washed 3 times with the same buffer, blotted, weighed and incubated in 1 N NaOH for 1 h at 65°C. Radioactivity of the NaOH extract was determined by scintillation counting. Glucose uptake is expressed per gram tissue weight. Tissues from male offspring were not tested due to time constraints.

2.3.7. Body composition. At 39 and 68 weeks of age, total fat tissue, lean tissue, and water were measured in live, conscious male and female offspring by nuclear magnetic resonance (EchoMRI; EchoMedical Systems; Houston, TX). The EchoMRI measures adipose tissue, lean mass, and free and total water. Although many tissues contribute to the lean mass output, there are undetectable components such as bone mineral content, hair, and claws.

2.3.8. Statistical Analysis. Repeated measures data were analyzed using repeated measures ANOVA, followed by Student's t-test, unless otherwise indicated. Repeated measures ANOVA analyses were performed using IBM SPSS statistics 20 software. For Figures 2.2C – F and Figures 2.3A – B repeated measurements data were analyzed using mixed models, a generalization of repeated measures ANOVA that uses as many observations as are available for each specimen (rather than requiring complete data from each specimen to be included in the analysis). Mixed models expressed mean scores as functions of both time and group membership, and mean scores at any particular time

point (in Figures 2.2.C, 2.2.D, 2.3.A, 2.3.B) were compared using approximate T tests corresponding to linear contrasts, as implemented in the ESTIMATE statement of PROC MIXED in SAS. Areas under the curve (as calculated by the trapezoidal rule) and mean scores at any particular time point were compared using linear contrasts embedded in the mixed models. Figures 2.3.C – D were also analyzed by mixed models with treatment replacing time as the within-subjects factor. Version 9.2 of SAS software was used for mixed model analyses. Non-repeated measurement data were analyzed by Student's t-test (Figure 2.4.D) or Mann-Whitney rank sum test when the data failed the Shapiro-Wilk normality test (Figures 2.4.A – C). These analyses were completed using SigmaPlot 11.0 software.

2.4. Results

2.4.1. Maternal body weight and food intake. After 7 days in sedentary or exercise groups, female ICR mice were bred to male mice for 10 days to ensure a maximum number of pregnancies. During breeding, males also had access to the running wheel. Depending on day of conception, exercise dams had running wheel access for a minimum of 7 and maximum of 17 days prior to conception. Eleven out of 18 exercise females conceived on the first night of mating while 14 out of 18 females conceived within the first two days of mating. Only 4 exercise females included in the study conceived after the first 2 days of mating meaning that the majority of female mice ran only 7 to 8 days prior to conception. Offspring were weaned at 21 days of age and were placed into cages that did not contain running wheels. Further details are provided in the methods.

Running data (Figure 2.1.A) were matched so that day 29 correlates to delivery day regardless of day of conception in relation to being placed on the running wheels.

Mean running distance per day increased over the first 7 days as the female mice grew accustomed to the running wheels (Figure 2.1.A). From days 8 – 17, a male mouse was also present in the cage for breeding, and running distance increased most likely due to male running. Mean running distance decreased as the female mice approached delivery on day 29 and was maintained at lower levels during lactation. Average running distance during nursing was significantly lower than average running distance prior to pregnancy ($P < 0.001$). Figures 2.1.B and 2.1.C show maternal body weight and food intake. These data were not matched for day of delivery because the values were only measured once per week. There were no significant differences in body weight due to maternal exercise prior to or during pregnancy and lactation (Figure 2.1.C). Figure 2.1.C shows the weekly food intake values divided by 7 as a measure of daily food intake. To determine food intake while males were in the cage, food intake was divided by 2 to account for two mice in the cage. There was a significant increase in food intake prior to and during mating and pregnancy ($P < 0.001$; repeated measures ANOVA) in the running dams at weeks 2, 3, and 4 ($P = 0.015$, $P < 0.001$, and $P < 0.001$, respectively) (Figure 2.1.C). There were no differences in food intake during nursing (weeks 5-7). Maternal running during pregnancy did not significantly affect pregnancy rate or litter size (Table 2.1). There were also no significant differences in mean pup body weight per litter on postnatal days (PND) 7, 14, or 21 (Table 1).

2.4.2. Glucose and insulin tolerance in offspring. The offspring born to sedentary and exercised dams (shown in Figures 2.1.A – C) were then used for further analyses. There were no significant differences in body weight in female or male offspring born to sedentary or exercised dams from 3 – 76 weeks of age (Figure 2.2.A, B). At 31 – 32

weeks of age, both female and male offspring born to sedentary and exercised dams were fasted and given an oral dose of glucose (2 g per kg body weight). Circulating blood glucose values were measured after the oral glucose challenge (data not shown). Overall glucose disposal was significantly improved in female and male offspring born to exercised dams compared to those from sedentary dams ($P = 0.023$ and $P = 0.005$, respectively). Oral glucose tolerance was again measured in the offspring at 36 – 37 weeks of age. Consistent with earlier results, overall glucose disposal was improved in female and male offspring born to exercised dams ($P = 0.004$ and $P = 0.011$, respectively). The female (Figure 2.2.C) and male (Figure 2.2.D) offspring born to exercised dams had significantly enhanced glucose disposal after 30 min compared to offspring from sedentary dams ($P < 0.001$ and $P = 0.010$, respectively). In addition, male offspring born to exercised dams also had significantly lower glucose levels 60 min after the glucose dose ($P = 0.019$). AUC was significantly decreased in female (Figure 2.2.E) offspring from exercised dams compared to those from sedentary dams ($P = 0.009$) with a similar effect observed in male (Figure 2.2.F) offspring born to exercised dams ($P = 0.026$). Oral glucose tolerance was tested for a final time in offspring at 71 – 72 weeks of age. Again, overall glucose tolerance was significantly improved in female ($P = 0.032$) and male ($P = 0.039$) offspring from exercised dams compared to those born to sedentary dams (Figure 2.3.A – D).

I then focused on the mechanism of enhanced glucose disposal in female and male offspring born to exercised dams by performing an insulin tolerance test. Blood glucose levels drop over time in response to the exogenous insulin and the rate of disposal provides an index of insulin sensitivity. At 33 – 34 weeks of age, mature female

offspring were fasted and insulin at 0.75 international units (IU) per kg body weight was injected. Offspring from both sedentary and exercised dams had consequential lowering of blood glucose in response to insulin. Offspring born to exercised dams had significantly improved overall glucose disposal ($P = 0.024$) compared to those from sedentary dams, with significantly enhanced disposal at 15, 30, and 60 min post-injection ($P = 0.005$, $P = 0.017$, and $P = 0.035$, respectively) suggesting that these mice were more insulin sensitive (Figure 2.4.A). Mature male offspring at 43 – 44 weeks of age were fasted and injected with a higher dose of insulin to ensure glucose uptake (1.25 IU per kg body weight). Offspring born to exercised dams had overall improved glucose disposal ($P = 0.003$) with significantly enhanced disposal at 15, 30, 60, and 120 minutes post injection ($P = 0.001$, $P = 0.010$, $P = 0.036$, and $P = 0.025$, respectively) compared to offspring from sedentary dams (Figure 2.4.B). These data confirmed that middle-aged offspring born to exercised dams had improved insulin sensitivity compared to offspring born to sedentary dams.

2.4.3. 2-DG uptake in offspring muscle and adipose. Skeletal muscle and adipose tissue are responsible for the majority of insulin-sensitive glucose uptake *in vivo* [170, 171]. I next set out to determine which tissues were responsible for the enhanced insulin sensitivity observed in the mature female offspring born to exercised dams. Therefore, soleus muscle was isolated and 2-deoxyglucose (2-DG) uptake was measured in the presence and absence of 100 nM insulin *in vitro* (Figure 2.4.C). Muscle collected from offspring born to exercised dams trended toward increased 2-DG uptake compared to muscle from sedentary dam offspring in the basal state ($P = 0.151$). Muscle isolated from offspring born to both sedentary and exercised dams showed significant increases in 2-

DG uptake in response to insulin compared to their basal uptake levels ($P < 0.001$ in both comparisons). Importantly, the muscles from exercised dam offspring exhibited significantly greater glucose uptake in response to the insulin dose when compared to muscle from sedentary dam offspring ($P = 0.007$). A similar design was used to measure 2-DG uptake into isolated adipose in a separate cohort of female offspring born to sedentary and exercised dams (Figure 2.4.D). The addition of 100 nM insulin to adipose explants isolated from sedentary offspring did not significantly affect 2-DG uptake ($P = 0.225$) which suggests a level of insulin resistance. However, adipose explants collected from offspring born to exercised dams were more sensitive and had significantly increased 2-DG uptake in response to insulin compared to their basal state ($P = 0.002$), as well as compared to the insulin-treated explants isolated from offspring born to sedentary dams ($P = 0.008$). Both skeletal muscle and adipose isolated from offspring born to exercised dams exhibited enhanced insulin sensitivity. These findings suggest that peripheral tissue insulin sensitivity is the mechanism largely responsible for enhanced glucose disposal following an oral glucose challenge.

2.4.4. Offspring body composition. Body composition was analyzed by quantitative magnetic resonance imaging (EchoMRI, Echo Medical Systems) in mature 39 week old female and male offspring. Fat and lean mass are expressed as a percent of body weight. There were no differences observed in female offspring fat (Figure 2.5.A) or lean content (Figure 2.5.C). There were also no differences in % total water between female offspring from exercised (41.1 ± 1.2) and sedentary dams (42.3 ± 1.2). There were, however, significant differences observed in male offspring body composition. Figure 2.5.B shows that male offspring from exercised dams had significantly reduced fat

content compared to male offspring from sedentary dams (P = 0.004). In addition, Figure 2.5.D shows that male offspring born to exercised dams also had significantly increased lean tissue composition compared to male offspring from sedentary dams (P < 0.001). Male offspring from exercised dams also had significantly increased % total water (53.9 ± 0.5) compared to male offspring from sedentary dams (51.2 ± 0.8) (P = 0.024). Offspring body composition was again analyzed at 68 weeks of age. Consistent with earlier analysis, there were no significant differences in female offspring fat or lean mass (Figure 2.6.A and C). At this age, however, female offspring from exercised dams had significantly increased % total water (P = 0.047) compared to female offspring from sedentary dams (data not shown). Again, there were significant differences in male body composition. Males from exercised dams had significantly reduced % fat content (P = 0.023) and significantly increased % lean tissue (P = 0.040) compared to males from sedentary dams (Figure 2.6.B and D). There were, however, no differences in male offspring % total water at the older age (data not shown). It appears that changes in body composition were not required for glucose tolerance improvements observed in the female offspring born to exercised dams but may play a role in changes in glucose disposal observed in male offspring.

2.5. Discussion

I have found that maternal exercise prior to and during pregnancy and lactation can improve long-term metabolic outcomes of offspring. Glucose disposal was significantly enhanced in both female and male offspring born to exercised dams compared to those from sedentary dams. In addition, male and female offspring were found to be more sensitive to exogenous insulin. In female offspring, I also found that

excised skeletal muscle and fat pads from offspring born to exercised dams were more sensitive to *in vitro* insulin stimulation compared to those from sedentary dams. Insulin stimulated glucose uptake is greater in type I fibres versus types IIa or IIb [172], and since the mouse soleus is made up of primarily type 1 fibres [173, 174], it has a higher glucose uptake in response to insulin stimulation compared to other skeletal muscles. Therefore, soleus muscle was used to investigate insulin stimulated glucose uptake. Exercise during pregnancy has been previously shown to result in acute and long-term reduction in glucose that reaches the fetus [90, 175]. Changes in glucose availability during development could have long-lasting effects in that mature offspring become more sensitive to glucose changes. Consistent with our findings, Vanheest and Rodgers used moderate speed treadmill running (20 m/min) in streptozotocin-induced diabetic pregnant rats and found their offspring had improved glucose tolerance compared to offspring born to sedentary diabetic and non-diabetic dams [141]. Although the connection is somewhat limited, this does suggest that the improvement of glucose regulation by maternal exercise will be observed in multiple species.

Previous studies by other laboratories have looked at different maternal and offspring outcomes resulting from exercise during pregnancy. In human studies, maternal exercise effects have mainly focused on pregnancies complicated by gestational diabetes. In a study of women with gestational diabetes it was found that resistance exercise was effective in lowering the number of women who needed insulin therapy in order to maintain normal blood glucose levels [125]. In a study of healthy pregnant women, mild physical activity was found to lower the risk of developing gestational diabetes [119]. In contrast, a recent randomized control trial in women of normal body

weight showed that exercise during pregnancy did not decrease the prevalence of gestational diabetes compared to sedentary controls [176]. Few human studies have investigated offspring effects of maternal exercise during pregnancy. These studies have focused mainly on birth weights, body composition, and cognitive outcomes. In several studies exercise during pregnancy reduced birth weights [129, 135]. Lower birth weights were found to coincide with reduced fat mass at birth as well as reduced cord serum concentrations of growth hormones. At 5 years old, children from mothers who exercised during pregnancy were shown to have improved scores on general intelligence and oral language skills tests [135]. Animal studies have detected neurological changes in offspring as a result of exercise during pregnancy. Maternal swimming in rats was found to increase hippocampal neurogenesis in rat pups which was then associated with improved memory in the pups [136]. A similar study found that, in mice, maternal running during pregnancy and nursing resulted in a 40% increase in total granule cells in the offspring hippocampus [160]. Recently, Herring et al. showed that short-term exercise during pregnancy was able to reduce Alzheimer pathology in offspring in a transgenic mouse model that is predisposed to the disease [140]. Exercise during pregnancy can clearly affect many maternal and offspring outcomes.

Finding the mechanisms behind the observed metabolic changes in offspring due to perinatal exercise will be an important focus of future studies. This will involve looking at all insulin sensitive tissues in the offspring, including other skeletal muscles and adipose depots since only the soleus and abdominal adipose were evaluated for insulin stimulated glucose uptake in the current study. This was beyond the scope of the current study given the number of possible epigenetic and sex-specific changes that are

associated with developmental programming models. Developmental programming of metabolism has been observed in human and animal models of intrauterine growth restriction (IUGR). IUGR in humans and animals is known to decrease glucose disposal and insulin sensitivity in mature offspring [33, 177]. Although the link between IUGR and decreases in insulin sensitivity has been known for decades, not until recently have researchers begun to investigate the mechanisms behind these metabolic derangements. Studies have found decreases in the expression and insulin-stimulated activation of many proteins involved in the insulin signalling pathway that correlate with decreases in whole body insulin sensitivity [178, 179]. Further complicating the elucidation of the mechanism is that many of the metabolic changes observed in developmental programming models due not occur until offspring are aged. Because of this it is unclear whether offspring from sedentary dams develop a dysfunction in insulin sensitivity as they age or if offspring from exercised dams are protected from a natural, age-related decline in insulin sensitivity. It is important to note that in the current study, glucose tolerance was first tested in 3 month old offspring, but differences were not detected until the offspring reached 7 months of age.

The effects of developmental programming on long-term offspring health have been shown to be sex-specific in several studies [180, 181]. A similar observation was made in this study when significant effects on the percentage of fat and lean tissue in response to maternal exercise were found only in male offspring despite an improvement in glucose tolerance in both females and males. Perhaps more important than the change in body composition in male offspring was the lack of changes in fat and lean tissue in female offspring, highlighting improved insulin sensitivity as the potential mechanism

contributing to enhanced glucose uptake in these offspring. It will be necessary to determine whether increased lean tissue plays a unique role in improved glucose uptake in male offspring to elucidate divergent mechanisms between males and females.

The findings from this study are an important step in discovering potential beneficial effects of maternal exercise for offspring. Future studies will look at the mechanism through which maternal exercise improves offspring glucose homeostasis; including investigating changes in milk content and production during lactation. Experiments can be designed to focus on periods of time before, during, and/or after pregnancy when maternal exercise is essential for beneficial effects in offspring. Further, cross-fostering strategies can be used to control multiple factors.

One recent paper showed that high-fat fed male rats produced female offspring with impaired β-cell function [181], and another showed that low protein diet consumption by male mice prior to mating affected metabolic gene expression in offspring [182]. Therefore, it will be necessary to determine whether paternal exercise prior to fertilization influences observed offspring outcomes in Figures 2 – 4. One possible way to control for paternal influence would be to use artificial insemination. It is also important to address that the presence of a running wheel in the cage may serve as enrichment for the exercising dams. Although no studies have been conducted to look at maternal environmental enrichment and its effects on the metabolic health of offspring, a few animal studies have shown that it can improve maternal and offspring cognitive functions [183-185]. It may be necessary in future studies to control for this as a possible confounding factor. Future studies to control for this would include controlled exercised paradigms in which there is no running wheel in the home cage. Rather, mice would be

removed from the home cage daily and would exercise for a predetermined amount of time using any one of a number of exercise paradigms. This design would also control for the vast amount of running seen in the voluntary exercise paradigm.

Much work is left to be done, but our studies provide new information on the positive impact that perinatal exercise can have on offspring metabolic health in a mouse model. Such an intervention provides a realistic mechanism to improve insulin sensitivity in the next generation and positively impact insulin-resistant states.

2.6. Figures

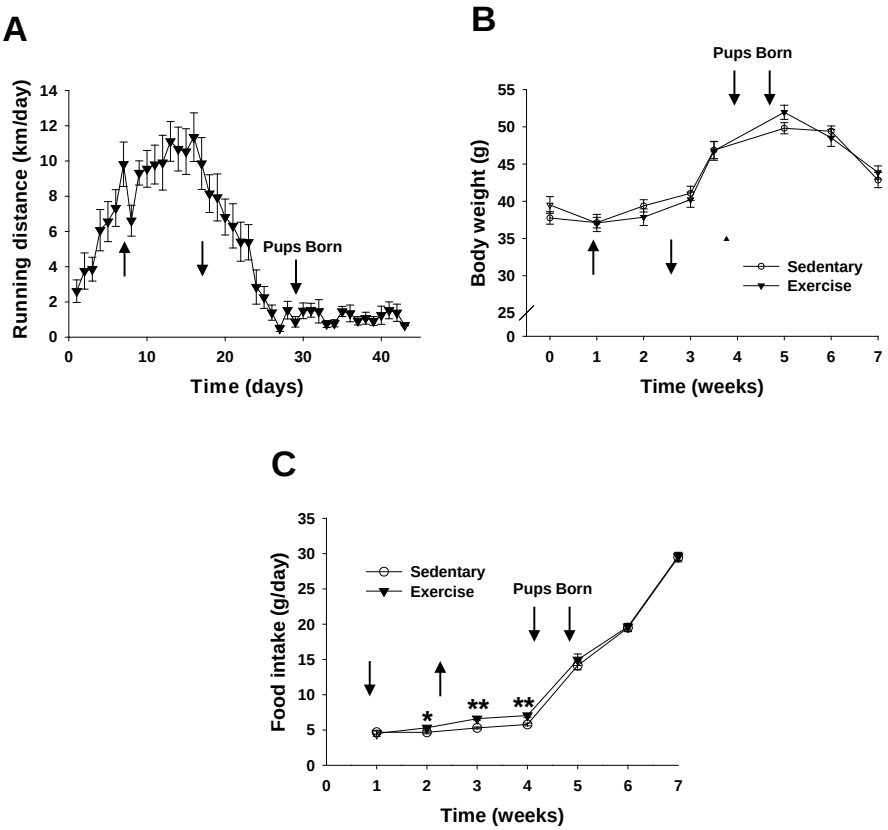


Figure 2.1. Maternal exercise affected food intake but not body weight in female mice that were set up in breeding scheme. (A) Maternal mean run distance per day. Arrows depict when males were present in the cages for breeding and when pups were born. Exercise decreased as dams approached delivery which is designated as day 29. Data for B and C were not matched for day of delivery. Arrows indicate when males were present for breeding and the range of days over which pups were born. (B) There were no differences in body weight between sedentary and exercised dams. There was however, a significant increase in food intake in pregnant exercise dams compared to

sedentary dams (C). n = 18 for *A*; n = 20 for sedentary and n = 18 for exercise for *B* and *C*. Error bars indicate s.e.m. Data in A – C collected and analyzed by L.G.C.

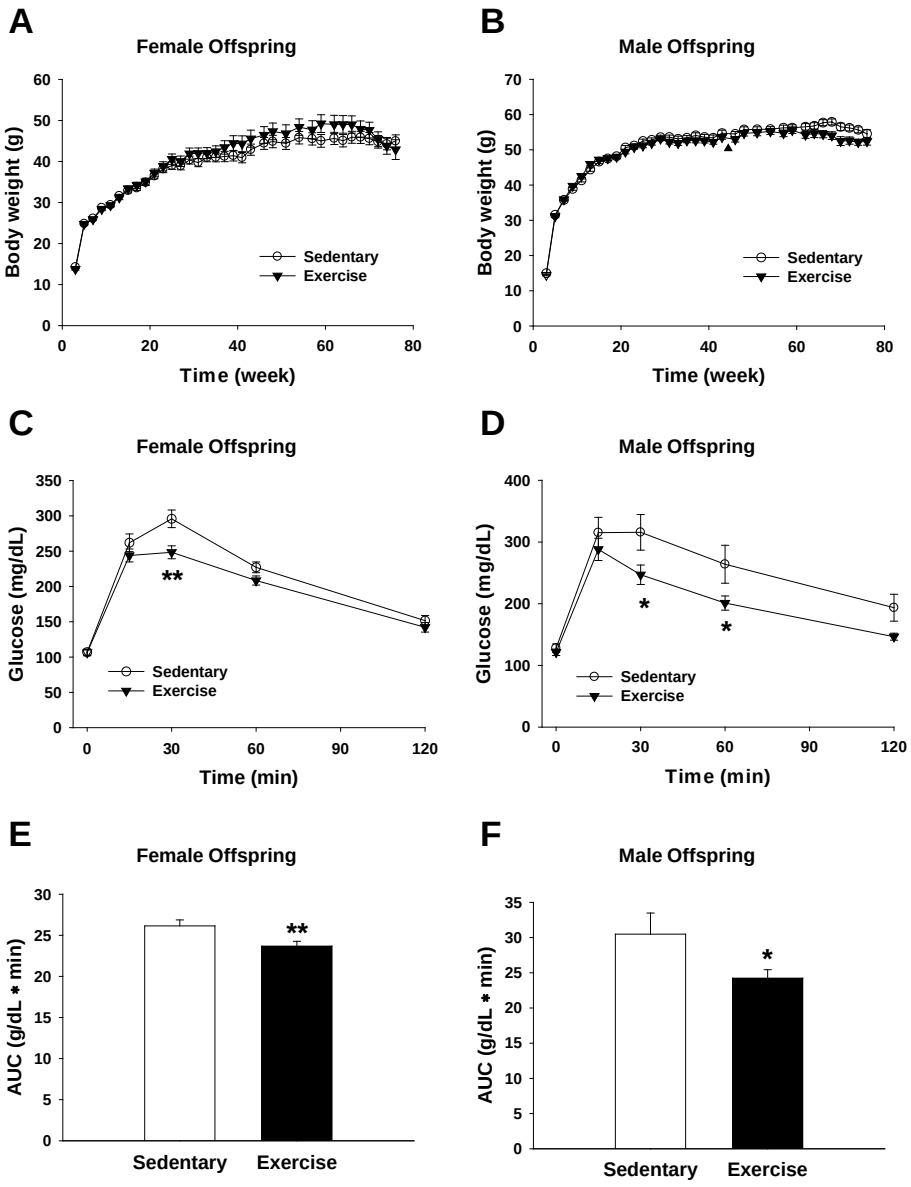
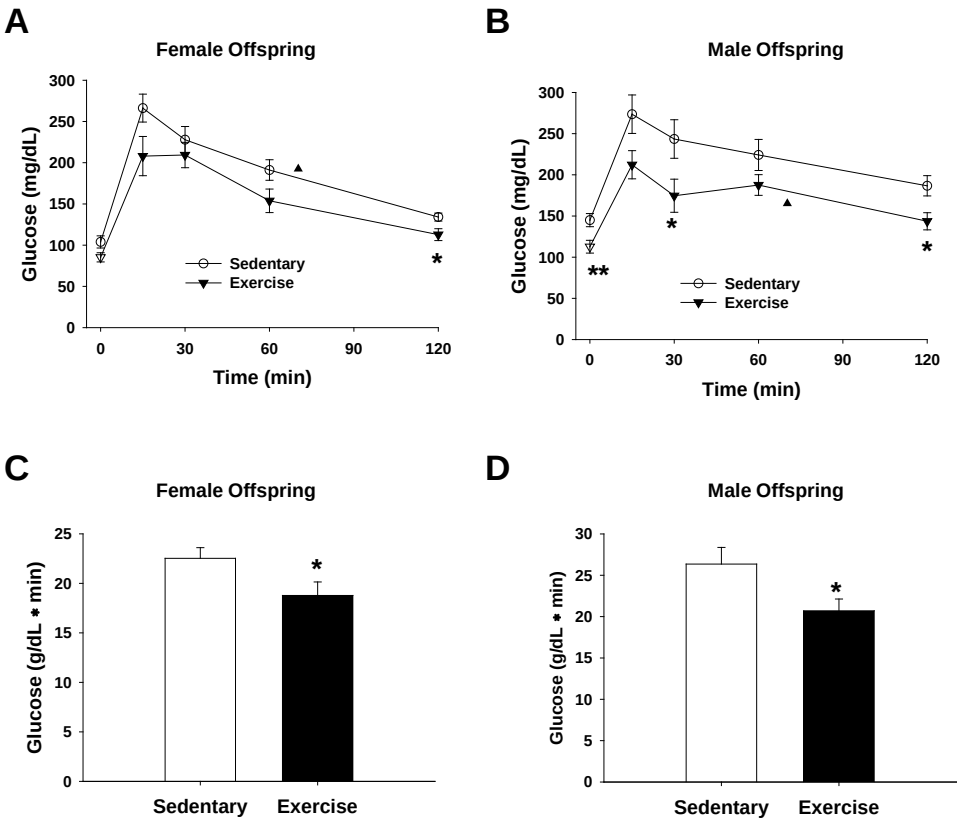
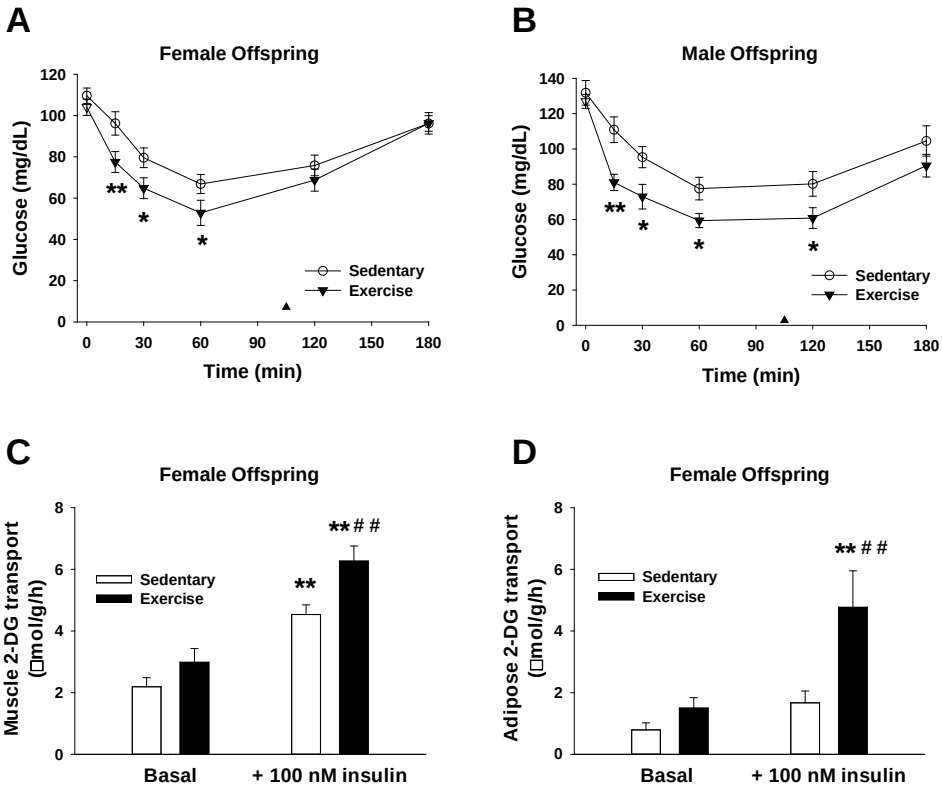


Figure 2.2. Thirty-seven week old female and male ICR offspring born to exercised dams had improved glucose disposal independent of body weight differences. There were no significant differences observed in female (A) or male (B) offspring body weight. Following an oral glucose challenge, blood glucose levels were significantly

reduced 30 min after glucose administration in female offspring (C) and 30 and 60 min after glucose administration in male offspring (D) from exercised dams compared to those from sedentary dams. AUC of circulating blood glucose was also significantly reduced in female (E) and male (F) offspring from exercised dams. *, $P < 0.05$; **, $P < 0.01$ compared to offspring born to sedentary dams; n = 85 for sedentary and n = 57 for exercise in A; n = 72 for sedentary and n = 84 for exercise in B; Sample size in A and B represent the number of female and male offspring that were originally weaned in week 3; n = 19 for sedentary and n = 18 for exercise in C and E; n = 18 for sedentary and n = 18 for exercise in D and F. Error bars indicate s.e.m. Data in A – F collected and analyzed by L.G.C.


A
Female Offspring
Glucose (mg/dL)
Time (min)
Sedentary
Exercise
B
Male Offspring
Glucose (mg/dL)
Time (min)
Sedentary
Exercise
C
Female Offspring
Glucose (g/dL * min)
Sedentary
Exercise
D
Male Offspring
Glucose (g/dL * min)
Sedentary
Exercise


A
Female Offspring
Glucose (mg/dL)
Time (min)
Sedentary
Exercise
B
Male Offspring
Glucose (mg/dL)
Time (min)
Sedentary
Exercise
C
Female Offspring
Muscle 2-DG transport (□mol/g/h)
Sedentary
Exercise
Basal
+ 100 nM insulin
D
Female Offspring
Adipose 2-DG transport (□mol/g/h)
Sedentary
Exercise
Basal
+ 100 nM insulin

uptake. In addition, muscle from offspring born to exercised dams showed significantly increased 2-DG uptake in response to insulin compared to offspring from sedentary dams. (D) Adipose from exercise dam offspring show significantly increased 2-DG uptake in response to insulin treatment compared to basal uptake and compared to insulin stimulated uptake in adipose from offspring born to sedentary dams. *, $P < 0.05$ and **, $P < 0.01$ compared to offspring born to sedentary dams (A, B) or insulin-treated compared to basal sedentary or exercise control (C, D); # #, $P < 0.01$ compared to insulin-treated sedentary; n = 20 for sedentary and n = 18 for exercise in A; n = 16 for sedentary and n = 14 for exercise in B; n = 6 for sedentary and exercise in C. n = 4 for sedentary and exercise in D. Error bars indicate s.e.m. Data in A and B collected and analyzed by L.G.C. Data in C and D collected with help from P.S. and M.G.C.; data analyzed by L.G.C.

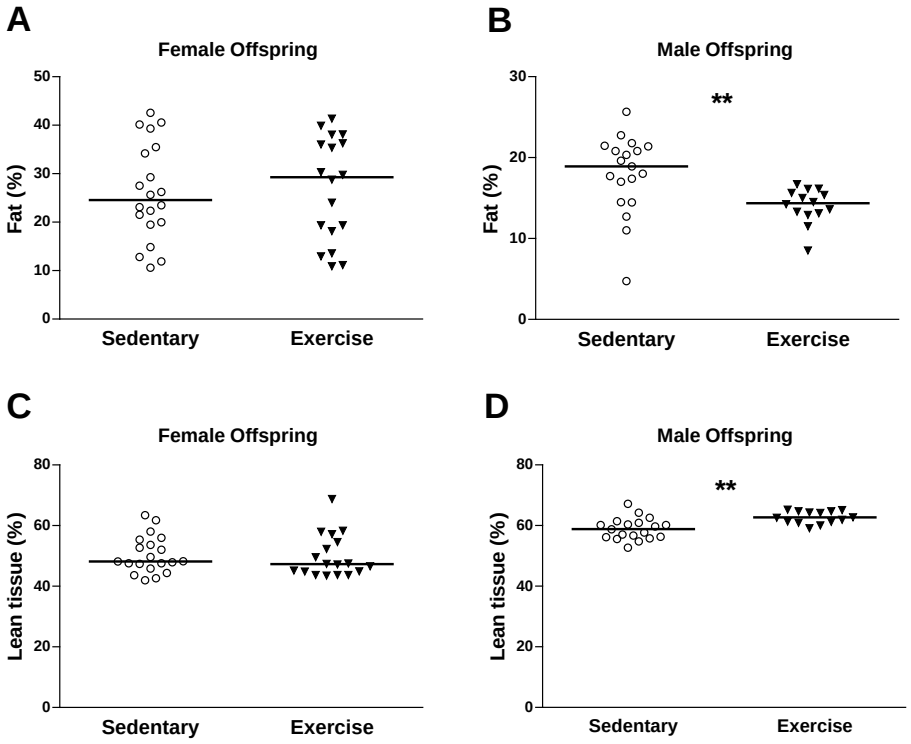
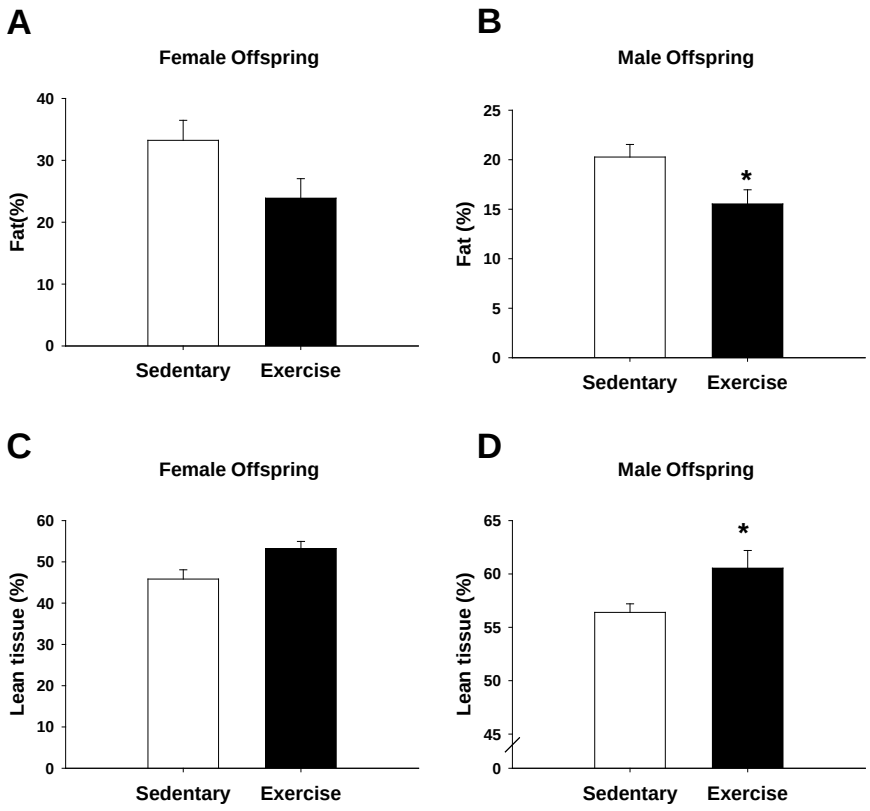


Figure 2.5. Maternal exercise significantly impacts body composition in thirty-nine week old male but not female offspring. Total body fat mass and lean mass were analyzed in mature female and male offspring using EchoMRI. Fat and lean mass results are shown as a percentage of body weight. There was no difference observed in female offspring percent fat (A) but male offspring born to exercised dams had significantly lower percent fat (B) compared to offspring from sedentary dams. Female offspring lean tissue percent was unchanged (C) while male lean tissue percent was significantly increased in offspring born to exercised dams compared to those born to sedentary dams. **, $P < 0.01$ compared to offspring born to sedentary dams; n = 20 for sedentary and n = 18 for exercise for A and C; n = 19 for sedentary and n = 14 for exercise for B and D.

Data were not normally distributed for panels A – C; horizontal line indicates median. Data were normally distributed for D; horizontal line indicates mean. Data in A – D collected and analyzed by L.G.C.


A
Female Offspring
Fat(%)
40
30
20
10
0
Sedentary
Exercise
B
Male Offspring
Fat (%)
25
20
15
10
5
0
*
Sedentary
Exercise
C
Female Offspring
Lean tissue (%)
60
50
40
30
20
10
0
Sedentary
Exercise
D
Male Offspring
Lean tissue (%)
65
60
55
50
45
0
*
Sedentary
Exercise

**, $P < 0.01$ compared to offspring born to sedentary dams; n = 10 for sedentary and n = 10 for exercise for A – D. Data in A – D collected and analyzed by L.G.C.

Table 2.1 | Pregnancy rates, litter size, and pup body weight.

Parameter	Sedentary (s.e.m.)	Exercise (s.e.m.)
Pregnancy rate	26/28	25/28
Subsequent analyses on litters (n)	20	18
Pups per litter	11.38 (0.71)	10.40 (0.74)
PND 7 pup body weight (g)*	5.67 (0.09)	5.42 (0.15)
PND 14 pup body weight (g)*	9.17 (0.20)	8.96 (0.25)
PND 21 pup body weight (g)*	14.55 (0.19)	14.14 (0.40)

*Pup body weights were calculated by averaging pup body weights per litter. Each litter average was then used to calculate the mean pup body weight per group.

Data collected and analyzed by L.G.C.

CHAPTER 3

MATERNAL EXERCISE IMPROVES INSULIN SENSITVITY IN MATURE OFFSPRING

3.1. Abstract

Recent findings have shown the intrauterine environment can negatively influence long-term insulin sensitivity in the offspring. I set out to test maternal voluntary exercise as an intervention in order to improve offspring insulin sensitivity and glucose homeostasis. Female Sprague Dawley rats were split into sedentary and exercise groups with the exercise group having voluntary access to a running wheel in the cage one week prior to mating and during mating, pregnancy, and nursing. Female offspring were weaned into sedentary cages. Glucose tolerance tests and hyperinsulinemic–euglycemic clamp were performed in adult offspring to evaluate glucose regulation and insulin sensitivity. Adult female offspring born to exercised dams had enhanced glucose disposal during glucose tolerance testing ($P < 0.05$) as well as increased glucose infusion rates ($P < 0.01$) and whole body glucose turnover rates ($P < 0.05$) during hyperinsulinemic–euglycemic clamp testing compared to offspring from sedentary dams. Offspring from exercised dams also had decreased insulin levels ($P < 0.01$) and hepatic glucose production ($P < 0.05$) during the clamp procedure compared to offspring born to sedentary dams. Offspring from exercised dams had increased glucose uptake in skeletal muscle ($P < 0.05$) and decreased heart glucose uptake ($P < 0.01$) compared to offspring from sedentary dams in response to insulin infusion during the clamp procedure. In conclusion, exercise during pregnancy enhances offspring insulin sensitivity and

improves offspring glucose homeostasis. This can decrease offspring susceptibility to insulin resistant related disease such as type 2 diabetes mellitus. Maternal exercise could be an easy, short–term, non–pharmacological method of preventing disease in future generations.

3.2. Introduction

Type 2 diabetes mellitus T2DM is characterized by peripheral tissue insulin resistance and dysfunctional insulin secretion caused by prolonged hyperglycemia. Genetic factors are also thought to play a role in susceptibility to the disease. There have also been many epidemiological studies that have shown a link between low birth weight and higher incidences of T2DM in adulthood [186, 187]. David Barker showed an association between prenatal environment and adulthood disease [18]. Hales and Barker coined the term 'thrifty phenotype' to describe the phenomenon in which low birth weight babies, exposed to low maternal nutrient intake during gestation, have higher incidences of T2DM and cardiovascular disease later in life [32]. They hypothesized that development is plastic and can shift depending on environmental cues received during gestation. In the case of poor maternal nutrient intake, the fetus receives cues that the environment outside the womb is low in nutrient availability, thus development shifts to adapt to survive in such an environment. When the postnatal environment, however, is rich in nutrient sources, contrary to what was predicted, the offspring are predisposed to diseases associated with excess nutrient intake such as cardiovascular disease and T2DM.

Many animal models have been created to study the mechanisms that cause late-life outcomes in low birth weight offspring. Maternal nutrient restriction and uterine artery ligation have been used to promote intrauterine growth restriction (IUGR) in the fetus [33, 49]. These studies have found impaired glucose regulation and insulin sensitivity in adult offspring exposed to IUGR [33]. Other studies have shown that offspring exposed to IUGR have decreased expression and activation of key insulin

signaling pathway proteins in skeletal muscle and adipose tissue suggesting a mechanism for the observed insulin insensitivity [40, 178].

Regular exercise is well known to decrease susceptibility to T2DM by enhancing insulin sensitivity and promoting non–insulin stimulated glucose uptake [102, 188]. In animal models, exercise reversed the negative metabolic impact of IUGR by improving offspring insulin sensitivity [189]. Still, given the numerous benefits of physical activity, seemingly few people have the time or motivation to maintain an active lifestyle. Pregnant women, however, might be more inclined to eat a healthy diet and remain physically active if these things could positively affect the long–term health of their developing child by decreasing susceptibility to T2DM. Physical activity during pregnancy is already known to have many maternal benefits including weight and body composition control and improved cardiovascular health [190]. Exercise during pregnancy has also been shown to have beneficial effects for offspring in humans. Maternal aerobic exercise has been found to decrease cord growth hormone levels and infant body mass index [129]. Other benefits of exercise during pregnancy include decreased fat mass at birth and in childhood as well as improved cognitive characteristics [135]. Changes in adiposity are thought, in part, to be the result of small decreases in fetal nutrient availability due to intermittent reduction in uterine blood flow during times of physical activity [90]. In animal studies, offspring of exercised dams show improved neurogenesis and memory [136]. Our laboratory has recently shown that perinatal exercise in chow fed mice enhances insulin sensitivity in adult male and female offspring [191]. One study also found that exercise during pregnancy in streptozotocin-induced diabetic rats affected 28 day old offspring glucose regulation compared to offspring from

sedentary diabetic rats [141]. However, no studies in rats have researched the long–term effects of exercise during healthy pregnancy on offspring glucose homeostasis and insulin sensitivity.

It has been well established in animal models and in humans that the intrauterine environment can negatively impact fetal development and future metabolic health. What needs further exploration is how certain maternal behaviors, such as exercise, can positively influence the perinatal environment and improve offspring long–term metabolic health outcomes. Maternal interventions during pregnancy are a novel and unique way of targeting and preventing T2DM and other diseases in future generations. Therefore the objective of the current study was to investigate the effects of maternal exercise prior to and during pregnancy and nursing on offspring insulin sensitivity using a rat model. The hypothesis for the current study was that adult offspring born to exercising dams would have enhanced insulin sensitivity compared to those offspring born to sedentary control dams.

3.3. Materials and Methods

3.3.1. Animals and diets for breeding scheme to follow offspring. Forty 12 week old female CD/IGS Sprague Dawley (SD) rats (Charles River Laboratories International, Wilmington, MA) arrived at the University of Kentucky animal facility and were housed 2 per cage for a one week acclimation period with unlimited access to food and water. The animal facility was on a 12 hour light/dark cycle and maintained at a temperature of 68 – 72^0F. After the acclimation period, females were split into sedentary and exercise cohorts, ensuring no initial weight differences between the two groups (n = 20 per group). Females were then single housed and placed on Formulab Diet #5008 (Labdiet®,

Cincinnati, OH). Female body weight and food intake were measured twice a week during breeding, pregnancy, and nursing. When males were present in the cage, food intake was divided by 2 to account for food intake of both animals. After 7-10 days in sedentary or exercise cohorts, 10 week old male SD rats were housed with the female rats for 10 days for mating. Females in the exercise group had unlimited access to a running wheel (Nalgene® Running Wheel) mounted in the cage prior to and during mating, throughout pregnancy and up through postnatal day 12. A magnetic counter recorded number of wheel turns onto a computer (VitalView Data Acquisition System, Mini Mitter Company Inc., Bend, OR).Wheels were removed on postnatal day 12 to prevent the pups from running and/or being injured. One exercise dam nested her litter too close to the wheel and this litter was removed from the study. Sedentary females were housed in identical cages without a running wheel. Males bred with exercising females had access to the running wheel during the breeding portion of the study. Although wheel turns due to male versus female running were not measured directly, a study comparing male and female SD rats found that females run significantly more than males [192]. On postnatal day 1, litters were culled to 10 pups per litter, ensuring 5 males and 5 females per litter when possible. On postnatal days 14 and 21 one male and one female from each litter was culled for serum and tissue collection. On postnatal day 21, remaining female pups were weaned into sedentary cages and housed 2 rats per cage and fed Teklad Global 18% Protein Rodent Diet #2018 (Harlan, Indianapolis, IN). Offspring did not have access to a running wheel for any portion of the study. Analyses in offspring were conducted such that only one female offspring per dam was included. Rats were shipped to the University of Michigan at 14 months of age where they were housed singly for the remainder of the

study. A previous study from our laboratory using mice found that perinatal exercise improves glucose disposal in offspring of both sexes [191]. Therefore, I chose to monitor insulin sensitivity in female rat offspring for this study as a way to limit costs.

3.3.2. Glucose tolerance test (GTT). An intraperitoneal GTT (IPGTT) and an oral GTT (OGTT) were performed in female offspring at 10 and 15 months of age, respectively. Rats were fasted overnight for 16 hours and given an injection or oral gavage of glucose at 2 g/kg body weight. Blood glucose readings were taken via tail prick prior to injection (minute 0) and 15, 30, 60, and 120 minutes post glucose administration using an Accu-Chek glucometer (Roche, Germany).

3.3.4. Body composition. Body composition was analyzed in 15 month old female offspring using a nuclear magnetic resonance-based analyzer (Minispec LF90II, Bruker Optics, TX, USA). Body fat, lean mass, and free water were measured.

3.3.5. Hyperinsulinemic-euglyemic clamp. The offspring that remained from the cohort used for glucose tolerance testing at 15 months of age underwent hyperinsulinemic-euglyemic clamping to test whole body insulin sensitivity at 17 months of age. Hyperinsulinemic-euglyemic clamp was performed by the University of Michigan Animal Phenotyping Core on conscious, unrestrained rats using the protocol adapted from the Vanderbilt Mouse Metabolic Phenotyping Center with some modifications (21). Clamp procedures could not be carried out at the University of Kentucky due to lack of funds and time to purchase and set up clamp equipment. The right jugular vein and carotid artery of the rats were surgically catheterized a week prior to the clamp and animals that had healthy appearance, normal activity, and body weight regained to or above 90% of their pre-surgery levels were used for the study. After an

overnight fast for 16 hours, rats underwent the clamp procedure consisting of a 90 min equilibration period, followed by a 120 min experimental period (t = 0 to 120 min). Insulin was infused at 4.0 mU/kg/min and euglycemia (120~130 mg/dL) was maintained during the clamp by infusing 50% [3-^{3}H]glucose at variable rates. To estimate insulin-stimulated glucose uptake in individual tissues, a bolus injection of [1-^{14}C]-2-deoxyglucose ([^{14}C]2-DG, PerkinElmer) (30 μCi) was given at t = 78 min while continuously maintaining the hyperinsulinemic-euglycemic steady-state. At the end of the experiment, animals were anesthetized with an intravenous infusion of sodium pentobarbital and tissues were collected and immediately frozen in liquid nitrogen for later analysis of tissue [^{14}C] radioactivity. Tissue [^{14}C]2-DG levels, plasma radioactivity of [^{14}C]2-DG and [3-^{3}H]glucose, and plasma insulin levels were analyzed as previously described in [193]. The body weight of one rat in each group was a significantly outlier compared to the rest of the group (body weight was higher, $P < 0.01$ by the Grubbs' test) and the data was removed for future analyses.

3.3.6. Timed pregnancy. Timed mating was performed in order to evaluate the effects of maternal exercise on the pregnant dams. The design was similar to the earlier experiment with differences highlighted below. After the acclimation period, female rats were split into sedentary and exercise cohorts, ensuring no initial weight differences between the two groups (n = 25 for sedentary and n = 20 for exercise). After 7-10 days in the sedentary or exercise cohorts, females were mated with 10 week old male SD rats. For timed mating, females were removed from their home cage and placed with a male in a wire cage to allow for plug detection. Females were removed from the home cage over multiple days (maximum of 4 days) until a plug was found. If there was no plug after 4

nights of breeding, the female was removed from the study. Only pregnant female rats that had plugs detected were included for further analyses, and the date the plug was found was designated gestation day 0. 18/25 sedentary and 14/20 exercise females became pregnant however the pregnant rats for which plugs were not detected were removed from the study.

On gestation day 14, rats were fasted overnight for 16 hours and a glucose tolerance test was performed. For the fasting period, exercise females remained in their home cage with running wheel access until 1 hour prior to testing. Exercise females were then placed in standard cages throughout the testing period. Blood glucose readings were taken via tail prick prior to administration (minute 0) and 15, 30, 60, and 120 minutes post glucose administration using an Ascensia Breeze 2 meter (Bayer, Mishawaka, IN).

On gestation day 18, rats were fasted overnight for 16 hours for blood and tissue collection. During the fasting period, exercise dams remained in their home cage with the running wheel until 1 hour prior to euthanasia. Heart, soleus, and parametrial and retroperitoneal fat pads were weighed at take–down. Gestation day 18 serum glucose and insulin were measured using a glucose assay kit (BioVision #K606–100, San Francisco, CA) and an insulin ELISA (Crystal Chem Inc #90080, Downers Grove, IL) respectively.

3.3.7. Animal care and use. Studies conducted at the University of Kentucky were approved by the Institutional Animal Care and Use Committee and adhered to American College of Sports Medicine (ACSM) animal care standards. Studies conducted at the University of Michigan Animal Phenotyping Core were approved by the University Committee on Use and Care of Animals and adhered to ACSM animal care standards.

3.3.8. Statistical analysis. Data were analyzed using a Student's t–test (Sigma Plot 11.0 software, Systat, Point Richmond, CA). Data that failed the Shapiro–Wilk normality test were transformed by calculating the natural log of the values. Area under the curves (AUC) for glucose was calculated using "Area Below Curves" function in Sigma Plot 11.0.

3.4. Results

3.4.1. Maternal and litter outcomes. Female SD rats were split into sedentary and running wheel cages for ~1 week prior to mating, throughout pregnancy, and the first 12 days of nursing. Running distance increased as the female rats became acclimated to the wheel and was highest when males were present in the cage for mating (Figure 3.1.A). While this study did not distinguish between male and female running, a previous study looking at running behavior of male and female SD rats found that females ran significantly more than males [192]. As pregnancy progressed, dams decreased running distance per day; distance was lowest on the day of delivery (set to day 33). Maternal running distance increased slightly during the nursing period.

Body weight and food intake were monitored for both groups during breeding. Maternal body weight trended toward a decrease in the exercise group compared to the sedentary group, but was significantly lower at only one time point (Figure 3.1.B). Exercise, however, had no effect on maternal food intake (Figure 3.1.C). Figures 1B and C are not matched for day of delivery.

There were no significant differences in pregnancy rates between sedentary (20/20) and exercise (18/20) dams (P = 0.4872 by Fisher Exact Test). At birth, the number of pups per litter was recorded. There was no significant difference in number of

pups per litter between sedentary (13.9 ± 0.39) and exercise (14.61 ± 0.59) dams (P = 0.312). Pup body weights were calculated by averaging pup body weights per litter then taking each litter mean. There were no significant differences in pup body weight when they were weighed at postnatal days 1, 7, 14, and 21. (Figure 3.1.D).

3.4.2. Offspring body weight and glucose tolerance. Body weight of offspring from sedentary and exercised dams was measured from weaning (week 3) up through 15 months of age with no recorded differences in body weight. Glucose tolerance testing was performed at several ages, starting at 2 months of age, however no significant differences were observed until offspring reached 10 months of age. At 10 months, rats were fasted for 16 hours and given an intraperitoneal injection of glucose. Offspring from exercise dams had significantly lower blood glucose levels at 60 and 120 minutes post glucose injection compared to those from sedentary dams (P = 0.004 and P = 0.001, respectively) (Figure 3.2.A). AUC, a measure of overall glucose disposal, was significantly lower in offspring from exercised dams (P = 0.033) (Figure 3.2.B). An oral glucose tolerance test was performed when offspring were 15 months of age. Rats were fasted for 16 hours and blood was collected to assess fasting insulin and glucose levels. Offspring from exercised dams had significantly lower plasma insulin levels compared to offspring from sedentary dams (P = 0.017) (Figure 3.3.A), but fasting glucose levels were not different (Figure 3.3.B). Rats were then given an oral glucose challenge, and blood glucose levels were significantly lower in offspring born to exercised dams compared to offspring from sedentary dams at the 30 and 120 minute time points compared to those from sedentary dams (P = 0.018 and P = 0.017, respectively) (Figure 3.3.B). AUC was significantly lower in offspring from exercised dams (P = 0.019) (Figure 3.3.C). At 15

months of age, female offspring body composition was analyzed and there were no significant differences in body fat, lean mass or free water between the two groups (data not shown). These data suggest that offspring born to exercised dams have enhanced glucose disposal compared to offspring from sedentary dams that is independent of body composition.

3.4.3. Offspring hyperinsulinemic-euglyemic clamp. At 17 months of age, offspring underwent hyperinsulinemic-euglyemic clamp testing to assess whole body insulin sensitivity. At this age point, a body weight difference developed between the two groups; offspring from exercised dams weighed significantly less than those born to sedentary dams (19.8% less, $P = 0.003$). The decrease in body weight in the offspring from the exercised dams was not detected earlier when the glucose disposal differences were observed. Prior to the clamp procedure, rats were fasted for 16 hours. They were then infused with insulin at a constant rate of 4 mU/kg/min for 120 minutes. Rats were simultaneously infused with glucose at varying rates in order to maintain blood glucose levels of approximately 120–130 mg/dL. Blood glucose and the glucose infusion rate (GIR) needed to maintain physiological blood glucose levels were monitored every 5-10 minutes for the 120 minute procedure (Figures 3.4.A and B, respectively). There were no differences in blood glucose levels in the offspring from sedentary or exercised dams during the procedure (Figure 3.4.A). The GIR was significantly higher in offspring born to exercised dams compared to offspring from sedentary dams at all time points after 40 min ($P < 0.05$) (Figure 3.4.B). The average steady–state GIR (80–120 minutes) was significantly higher in offspring born to exercised dams ($P < 0.001$) (Figure 3.4.C).

Both basal and clamp plasma insulin levels were significantly lower in offspring from exercised dams compared to offspring from sedentary dams ($P < 0.001$ for both) (Figure 3.4.D). There were no differences in basal whole body glucose turnover rates however during the clamp, offspring from exercised dams had significantly increased glucose turnover rates compared to offspring from sedentary dams ($P = 0.011$) (Figure 3.4.E). Also, there were no differences in basal hepatic glucose production (HGP) however in response to insulin infusion during clamp, offspring from exercised dams had significantly lower HGP compared to those from sedentary dams ($P = 0.037$) (Figure 3.4.F). Suppression of HGP was also significantly increased in offspring from exercised dams ($P = 0.013$) (data not shown). These data suggest that mature offspring born to exercised dams have enhanced insulin sensitivity compared to offspring from sedentary dams.

3.4.4. Offspring tissue specific glucose uptake. At the end of the clamp procedure, the aged offspring were euthanized and insulin sensitive tissues were analyzed for 2–deoxyglucose uptake. Under the hyperinsulinemic-euglyemic steady– state, both the extensor digitorum longus (EDL) ($P = 0.035$) and gastrocnemius (gastroc) ($P < 0.001$) muscles from offspring born to exercised dams had significantly increased glucose uptake (Figures 3.5.A and B, respectively), while soleus muscle uptake was unchanged (Figure 3.5.C). There were no differences in white adipose glucose uptake as represented by visceral and subcutaneous fat pads (Figures 3.5.D and E, respectively). Interestingly, offspring from exercise dams had significantly decreased glucose uptake in the heart compared to offspring from sedentary dams ($P = 0.006$) (Figure 3.5.F). These data

suggest that there are tissue specific effects in insulin sensitivity in offspring that result from maternal exercise.

3.4.5. Timed mating maternal outcomes. There were no significant differences in body weight between sedentary and exercising dams prior to or during pregnancy. Further, glucose tolerance was not significantly affected by exercise in the pregnant dams at gestation day 14 (data not shown). Table 3.1 provides a summary of maternal outcomes after tissue and blood collection at gestation day 18. There were no differences in heart or soleus muscle weight between sedentary and exercise dams. Exercise females, however, had significantly smaller parametrial and retroperitoneal fat pads at gestation day 18 compared to sedentary dams ($P = 0.017$ and $P < 0.001$, respectively). Similar to the findings with glucose tolerance testing on gestation day 14, there were no differences in serum glucose levels at gestation day 18. Serum insulin levels, however, were significantly reduced in exercise dams compared to sedentary dams which could suggest a level of heightened insulin sensitivity.

3.5. Discussion

This study has shown that maternal running during pregnancy can improve glucose homeostasis and enhance insulin sensitivity in adult offspring. I found that mature offspring born to exercised dams had improved glucose disposal following a glucose challenge and enhanced whole body insulin sensitivity as determined by hyperinsulinemic-euglyemic clamp. Although glucose tolerance testing was conducted in younger animals, differences were not observed until approximately ten months of age. This is not surprising given that numerous developmental programming studies, in animals as well as humans, do not detect differences in offspring until advanced age [40,

194]. This could be due to the dysregulation of metabolic processes that occur with age or a number of other factors. Regardless, I have shown that a maternal intervention, exercise during healthy pregnancy, can have a long-lasting positive impact on offspring metabolic health.

In this study I also looked at tissue specific glucose uptake in response to insulin infusion during clamp. Skeletal muscle and white adipose tissues are the main sites of insulin stimulated glucose uptake in the body [5, 6]. Compared to offspring from sedentary dams, those born exercised dams had significantly increased glucose uptake in response to insulin in skeletal muscle but not adipose tissue. Differences in skeletal muscle glucose uptake were detected in the EDL and gastroc muscles while no differences were observed in the soleus muscle. This may be due to different fibre types in the various muscles. Type I fibres (slow twitch) are used for slow contractions and have a high oxidative capacity but low glycolytic capacity [195]. Type II fibers (fast twitch), used for fast contractions, have a high glycolytic capacity [195]. In the rat, the EDL muscle is primarily composed of type IIB fibers, while the soleus is mostly made up of type I fibres [196, 197]. The gastroc muscle is comprised of a mixture of both muscle fibre types [196]. Taking this into account, it is not surprising that glucose uptake differences were observed in the EDL and gastroc as opposed to the soleus. It will be necessary in upcoming studies to investigate whether there are changes in expression or activation of insulin signaling pathway proteins in the EDL and gastroc muscles.

In addition to differences in skeletal muscle glucose uptake during insulin infusion, I observed that heart glucose uptake was significantly decreased in offspring born to exercised dams compared to those from sedentary dams. Fatty acids are the main

substrate for metabolism in the heart and oxidation of fatty acids prevents glucose metabolism [198]. Substrate utilization in the cardiac tissue shifts from primarily fatty acids to glucose when there is increased workload on the heart [199]. Glucose also becomes the main substrate of metabolism when there are high levels of circulating glucose and insulin [198, 200]. This may suggest that the decreased glucose uptake in the heart observed in offspring from exercised dams is actually indicative of improved cardiovascular and metabolic health. Future studies in the lab will explore in depth the cardiovascular effects of maternal exercise in offspring.

Through the clamp procedure I was also able to evaluate fasting and insulin stimulated HGP. Although there were no differences in fasting HGP, insulin stimulated HGP was significantly decreased in offspring from exercised dams compared to those from sedentary dams suggesting the liver in offspring from exercised dams had enhanced insulin sensitivity. Insulin is a major signaling hormone released in the fed state that suppresses endogenous energy (glucose) production while promoting energy storage. After skeletal muscle and white adipose tissue, the liver is a main site of insulin action and glucose uptake [201]. With insulin resistance, there is increased gluconeogenesis and decreased glucose uptake which contributes significantly to hyperglycemia seen with T2DM [202]. Given the importance of insulin action on the liver, enhanced hepatic insulin sensitivity, as observed in the offspring from exercised dams, is essential to improving overall metabolic health. Future studies will look at hepatic expression of insulin pathway proteins and markers of hepatic function such as glycogen storage and expression and activation of proteins involved in glycogen synthesis and gluconeogenesis.

It is important to note that throughout the majority of the offspring lifespan, there were no body weight differences between the two groups. When the offspring were shipped to the University of Michigan at 14 months of age and during the OGTT at 15 months of age, there were still no differences in body weight. However, at the time of clamp testing, when the offspring were 17 months old, offspring from exercised dams weighed significantly less than those from sedentary dams. There could be several reasons for this observed difference in body weight. Weight loss is a well known response in rats to stress [203]. The two groups may have responded differently to the shipping and transition from group to single housing conditions resulting in weight differences between the two groups. Also, an equal number of rats per group (n = 5) had to be removed from the study due to health problems (such as labored breathing), and this could have led to unintentional differences in body weight. Regardless, the clamp procedure corrects for differences in body weight because insulin is infused at a rate relative to the rat body weight.

This study provides strong evidence that maternal exercise during pregnancy can improve insulin sensitivity and glucose homeostasis in adult offspring. There are, however, several limitations in the current study that should be considered. The running wheel in the cage of exercise group dams could be a form of environmental enrichment. Maternal environmental enrichment has been found to have developmental programming effects in offspring [184, 185]; however, none of these studies have shown maternal environmental enrichment affects offspring insulin sensitivity or glucose regulation. In addition, male rats had access to the running wheel during breeding so paternal running could have an influence on the outcomes observed in offspring. Recent papers have

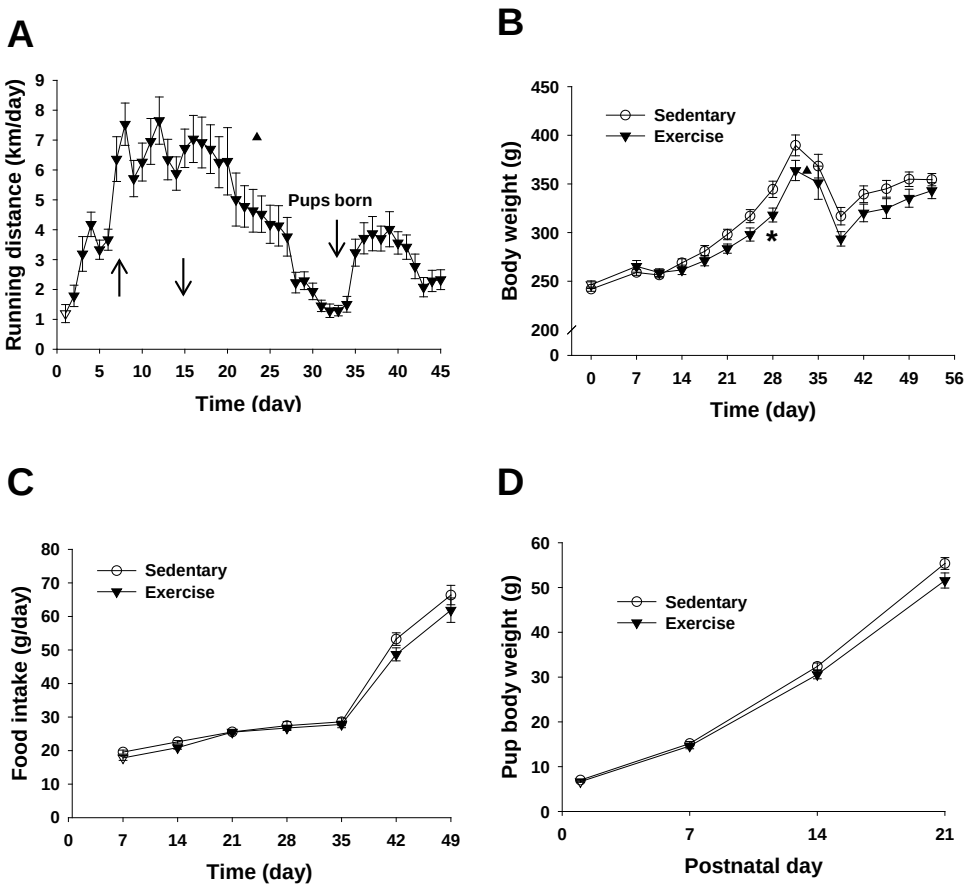
shown that a paternal low protein diet can cause changes in offspring gene methylation [182] and that paternal high fat diet consumption can induce glucose intolerance in female offspring [181]. Finally, only female offspring were used in the current study although developmental programming models have been shown to have sex specific effects in offspring [180]. Regardless, I have previously demonstarted that exercise during pregnancy enhances insulin sensitivity in both male and female offspring in mice suggesting that maternal exercise can positively impact metabolic health in both sexes [191]. It is important to note that female offspring estrous cycle was not monitored during testing and this may have impacted results as sex hormones are known to affect insulin sensitivity and glucose tolerance [204]. It will be necessary to take all of these confounding factors into consideration in future studies.

Other upcoming studies will focus on the timing (prior to conception, during gestation, and/or during nursing) and intensity of maternal exercise that is key for enhancing insulin sensitivity in offspring. I speculate however, that exercise during gestation, more so than prior to conception or during nursing, is essential for creating the life–long changes in offspring sensitivity. Acute exercise during pregnancy causes intermittent decreases in oxygen and glucose availability to the fetus [90] which most likely impacts fetal metabolic development. Results from the timed breeding in this study suggest that maternal insulin levels and fat mass, both affected by exercise prior to and during pregnancy, may also be impacting fetal metabolic development. They also suggest that maternal insulin sensitivity is increased with exercise, which could be playing a role in glucose levels reaching the fetus. Thus, it will also be necessary to look at epigenetic modifications in offspring, specifically of genes involved in insulin and glucose

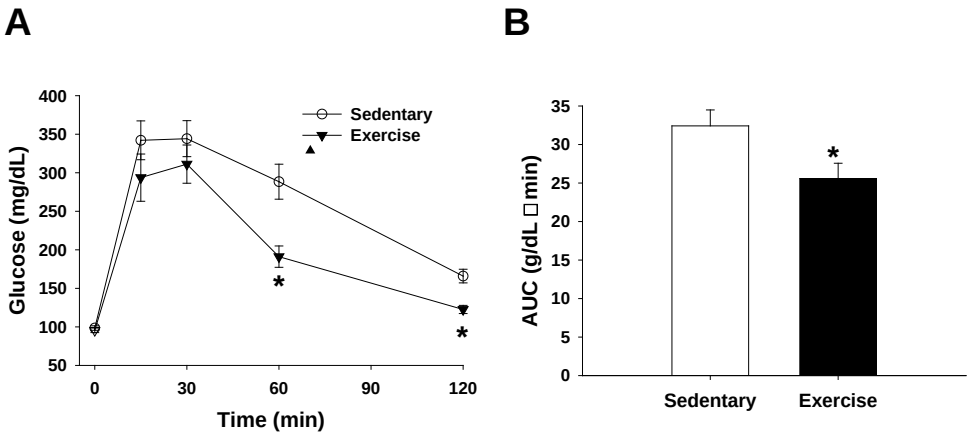
regulation in an attempt to elucidate the mechanism by which exercise during pregnancy improves adult offspring insulin sensitivity. Many studies, in particular those looking at the effects of IUGR on offspring, have already shown that altering the intrauterine environment can influence epigenetic modifications of genes involved in glucose homeostasis. For example, histone modifications have been found to be responsible for the decrease in glucose transporter type 4 expression in offspring from protein restricted dams [205]. IUGR has also been associated with methylation and silencing of the pancreatic and duodenal homeobox 1 (*Pdx1*) gene which is involved in pancreatic development and function in rats [49]. A recent human study has found that offspring exposed to nutrient restriction during gestation have changes in methylation patterns of the maternally imprinted insulin – like growth factor 2 (*IGF2*) gene that is involved in human development [206].

More work needs to be done in this area, but our results are particularly exciting because of the translational relevance that maternal exercise can improve offspring health. The incidence of T2DM is increasing worldwide, and it is necessary to find novel ways to prevent the disease. Exercise during pregnancy could be a short–term, easily accessible and achievable way to target T2DM in future generations.

3.6. Figures


A
Running distance (km/day)
Pups born
Time (day)
B
Body weight (g)
Sedentary
Exercise
*
Time (day)
C
Food intake (g/day)
Sedentary
Exercise
Time (day)
D
Pup body weight (g)
Sedentary
Exercise
Postnatal day

delivery in B and C. * $P < 0.05$ compared to sedentary dams; n = 20 for sedentary and n = 17 for exercise in A – D. Error bars indicate s.e.m. Data collected and analyzed by L.G.C.


A
B
Glucose (mg/dL)
400
350
300
250
200
150
100
50
0
30
60
90
120
Time (min)
Sedentary
Exercise
*
*
AUC (g/dL □ min)
35
30
25
20
15
10
5
0
Sedentary
Exercise
*

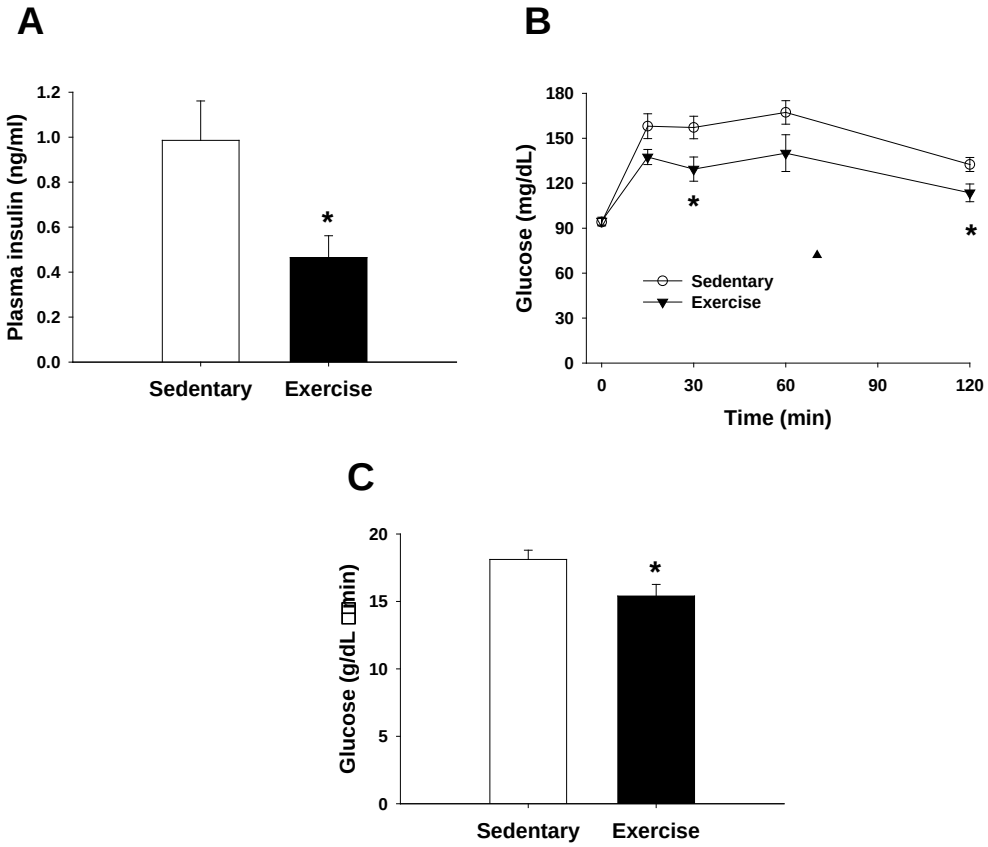
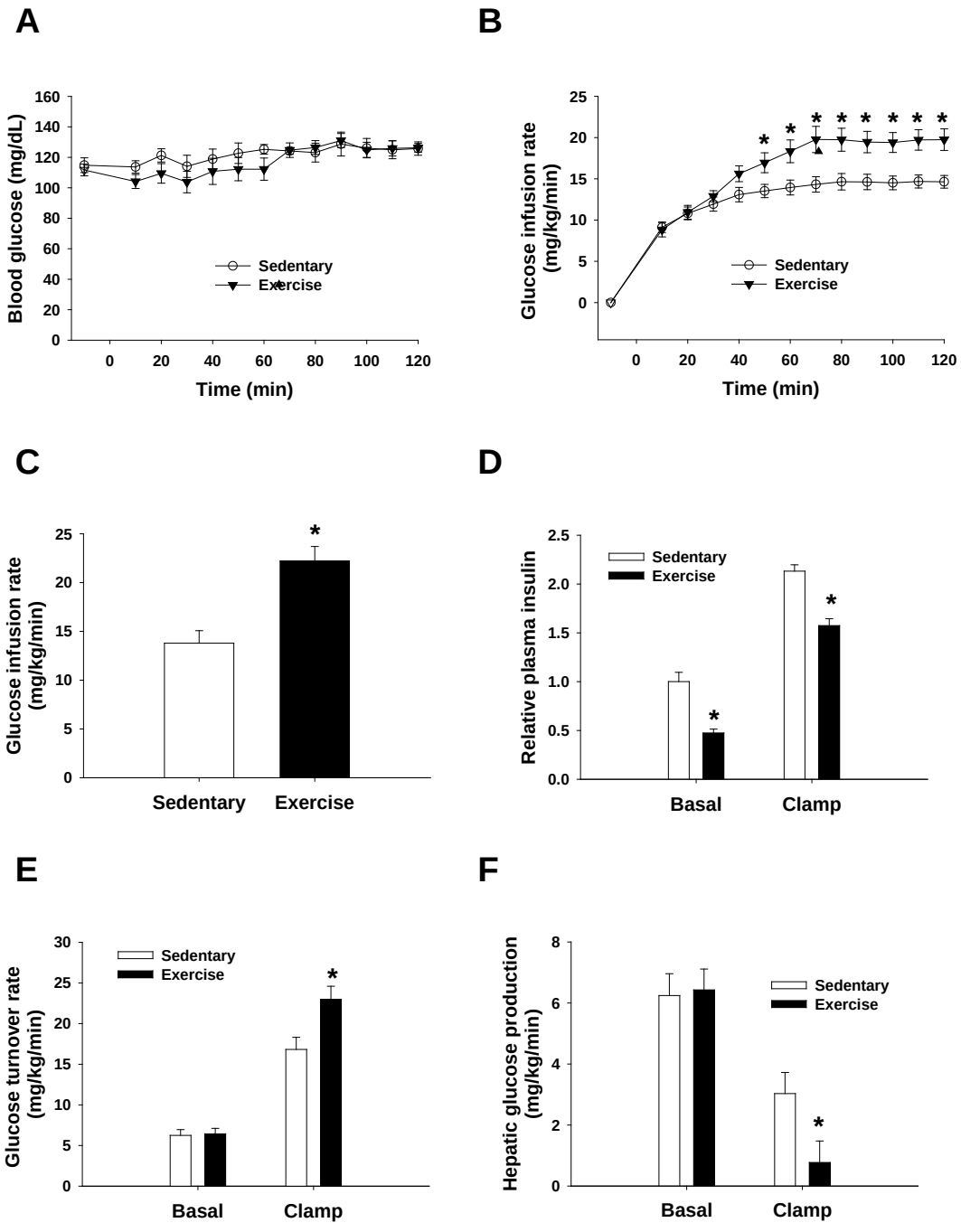
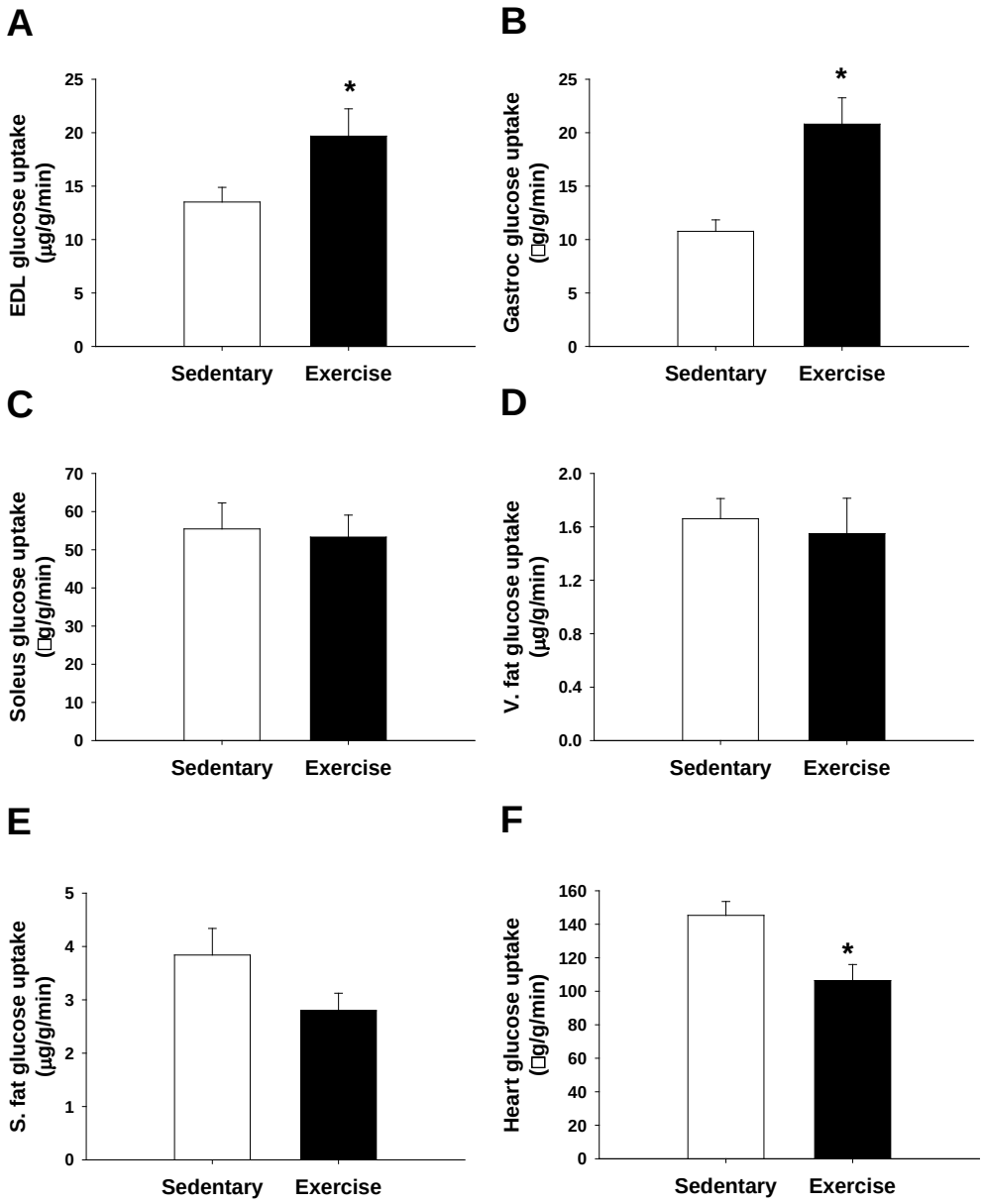


Figure 3.3. Female offspring born to exercised dams had improved glucose disposal following a glucose challenge at fifteen months. Fifteen month old female offspring were fasted for 16 hours then given an oral gavage of glucose (2 g/kg body weight). (A) Offspring from exercised dams had significantly decreased fasting insulin compared to offspring from sedentary dams. (B) At 30 and 120 minutes post glucose administration, blood glucose was significantly lower in offspring from exercised dams compared to offspring from sedentary dams. (C) Area under the curve (AUC) of blood glucose levels during the glucose tolerance test was also significantly lower in offspring from exercised dams compared to those from sedentary dams. * $P < 0.05$ compared to sedentary control;

n = 13 for sedentary and n = 10 for exercise in A; n = 16 for sedentary and n = 14 for exercise in B and C. Error bars indicate s.e.m. Data collected at the Michigan Metabolomics and Obesity Center and analyzed by L.G.C.


A
Blood glucose (mg/dL)
Time (min)
Sedentary
Exercise
B
Glucose infusion rate (mg/kg/min)
Time (min)
Sedentary
Exercise
C
Glucose infusion rate (mg/kg/min)
Sedentary
Exercise
D
Relative plasma insulin
Sedentary
Exercise
Basal
Clamp
E
Glucose turnover rate (mg/kg/min)
Sedentary
Exercise
Basal
Clamp
F
Hepatic glucose production (mg/kg/min)
Sedentary
Exercise
Basal
Clamp

body insulin sensitivity. Following a 16 hour fast, insulin was infused at a constant rate of 4.0 mU/kg/min for 120 minutes. Glucose was infused simultaneously at varying rates in order to maintain a 120 – 130 mg/dl blood glucose level in the animal. (A) There were no differences in blood glucose levels during the procedure between the two groups. (B) Glucose infusion rate (GIR) needed to maintain target body blood glucose levels was significantly increased in offspring from exercised dams compared to those from sedentary dams. (C) Steady – state GIR (average of the 80 – 120 min GIR) was significantly increased in offspring from exercised dams compared sedentary dams. (D) Plasma insulin levels were significantly lower under basal and clamp conditions in offspring born to exercised dams compared to offspring from sedentary dams. (E) There were no differences in basal condition whole body glucose turnover rates. Under the clamp condition, glucose turnover rate was significantly increased in offspring from exercised dams compared to those from sedentary dams. (F) There were no differences in basal condition hepatic glucose production (HGP). In response to insulin stimulation during the clamp HGP was significantly decreased in offspring from exercised dams compared to offspring from sedentary dams.* $P < 0.05$ compared to sedentary control; n = 12 for sedentary and n = 9 for exercise in A – F. Error bars indicate s.e.m. Data collected at the Michigan Metabolomics and Obesity Center and analyzed by L.G.C.


A
EDL glucose uptake (µg/g/min)
25
20
15
10
5
0
*
Sedentary
Exercise
B
Gastroc glucose uptake (µg/g/min)
25
20
15
10
5
0
*
Sedentary
Exercise
C
Soleus glucose uptake (µg/g/min)
70
60
50
40
30
20
10
0
Sedentary
Exercise
D
V. fat glucose uptake (µg/g/min)
2.0
1.6
1.2
0.8
0.4
0.0
Sedentary
Exercise
E
S. fat glucose uptake (µg/g/min)
5
4
3
2
1
0
Sedentary
Exercise
F
Heart glucose uptake (µg/g/min)
160
140
120
100
80
60
40
20
0
*
Sedentary
Exercise

from sedentary dams. There were no differences in (C) soleus muscle, (D) visceral (V.) adipose, or (E) subcutaneous (S.) adipose glucose uptake. Offspring from exercised dams had significantly decreased (F) heart glucose uptake compared to those from sedentary dams. * $P < 0.05$ compared to sedentary control; $n = 12$ for sedentary and $n = 9$ for exercise in A – F. Error bars indicate s.e.m. Data collected at the Michigan Metabolomics and Obesity Center and analyzed by L.G.C.

Table 3.1 | Maternal outcomes on gestation day 18

Parameter	Sedentary (s.e.m.)	Exercise (s.e.m.)	P value
Heart/body weight (x1000)[a]	3.769 (0.092)	3.777 (0.123)	0.952
Soleus muscle/body weight (x1000)[a]	0.407 (0.031)	0.395 (0.063)	0.551
Parametrial fat/body weight (x1000)[a]	8.219 (0.723)	5.288 (0.797)	0.017
Retroperitoneal fat/body weight (x1000)[a]	7.417 (0.645)	4.039 (0.311)	< 0.001
Serum glucose (nmol/µl)[b]	4.347 (0.356)	4.571 (0.359)	0.672
Serum insulin (ng/ml)[b]	0.866 (0.136)	0.385 (0.035)	< 0.001

[a] sedentary n = 17 and exercise n = 9; [b] sedentary n = 13 and exercise n = 9.

Data collected and analyzed by L.G.C.

CHAPTER 4

EXERCISE IMPROVES GLUCOSE DISPOSAL IN PREGNANCT MICE FED A HIGH FAT DIET

4.1. Abstract

Though all pregnant women are susceptible to the development of gestational diabetes mellitus (GDM), obese women are at a particularly high risk. Physical activity has been suggested as a non – pharmacological intervention that can be used to prevent the development of GDM and/or to improve glucose homeostasis in women with GDM. The purpose of this study was to study the effects of voluntary exercise on glucose tolerance and body composition in pregnant high fat diet fed mice. Female ICR mice were put on a standard diet (SD) or high fat diet (HFD) for two weeks. Glucose tolerance was measured, and the mice were then split into 4 groups; control SD, exercise SD, control HFD, and exercise HFD. Exercise mice had voluntary access to a running wheel in the home cage one week prior to mating, during mating, and throughout pregnancy. Glucose tolerance and body composition were measured late in pregnancy. Akt levels were measured in skeletal muscle and adipose tissue isolated from saline or insulin injected pregnant dams as a marker for insulin signaling. Consumption of the HFD led to significantly increased body weight, fat mass, and impaired glucose tolerance in control mice. However, voluntary running in the HFD fed dams significantly reduced weight gain and fat mass and ultimately improved glucose tolerance compared to control HFD dams. Further, body weight, fat mass, and glucose disposal in exercise HFD dams were indistinguishable from dams fed the SD. HFD fed exercise dams also had significantly increased insulin stimulated phosphorylated Akt expression in adipose tissue, but not

skeletal muscle, compared to control dams on HFD. These results show that voluntary exercise can reduce body weight and fat mass and improve glucose disposal and adipose tissue insulin signaling in pregnant HFD fed female mice. This model may be effective to study whether exercise can provide long-term benefits to offspring born to obese or GDM dams.

4.2. Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance first recognized during pregnancy, and women with GDM have a 35 – 60% chance of developing T2DM within 10 to 20 years postpartum [145, 207]. Recently, GDM rates have been increasing with approximately 2 – 8% of pregnancies in the U.S. affected by GDM [208, 209]. Though a natural insulin resistance develops to ensure adequate glucose supply to the fetus in all pregnancies, this further develops into GDM in some women, especially those who are obese [210, 211]. Babies born to diabetic mothers have the potential risk of hypoglycemia after birth and increased body weight due to excessive fat deposition. In a well studied population of Pima Indians, known to have high rates of T2DM and GDM, offspring exposed to diabetes during gestation have a higher incidence of obesity and T2DM [64, 68]. In another human study, it was found that high gestational glucose concentration is positively correlated with insulin resistance in offspring at approximately 7 years of age [212]. An animal model of GDM also showed higher body weights and impaired glucose regulation in offspring exposed to diabetes during gestation compared to offspring from non – obese, control dams [72]. Many other studies have found similar results [71, 213, 214].

GDM is associated with many risk factors. Non – modifiable risk factors include age, ethnicity, and family history of diabetes (genetics) [215, 216]. There are, however, modifiable risk factors that can be targeted to help prevent GDM including body mass index (BMI), diet, and physical activity [52, 217]. Since traditional medications used to treat diabetes such as insulin or oral drugs used to improve insulin sensitivity can

potentially be harmful to the fetus, it is important to look at the modifiable risk factors as treatment options.

There have been human studies conducted to look at physical activity and the risk and management of GDM. Liu et al. have shown that physical activity during pregnancy can reduce the incidence of GDM [119]. Other studies looking at women who already have GDM have found that moderate exercise can reduce the need for other treatments such as insulin [124, 125]. A recent randomized control trial has found, however, that exercise during pregnancy did not reduce the risk of developing GDM [176]. Exercise is known to improve glucose uptake in non – pregnant women by increasing insulin sensitivity as well as stimulating non – insulin dependent glucose uptake in skeletal muscle. However, the effects of exercise on insulin sensitivity and insulin independent glucose uptake in pregnant women as well as potential offspring benefits of maternal exercise have not been well studied. This makes research focusing on these pathways in pregnant women necessary. For example, Hopkins et al. have shown that exercise during pregnancy does not improve maternal insulin sensitivity but still impacts offspring birth weight [129].

Despite the promising results observed in human studies, it is necessary to study maternal and offspring effects of exercise during pregnancy in animals models as they allow for more extensive research to be performed, including elucidating tissue specific mechanisms. The purpose of this study was to develop a HFD induced animal model of GDM with the follow-up hypothesis that exercise prior to and during pregnancy would improve obesity and glucose tolerance outcomes in female HFD fed pregnant mice.

4.3. Materials and Methods

4.3.1. Animals and diets. Studies conducted at the University of Kentucky were approved by the Institutional Animal Care and Use Committee. Sixty female ICR mice were ordered from Taconic after delivering one litter prior to their arrival at the University of Kentucky at 4 months of age. Females were group housed (4 per cage) for a 2 week acclimation period following their arrival. Mice were housed in an environmentally controlled vivarium with temperature ranging from between 68 – 72 ^{0} F and a 14 hour light/10 hour dark light cycle. After acclimation, females were individually housed for the remainder of the study. Females were placed on either a standard diet (SD) (14.6% kcal diet from fat; Mod TestDiet® 5342 5SSG) or high fat diet (HFD) (40.5% kcal diet from fat; Mod TestDiet® 5342 5SSH). The HFD also contained higher levels of sucrose, salt, and cholesterol. More detailed diet information is available in Table 1. Females had unlimited access to food and water. After 2 weeks on the diets, females were separated into 4 groups; control SD, exercise SD, control HFD, and exercise HFD. Mice in the exercise cohorts were housed in cages with voluntary access to a running wheel (Phenome Technologies, Inc. Lincolnshire, IL) mounted in the cage for 1 week prior to mating, during mating, and during gestation. Running distance per day was monitored for the duration of the study using a magnetic counter (ClockLab software, Actimetrics, Wilmette, IL). Control females were housed in identical cages that did not contain a running wheel. After 1 week in the control or exercise groups, male ICR mice were introduced into the female cage for mating. After 4 days, males were removed and female mice that did not conceive were removed from the study. During all portions of the study, female body weight and food intake was monitored weekly. Due to mating

over the course of 4 days, gestation days were estimated by weight gain for analyses in pregnant mice. Days of the experiment were made relative to mating so that day 0 corresponds to first day of mating.

4.3.2. Glucose tolerance tests. Glucose tolerance tests were performed in female mice, prior to gestation, 2 weeks after being placed on the SD or HFD and in dams at gestation day ~14. Mice were fasted for 3 hours then given an intraperitoneal injection of D-(+)-glucose (Sigma-Aldrich, St. Louis, MO) at 2 g per kg body weight. Glucose levels were measured in whole blood using an Ascensia Breeze 2 meter (Bayer, Mishawaka, IN) following a tail vein prick prior to injection (0 min) and 15, 30, 60, and 120 min post – injection.

4.3.3. Body composition analysis. Body composition was analyzed in live, pregnant mice at gestation day ~17 using nuclear magnetic resonance (EchoMRI; EchoMedical Systems; Houston, TX). The EchoMRI measures adipose tissue, lean mass, and free and total water. Although many tissues contribute to the lean mass output, there are undetectable components such as bone mineral content, hair, and claws.

4.3.4. Western blot analysis. Western blotting was used to analyze total and phosphorylated Akt levels in adipose (parametrial) and skeletal muscle (soleus) tissue excised from pregnant mice at gestation day ~18. Dams on the HFD were fasted 3 h and injected with saline or porcine insulin (Sigma, I-5523) (1.25 IU/kg body weight). Fifteen min later, mice were euthanized and tissues were collected. Total and phosphorylated Akt primary antibodies were purchased from Cell Signaling Technology® (#4685 and #4058, respectively). Peroxidase conjugated immunoglobulin G secondary antibody was ordered from Millipore (Billerica, MA) (goat anti – rabbit #AP132P). Tissues were

homogenized in cell lysis buffer and a Bradford assay was used to determine sample protein concentrations prior to western blot analysis. For western blots, 12 μg of protein was loaded into 4 – 20 % gradient SDS – PAGE gel and proteins separated. Protein was transferred to nitrocellulose membranes which were then incubated in primary antibody overnight at 4^0C (1:1000 in 5% BSA for total Akt; 1:1000 in 5% milk for phosphorylated Akt). Membranes were washed then incubated in the secondary antibody (1:5000) for 1 hour then washed again. Bands were imaged using enhanced chemiluminescence (ECL) detection reagents (FEMTOMAX – 110, Rockland, Gilbertsville, PA) using a BioRad ChemiDoc system. Intensity of bands was determined using Quantity One Analysis Software version 4.6.1 (BioRad, Hercules, CA). Data are represented as insulin-stimuated and non-stimulated phosphorylation as a ratio of phosphorylated Akt to total Akt.

4.3.5. Statistical analysis. Two–way ANOVAs were conducted in SPSS 20 (IBM SPSS Statistics Software, Armonk, NY). For comparisons between 3 or more groups, one–way ANOVA was performed followed by Fisher LSD post–hoc analysis. Student's t tests were used for comparisons between 2 groups. These analyses were performed using Sigma Plot 11.0 software (Systat, Point Richmond, CA). Data that failed the Shapiro–Wilk normality test was transformed by calculating the natural log of the values. Area under the curve (AUC) for blood glucose levels were calculated using "Area Below Curves" function in Sigma Plot 11.0.

4.4. Results

4.4.1. Nonpregnant female body weight and glucose tolerance. After 7 and 14 days of dietary treatment, mice on the HFD had significantly increased body weight compared to mice on the SD ($P = 0.013$ and $P < 0.001$, respectively) (Figure 4.1.A). After 2 weeks on the diet, a glucose tolerance test showed that female mice on HFD had significantly increased blood glucose levels (Figure 4.1.B). At 15, 30, 60, and 120 min post glucose injection compared to female mice on SD ($P = 0.027$, $P < 0.001$, $P < 0.001$, and $P = 0.002$, respectively). Area under the curve (AUC) of blood glucose levels during the GTT was also significantly increased in mice on HFD compared to mice those fed SD ($P < 0.001$) (Figure 4.1.C).

4.4.2. Female body weights and energy intake. One week of voluntary running significantly decreased body weight in HFD fed mice compared to HFD fed controls ($P = 0.010$), while control HFD mice weighed significantly more compared to control mice on SD ($P < 0.001$) (Figure 4.2.A). Females began mating on day 0 and after conception (days 7, 14, and 18) control dams on HFD continued to weigh significantly more than control dams on SD ($P = 0.002$, $P < 0.001$, and $P = 0.017$, respectively). Exercise attenuated the weight gain in the HFD fed dams (days 7 and 14) ($P = 0.008$ and $P = 0.011$, respectively) while toward the end of gestation (day 18) body weight differences between these two groups became insignificant ($P = 0.337$). Mean energy intake per day per mouse was significantly increased in control and exercise HFD dams compared to control dams on SD ($P < 0.001$ and $P = 0.001$, respectively) (Figure 4.2.B). However, there were no differences in calorie intake between control or exercising HFD fed dams.

4.4.3. Pregnant female glucose tolerance. During mid gestation, a glucose tolerance test was performed in the pregnant dams. Exercise in the SD fed dams was also able to decrease blood glucose at 30 min post glucose injection compared to SD control dams ($P = 0.041$) (Figure 4.2.C). HFD fed control dams had significantly increased blood glucose levels compared to SD fed control dams at 60 and 120 min post glucose injection ($P = 0.003$ and $P = 0.001$, respectively). Exercise by the HFD fed dams attenuated this increase in blood glucose at 60 and 120 min post glucose injection ($P = 0.027$ and $P = 0.011$, respectively) such that these values did not differ from control SD fed dams. Control dams on HFD had significantly increased AUC of blood glucose levels compared to SD fed control dams ($P = 0.007$) (Figure 4.2.D) while AUC for exercise HFD dams did not significantly differ from control SD dams. These data suggest that exercise during pregnancy can protect against impaired glucose disposal caused by HFD consumption.

4.4.4. Pregnant female body composition. Body composition was measured late in pregnancy, and there were no significant differences in total lean mass between the groups (Figure 3A). Control dams on HFD had significantly increased fat mass compared to SD fed control dams ($P < 0.001$) (Figure 4.3.B). Exercise protected HFD fed dams against the increase in fat mass ($P < 0.001$) such that fat mass was not significantly different than SD fed control dams. These data suggest that exercise during pregnancy can prevent increases in fat mass caused by HFD consumption.

4.4.5. Adipose tissue and skeletal muscle Akt phosphorylation. In order to assess whether glucose disposal was improved as a result of improved insulin signaling, basal and insulin stimulated phosphorylated Akt expression was analyzed in adipose and skeletal muscle isolated from dams late in pregnancy. Biochemical analysis focused on

tissues from HFD fed dams since differences in glucose tolerance and body composition were most obvious in these two groups. No differences were observed in total Akt levels between the two groups. There were no differences in basal expression of phosphorylated Akt in adipose tissue between control and exercise HFD fed dams (Figure 4.4.A). After insulin stimulation however, adipose tissue from exercise dams showed significantly increased levels of phosphorylated Akt compared to adipose from HFD fed controls (P = 0.010) (Figure 4.4.B). In addition, there were no significant differences between basal and insulin stimulated phosphorylated Akt in adipose tissue from control HFD dams (P = 1.000). Adipose tissue from exercising HFD dams, however, showed a significant increase in phosphorylated Akt levels after insulin stimulation compared basal phosphorylated Akt levels (P = 0.011). There were no differences in either basal (Figure 4.4.C) or insulin stimulated (Figure 4.4.D) phosphorylated Akt expression in skeletal muscle from control dams and exercise dams on HFD. These data suggest that exercise during pregnancy improved insulin signaling in adipose tissue of HFD fed pregnant mice.

4.5. Discussion

This study demonstrates that voluntary exercise can improve glucose tolerance in HFD fed pregnant mice. I found that exercise during pregnancy was able to significantly increase insulin stimulated Akt phosphorylation in adipose tissue in exercising dams fed a HFD compared to control dams fed a HFD. This suggests thatexercising HFD fed dams are more insulin sensitive than HFD fed controls. The observed decreases in fat mass in exercising dams on a HFD compared to HFD fed controls could contribute to adipose tissue insulin sensitivity. It is well known that fat accumulation can lead to insulin resistance. As fat mass increases, macrophage accumulation within the tissue increases,

leading to a pro-inflammatory environment. The adipose tissue releases numerous pro-inflammatory hormones and cyto/adipokines that can decrease whole body insulin sensitivity [8] through inactivation of key proteins involved in the insulin signaling pathway in insulin sensitive tissues (white adipose tissue and skeletal muscle) [60]. Decreasing fat mass during pregnancy through physical activity could be critical to increasing insulin sensitivity, especially in women with diet induced obesity.

It is important to note that insulin independent glucose uptake most likely contributed to the increases in glucose uptake observed in the exercising dams. Contraction can stimulate glucose uptake in the absence of insulin. As muscles contract and energy is depleted, AMP–activated protein kinase (AMPK) can be activated to stimulate glucose transporter type 4 (GLUT4) translocation to the cellular surface to promote glucose uptake into muscle [218, 219]. Future studies using this animal model will look at skeletal muscle expression and activation of AMPK and other proteins involved in insulin independent glucose transport. Also, further investigation into tissue specific changes in insulin sensitivity will be required. In the current study, only soleus muscle and abdominal adipose tissue were used to evaluate insulin sensitivity which may not be representative of whole body skeletal muscle and adipose tissue insulin sensitivity.

High fat feeding has been shown to be a risk factor for developing GDM in animals and humans [52, 220, 221]. Previous studies pertaining to exercise during pregnancy, especially those focused on women at risk for developing GDM and those with GDM, have yielded conflicting results. Many have shown that exercise prior to pregnancy and during early pregnancy can reduce risk of developing GDM while others have shown that physical activity can reduce the need for insulin to control blood glucose

in women already diagnosed with GDM [119, 124, 222, 223]. A recent randomized control trial has found however, that exercise during pregnancy did not reduce the risk of developing GDM [176]. These differing results support the need for the use of animal models to study exercise during pregnancy, especially those complicated by HFD consumption and GDM. Using a HFD diet in this study I induced a GDM like state of impaired glucose tolerance in mice and furthered studied the protective effects of exercise.

GDM is not only detrimental to the pregnant female, but can also lead to developmental programming of the offspring. As little data exist as to how this can come about, our model will be a useful tool in which to investigate interventions used to reverse the long-term offspring outcomes that result from maternal obesity. Previous animal studies have shown that high fat feeding during pregnancy and nursing can cause endothelial dysfunction, hypertension, impaired glucose tolerance and insulin resistance, as well as fatty liver in offspring [71, 73, 74, 224]. Adiposity has also been shown to be increased in adult offspring from dams fed a high fat diet [69]. In humans, exposure to obesity and GDM *in utero* has been found to result in increased offspring incidence of diabetes and obesity in adolescences and adulthood [67, 225]. Lesser studied are the effects of exercise during pregnancy and offspring health outcomes, especially in mothers who are or obese or gestational diabetic. In healthy pregnancies, exercise has been found to decrease birth weights and adiposity [135]. Previously published data from our lab indicates that exercise during pregnancy can have long – term effects on offspring metabolism such that adult offspring from exercised dams show improved glucose tolerance as well as increased insulin sensitivity compared to offspring from control dams

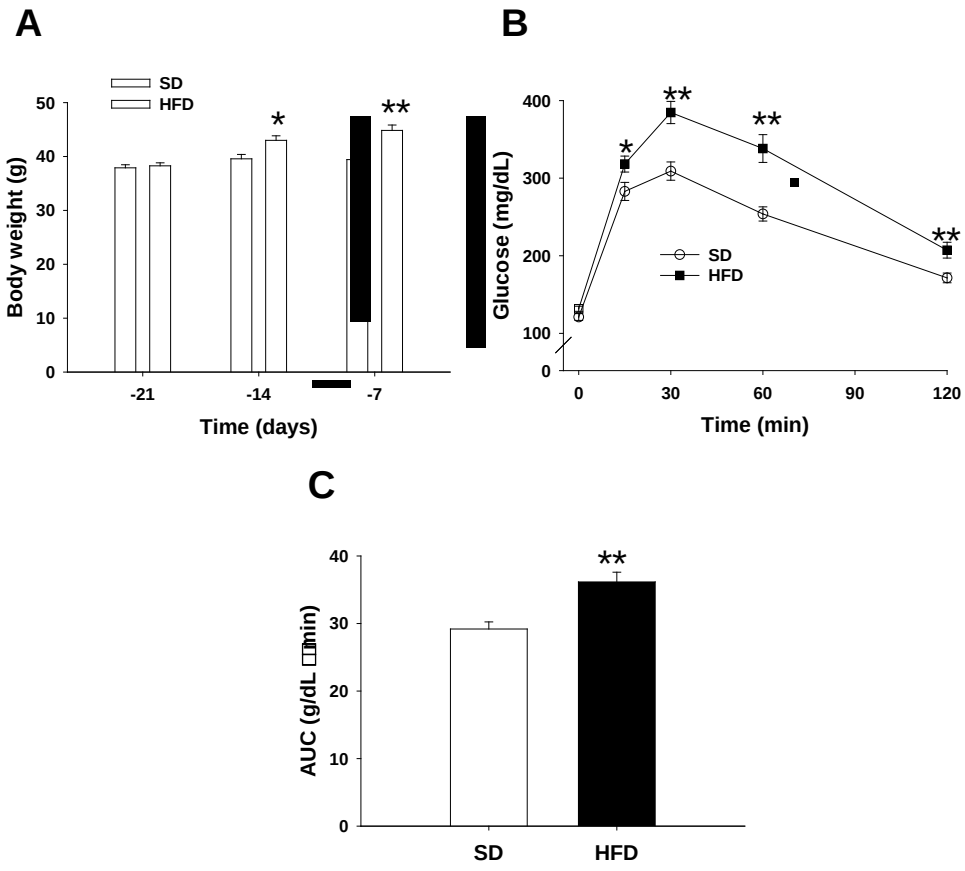
[191]. Therefore, upcoming studies will also focus on how exercise can protect offspring from the harmful effects of high fat feeding and GDM during pregnancy and nursing.

Several factors should be considered when reviewing these data. Given the mating scheme used in this study, it was impossible to time the exact day of conception and therefore I was unable to get accurate fetal body weights at the termination of the experiment. Regardless, litter size was unaffected by maternal exercise, however there was a significant effect of diet when a two – way ANOVA was conducted such that HFD dams had significantly larger litter sizes than SD dams ($P = 0.023$) (data not shown). Alternatives to this breeding approach would include using artificial insemination or timed mating so that the exact date of conception would be known. The amount of running observed when mice have voluntary access to a running wheel could be considered another limitation. The mice from this study ran up to 14 km/day which may not be realistic for translation to human studies. An alternative approach would be to use a controlled exercise model in which the mice are taken out of a home cage and placed into a running wheel for a predetermined amount of time per day. The controlled exercise model could also be used to determine the amount of physical activity required for maternal and offspring beneficial effects to be observed. Both the controlled and voluntary exercise model can be used to determine the stage of pregnancy or nursing that physical activity is most beneficial. This animal model provides important information on the effects of high fat feeding and exercise during pregnancy that are independent of the current limitations.

The findings from the present study, if translated to humans, could help promote the use of physical activity interventions for pregnancies in obese or GDM women. The

animal model used in this study could also be used to further study the maternal and offspring effects and mechanisms of exercise during an unhealthy pregnancy.

4.6. Figures



Figure

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for the glucose tolerance curves was significantly increased in females on the HFD compared to those on the SD. *P < 0.05 and **P < 0.01 compared to control SD; n = 30 for control and n = 30 for exercise in A – C. Error bars indicate s.e.m. Data collected and analyzed by L.G.C.

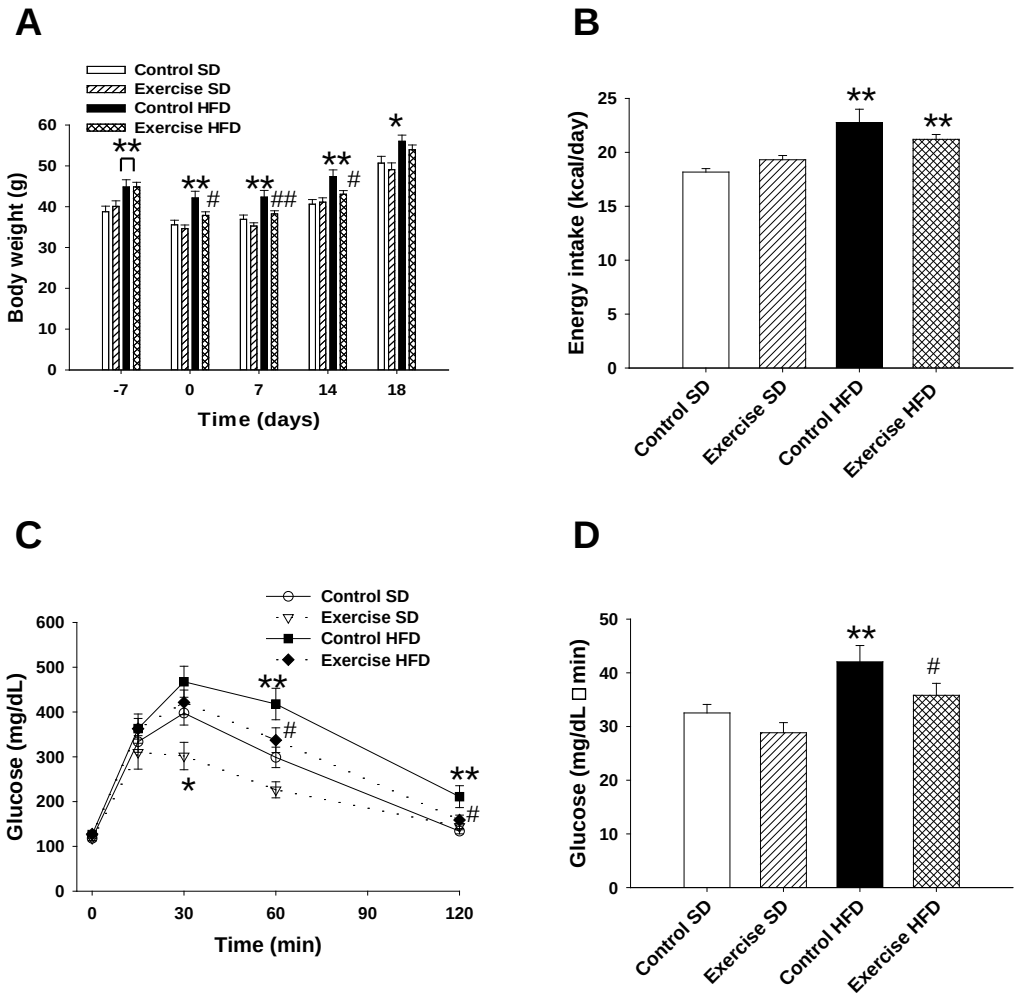
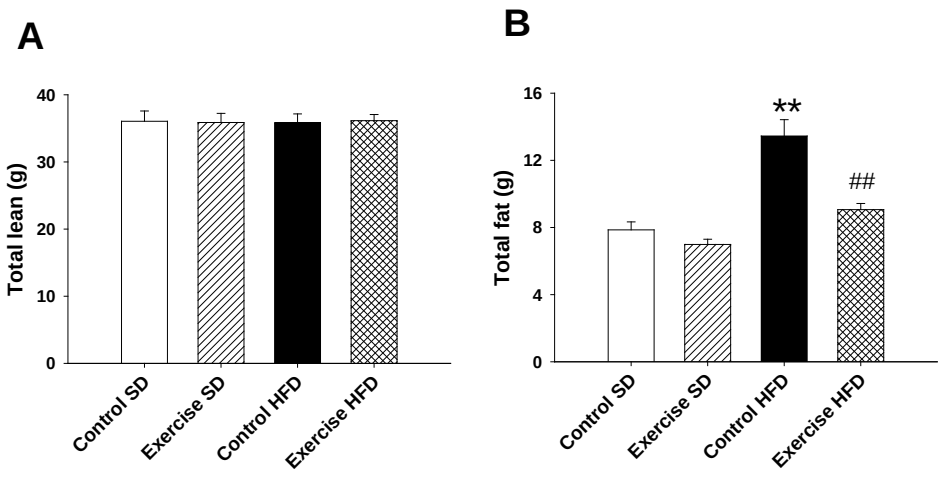


Figure 4.2. Voluntary exercise decreases weight gain and improves glucose tolerance during pregnancy in female mice on a high fat diet. After two weeks on the SD or HFD, female mice were split into control SD, exercise SD, control HFD, and exercise HFD groups. (A) Exercise HFD mice weighed significantly less than control HFD mice during mating and gestation. (B) Female mice on HFD had significantly increased daily energy intake compared to those on SD. (C) At mid gestation, pregnant dams underwent

glucose tolerance testing. Control dams on HFD had significantly higher blood glucose levels compared to control mice on the SD after glucose injection. Exercise significantly improved glucose disposal in HFD fed dams when compared to HFD fed control dams. (D) Area under the curve (AUC) of blood glucose levels during the glucose tolerance test was significantly increased in control HFD dams compared to SD dams. Exercise dams on HFD had significantly decreased AUC compared to control HFD dams. *$P < 0.05$ and **$P < 0.01$ compared to control SD; #$P < 0.05$ and ##$P < 0.01$ compared to control HFD; n = 11 for control SD, n = 11 for exercise SD, n = 12 for control HFD, and n = 15 for exercise HFD in A – D. Error bars indicate s.e.m. Data collected and analyzed by L.G.C.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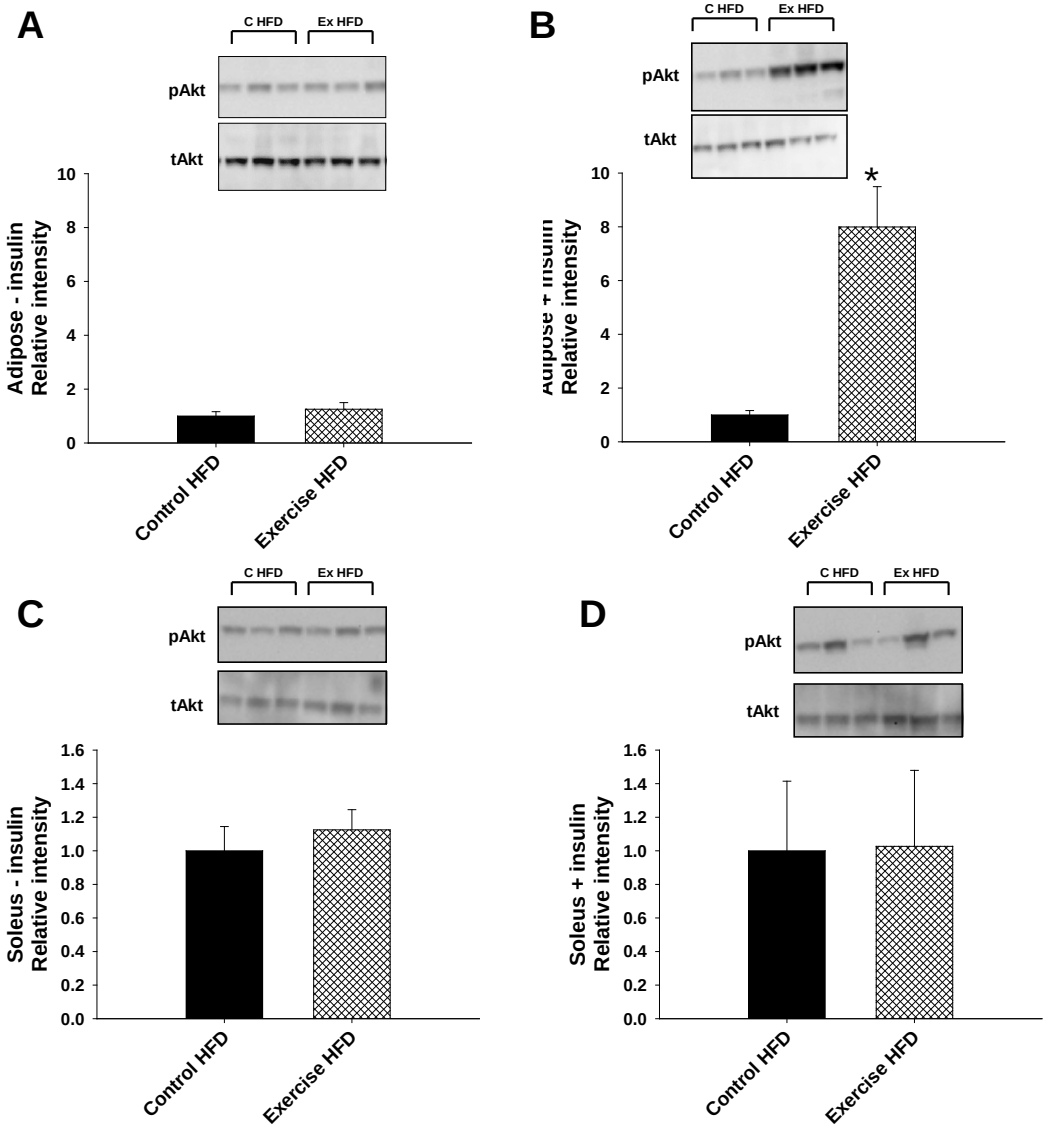
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dams. There were no differences between the two groups in basal (C) or insulin stimulated (D) skeletal muscle levels of phosphorylated Akt. *P < 0.05 compared to control HFD; n = 3 for control HFD and n = 3 for exercise HFD in A – D. Error bars indicate s.e.m. Data collected and analyzed by L.G.C.

Table 4.1 | Maternal diet

Standard diet (Mod TestDiet® 5342 5SSG)	
Ingredient	**%**
Corn starch	26.5507
Dextrin	25.0000
Casein	21.3567
Maltodextrin	9.9846
Milk fat	5.4023
Powered cellulose	4.9923
AIN – 76 mineral mix	3.4946
AIN – 76 vitamin mix	0.9985
Corn oil	0.9985
Calcium carbonate	0.3994
Salt	0.3068
DL – methionine	0.2995
Choline bitartrate	0.1997
Cholesterol	0.0097
Ethoxyquin (preservative)	0.0040
Vitamin A palmitate*	0.0018
	kcal/g kcal%
Fat	0.558 14.6
Protein	0.772 20.2
Carbohydrate	2.495 65.2

Carbohydrate	
Carbohydrate	
High fat diet (Mod TestDiet® 5342 5SSH)	
Ingredient	**%**
Corn starch	4.9923
Sucrose	31.2186
Casein	21.6017
Maltodextrin	9.9846
Milk fat	19.9692
Powered cellulose	4.9923
AIN – 76 mineral mix	3.4946
AIN – 76 vitamin mix	0.9985
Corn oil	0.9985
Calcium carbonate	0.3994
Salt	0.6973
DL – methionine	0.2995
Choline bitartrate	0.1997
Cholesterol	0.1498
Ethoxyquin (preservative)	0.0040
	kcal/g kcal%
Fat	1.804 40.5
Protein	0.772 17.3
Carbohydrate	1.882 42.2

CHAPTER 5

DISCUSSION

5.1. General Discussion

5.1.1. *Aim 1.* The main findings of these studies were that maternal voluntary exercise could improve offspring insulin sensitivity and glucose regulation in mouse and rat models. These results suggest that maternal exercise could potentially be used as a method for preventing or decreasing insulin resistance and type 2 diabetes in future generations. In the mouse model, exercise during pregnancy did not change maternal body weight; however it did cause an increase in maternal food intake. Exercise did not affect litter size or offspring body weights during nursing or throughout lifespan. At approximately 7 months of age and up through 16 months of age when the study was ended, male and female offspring from exercise dams had increased glucose disposal compared to offspring from control dams as measured by glucose tolerance tests. Offspring from exercise dams also showed enhanced glucose uptake into peripheral tissues following injection of exogenous insulin, indicating enhanced insulin sensitivity in adipose and skeletal muscle compared to offspring from control dams. In female offspring, insulin stimulated glucose uptake was measured ex vivo in an adipose depot and soleus muscle. Unfortunately, tissues from male offspring were not tested. Offspring from exercise dams had increased glucose uptake in both tissues in response to insulin treatment compared to tissues from offspring born to control dams. Finally, body composition in female offspring was unaffected by maternal exercise, but males from exercise dams had decreased fat mass and increased lean mass at several age points (starting at 9 months) compared to males from control dams.

Similar results were found in the rat model. Maternal exercise did not affect food intake but did cause a small decrease in maternal body weight. Again, litter size and offspring body weights during nursing were unchanged with the exercise intervention. Female offspring from exercise dams showed enhanced glucose disposal compared to offspring from control dams at approximately 10 months of age and these improvements were seen again at 12 and 14 months of age. At 17 months, female offspring from exercise dams had enhanced whole body insulin sensitivity compared to those from control dams as measured by hyperinsulinemic-euglycemic clamp. These females required a higher glucose infusion rate to maintain target blood glucose levels during insulin infusion and had higher glucose uptake into skeletal muscle compared to females from control dams. A timed breeding in the rats also revealed that voluntary exercise did not change maternal glucose tolerance mid-gestation; however exercise did reduce maternal fasting insulin and decreased fat pad mass compared to control dams.

These results are the first to show that exercise during healthy pregnancy can impact long-term offspring insulin sensitivity and glucose regulation; however the mechanisms behind these improvements are still unknown. The main question is what is happening with maternal exercise that could be influencing fetal development that results in long-term metabolic changes in offspring. To begin, sustained, weight-bearing endurance exercise, such as running, increases placental growth, size, and blood flow in early pregnancy, although in our mice, we do not see differences in placental size with exercise at the end of gestation [127, 132, 143]. These changes in placenta size and blood flow can increase glucose and oxygen levels reaching the fetus, however bouts of sustained weight-bearing endurance exercises cause intermittent reduction in blood flow

to placenta in favor of maternal muscle and skin [226, 227]. These alterations in maternal glucose flow could be important for several reasons. Maternal glucose signals to the fetus to release insulin; acute increases in glucose cause an increase in fetal insulin secretion while chronic overexposure to glucose can actually blunt fetal glucose stimulated insulin secretion [228]. Past research has also shown that maternal glucose can modify expression of glucose transporters in the fetus. Acute hyperglycemia causes an increase in GLUT1 in the fetal brain along with a decrease of GLUT4 in fetal skeletal muscle and adipose tissue, and this correlates with insulin resistance in the offspring [229, 230]. In a model of fetal hypoglycemia brought about by maternal insulin infusion, there is a decline in fetal liver GLUT1 but no changes in skeletal muscle or adipose tissue GLUT4 [229]. These finding indicate that fetal tissues respond and adapt to changes in maternal glucose. Changes in glucose flow to the fetus due to bouts of maternal exercise could be a potential programming mechanism behind the observed results in our studies. Intermittent reductions in glucose reaching the fetus could be altering fetal insulin secretion patterns and/or altering how the pancreas will respond to glucose stimulation; thereby modifying adipose tissue and skeletal muscle expression of insulin signaling proteins. In my mouse and rat model, changes in fetal exposure to glucose levels would be particularly important late in gestation when the rodent fetal pancreas exhibits increased glucose stimulated insulin release [231, 232]. Insulin signaling proteins may be up-regulated in these tissues and therefore making the tissues more sensitive to insulin stimulation. A next step for this project will be to look more extensively at insulin signaling proteins in the skeletal muscle and adipose tissue. Future studies will look at

possible epigenetic changes in offspring genes associated with glucose metabolism and insulin signalling.

In these studies, differences in offspring insulin sensitivity and glucose regulation were not detected until the animals were aged, in mice differences were first observed around 7 months of age and in rats, differences were detected starting at 10 months of age. This is not necessarily surprising given that much of the developmental programming research investigating glucose metabolism in offspring do not see differences until animals are aged [40, 44]. It may be that modifications in insulin sensitivity created by maternal malnutrition, or in the case of this project, maternal exercise, are subtle and cannot be detected until an "insult" such as age, occurs. It has been well documented that aging, even in healthy subjects, can lead to impaired glucose tolerance and peripheral insulin resistance [233-237]. In elderly subjects with impaired glucose uptake into skeletal muscle and adipose tissue, there is normal binding of insulin to insulin receptor in these tissues, indicating impaired post-receptor signaling [233]. Changes induced in offspring by maternal exercise could be protecting the offspring from this age related decline in insulin sensitivity. It will be important to look insulin signaling protein expression and activation both before and after there are noticeable differences in glucose regulation (at young and older ages) in offspring. A goal of a future study may also be to examine whether or not maternal exercise can protect offspring from the harmful effects of high fat feeding, similar to its effects seen in the aging offspring.

Timing and intensity of exercise required to produce beneficial effects in offspring will also be a focus of upcoming studies. In the current rodent model, mice and rats had voluntary access to running wheels before and during pregnancy and nursing.

Once acclimated to the wheels, both mice and rats ran up to an average of 10 km per day. In order to reduce and control the amount of running, a controlled exercise paradigm could be used in which mice or rats are removed from the home cage and placed on a treadmill or wheel for a prescribed amount of time per day.

Rodents can be offered an exercise intervention prior to and during pregnancy or start exercise right at conception as a means to investigate the critical window of exercise exposure. It is most likely that exercise prior to and during pregnancy will produce the most robust results in offspring since, in humans, the greatest reduction in fat mass at birth occurred when women exercise before and during pregnancy [126]. This particular timing of exercise may bring about the greatest changes in placental blood flow and therefore the greatest change in glucose flow to fetus, leading to the biggest changes in fetal insulin secretion and possibly programming of insulin sensitivity. Exercise training can increase the growth and density of blood vessels in skeletal muscle [238, 239], and perhaps in women who are already trained this contributes to greater changes in blood flow during exercise bouts during pregnancy than in women who are only starting an exercise regimen at the onset of pregnancy. The evidence from humans also suggests that it may be detrimental to stop exercise during pregnancy because of the impact exercise can have on placental growth. Again, physical activity in early pregnancy can increase placenta size and blood flow [127, 132, 143], so stopping exercise after this period can actually cause excess nutrient flow to the fetus resulting in higher fat mass and weight at birth compared to infants from mothers who were not active throughout their entire pregnancy [143]. Therefore it will be most interesting to see if exercise initiated at the beginning of pregnancy as opposed to before can have the same effects in offspring as

seen in our studies. This might also be more relevant in the human population since many women who become pregnant are not actively trying to become pregnant and would not have an opportunity to start an exercise regimen before conception [240].

Cross-fostering can also be used to evaluate the importance of maternal exercise during nursing on offspring. Pups from control dams would be cross-fostered to exercise dams and vice versa. Offspring would then be tested for differences between those exposed to maternal exercise during only gestation and those exposed only during the nursing period. In humans, moderate to intense endurance training during lactation does not impact lipid or lactose concentrations of energy density in breast-milk but can significantly increase protein content [241]. This level of exercise can also increase milk volume compared with volume from control mothers [242]. In rats, swim training does not change milk protein or fat content but can lower lactose concentrations [243]. Since exercise can somewhat alter milk composition, it will important to assess its influence on offspring in upcoming studies.

Given that data from both human and animal studies seems to suggest that even fairly intense weight-bearing exercises (such as running and aerobics) are not harmful to the fetus [244-246], and that many women of childbearing age are already exercise regularly [247], a feasible and important future goal of this project is to investigate the offspring metabolic effects of exercise during pregnancy in humans [248, 249]. There would be several factors to consider when designing a human trial for this project such as subject characteristics and exercise type/regimen. Since the initial goal of this study would be to follow metabolic outcomes in offspring as opposed to improving maternal health, it would be easiest to choose a population of healthy women who are already

performing some type of regular endurance exercise. Recruiting women who already exercise on a regular basis could also help with compliance throughout the study. Endurance exercise would be preferable over only resistance training since the evidence thus far in humans indicates that this type of physical activity result in some offspring differences like reduced fat mass at birth and reduced IGF-1 in cord blood [126, 129, 134]. Resistance training may be more desirable in pregnancies complicated by obesity and/or diabetes which will be discussed in the next section.

Women considered for the exercise cohort should maintain similar levels of physical activity (target could be 30 – 60 minutes a bout, 3 or 4 times a week at a moderate intensity) during pregnancy that she performed prior to pregnancy. Previous research has found that maintaining pre-pregnancy levels of physical activity are the most effective at changing offspring parameters such as birth weight [132]. In the offspring birth weight and body composition could be measured while cord blood could be collected to look at hormone levels such as leptin and IGF-1. At several age points throughout the first years of life, body weight, body composition, insulin, and glucose could be measured. These outcomes would all be compared to offspring from healthy mothers who did not exercise during their pregnancies to see if maternal exercise impacted offspring metabolic parameters at an early age.

To follow these offspring through adulthood and perform glucose tolerance tests and measure other parameters would be ideal, however it is not very realistic to expect that these offspring would remain in close geographical proximity or maintain compliance with such a long-term study. Funding for such a long-term project would also be difficult to obtain. In order to observe long-term effects of maternal exercise, it may be

more feasible to find an adult population that either: (1) still has a living mother who is available and able to give information about her level of physical activity or (2) who has good knowledge of how active their mother was during her pregnancy. After adults are separated into control or exercise groups based on the population spectrum, these subjects could be used to evaluate the impact of physical activity during pregnancy on outcomes such as adulthood diseases (like cancer or type 2 diabetes), insulin sensitivity, and glucose regulation. To date, no population studies looking at these outcomes have been performed.

The primary goal of this dissertation project is to provide evidence that will encourage women to be physically active during their pregnancy. Hopefully, women will be motivated to be active throughout their pregnancy if they are aware of the potential health benefits it can provide for their children.

5.1.2. *Aim 2.* The second aim of this dissertation was to evaluate the use of exercise in preventing maternal glucose intolerance during pregnancy in a model of diet-induced obesity. Two weeks of high fat feeding resulted in impaired glucose tolerance before mating. One week of exercise was effective in lowering body weight prior to mating. In mice fed a high fat diet, voluntary exercise improved glucose tolerance mid-gestation. Exercise also reduced fat mass and improved insulin sensitivity in adipose tissue. This suggests that exercise could be used to prevent the development of gestational diabetes. The most likely causes of improved glucose regulation in the current study are decreases in fat mass and insulin independent glucose uptake in skeletal muscle. Decreases in fat mass could be reducing inflammation-induced insulin resistance. Inflammatory cytokines, such as TNFα, are increased with obesity and pregnancy and

can inactivate proteins in the insulin signaling pathway and these levels can be even higher in obese pregnant women [250-252], while exercise training can decrease circulating cytokine levels [106]. In our model we do see decreased Akt phosphorylation with high fat feeding in adipose tissue after insulin stimulation and exercise returns phosphorylated Akt to control levels. This indicates that there is improvement in tissue insulin sensitivity with exercise. Exercise, which promotes muscle contractions, is also probably activating AMPK and therefore stimulating insulin independent glucose uptake. Future studies using this animal model will focus on the mechanisms of improved insulin sensitivity and glucose metabolism.

Translating this project to humans should be done with caution. First, the week of exercise prior to pregnancy is most likely not directly related to a clinical population of obese women unless they are advised/follow advice to start an exercise regimen before pregnancy. Although for some overweight women with infertility issues, diet and exercise has been effective in helping to conceive [253]. It is perhaps more relevant to study exercise at the onset of pregnancy when targeting maternal obesity and gestational diabetes. Also, the type of exercise used in this model may not be safe for a human population of overweight and/or diabetic women and their developing child. In humans, the intense weight-bearing exercises that have resulted in offspring benefits were performed by healthy women [85, 133]. For previously sedentary and overweight women, high levels of endurance training could be stressful and potentially harmful for the fetus [254, 255]. If the goal is to maintain normal glucose homeostasis, less rigorous cardiovascular exercises like resistance training that still promote muscle contraction and thereby stimulate glucose uptake into muscle, may be more reasonable and less stressful.

Previous studies have shown that resistance training, such as use of resistance bands, can be effective in helping to control hyperglycemia associated with gestational diabetes [124].

As stated in the introduction, high fat feeding during pregnancy and gestational diabetes can lead to offspring obesity and type 2 diabetes [68, 74, 208, 256]. Therefore, the ultimate goal with this animal model will be to follow offspring from dams set up in a similar breeding experiment to see if exercise can protect offspring from the detrimental effects of a maternal high fat diet. If exercise is effective in reducing high levels of glucose reaching the fetus by increasing maternal glucose uptake, it may be a desirable invention for pregnancies complicated by maternal obesity or diabetes.

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EDUCATION

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GRANTS AND AWARDS

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2005-2007	Dean's List, University of Kentucky
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PUBLICATIO NS

Carter, L.G., D'Ozario, J., and K.J. Pearson. Resveratrol and Cancer –Focus on In Vivo Evidence. *Endocrine-Related Cancer*. Invited review, Submitted May 2013.

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PLATFORM PRESENTATIONS AND SEMINARS

Carter, L.G. "Exercise during Pregnancy; Offspring Health Benefits" Gill Heart Institute Cardiovascular Research Day, University of Kentucky, Lexington, KY 2012.

Carter, L.G. "Exercise during Pregnancy Improves Glucose Regulation in Mature Offspring" Graduate Center for Nutritional Science Seminar Series, University of Kentucky, Lexington, KY. 2012.

Carter, L.G. "Maternal and Offspring Health Benefits of Perinatal Exercise" Graduate Center for Nutritional Science Seminar Series, University of Kentucky, Lexington, KY. 2011.

PUBLISHED ABSTRACTS
Carter, L.G., Tobia, C.M., Pearson, K.J. (2011) Maternal Running during Pregnancy Improves Glucose Disposal in Obese Mice. Obesity Society Annual Scientific Meeting, Orlando, FL.

Wellmann, K.A., Lewis, J.B., Carter, L.G., Carter, M., and S. Barron. Acute agmatine administration during ethanol withdrawal ameliorates ultrasonic vocalization deficits in pups exposed to ethanol during the first neonatal week. International Society for Developmental Psychobiology, November, 2007. Abstract published in *Developmental Psychobiology* 49: 744.

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ABSTRACTS AND POSTER PRESENTATIONS

Carter, L.G., Platt, K.M., Kinney, B, and Pearson, K.J. (2013) Maternal Controlled Exercise Improves Glucose Disposal in Mouse Offspring. Society for Gynecological Investigation Annual Meeting. Orlando, FL.

Carter, L.G., Qi, N., and Pearson, K.J. (2012). Exercise during Pregnancy Enhances Insulin Sensitivity in Adult Offspring. Barnstable Brown Obesity and Diabetes Research Day, University of Kentucky, Lexington, KY.

Carter, L.G., Platt, K.M., and Pearson, K.J. (2012). Maternal Running Improves Glucose Disposal in Obese Female Mice. Society for Gynecological Investigation Annual Meeting. San Diego, CA.

Carter, L.G., Tobia, C.M., Shridas, P., Garcia-Cazarin, M.L., Wolff, G., Esser, K.A., and Pearson, K.J. (2011). Exercise during Pregnancy Improves Glucose Disposal in Mice Offspring. Gill Heart Institute Cardiovascular Research Day, University of Kentucky, Lexington, KY.

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Kehrberg H.A., Carter L.G., Wellmann K.A., Prendergast M.A., Crooks P.A., Littleton J.M., and S. Barron. (2008) A Novel Compound, JR-220, Reduces Ethanol Withdrawal-Induced Neurotoxicity in Organotypic Hippocampal Slice Cultures. Society for Neuroscience, Washington DC.

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Carter, L.G., Carter, M.L., Wellmann, K.A., and S. Barron. (2007) Agmatine reduces ultrasonic vocalization deficits in female rat pups exposed neonatally to ethanol. The Society for Neuroscience: Blue Grass Chapter Neuroscience Day, Lexington, KY.