PROGRAMMING OF ADIPOSITY IN FEMALE OFFSPRING BY MATERNAL OBESITY AND EXERCISE

by

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Abstract

INTRODUCTION: In Canada, nearly half of women of child-bearing age are overweight (BMI 25-29.9 kg/m²) or obese (BMI ≥30 kg/m²). Current guidelines recommend moderate exercise for all pregnant women. However, little is known about the effect of exercise during pregnancy on the developing offspring. Rodent studies demonstrate that offspring from dams fed a high-fat diet have vessel-specific impairment in endothelium-dependent vascular function, greater adiposity, and impairments in glucose homeostasis. Further, offspring from exercised dams have improved glucose homeostasis.

OBJECTIVE: In this thesis, I determined if maternal exercise during pregnancy mitigates the adverse effects of maternal obesity on offspring adiposity, glucose homeostasis, and vascular health in female offspring.

METHODS: Dams (C57BL/6) were fed a control (10% energy from fat) or western diet (45% energy from fat) from weaning for 13 weeks and through breeding, pregnancy, and lactation. Just prior to breeding, dams were put into cages with or without a running wheel for voluntary exercise through breeding, pregnancy, and lactation. Offspring were weaned onto the control or western diet and maintained on the diet for 20 weeks; only female offspring were studied. Lean and fat mass were quantified by ^{1}H-NMR. Intraperitoneal insulin tolerance (IPITT), glucose tolerance (IPGTT) and insulin secretion tests (IPIST) were performed prior to the end of the feeding period. Vascular endothelial-dependent and independent dilatation were assessed *ex vivo*

by isometric force measurements of aortic rings in response to phenylephrine, acetylcholine, and sodium nitroprusside.

RESULTS: Weaning weights were greater in offspring from western-fed dams than control-fed dams. Control-fed offspring from western-fed dams had higher heart and kidney weights than those from control-fed dams. Control-fed offspring from exercised dams had higher blood glucose concentrations at $IPGTT_{30minutes}$ than those from sedentary dams. Western-fed offspring from exercised dams had greater body weights, retroperitoneal fat weights, and *Il10* mRNA in retroperitoneal adipose tissue, and lower blood glucose concentrations at $IPGTT_{90minutes}$ than those from sedentary dams. Endothelium-dependent vasorelaxation of thoracic aortae was likely unaffected by maternal western diet or maternal exercise in control-fed and western-fed offspring.

CONCLUSION: These findings suggest maternal exercise is beneficial in improving glucose homeostasis and adiposity in western-fed female offspring.

Lay Abstract

In Canada, nearly half of women of childbearing age are overweight or obese. Further, the children of overweight or obese women have a higher risk of hospitalization from heart disease and a higher risk of death from any cause. Current guidelines recommend moderate exercise for all pregnant women to maintain a healthy lifestyle. However, little is known about the effect of maternal exercise on the developing child.

This work aims to understand whether maternal exercise during pregnancy is beneficial in reducing the effects of maternal obesity on their offspring's risk for heart disease. The health of female offspring was assessed by an indicator of blood vessel health that precedes heart disease and their ability to handle changes in blood sugar. This work could allow us to better inform the recommendation of maternal exercise during pregnancy and anticipate the future metabolic health of Canadians.

Preface

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Experimental Medicine and was prepared under the direction and supervision of Dr. Angela Devlin and Dr. Alexander Beristain. This thesis was reviewed by Dr. Angela Devlin, Dr. Alexander Beristain, Dr. Ismail Laher, and Dr. Louis LeFebvre. All work contained herein is unpublished.

All procedures were approved by the University of British Columbia Animal Care Committee under the following certificate numbers: A14-0246, A14-0030.

All molecular biology experiments were performed by me within Dr. Angela Devlin's laboratory at British Columbia Children's Hospital Research Institute (BCCHRI). All animal work was performed in the animal facility located at BCCHRI. Both Nicha Boonpattrawong and I were involved in the feeding of dams and weaning of offspring mice. Feeding, weighing, IPITTs, IPGTTs, ISTs and dissections of the female offspring discussed in this thesis were performed by me.

Functional assessment of the thoracic aorta was performed in Dr. Ismail Laher's laboratory; located on the Point Grey campus of the University of British Columbia, Vancouver, BC. Dissection, mounting, and functional assessment of the aorta was performed by Dr. Saeid Golbidi.

All analyses of data produced were performed by me under the supervision and guidance of Dr. Angela Devlin. The overall hypothesis was designed by Dr. Angela Devlin, Dr. Alexander Beristain, and Dr. Ismail Laher.

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List of Abbreviations

18S rRNA	18S ribosomal RNA
ACh	acetylcholine
ACTH	adrenocoricotropic hormone
ANOVA	analysis of variance
BCCHRI	BC Children's Hospital Research Institute
BH_4	tetrahydrobiopterin
BMI	body mass index
CCL2	C-C motif chemokine ligand 2
CCR2	C-C motif chemokine receptor 2
CDC	Centers for Disease Control and Prevention
cGMP	cyclic guanine monophosphate
CRH	corticotropin-releasing hormone
CSEP	Canadian Society for Exercise Physiology
CVD	cardiovascular disease
CX_3CR1	C-X3-C motif chemokine receptor 1
DIO	diet-induced obesity
DOHaD	Developmental Origins of Health and Disease
eNOS	endothelial nitric oxide synthase
HPLC	high performance liquid chromatography
ICR	Institute for Cancer Research
IL-10	interleukin 10

IL-13	interleukin 13
IL-1Ra	interleukin 1 receptor antagonist
IL-1β	interleukin 1 beta
IL-4	interleukin 4
IL-6	interleukin 6
IP	intraperitoneal
IPGTT	intraperitoneal glucose tolerance test
IPIST	intraperitoneal insulin secretion test
IPITT	intraperitoneal insulin tolerance test
L-NAME	N^G-nitro-L-arginine methyl ester
L-NMMA	N^G-monomethyl-L-arginine
LPS	lipopolysaccharide
MCP-1	monocyte chemoattractant protein 1
MRI	magnetic resonance imaging
MSP	methylation-specific PCR
NADPH	reduced form of nicotinamide adenine dinucleotide phosphate
Ncoa6	nuclear receptor coactivator 6
NMR	nuclear magnetic resonance
NO	nitric oxide
NOD	non-obese diabetic
$ONOO^-$	peroxynitrite
PE	phenylephrine
PSIA	pounds per square inch absolute

PSS	physiological saline solution
rRNA	ribosomal RNA
RT-qPCR	reverse transcription quantitative polymerase chain reaction
SD	standard deviation
SNP	sodium nitroprusside
SOGC	Society of Obstetricians and Gynaecologists of Canada
STZ	streptozotocin
Tnf	tumour necrosis factor alpha
TSS	transcriptional start site
VSMC	vascular smooth muscle cell
WAT	white adipose tissue

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Chapter 1: Introduction

1.1 Obesity

Obesity is a complex disease that fundamentally, involves an imbalance between energy intake and energy expenditure. It is defined as a body mass index (BMI) greater than or equal to 30 kg/m^2 by the Centers for Disease Control and Prevention (CDC)[1]. Further, the overweight range is defined by the CDC as a BMI greater than or equal to 25 kg/m^2 but less than or equal to 29.9 kg/m^2. Importantly, being overweight or obese is associated with a higher risk for several comorbidities, such as: coronary heart disease, type 2 diabetes, certain cancers, hypertension, blood lipid disorders, stroke, liver disease, gallbladder disease, abnormal menstrual cycles, and infertility[2].

An overwhelming amount of evidence exists for the association between a sedentary lifestyle and cardiovascular disease (CVD) risk. Additionally, although heterogeneous in its effect on the individual level, physical activity in adult men and women is associated with lower visceral adiposity, lower blood pressure, improved glucose homeostasis[3]. Further, physical activity may represent a non-pharmacological intervention to both reduce adipose stores within the body and mitigate changes in metabolism that are associated with excess adiposity.

Other interventions for reducing adiposity include weight loss through dieting and bariatric surgery. In a meta-analysis of 22, 094 subjects who underwent bariatric surgery, significant improvements were made in blood pressure, type 2 diabetes, and dyslipidemia[4]. However, it is currently recommended that bariatric surgery to be reserved for individuals with severe obesity[5], defined as a BMI greater than 40 kg/m^2. Additionally, in a single-centre randomized trial involving 160 overweight or obese subjects with hypertension, dyslipidemia, or

fasting hyperglycemia, higher diet adherence was associated with higher weight reduction and less CVD risk[6].

In older adults with coronary artery disease physical activity is associated with improved endothelial function, as assessed via flow-mediated vasodilation in the posterior tibial artery[7] and endothelium-dependent vasodilation in the left mammary artery in response to acetylcholine (ACh)[8]. Additionally, maternal exercise is associated with several benefits for the mother. Specifically, maternal exercise is associated with less gestational weight gain, as well as improved glycemic control in women with gestational diabetes mellitus[9].

Maternal obesity during pregnancy is linked with several adverse outcomes, such as lowered fertility, gestational diabetes mellitus, preeclampsia, a higher risk of preterm birth, as well as higher risk for neural tube defects and macrosomia in their offspring[10]. Gestational diabetes mellitus is defined as any degree of glucose intolerance with onset or first recognition during pregnancy[11]. Gestational hypertension is raised blood pressure in the absence of proteinuria that occurs in the second half of pregnancy[12]. Conversely, preeclampsia involves the presence of proteinuria in addition to raised blood pressure, and usually occurs after 20 weeks of gestation.

Interestingly, higher fat mass and percent body fat is seen in neonates from overweight or obese mothers compared to those from mothers with a healthy BMI[13,14]. Differences are not seen in neonate fat-free mass and birth weight; highlighting the importance of assessing body composition. In a study involving 33 neonates from overweight or obese mothers and 33 neonates from mothers with a healthy BMI, offspring from overweight or obese mothers had higher fat mass and percent body fat mass, as assessed by air-displacement plethysmography, compared to offspring from mothers with a healthy BMI[13]. In another study involving 76

neonates from overweight or obese mothers and 144 neonates from mothers with a healthy BMI, offspring from overweight or obese mothers had higher fat mass and percent body fat as compared to offspring from mothers with a healthy BMI[14].

Additionally, the Healthy Start study was a prospective observational cohort that analyzed 1040 mother-offspring pairs to determine the effect of maternal diet during pregnancy on infant body size and body composition[15]. Neonatal adiposity, as determined by air displacement plethysmography, was associated with higher maternal intake of total fat, saturated fat, unsaturated fat, and total carbohydrates compared to protein; however, fat-free mass and birth weight were not.

Interestingly, the higher adiposity exhibited by offspring of mothers with higher BMIs is seen in neonates, children, and adolescents. The Avon Longitudinal Study of Pregnancy and Childhood, a longitudinal cohort study in the United Kingdom of 889 children representative of their population, found that the point at which BMI increases after its low point in childhood, termed adiposity rebound, was earlier in offspring of parents with higher BMI[16]. Further, a retrospective study involving the calculation of BMIs for 390 Group Health Cooperative of Puget Sound members at three intervals encompassing a portion of their childhood and adolescence determined that early adiposity rebound was associated with a higher risk of adult obesity[17].

1.2 Programming of Offspring Cardiovascular Disease by Maternal Obesity in Humans

The developmental origins of health and disease (DOHaD) theory, which has evolved from the fetal origins hypothesis originally formulated by Dr. David Barker[18], posits that the conditions to which one is exposed either *in utero* or in early life have the capacity to influence

one's health in adulthood. It is currently known that maternal obesity in humans have been associated with adverse health outcomes for their adult offspring[19–22]. These adverse health outcomes mainly pertain to their cardiovascular health as well as disturbances in glucose homeostasis. For example, epidemiological studies have reported that maternal obesity and/or greater than recommended weight gain during pregnancy have been associated with higher adiposity, higher BMI, and risk factors for CVD both in childhood and adulthood[19–22]. Further, in a record linkage cohort analysis of 37 709 people based in the United Kingdom, the risk of a cardiovascular event as well as all-cause mortality was higher for individuals whose mothers were obese compared to mothers with a healthy BMI[22]. These effects were seen after adjusting for possible confounders such as maternal age at delivery, socioeconomic status, sex, current age, birth weight, gestational age at delivery, and gestational age at measurement of maternal BMI.

There is evidence to suggest that there are critical periods in development within which developmental programming may occur. For example, the Dutch Famine Birth Cohort consists of 2414 individuals who were conceived at a university hospital in Amsterdam, the Netherlands between November 1st, 1943 and February 28th, 1947[(Ref. 23)]. Extreme stress and undernutrition occurred in a subset of these individuals' mothers during pregnancy because of the Dutch famine, which occurred from November 1944 until April 1945. Thus, this allowed for the study of individuals who *in utero*, were exposed to the Dutch famine at different points in gestation[23]. Interestingly, the effects seen in the adult offspring of these mothers were different depending on when in gestation they were exposed to the Dutch famine. Individuals exposed to the famine in late or mid gestation, were found to have glucose intolerance[24]. This was evidenced by higher plasma glucose concentrations 2 hours post-administration of a 75 gram glucose load taken

orally[24]. However, individuals who were exposed to the famine in early gestation were found to have an atherogenic lipid profile consisting of a higher LDL to HDL cholesterol ratio in both sexes[25], as well as higher BMIs only in females[26] compared to those not exposed to the Dutch famine *in utero*.

Currently, although moderate exercise is recommended for all pregnant women to maintain a healthy lifestyle[27], very little is known about its effect on the developing offspring. However, it is important to note that maternal exercise does not appear to have adverse effects on the fetus between 28 and 32 weeks of age, as assessed by measures of fetal well-being including: umbilical artery Dopplers, fetal heart tracing, fetal heart rate, and biophysical profile[28].

1.3 Programming of Offspring Cardiovascular Disease by Maternal Excess Adiposity in Rodents

Rodent models of diet-induced obesity (DIO) in dams have served as an indispensable tool in complementing epidemiological studies. While informative about risk factors that may contribute to a disease, epidemiological studies are associative in nature. Thus, they do not allow one to investigate risk factors that may be causative. In contrast, rodent studies allow the scientific community to investigate the possible mechanisms by which offspring of obese mothers are predisposed to metabolic consequences later in life. By using rodent models to gain an improved understanding of the biology underlying the programming of offspring from dams with DIO, therapeutic interventions may be tested to determine their safety, efficacy, and potential utility in treating maternal DIO in humans.

Further, there are many benefits to working with rodents that relate primarily to the feasibility of conducting a study. Mice have a gestational period of 21 days compared to

approximately 40 weeks in humans. Issues of cost, logistics, ethics, treatment adherence, and variance due to genetic and environmental heterogeneity are reduced when working with mice compared to humans. However, although rodent models are conducive to performing experiments the results of these studies must be interpreted carefully in informing potential links to human biology.

Rodent models of DIO in dams have been useful in elucidating the mechanisms that may underlie the programming of their offspring. In many studies, offspring from dams with DIO are studied in relation to those from control diet-fed dams to determine the effect of maternal diet on offspring health. Interestingly, offspring from Sprague Dawley rat dams with DIO have higher adiposity, hypertension, insulin resistance, and risk factors for CVD, including impairments in endothelium-dependent vasorelaxation in third-order branches of the mesenteric arcade[29–34]. Offspring from C57BL/6J mouse dams with DIO have higher adiposity, hyperphagia, hypertension, insulin resistance and cardiac hypertrophy[35,36].

Further, there is a role for maternal exercise in the programming of their offspring. Namely, glucose homeostasis is improved in offspring from exercised Institute for Cancer Research (ICR) mice dams compared to sedentary dams[37]. Specifically, male and female offspring from exercised ICR dams exhibit lower blood glucose concentrations at 15, 30, 60, and 90 minutes post-administration of insulin in an intraperitoneal insulin tolerance test (IPITT). Male offspring also have lower blood glucose concentrations at 120 minutes post-administration of insulin in an IPITT. These findings indicate that offspring from exercised mouse dams are more sensitive to the effects of insulin compared to those from sedentary dams.

In addition, glucose tolerance is improved in offspring from exercised ICR dams compared to those from sedentary ICR dams; as indicated by lower area under the curve

calculations from oral glucose tolerance tests. Although this supports maternal exercise as being beneficial for their offspring, a knowledge gap remains with respect to whether maternal exercise is beneficial for their offspring in the context of maternal DIO. Interestingly, paternal exercise has been demonstrated to be deleterious to offspring body composition and glucose homeostasis. Greater body weight, adiposity, and insulin concentrations, and glucose intolerance were reported in post-weaning high-fat diet-fed offspring from exercised C57BL/6J sires compared to sedentary sires[38].

1.4 Endothelial Dysfunction and Endothelial Nitric Oxide Synthase Uncoupling

The vascular endothelium has roles in regulating vascular tone, leukocyte adhesion, as well as the clotting cascade[39], with vascular endothelial dysfunction characterized as impairments in any these processes[39]. Comprised of a single-cell layer facing the lumen of the vasculature, the vascular endothelium provides an interface between the blood and the underlying smooth muscle. It is understood that endothelial dysfunction is one of the earliest markers of vascular disease[40–43] and is predictive of the future risk of a cardiovascular event[44–47].

Nitric oxide (NO) is a key molecule produced by the endothelium involved in relaxing vascular tone. Further, endothelial nitric oxide synthase (eNOS) is responsible for the majority of NO produced within the vasculature[48]. Nitric oxide produced within the endothelium diffuses within the vascular wall where it may stimulate the production of cyclic guanine monophosphate (cGMP) by soluble guanylyl cyclase present in vascular smooth muscle cells (VSMCs). Finally, regulation of vascular tone is achieved by the endothelium through VSMC relaxation induced by cGMP. Briefly, nitric oxide synthases consist of two monomers, each consisting of a reductase domain and an oxygenase domain. In the presence of O_2, nitric oxide synthases catalyze the

oxidation of L-arginine and require the following co-factors: calmodulin, flavin adenine dinucleotide, flavin mononucleotide, heme, the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), and tetrahydrobiopterin (BH_4). Heme is required for the formation of the nitric oxide synthase homodimer whereas BH_4 stabilizes the homodimer.

The production of superoxide within endothelial cells, whether through eNOS uncoupling or higher production by NADPH oxidases, can result in the formation of peroxynitrite ($ONOO^-$) from the reaction of superoxide with NO. Further, BH_4 is a primary target for oxidation by $ONOO^-$ wherein it is converted to dihydrobiopterin and can no longer act as a cofactor for eNOS[49]; promoting a cycle wherein BH_4 is degraded and the production of superoxide by eNOS is higher, owing to eNOS uncoupling[48,49]. Thus, BH_4 supplementation has been explored in both humans and rodent models to determine whether it is possible to restore endothelium-dependent vasorelaxation through BH_4 supplementation. Interestingly, in Sprague-Dawley rats rendered diabetic through the administration of streptozotocin (STZ), a beta-cell toxin, endothelium-dependent vasorelaxation in response to ACh was improved after aortic rings were incubated with BH_4 compared to aortic rings that had not been incubated with BH_4[(Ref. 50)]. Further, BH_4 administration did not have an effect on control rats that had not been treated with STZ.

Tetrahydrobiopterin supplementation has been reported to improve endothelium-dependent vasorelaxation in response to ACh in a controlled, clinical trial involving subjects with type 2 diabetes[51]. The trial consisted of 23 subjects with diabetes aged 52 ± 2 years (mean ± SEM), of which 17 were male and 7 were female, and 12 controls aged 50 ± 3 years (mean ± SEM), of which 8 were male and 4 were female. Venous occlusion plethysmography measurements of forearm blood flow were measured in response to intra-brachial artery infusion of ACh and sodium nitroprusside (SNP) to assess endothelium-dependent vasodilation and

endothelium-independent vasodilation, respectively. Following an ACh dose-response curve in the presence of NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) and a 30-minute rest, subjects were infused with BH_4. Acetylcholine and SNP dose-response curves were then repeated to determine the effect of BH_4. An ACh dose-response curve was subsequently repeated with co-infusion of BH_4 and L-NMMA. Subjects with hypertension, increased LDL-cholesterol, cardiac or cerebrovascular disease, renal impairment, insulin use, antioxidant use were excluded from this study. Subjects who were smokers or were undergoing hormone replacement therapy were also excluded.

Additionally, exercise has been demonstrated to have a beneficial effect on vascular function. It has been reported that exercise can restore endothelium-dependent vasorelaxation in response to ACh in hypercholesterolemic rabbit femoral arteries[52]. Briefly, male New Zealand white rabbits were fed a chow diet including 2% cholesterol for eight weeks and were exercised five days per week for a total of eight weeks by running on a treadmill. Additionally, Oil Red O staining on longitudinally-split 5-millimetre-long femoral artery segments showed higher lipid deposition on the vascular surface in rabbits fed a chow diet containing 2% cholesterol compared to those fed a chow diet. Importantly, lipid deposition on the vascular surface was lower in exercised rabbits compared to sedentary rabbits.

1.5 Adipose Tissue Inflammation

It is important to contrast chronic low-grade systemic inflammation, which is seen to occur in the context of excess adiposity, against acute inflammation. The circulating concentrations of cytokines such as TNF-α, IL-1β, IL-6, IL-1 receptor antagonist, and soluble TNF-α receptors may increase several-fold in acute inflammation. However, they also decrease

following the resolution of acute infection or trauma. In contrast, these cytokines and cytokine antagonists exhibit a two to three-fold increase circulating concentrations in chronic low-grade systemic inflammation and do not appear to decrease or resolve[53–55]. Further, chronic low-grade systemic inflammation is distinct from acute inflammation, which is defined by the presence of redness, swelling, pain, and heat[55].

It is generally understood that excess adiposity is associated with inflammation in adipose tissue, as well as the brain, liver, and pancreas[55]. Adipocyte cell death, resulting from adipocyte hypertrophy may be a precipitating factor in the initiation of adipose tissue inflammation. Further, adipocyte cell death may occur as a result of local hypoxic conditions[56–58]; potentially, as a result of the expansion of the adipose tissue beyond the capabilities of the vasculature that supplies it[59].

The formation of crown-like structures, consisting of a dead adipocyte within its center surrounded by inflammatory macrophages, are seen in adipose tissue expansion in mice fed either a diet consisting of 45% kcal from fat[60] or 60% kcal from fat[61,62]. Further, these crown-like structures are also seen in biopsies of abdominal and gluteal subcutaneous white adipose tissue (WAT) and omental visceral WAT of obese humans[63]. Adipose tissue biopsies were taken from male (n = 12) and female (n = 9) subjects without ongoing infection or cancer undergoing elective surgery at Ancona General Hospital, located in Ancona, Italy. Subjects were 44.7 ± 16.2 (mean ± SD) years of age, and samples were taken from 12 obese subjects, 2 overweight subjects, and 7 lean subjects; with BMI not being statistically different across sex. Crown-like structures serve to scavenge the remnants of a dead adipocyte and its associated lipid droplet, which has the potential to be cytotoxic[63]. However, these inflammatory macrophages also release factors, such as TNF-α, that influence the development of insulin resistance[64].

1.6 Anti-Inflammatory Effects of Exercise

Activation of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis occurs in response to exercise, leading to higher secretion of cortisol and epinephrine[65]. Further, afferent impulses from working muscles induce an intensity-dependent increase in sympathetic nervous system activity to the adrenal glands.

Briefly, corticotropin-releasing hormone (CRH) is synthesized and released from the paraventricular nucleus of the hypothalamus into the hypophyseal portal system to be transported to the anterior pituitary. Subsequently, adrenocorticotropic hormone (ACTH) is released into the systemic circulation following binding of CRH to pituitary corticotropes. Finally, glucocorticoid synthesis and secretion into the systemic circulation is stimulated following ACTH stimulation. Glucocorticoids produced by the adrenal glands then inhibit the production of CRH and ACTH in the hypothalamus and anterior pituitary, respectively, through a negative feedback cycle.

Cortisol is a glucocorticoid released by the adrenal cortex following ACTH stimulation in response to exercise; and may exert its effects via transcriptional or non-transcriptional mechanisms[66,67]. Cortisol is known to be a potent anti-inflammatory. Further, norepinephrine and epinephrine are catecholamines released from the adrenal medulla following sympathetic nervous system stimulation. In a study including 19 adult males, epinephrine was determined to inhibit the appearance of TNF-α in the circulation following lipopolysaccharide (LPS) stimulation and promote the release of IL-10[68]. When pretreated with a three-hour infusion of epinephrine (n = 5) the appearance of TNF-α within the circulation following LPS stimulation in males was lower compared to males who received an infusion of saline (n = 6)[68]. Additionally, the appearance of IL-10 within the circulation following LPS stimulation in males was higher

when pretreated with a three-hour infusion of epinephrine. However, pre-treatment with a 24-hour infusion of epinephrine (n = 6) attenuated the appearance of TNF-α within the circulation.

Thus, the anti-inflammatory effects of exercise are multi-faceted. Higher amounts of cortisol and epinephrine due to the activation of the sympathetic nervous system result in lower TNF-α within the circulation[66,67]. Potentially, a lower proportion of inflammatory to anti-inflammatory monocytes within the circulation[69], leads to lower levels of inflammatory cytokines and adipose tissue infiltration of inflammatory monocytes. Finally, higher levels of IL-6 being released from working muscle, stimulating the release of IL-1 receptor antagonist from monocytes and macrophages, inhibits the action of the inflammatory cytokine IL-1β. Studies have reported that the higher concentrations of IL-6 within the circulation, whether as a function of exercise or administration of recombinant human IL-6, results in the induction of higher IL-1 receptor antagonist, IL-10, and cortisol concentrations within the plasma of human males[70,71].

Chapter 2: Rationale and Hypothesis

In Canada, nearly half of men and women between the ages of 25 and 44 are overweight or obese; between the ages of 18 and 24, 36.2% of men and women are overweight or obese[72]. Importantly, maternal obesity and/or greater than recommended weight gain during pregnancy have been associated with higher adiposity, higher BMI, and risk factors for CVD both in childhood and adulthood[19–22]. Currently, it is recommended by the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Canadian Society for Exercise Physiology (CSEP) for all pregnant women to participate in aerobic and strength-conditioning exercises to promote a healthy lifestyle[27]. Maternal exercise during pregnancy is associated with less gestational weight gain in overweight women as well as improved glucose tolerance in women with gestational diabetes[9]. However, the consequences of maternal exercise in the context of maternal obesity on the metabolic health of their offspring in adulthood are largely unknown.

Thus, the overall hypothesis of my thesis states: maternal exercise during pregnancy is beneficial in mitigating the adverse effects of maternal obesity on offspring health.

I tested this hypothesis through the following specific aims:

AIM 1

A. To determine whether exercise can mitigate the adverse effect of maternal obesity on offspring adiposity, glucose homeostasis, and endothelium-dependent vasorelaxation in adult female offspring fed a post-weaning control diet.

B. To determine whether there is an association between maternal exercise and maternal obesity on the expression of inflammatory cytokines in the retroperitoneal adipose tissue of adult female offspring fed a post-weaning control diet.

AIM 2

A. To determine whether exercise can mitigate the adverse effect of maternal obesity on offspring adiposity, glucose homeostasis, and endothelium-dependent vasorelaxation in adult female offspring fed a post-weaning western diet.

B. To determine whether these is an association between maternal exercise and maternal obesity on the expression of inflammatory cytokines in the retroperitoneal adipose tissue of adult female offspring fed a post-weaning western diet.

Chapter 3: Methods

3.1 Animals and Animal Breeding Scheme

C57BL/6 mice were purchased from Charles River Laboratories to breed for experimental mice. Mice were bred, housed, and maintained in the animal care facility located at BCCHRI. Daily observation of animals was performed by animal care facility staff.

Mice obtained from Charles River Laboratories were bred at six weeks of age; offspring of these mice were used as the dams for the current study. Dams were assessed and taken care of by a PhD student in the Devlin laboratory (Nicha Boonpattrawong). Dams were weaned at three weeks of age and were randomly assigned to either a control diet (Catalog Number: D12450K, Research Diets Incorporated) or a western diet (Catalog Number: D12451, Research Diets Incorporated) to induce excess adiposity; consisting of 10% energy from fat and 45% energy from fat, respectively (see **Table 3.1** for diet composition). Dams were fed for 13 weeks post-weaning and through breeding, pregnancy, and lactation. Immediately prior to breeding, dams were placed into cages with or without an exercise wheel to allow for voluntary exercise through breeding, pregnancy, and lactation. Female offspring were weaned at three weeks of age and were randomly assigned to either the control diet or western diet for 20 weeks. Glucose homeostasis was assessed prior to the end of the feeding period by IPITTs, IPGTTs, and ISTs at 19, 20, and 21 weeks post-weaning, respectively.

Isometric force measurements of contraction and relaxation were measured in aortic rings in response to phenylephrine (PE), ACh, and SNP (vasoconstriction, endothelium-dependent vasodilation, and endothelium-independent vasodilation, respectively) described below.

Eight offspring groups were studied to discern the effects of maternal obesity and maternal exercise during pregnancy:

CSC, Maternal Control/Sedentary - Post-Weaning Control-Fed (N=7),

CEC, Maternal Control/Exercise - Post-Weaning Control-Fed (N=7),

WSC, Maternal Western/Sedentary - Post-Weaning Control-Fed (N=5),

WEC, Maternal Western/Exercise - Post-Weaning Control-Fed (N=8),

CSW, Maternal Control/Sedentary - Post-Weaning Western-Fed (N=7),

CEW, Maternal Control/Exercise - Post-Weaning Western-Fed (N=10),

WSW, Maternal Western/Sedentary - Post-Weaning Western-Fed (N=6),

and WEW, Maternal Western/Exercise - Post-Weaning Western-Fed (N=8).

A total of 143 offspring mice were used in this study. However, only 134 mice could be used within the experiment. This is because eight mice were sacrificed due to malocclusion. Further, one mouse was found dead due to unknown cause: despite lower body weight as compared to its sister, this was not due to malocclusion. Two mice per litter were used for the study; one for each post-weaning diet. However, littermates were not culled to provide ample tissue for molecular biology experiments. Mice were housed in groups of 2-4 per cage; with littermates when possible.

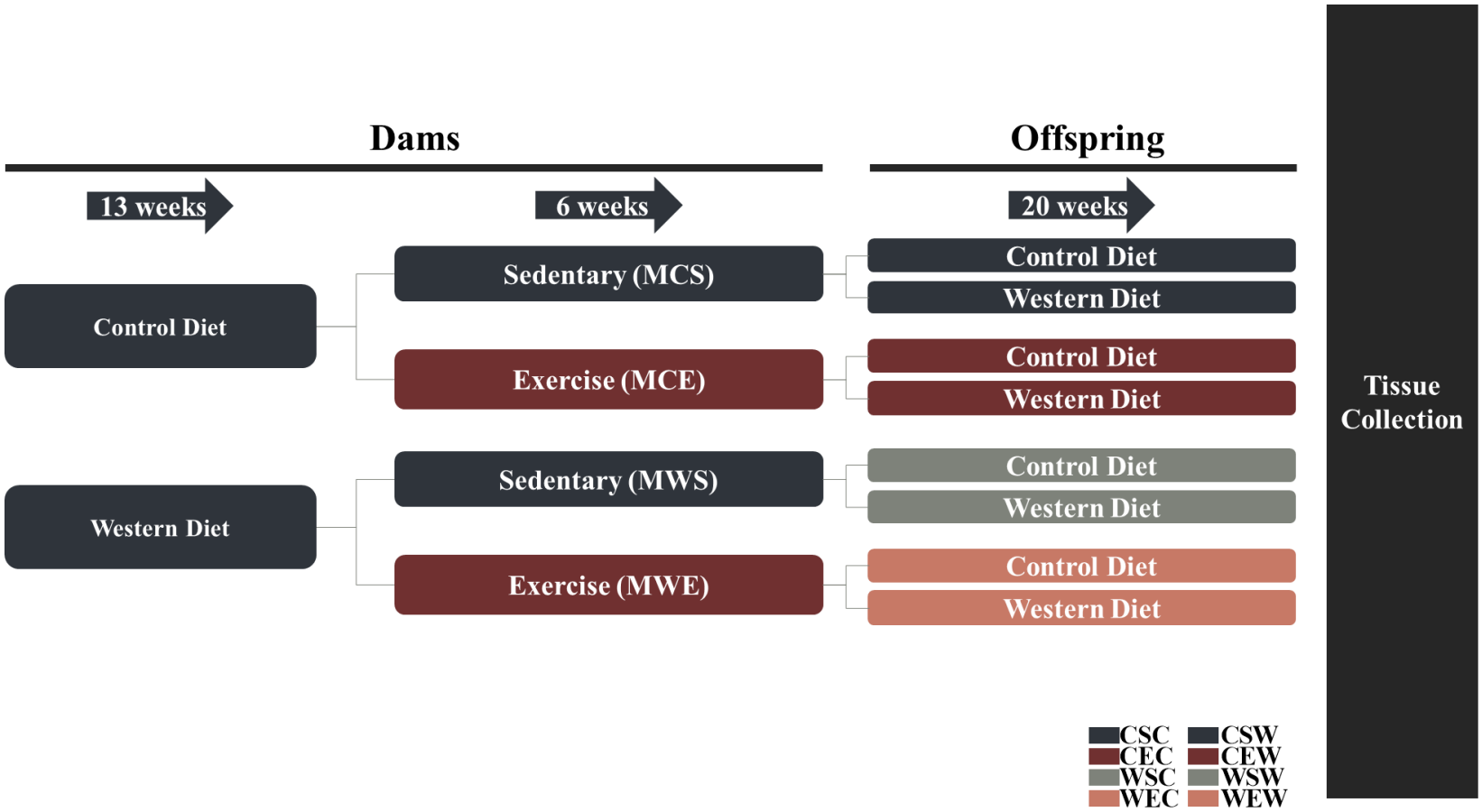

Figure 3.1 Animal Breeding Scheme

The animal breeding scheme resulted in eight offspring groups: maternal **w**estern/**e**xercise, maternal western/sedentary, maternal control/exercise, and maternal control/sedentary x 2 post-weaning diet groups (WEW (N=8), WSW (N=6), CEW (N=10), CSW (N=7), WEC (N=8), WSC (N=5), CEC (N=7), and CSC (N=7)).

3.2 Diet Composition and Exercise Treatment

	D12450K (Control)		D12451 (Western)	
	g%	kcal%	g%	kcal%
Protein	19.2	20	24	20
Carbohydrate	67.3	70	41	35
Fat	4.3	10	24	45
Total		100		100
Total kcal/gm	3.85		4.73	
Ingredient	**g**	**kcal**	**g**	**kcal**
Casein	200	800	200	800
L-Cystine	3	12	3	12
Corn Starch	550	2200	72.8	291
Maltodextrin 10	150	600	100	400
Sucrose	0	0	172.8	691
Cellulose, BW200	50	0	50	0
Soybean Oil	25	225	25	225
Lard	20	180	177.5	1598
Mineral Mix S10026	10	0	10	0
DiCalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate, 1 H2O	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40
Choline Bitartrate	2	0	2	0
FD&C Red Dye #40	0.025	0	0.05	0
FD&C Blue Dye #1	0.025	0	0	0
Total	**1055.05**	**4057**	**858.15**	**4057**

Table 3.1 - Diet Composition

3.3 Body Composition Assessment via Echo Magnetic Resonance Imaging (EchoMRI)

Body composition was assessed in female offspring mice at 19 weeks of age via ^{1}H-NMR using the EchoMRI-095 (Catalog Number: EMR-095, EchoMRI) and EchoMRI Software Version 120518[73]. The EchoMRI machine is located in the animal facility at BCCHRI and its use kindly provided by Dr. William Gibson's laboratory. Measurements of fat mass, lean mass, free water mass, and total water mass were obtained in triplicate for each animal which were then averaged for analysis.

3.4 Intraperitoneal Insulin Tolerance Test (IPITT)

The IPITT was performed on female offspring mice at 19 weeks post-weaning. Insulin (Novolin®ge Toronto, Insulin Injection, Human Biosynthetic (Regular), rDNA, DIN 02024233) was diluted 1:1000 in PBS (Catalog number: 70013032, ThermoFisher Scientific) that was filtered through a sterile, 25 mm Acrodisc syringe filter with a 0.2 μm Supor membrane (Catalog Number: 4612, Pall Corporation). Mice were administered 0.75 units of insulin per kg of lean mass as determined by EchoMRI at 19 weeks of age. Mice were fasted for five hours prior to the start of the IPITT. The lateral tip of the tail was lanced with a 25-gauge needle (Catalog Number: 305122, Becton, Dickinson and Company) to produce blood samples for blood glucose concentration measurements for the duration of the IPITT. Blood glucose was measured at 0, 15, 30, 60, 90, and 120 minutes post-administration of insulin. Blood glucose concentrations were measured using the OneTouch Verio® Blood Glucose Meter (LifeScan).

3.5 Intraperitoneal Glucose Tolerance Test (IPGTT)

The IPGTT was performed on female offspring mice at 20 weeks post-weaning. A 25% solution of dextrose (Catalog Number: D9434, Sigma) was prepared in water that was subsequently filtered through a sterile, 25 mm Acrodisc syringe filter with a 0.2 μm Supor membrane (Catalog Number: 4612, Pall Corporation). Mice were administered 0.75 grams of dextrose per kilogram of lean mass as determined by EchoMRI at 19 weeks of age. Mice were fasted for five hours prior to the start of the IPGTT. The lateral tip of the tail was lanced with a 25-gauge needle to produce blood samples for blood glucose concentration measurements for the duration of the IPGTT. Blood glucose was measured at 0, 15, 30, 60, and 90 minutes post-administration of dextrose solution. Blood glucose concentrations were measured using the OneTouch Verio® Blood Glucose Meter (LifeScan). Immediately prior to the start of the IPGTT, mice were restrained in a 50 mL conical tube with holes to provide ventilation. A small section above the lateral saphenous vein of the left hind leg was trimmed. This section was swabbed with gauze soaked in 70% ethanol prior to a thin film of petroleum jelly being applied with a cotton-tipped swab. Blood was collected by lancing the lateral saphenous vein with a sterile, 25-gauge needle. Approximately 50-70 μL of blood was collected into a microhematocrit capillary tube (Catalog Number: 22-362-574, Fisher Scientific) before being transferred to a microcentrifuge tube to prepare serum.

3.6 Intraperitoneal Insulin Secretion Test (IPIST)

The IPIST was performed on female offspring mice at 21 weeks post-weaning. A 27% solution of dextrose (Catalog Number: D9434, Sigma) was prepared in 0.9% NaCl and subsequently filtered through a sterile, 25 mm Acrodisc syringe filter with a 0.2 μm Supor

membrane (Catalog Number: 4612, Pall Corporation). 0.9% NaCl was prepared with crystalline NaCl (Catalog Number: S271-3, Fischer Scientific Company). Mice were administered 2 grams of dextrose per kilogram of body weight. Mice were fasted for five hours prior to the start of the IPIST. A small section above the lateral saphenous vein of the left and right hind legs was trimmed immediately after restricting access to food. Blood glucose concentration was measured at 0, 15, and 30 minutes post-administration of dextrose solution using blood samples produced from the saphenous vein. Blood glucose concentrations were measured using the OneTouch Verio® Blood Glucose Meter (LifeScan). Mice were restrained in a 50 mL conical tube with holes to provide ventilation for all blood collections. Immediately prior to the start of the IPIST, sections previously trimmed on the hind legs were swabbed with gauze soaked in 70% ethanol prior to a thin film of petroleum jelly being applied with a cotton-tipped swab. Blood was collected by lancing the lateral saphenous vein with a sterile, 25-gauge needle. Approximately 40 μL of blood was collected into a microhematocrit capillary tube (Catalog Number: 22-362-574, Fisher Scientific) before being transferred to a microcentrifuge tube on ice to prepare serum. Blood was collected at 0, 2, 15, and 30 minutes post-administration of dextrose solution.

3.7 Preparation of Serum

3.7.1 IPITT and IPGTT

Following blood collection blood was allowed to clot at room temperature for 30-45 minutes prior to being spun in a microcentrifuge for 10 minutes at 4°C at 2000 x g. Serum was removed and stored at -80°C.

3.7.2 IPIST

Following blood collection blood was allowed to clot on ice for 30-45 minutes prior to being spun in a microcentrifuge for 10 minutes at 4°C at 9300 x g. Serum was removed and stored at -80°C.

3.8 Euthanasia

Mice were exposed in a chamber to isoflurane mixed with oxygen to induce anaesthesia. The mixture of anaesthetic used was 3.75% isoflurane in 2 litres per minute oxygen flow. Oxygen flow was calibrated at 14.7 pounds per square inch absolute (PSIA) at 21°C. Following loss of the toe pinch reflex, anaesthesia was continued following transfer to a nose cone. Exsanguination was performed via cardiac puncture using a 25-gauge needle affixed to a 1 mL syringe. Following this, euthanasia was confirmed via cervical dislocation.

3.9 Vascular Endothelial Function

Isometric force measurements of contraction and relaxation[74] were measured in aortic rings in response to PE, ACh, and SNP (vasoconstriction, endothelium-dependent vasodilation, and endothelium-independent vasodilation, respectively). Aortic rings measuring two millimetres in length were prepared from the thoracic aorta and were cleaned of surrounding fat in ice-cold physiological saline solution (PSS: 119 mM NaCl, 4.7 mM KCl, 1.18 mM KH_2PO_4, 1.17 mM $MgSO_4$, 24.9 $NaHCO_3$, 0.023 mM EDTA, 1.6 mM $CaCl_2$ and 11.1 mM dextrose) prior to being mounted on a four-channel wire myograph (Danish Myotechnology, Aarhus, Denmark). Aortic rings were gradually stretched over the period of an hour to a basal tone of 5.5 millinewtons; with 3 washes of PSS every 20 minutes. Aortic rings were then challenged with PSS containing

an equimolar substitution of 80 mM KCl for NaCl over a period of 10 minutes to ascertain maximal contraction and tissue viability. Following washout with PSS and development of a basal tone, aortic rings were contracted with cumulative additions of PE from 1 nmol/L to 10 µmol/L. Following washout with PSS and development of a basal tone, aortic rings were constricted with PE at 10 µmol/L and challenged with cumulative additions of ACh from 1 nmol/L to 10 µmol/L to assess endothelium-dependent vasorelaxation. Following washout with PSS aortic rings were incubated with 200 mM N^G-nitro-L-arginine methyl ester (L-NAME) for 20 minutes prior to constricting with PE from 1 nmol/L to 10 µmol/L. L-NAME is a pharmacological inhibitor of the inducible, endothelial, and neuronal isoforms of nitric oxide synthase. Following washout with PSS for 20 minutes aortic rings were again incubated with 200 mM L-NAME for 20 minutes. Aortic rings were then constricted with PE 10 µmol/L and challenged with cumulative additions of ACh from 1 nmol/L to 10 µmol/L. Finally, following a 10 minute washout with PSS aortic rings were constricted with PE at 10 µmol/L and challenged with cumulative additions of SNP from 0.1 nmol/L to 10 µmol/L to assess endothelium-independent vasorelaxation. Phenylephrine contraction, both in the presence and absence of L-NAME was calculated as the percent contraction relative to the KCl contraction. Acetylcholine vasorelaxation, both in the presence and absence of L-NAME was calculated as the percent relaxation relative to the immediately preceding PE contraction.

3.10 Tissue Harvest

The following tissues were collected and weighed using an analytical balance in the following order, following euthanasia: heart, aorta, gonadal adipose tissue, pancreas, mesenteric adipose tissue, liver, kidneys, retroperitoneal adipose tissue, inguinal adipose tissue,

interscapular brown adipose tissue. In the results section, tissue weights are expressed as a percentage relative to total body weight. Tissues were snap-frozen in liquid nitrogen prior to storage at -80°C.

3.11 RNA Extraction

RNA was extracted from a sample of retroperitoneal adipose tissue weighing approximately 80 to 100 milligrams using the RNeasy Lipid Tissue Mini Kit (Qiagen) following the manufacturer's protocol. Tissue was homogenized in QIAzol Lysis Reagent (Catalog Number: 79306, QIAGEN) using a blend of stainless steel beads, 0.9 to 2.0 mm (Catalog Number: SSB14B-RNA, Next Advance), at speed eight for four minutes in a Bullet Blender Blue (Catalog Number: BBX24B, Next Advance). On-column DNase digestion was performed using the RNase-Free DNase set (Catalog Number: 79254, QIAGEN). RNA was eluted in 30 microlitres of RNase-free water after incubation for five minutes at room temperature. Eluate was incubated on the column for five minutes at room temperature prior to a second elution. RNA was quantified using the Qubit RNA BR Assay Kit (Catalog Number: Q10210, ThermoFisher Scientific) as per manufacturer's instructions using the Qubit fluorometer (Catalog Number: Q32857, Invitrogen). Two microlitres of the RNA preparation were used for quantification in a 200 microlitre reaction. RNA integrity was assessed by the visualization of intact 28S and 18S rRNA bands on a 1.8% TAE (Catalog Number: 15558-026, ThermoFisher Scientific) agarose gel (Catalog Number: 16500-100, ThermoFisher Scientific) containing SYBR Safe DNA Gel Stain (Catalog Number: S33012, ThermoFisher Scientific). Agarose gel electrophoresis was performed at 100 V for 30 minutes. Gels were visualized via UV

transillumination with 1 second exposure using a ChemiGenius^2 Bio Imaging System (SynGene) using GeneSnap Software Version 7.12.

3.12 RT-qPCR

First-strand cDNA synthesis was performed with the High Capacity cDNA Reverse Transcription Kit (Catalog Number: 4368814, ThermoFisher Scientific) using 1 µg of RNA. RT-qPCR was performed using the 7500 Fast Real-Time PCR System (Applied Biosystems) using 7500 Software v2.3. All RT-qPCR reactions were prepared in a PCR workstation preirradiated for five minutes with UV illumination (Catalog Number: PCR WORKSTATION (UV/HP), Bigneat Limited). Taqman Gene Expression Assays Mm01288386_m1 and Mm00443258_m1 were used to quantify *Il10* and *Tnf* mRNA abundance, respectively. Taqman Gene Expression Assays Hs99999901_s1 and Mm01249935_m1 were used to quantify *18S* rRNA and *Ncoa6* mRNA abundance, respectively, and were used as reference genes. Five microlitres of cDNA template were used for *Il10* and *Tnf* mRNA abundance measurement. Two and a half microlitres of cDNA template were used for *18S* rRNA and *Ncoa6* mRNA abundance measurement. qRT-PCR reactions were performed using TaqMan Gene Expression Master Mix (Catalog Number: 4369016, ThermoFisher Scientific) in MicroAmp Fast 96-Well Reaction Plates (Catalog Number: 4346907, ThermoFisher Scientific) in a 20 microlitre reaction volume as per manufacturer's instructions. Relative mRNA abundance of *Il10* and *Tnf* were calculated using the ΔΔCt method of relative quantification[75]. Samples were analyzed in duplicate on the same plate. Two inter-run calibrators, one for post-weaning control diet-fed female offspring and one for post-weaning western diet-fed female offspring, were used to calculate ΔΔCt's. Inter-run calibrators consisted of a mixture of cDNA from the Maternal Control Sedentary group: two

microlitres each from six preparations of cDNA diluted with 84 microlitres of DNase-free and RNase-free water (Catalog Number: 10977-015, ThermoFisher Scientific).

3.13 Statistical Analysis

Control-fed offspring and western-fed offspring were analyzed separately in all analyses. This is to ascertain the effect of maternal diet and maternal exercise; recognizing that differences in adiposity and metabolic phenotypes will arise because of post-weaning diet. The only exception to separating the analysis by post-weaning diet groups is the analysis of offspring weight at weaning. This is because the offspring have not yet been weaned onto their post-weaning diet. Significance of difference between group means was assessed by two-way analysis of variance (ANOVA). Maternal diet and maternal exercise were used as the independent variables for the ANOVAs. Homogeneity of variance was assessed using Levene's test for equality of variances. Normality was assessed using the Shapiro-Wilk normality test. Results are expressed as individual data points with mean ± SD, or mean ± SD without individual data points, where appropriate. Results were determined to be significant if the p-value was less than the alpha level of 0.05. Statistical analysis was performed using R version 3.4.0.

3.14 Beta Cell Death Assay

3.14.1 Cell-Free DNA Isolation, Quantification, and Bisulfite Conversion

Cell-free DNA was isolated from 50 microlitres of serum. Cell-free DNA was extracted according to the "Purification of Viral Nucleic Acids from Plasma or Serum" protocol listed on Page 19 of the QIAamp MinElute Virus Spin Handbook (QIAGEN). Carrier RNA was added to

Buffer AL to aid in nucleic acid binding to the membrane and to prevent nucleic acid degradation as per manufacturer's instructions. Reagents were procured from the QIAamp MinElute Virus Spin Kit (Catalog Number: 57704, QIAGEN). Cell-free DNA was eluted with 25 microlitres of RNase-free water after incubation for five minutes at room temperature. Eluate was incubated on the column for five minutes at room temperature prior to a second elution. Cell-free DNA was quantified using the Qubit dsDNA HS Assay Kit (Catalog Number: Q32851, Invitrogen). Two microlitres of the cell-free DNA preparation were used for quantification in a 200 microlitre reaction; note that it was determined that the fluorescent dye used to quantify DNA concentration was not specific to DNA and that the artificially induced loading of carrier RNA to aid in cell-free DNA extraction interfered with DNA quantification. 15 nanograms as determined by the Qubit dsDNA HS assay were bisulfite-converted using the EZ DNA Methylation Gold Kit (Catalog Number: D5006, Zymo Research); which also caused degradation of the carrier RNA due to high pH during desulphonation.

3.14.2 PCR and Bisulfite Pyrosequencing

A region of exon 2 of *Ins1* containing a CpG at +177, relative to the transcriptional start site (TSS), was amplified by PCR of bisulfite-treated cfDNA. The forward primer is as follows: 5' AGTATTTTGTGGTTTTTATTTGGTAGAGG 3'. The reverse primer was biotinylated to allow for binding to streptavidin-coated beads to facilitate strand-specific sequencing and purified by high performance liquid chromatography (HPLC) to reduce free biotin; which would compete with biotin-bound template for streptavidin binding. The reverse primer, which was biotinylated and purified by HPLC is as follows: 5' /5BiosG/TCCCAACTCCAATTATTCCACTTATAAATC 3'. The sequencing primer which was

used to prime bisulfite pyrosequencing was as follows: 5' TTTGGTGTGTGGGGA 3'. PCR primers were nonspecific with respect to CpG sites. PCR reaction master mix for one reaction was as follows: 5 microlitres of 10X PCR Buffer, 1 microlitre of 100 mM dNTP mix diluted 1:10 (Catalog Number: 362271, ThermoFisher Scientific), 1 microlitre of forward primer at 10 micromolar stock, 1 microlitre of reverse primer at 10 micromolar stock, 0.32 microlitres of HotStarTaq polymerase (Catalog Number: 203205, QIAGEN), 39.68 microlitres of Ultrapure DNase-free and RNase-free water (Catalog Number: 10977-015, ThermoFisher Scientific), and 2 microlitres of bisulfite-converted template DNA. Thermal cycling conditions were as follows: 95°C for 15 minutes, 37 cycles of 94°C for 1 minute, 57°C for 1 minute, and 72°C for 1 minute; followed by a final extension at 72°C for 10 minutes and a hold at 4°C. Successful PCR was assessed by the visualization of a single band on a 1.8% TAE (Catalog Number: 15558-026, ThermoFisher Scientific) agarose gel (Catalog Number: 16500-100, ThermoFisher Scientific) containing SYBR Safe DNA Gel Stain (Catalog Number: S33012, ThermoFisher Scientific). Agarose gel electrophoresis was performed at 100 V for 30 minutes. Gels were visualized via UV transillumination with 1 second exposure using a ChemiGenius^2 Bio Imaging System (SynGene) using GeneSnap Software Version 7.12. Single stranded templates were prepared for one reaction as follows: 25 microlitres Ultrapure DNase-free and RNase-free free water (Catalog Number: 10977-015, ThermoFisher Scientific), 38 microlitres Binding Buffer (Catalog Number: 979006, QIAGEN), 2 microlitres streptavidin sepharose high performance beads (Catalog Number: 17-5113-0, GE Healthcare), and 15 microlitres of bisulfite PCR product. Single stranded templates were prepared using the PyroMark Q96 Vacuum Workstation (Biotage) as per manufacturer's instructions. Single stranded templates were released into 0.36 microlitres sequencing primer (10 micromolar) plus 11.64 microlitres of annealing buffer per sample.

Bisulfite pyrosequencing was performed with PyroMark GoldQ96 Reagents (Catalog Number: 972804, QIAGEN). Liver DNA was used as a high methylation control and did not contain carrier RNA; and was generously gifted by Abeer Aljaadi. MIN6 DNA was used as a low methylation control and did not contain carrier RNA.

3.14.3 Non-Obese Diabetic Mice

Male and female mice (NOD/ShiLtJ) were obtained at 12 weeks of age and were gifted from Sigrid Alvarez from the Verchere laboratory. Serum was collected weekly from the saphenous vein. Following blood collection blood was allowed to clot at room temperature for 30-45 minutes prior to being spun in a microcentrifuge for 10 minutes at 4°C at 2000 x g. Serum was removed and stored at -80°C. Diabetes was characterized as having a unfasted blood glucose concentration greater than 20 mmol/L.

Chapter 4: Aim 1: Tissue Weights, Body Composition, Glucose Homeostasis, Vascular Function, and Inflammatory Cytokine Expression in Control Diet-Fed Female Offspring

AIM 1

A. To determine whether exercise can mitigate the adverse effect of maternal obesity on offspring adiposity, glucose homeostasis, and endothelium-dependent vasorelaxation in adult female offspring fed a post-weaning control diet.

As mentioned previously in Chapter 1, the DOHaD theory posits that the environmental conditions to which one is exposed *in utero* or in early life have the capacity to influence one's health in adulthood. It is known that the offspring from overweight and obese mothers have a higher risk of hospitalization from a cardiovascular event in addition to higher all-cause mortality compared to those from mothers with a healthy BMI[22]. Further, epidemiological studies have shown that the offspring of obese mothers have higher adiposity, higher BMIs, and risk factors for the development of CVD compared to those from mothers with a healthy BMI[19–22]. Many of the risk factors that are predictive for the development of CVD are encompassed within the definition of the metabolic syndrome. Thus, in a mouse model these are primarily the measures by which I had evaluated the efficacy of maternal exercise in mitigating the effects of maternal obesity on their offspring.

Many of the adverse effects of maternal obesity on their offspring have been reproduced in rodent models. Higher adiposity, higher fasting blood glucose, higher fasting blood insulin,

hypertension, and impairments in endothelium-dependent vasorelaxation are seen within the offspring of obese dams compared to those from control diet-fed or chow diet-fed dams[29–33,35,36]. Interestingly, maternal exercise has been shown to improve insulin tolerance and glucose tolerance in their offspring compared to sedentary dams. However, whether the beneficial effects of maternal exercise may be used to mitigate the adverse effects of maternal obesity on the developing offspring is not known. Thus, in Aim 1 I assessed the effects of maternal exercise and maternal diet on insulin tolerance, glucose tolerance, endothelium-dependent vasorelaxation, and adipose tissue distribution of post-weaning control diet-fed mice. Given that I did not see biologically significant effects of maternal diet or maternal exercise on endothelium-dependent vasorelaxation, this portion of the experiment was stopped prior to completion to focus on other portions of the experiment.

4.1 Body Weight in Post-Weaning Control Diet-Fed Female Offspring

Female offspring mice were weighed at weaning to ascertain the effect of maternal diet and maternal exercise prior to their introduction to a post-weaning diet. Female offspring mice from western diet-fed dams had higher ($p \leq 0.05$) body weight at weaning compared to control diet-fed dams (**Figure 4.1B)**. There was no significant effect of maternal exercise on female offspring mice body weight at weaning. There were no significant effects of maternal diet or maternal exercise on body weight in post-weaning control diet-fed female offspring at 5, 10, 15, or 20 weeks post-weaning (**Figure 4.1A)**.

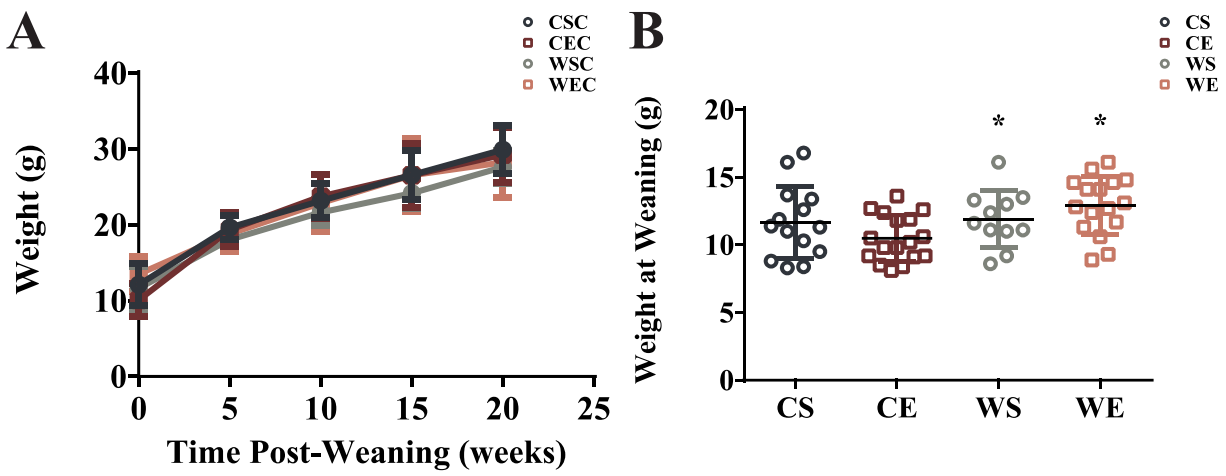

Figure 4.1 Body Weights in Post-Weaning Control Diet-Fed Female Offspring

A. Body weight in post-weaning control diet-fed female offspring. Weights were measured weekly in grams from weaning to 20 weeks post-weaning. Data are presented from weeks 0, 5, 10, 15, and 20. Data presented as mean ± standard deviation, n = 5-8 per diet/exercise group. **B.** Body weight in female offspring prior to introduction to a post-weaning offspring diet. Data presented as individual points with mean ± standard deviation, n = 11-17 per diet/exercise group, ***** $p \leq 0.05$ - effect of maternal diet.

4.2 Adiposity in Control Diet-Fed Female Offspring

Post-weaning control diet-fed female offspring tissues were harvested at approximately 21 weeks post-weaning. Body composition was assessed by EchoMRI at 19 weeks post-weaning. Gonadal adipose tissue weights were lower (p=0.09) in post-weaning control diet-fed female offspring from western diet-fed dams compared to control diet-fed dams (**Figure 4.2A**). There were no effects of maternal diet or maternal exercise on retroperitoneal, inguinal, and mesenteric adipose tissue weights (**Figure 4.2B-D**). There were no effects of maternal diet or exercise on interscapular brown adipose tissue weights (**Figure 4.2E**) and percent total body adipose as assessed by EchoMRI (**Figure 4.2F**) in post-weaning control diet-fed female offspring.

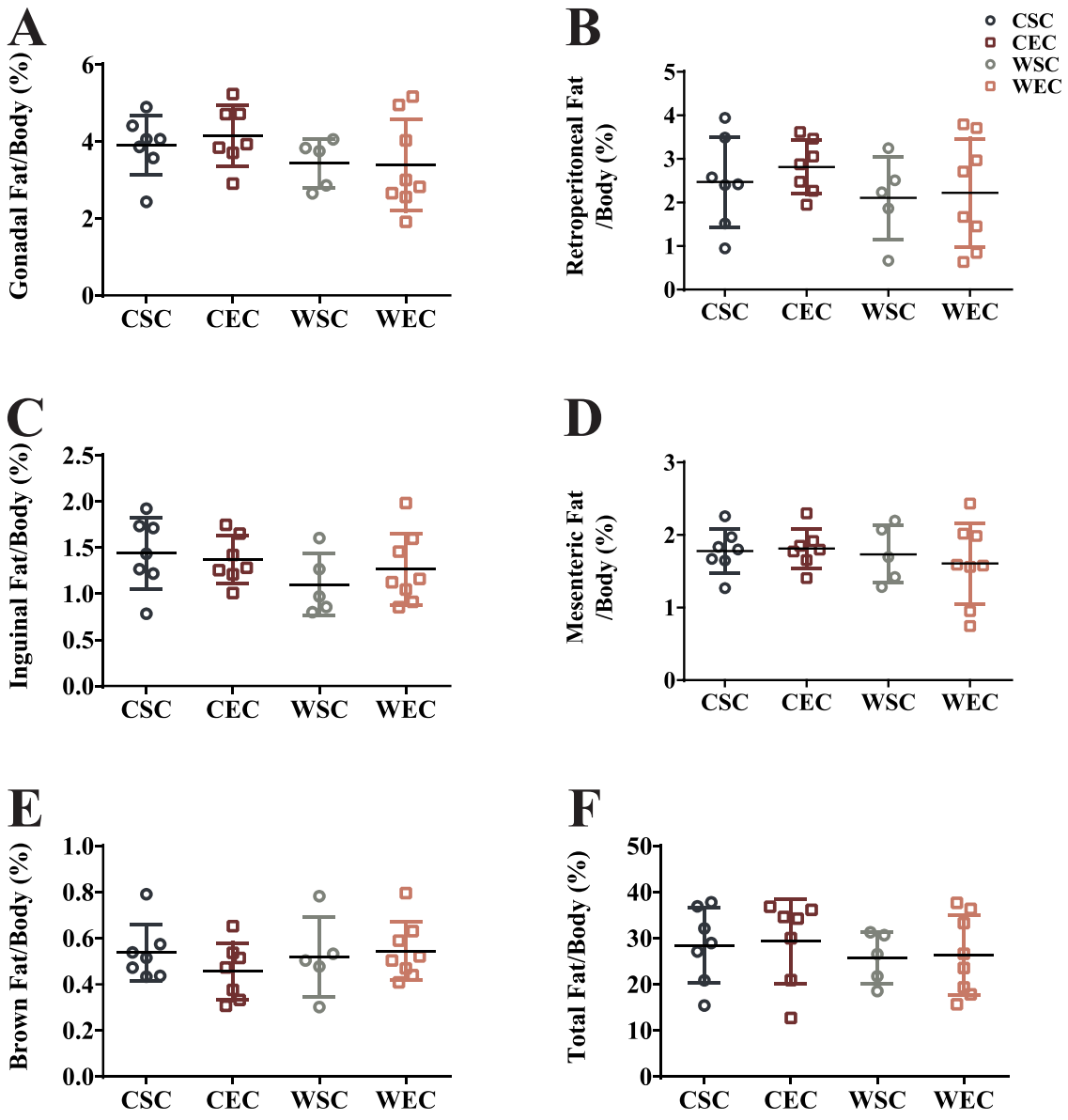

Figure 4.2 Normalized Adipose Tissue Weights in Post-Weaning Control Diet-Fed Female Offspring

A. Gonadal adipose tissue/body weight (%). **B.** Retroperitoneal adipose tissue/body weight (%). **C.** Inguinal adipose tissue/body weight (%). **D.** Mesenteric adipose tissue/body weight (%). **E.** Interscapular brown adipose tissue/body weight (%). **F.** Total fat/body weight (%). Data presented as individual points with mean ± standard deviation, n = 5-8 mice per diet/exercise group.

4.3 Lean Mass in Control Diet-Fed Female Offspring

Post-weaning control diet-fed female offspring tissues were harvested at approximately 21 weeks post-weaning. Body composition was assessed by EchoMRI at 19 weeks post-weaning. Heart weights were higher ($p \leq 0.05$) in post-weaning control diet-fed female offspring from dams fed a western diet compared to those from control diet-fed dams (**Figure 4.3A**). Kidney weights were higher ($p \leq 0.05$) in post-weaning control diet-fed female offspring from western diet-fed dams compared to those from control diet-fed dams (**Figure 4.3C**). Significant effects of maternal diet and maternal exercise were not observed on the following tissue weights in post-weaning control diet-fed female offspring: liver weight (**Figure 4.3B**), pancreas weight (**Figure 4.3D**), and lean mass (**Figure 4.3E**). Tissue weights are expressed as a percentage relative to total body weight of each animal.

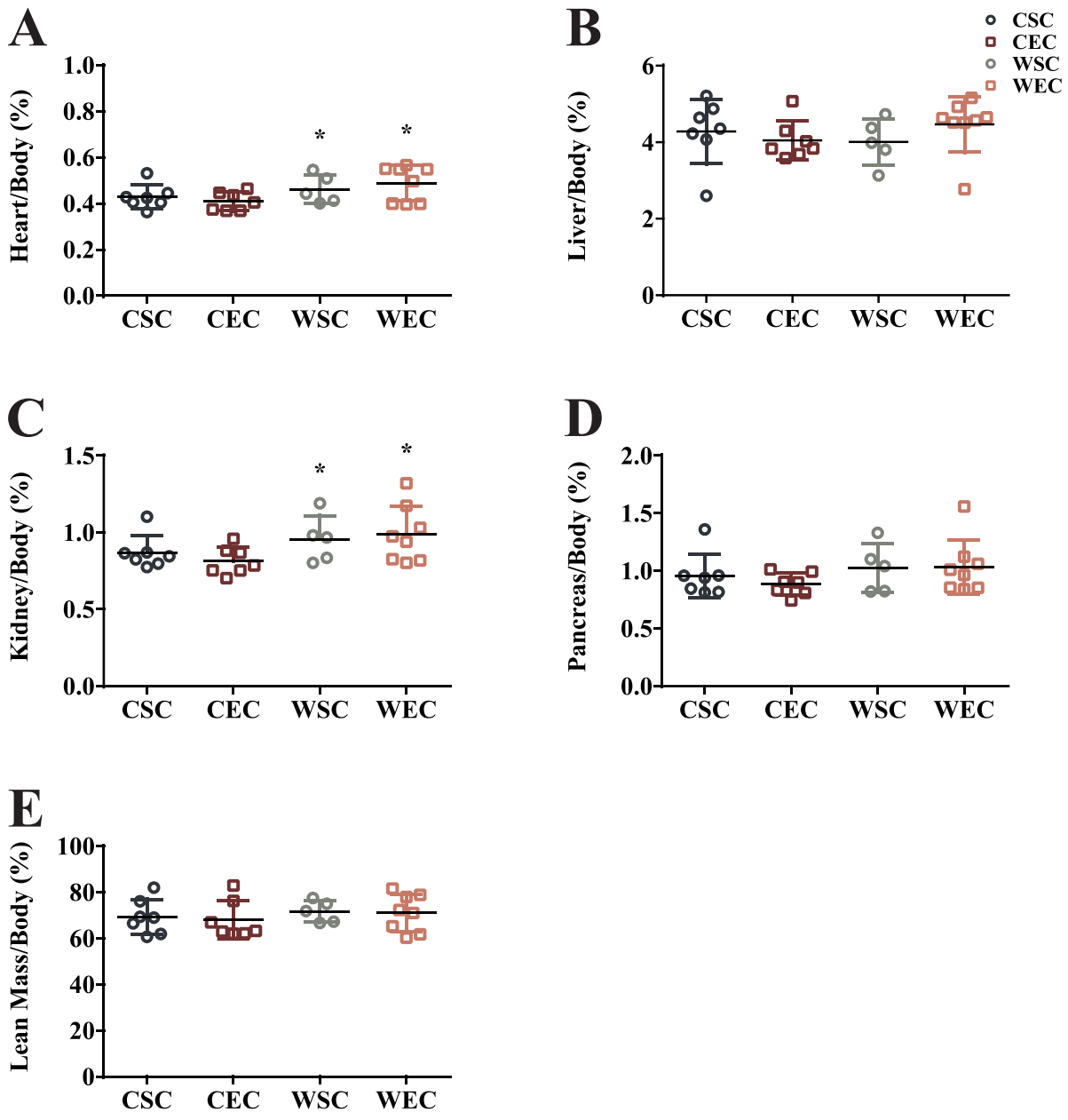

Figure 4.3 Normalized Lean Mass Weights in Post-Weaning Control Diet-Fed Female Offspring

A. Heart/body weight (%). **B.** Liver/body weight (%). **C.** Kidney/body weight (%). **D.** Pancreas/body weight (%). **E.** Lean mass/body weight (%). Data presented as individual points with mean ± standard deviation, n = 5-8 mice per diet/exercise group, ***** $p \leq 0.05$ - effect of maternal diet.

4.4 Insulin Tolerance in Post-Weaning Control Diet-Fed Female Offspring

Insulin tolerance tests were conducted on post-weaning control diet-fed female offspring at 19 weeks post-weaning. Dosage of insulin solution was based on the lean mass of each animal assessed at approximately 19 weeks post-weaning. There was no significant effect of maternal diet or maternal exercise on blood glucose concentration at 15 minutes, 30 minutes, 60 minutes, 90 minutes, or 120 minutes post-administration of insulin (**Figure 4.4A**); IPITT area under the curve calculations (**Figure 4.4B**); or fasting blood glucose concentrations prior to the administration of insulin (**Figure 4.4C**) in post-weaning control diet-fed female offspring.

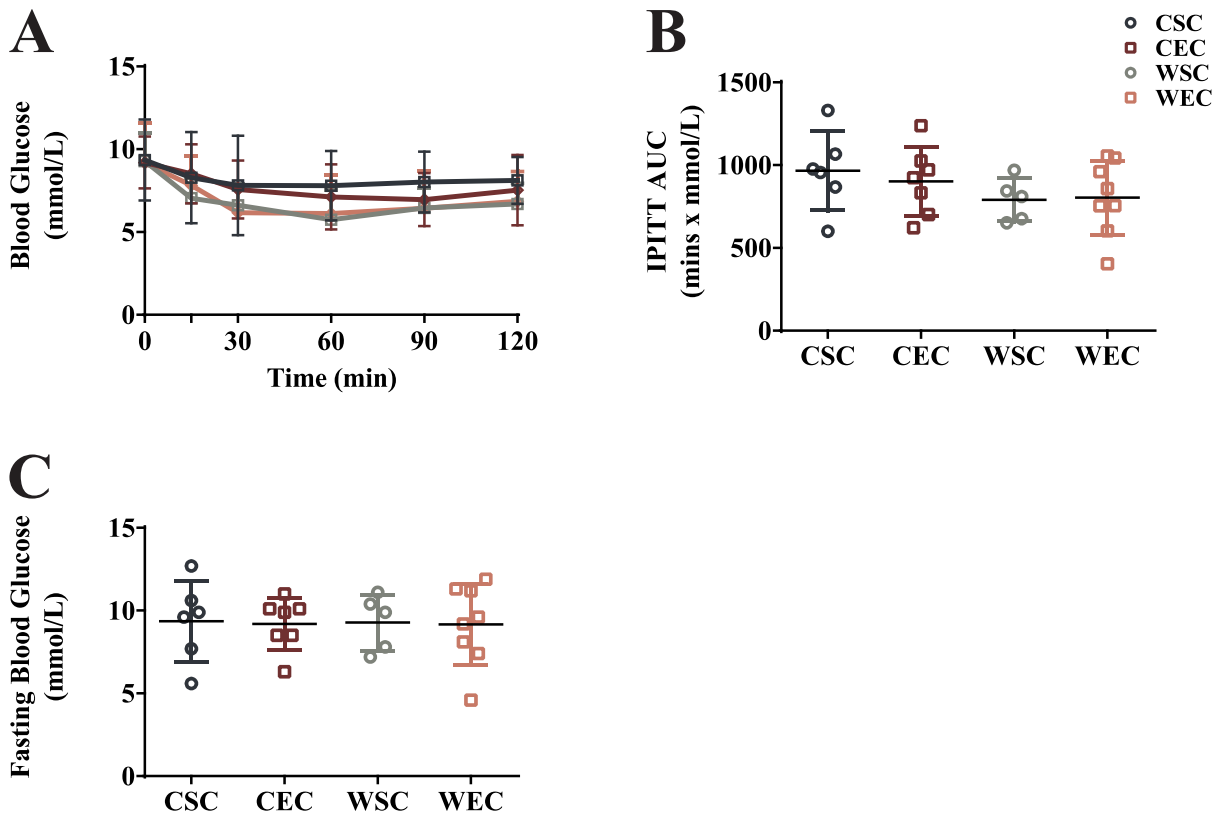

Figure 4.4 Insulin Tolerance in Post-Weaning Control Diet-Fed Female Offspring at 19 Weeks Post-Weaning

A. Blood glucose (mmol/L) versus time post-administration of insulin. Data presented as mean ± standard deviation, n = 5-8 per diet/exercise group. **B.** Intraperitoneal insulin tolerance test area under the curve. **C.** Fasting blood glucose (mmol/L) prior to administration of insulin. Data presented as mean ± standard deviation, n = 5-8 per diet/exercise group.

4.5 Glucose Tolerance in Post-Weaning Control Diet-Fed Female Offspring

Glucose tolerance tests were conducted on post-weaning control diet-fed female offspring at 20 weeks post-weaning. Dosage of dextrose solution was based on the lean mass of each animal as assessed at approximately 19 weeks post-weaning. Blood glucose concentrations were higher ($p \leq 0.05$) at 30 minutes post-administration of dextrose solution in post-weaning control diet-fed female offspring from exercised dams compared to those from sedentary dams (**Figure 4.5A**). Blood glucose concentrations were lower ($p=0.08$) at 120 minutes post-administration of dextrose solution in post-weaning control diet-fed female offspring from western diet-fed dams compared to those from control diet-fed dams (**Figure 4.5A**). There was no significant effect of maternal diet or maternal exercise on IPGTT area under the curve calculations (**Figure 4.5B**), or fasting blood glucose concentrations prior to the administration of dextrose solution (**Figure 4.5C**) in post-weaning control diet-fed female offspring.

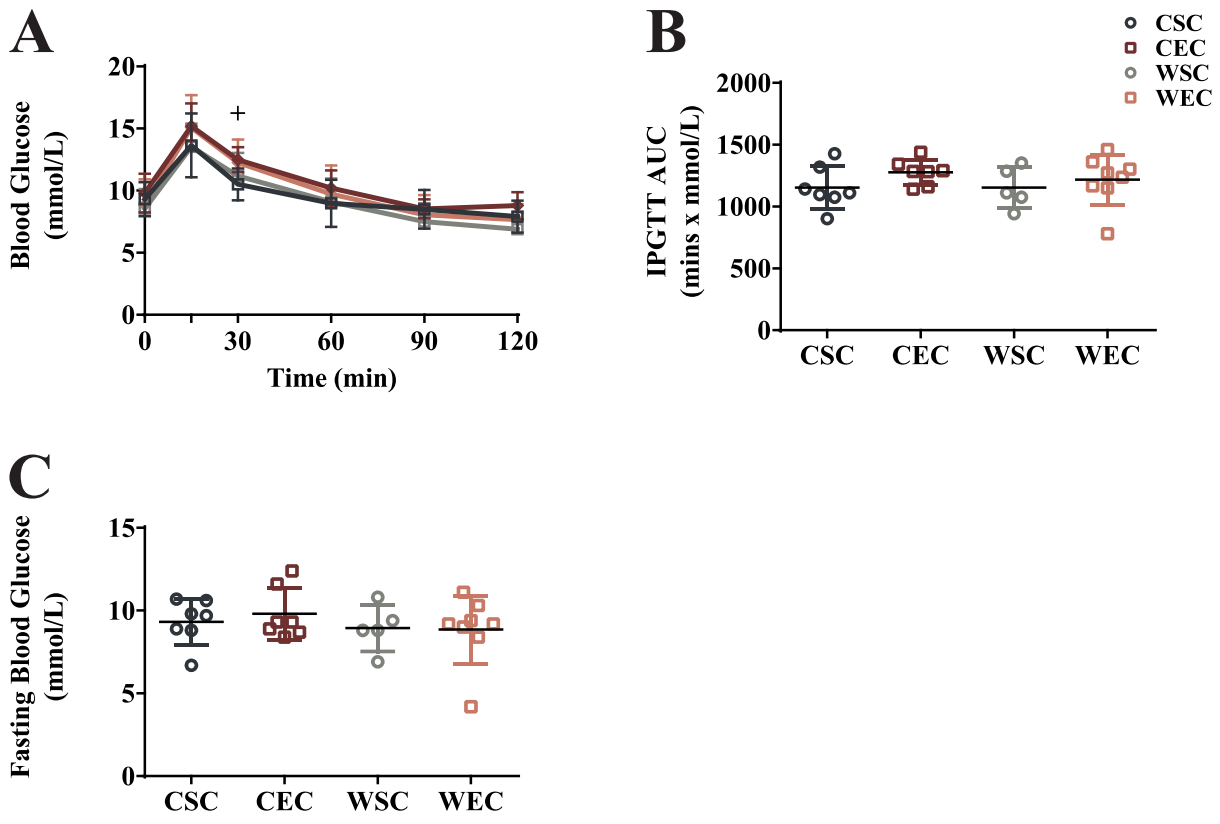

Figure 4.5 Glucose Tolerance in Post-Weaning Control Diet-Fed Female Offspring at 20 Weeks Post-Weaning

A. Blood glucose (mmol/L) versus time post-administration of dextrose solution. Data presented as mean ± standard deviation, n = 5-8 mice per diet/exercise group, + $p \leq 0.05$ - effect of maternal exercise. **B.** Intraperitoneal glucose tolerance test area under the curve. **C.** Fasting blood glucose (mmol/L) prior to administration of dextrose solution. Data presented as individual points with mean ± standard deviation, n = 5-8 mice per diet/exercise group.

4.6 *Ex Vivo* Functional Assessment of Thoracic Aortae in Post-Weaning Control Diet-Fed Female Offspring

4.6.1 Acetylcholine-Mediated Vasorelaxation of Thoracic Aortae in Post-Weaning Control-Diet Fed Female Offspring

Acetylcholine dose-response curves were constructed to assess endothelial-dependent vasorelaxation in post-weaning control diet-fed female offspring. Acetylcholine-mediated vasorelaxation of thoracic aortae were lower ($p=0.08$) in response to 10^{-8} M ACh in post-weaning western diet-fed female offspring from western diet-fed dams compared to those from control diet-fed dams (**Figure 4.6A**). There were no effects of maternal exercise or maternal diet on endothelium-dependent vasorelaxation of thoracic aortae in response to 10^{-10} M ACh, 10^{-9} M ACh, 10^{-7} M ACh, and 10^{-6} M ACh in post-weaning control diet-fed female offspring (**Figure 4.6A**). There were no effects of maternal diet or maternal exercise on maximal ACh-mediated vasorelaxation at 10^{-5} M ACh in post-weaning western diet-fed female offspring (**Figure 4.6A**). There were no effects of maternal diet or maternal exercise on the $logEC_{50}$ values calculated from the ACh dose-response curves from post-weaning control diet-fed female offspring (**Figure 4.6C**). However, two $logEC_{50}$ values could not be calculated by Graphpad Prism as the 95% confidence intervals were very wide for these specific curves.

4.6.2 Phenylephrine-Mediated Vasoconstriction of Thoracic Aortae in Post-Weaning Control Diet-Fed Female Offspring

Phenylephrine dose-response curves were constructed to assess vasoconstriction in post-weaning control diet-fed female offspring. There were no effects of maternal exercise or

maternal diet on PE-mediated vasoconstriction of thoracic aortae at 10^{-9} M PE, 10^{-8} M PE, 10^{-7} M PE, and 10^{-6} M PE in post-weaning control diet-fed female offspring **(Figure 4.6B)**. There were no effects of maternal exercise or maternal diet on maximal PE-mediated vasoconstriction of thoracic aortae at 10^{-5} M PE in post-weaning control diet-fed female offspring **(Figure 4.6B)**. There were no effects of maternal diet or maternal exercise observed on the $\log EC_{50}$ values calculated from the PE dose-response curves in post-weaning control diet-fed female offspring **(Figure 4.6D)**.

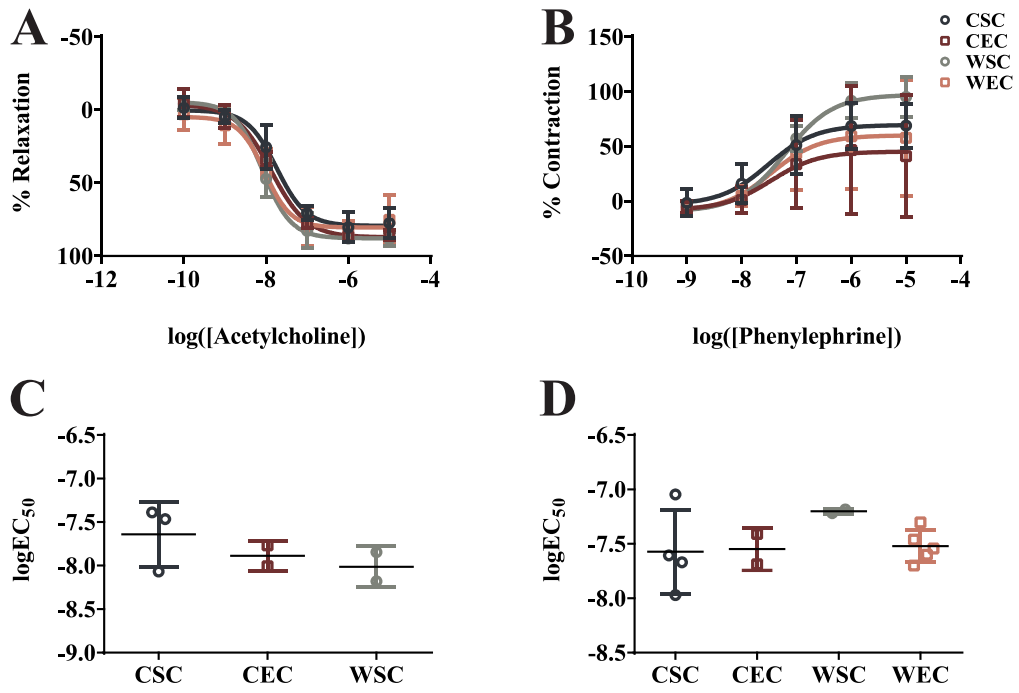

Figure 4.6 Acetylcholine Dose-Response Curves and Phenylephrine Dose-Response Curves in Post-Weaning Control Diet-Fed Female Offspring

A. Acetylcholine dose-response curves of thoracic aortae from post-weaning control diet-fed female offspring. **B.** Phenylephrine dose-response curves of thoracic aortae from post-weaning control diet-fed female offspring. **C.** $logEC_{50}$ calculations of ACh dose-response curves from post-weaning control diet-fed female offspring. **D.** $logEC_{50}$ calculations of PE dose-response curves from post-weaning control diet-fed female offspring. Phenylephrine contraction is measured as % contraction relative to a KCl curve. Acetylcholine-mediated vasorelaxation is measured as % relaxation relative to a PE contraction. Data presented as nonlinear regression with mean ± standard deviation (**A, B)** and individual points with mean ± standard deviation (**C, D)**; n = 2-5 mice per diet/exercise group in **A** and **B**, n = 2-3 mice per diet/exercise group in **C** and n = 2-5 mice per diet/exercise group in **D**. Interactions between maternal diet and maternal exercise were not assessed in **C**.

AIM 1

B. To determine whether there is an association between maternal exercise and maternal obesity on the expression of inflammatory cytokines in the retroperitoneal adipose tissue of adult female offspring fed a post-weaning control diet.

The effects of maternal exercise on the cardiovascular health of their offspring in humans are largely unknown. In Aim 2A, I found that retroperitoneal adipose tissue weights were higher in post-weaning western diet-fed female offspring from exercised dams compared to those from sedentary dams. Further, I also found that blood glucose concentrations were lower ($p \leq 0.05$) at 90 minutes post-administration of dextrose solution in post-weaning western diet-fed female offspring. This led to the hypothesis that the retroperitoneal adipose tissue in post-weaning western diet-fed female offspring from exercised dams is of a healthier phenotype compared to that from sedentary dams. Given that I did not see these specific changes in post-weaning control diet-fed female offspring, the retroperitoneal adipose tissue from post-weaning control diet-fed mice served as a complement and verification for the study in Aim 2B.

4.7 Inflammatory Cytokine Expression in the Retroperitoneal Adipose Tissue of Post-Weaning Control Diet-Fed Female Offspring

There were no effects of maternal diet or maternal exercise on the mRNA abundance of *Tnf* (**Figure 4.7A)** and *Il10* (**Figure 4.7B**) in the retroperitoneal adipose tissue of post-weaning control diet-fed female offspring.

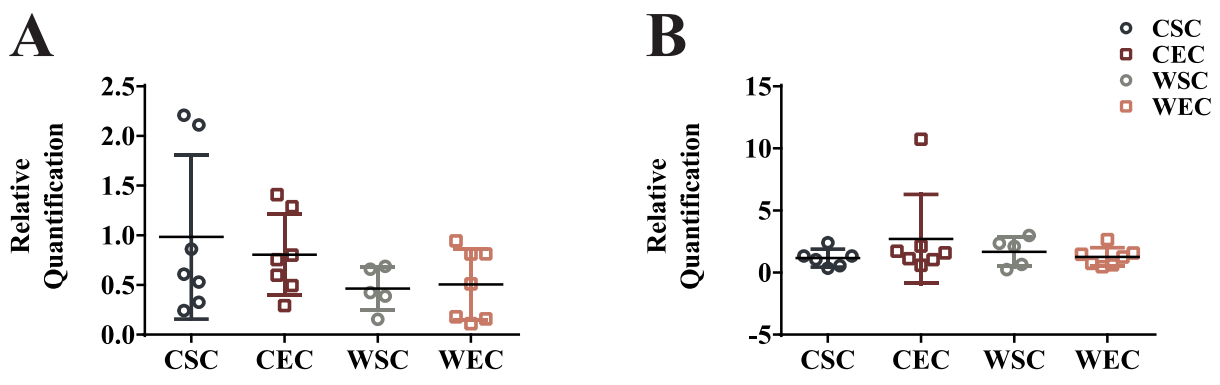

Figure 4.7 Retroperitoneal Adipose Tissue mRNA Abundance of *Tnf* and *Il10* in Post-Weaning Control Diet-Fed Female Offspring

A. Relative mRNA abundance of *Tnf* normalized to abundance of *18S* rRNA and *Ncoa6* mRNA.

B. Relative mRNA abundance of *Il10* normalized to abundance to *18S* rRNA and *Ncoa6* mRNA.

Data presented as individual points with mean ± standard deviation, n = 5-7 mice per diet/exercise group.

4.8 Beta Cell Death Assay

While I was waiting for the female offspring to mature to an age where I could perform phenotyping, I developed a bisulfite pyrosequencing assay to quantify *Ins1* methylation in serum from non-obese diabetic (NOD) mice as a side project. Type 1 diabetes is an autoimmune disease characterized by destruction of the beta cells of the pancreas. Unfortunately, the presentation of hyperglycemia and a diagnosis of type 1 diabetes occurs following the destruction of the majority of beta cells present within an individual; reducing the amount of interventions available to the patient[76].

Previous studies have reported that quantification of serum insulin gene (*Ins1*) methylation by methylation-specific PCR (MSP) is an indicator of beta-cell death[77]. *Ins1* is unmethylated at CpG +177 relative to the TSS in pancreatic beta cells and methylated at this CpG site in tissues where it is not expressed[77]. The cell-free DNA present in serum comes from beta cell death; as demonstrated by experiments involving STZ, a beta cell toxin. However, the mechanism underlying the transfer of the genetic material into the circulation is not known.

Bisulfite pyrosequencing is a more robust method for quantifying site-specific DNA methylation as compared to MSP. Thus, I sought to replicate the assay using bisulfite pyrosequencing. The NOD mouse model spontaneously develops diabetes and is used as a model of Type 1 diabetes. Thus, the NOD mouse model was used to assess the utility of the bisulfite pyrosequencing assay.

4.9 Assessment of Beta Cell Death Assay Linearity

The linearity of the assay was confirmed by assessing standards that had been created using defined mixtures of liver DNA and MIN6 DNA. Pure liver DNA was measured to have 80% ± 1.75% (mean ± SD) methylation at CpG +177 relative to the TSS of *Ins1*by bisulfite pyrosequencing. Pure MIN6 DNA was measured to have 50% ± 0.73% (mean ± SD) methylation at CpG +177 relative to the TSS of *Ins1* by bisulfite pyrosequencing. Methylation standards were created by mixing pure liver DNA and MIN6 DNA in defined quantities: 25% liver DNA:75% MIN% DNA, 50% liver DNA:50% MIN6 DNA, 75% liver DNA:25% MIN6 DNA. Thus, these methylation standards were expected to have 57.5%, 65%, and 72.5% methylation at CpG +177 relative to the TSS of *Ins1*. The methylation standards were measured to have 58.28% ± 1.32%, 64.59 ± 2.34%, and 72.11 ± 0.47% methylation at CpG +177 relative to the TSS of *Ins1* in the 25% liver DNA:75% MIN% DNA, 50% liver DNA:50% MIN6 DNA, and 75% liver DNA:25% MIN6 DNA methylation standards, respectively. Thus, the corresponding curve of expected methylation versus the observed methylation at CpG +177 relative to the TSS of *Ins1* corresponded to a R^2 value of 0.9768. The percent methylation at CpG +177 of *Ins1* was assessed in three separate PCR reactions of each mixture.

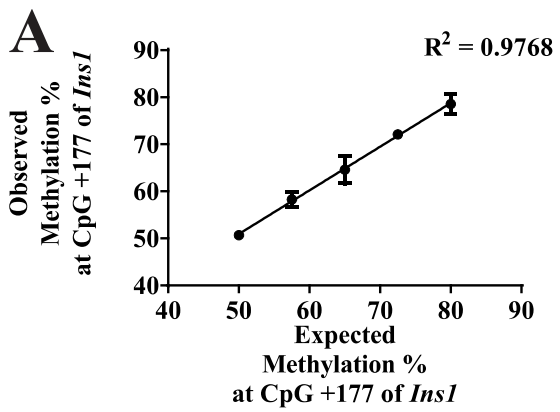

Figure 4.8 Beta Cell Death Assay Linearity

A. Observed methylation versus expected methylation percentage at CpG +177 of *Ins1*. Data presented as linear regression with mean ± standard deviation of three replicates per mixture.

4.10 Beta Cell Death Assay in Serum from Non-Obese Diabetic Mice

Blood glucose concentrations were obtained weekly in unfasted NOD mice to verify the development of hyperglycemia. Average methylation at *Ins1* CpG +177 (N=4) was 77.84 ± 6.58% (mean ± SD) at baseline, defined as the earliest timepoint that sera was available for each animal, 51.89 ± 18.86% (mean ± SD) 4 weeks prior to blood glucose rising >20 mmol/L, and 67.29 ± 7.00% (mean ± SD) 1 week prior to blood glucose rising >20mmol/L. Blood glucose concentrations were 8.2 ± 1.0 mmol/L (mean ± SD) at baseline, 7.7 ± 0.4 mmol/L at 4 weeks prior to blood glucose rising >20mmol/L, and 14.1 ± 3.5 mmol/L at 1 week prior to blood glucose rising >20mmol/L. Currently, the progression of the autoimmune destruction of beta cells in type 1 diabetes with respect to time is not known. Further, the amount of blood required for the assay allowed for at most, weekly sampling. Thus, three blood samples per mouse were analyzed. The earliest blood sample was analyzed to establish a baseline where no beta cell death should be present. The latest blood sample was analyzed to serve as another baseline which would be reached following the clearance of beta cell DNA from the circulation. Further, the second blood sample was analyzed to verify a spike of beta cell DNA in the circulation, presumably from beta cell death.

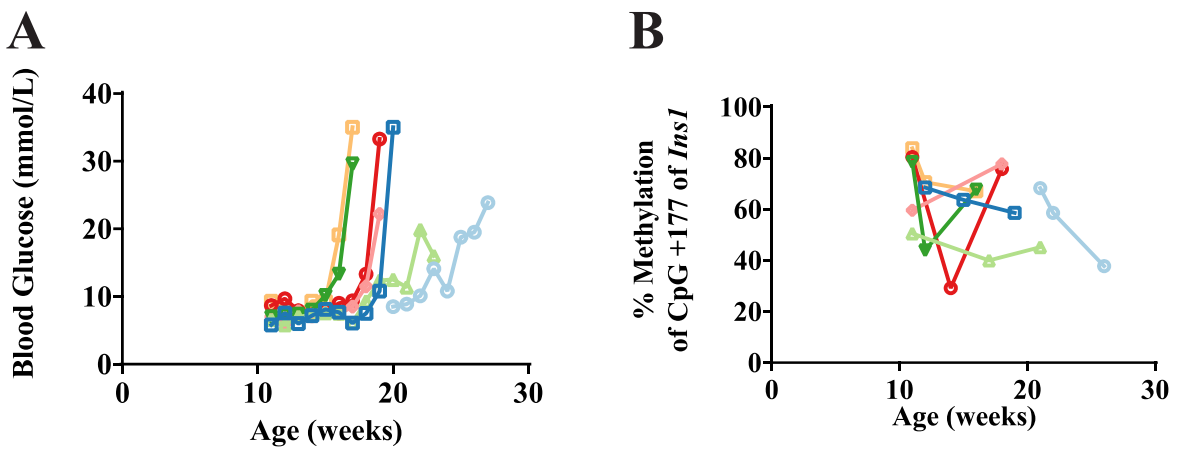

Figure 4.9 Beta Cell Death Assay in Sera from Non-Obese Diabetic Mice

A. Blood glucose concentrations in mmol/L versus age of non-obese diabetic mice in weeks. **B.** Percent methylation of CpG +177 relative to the transcriptional start site of *Ins1* in cell-free DNA isolated from the sera of non-obese diabetic mice. Data presented as mean with connecting line for each mouse; n = 7.

Chapter 5: Aim 2: Tissue Weights, Body Composition, Glucose Homeostasis, Vascular Function, and Inflammatory Cytokine Expression in Western Diet-Fed Female Offspring

AIM 2

A. To determine whether exercise can mitigate the adverse effect of maternal obesity on offspring adiposity, glucose homeostasis, and endothelium-dependent vasorelaxation in adult female offspring fed a post-weaning western diet.

The adverse effects of maternal obesity on their developing offspring are known[19–22]. However, most rodent studies have focused on the effect of maternal obesity on post-weaning control diet-fed or post-weaning chow diet-fed offspring. Thus, to be able to compare whether the effects of maternal exercise and maternal diet are different depending on the diet fed to their offspring, these effects were assessed in post-weaning western diet-fed female offspring. Nearly half of men and women between the ages of 25 and 44 in Canada are overweight or obese[72]. Between the ages of 18 and 24, 36.2% of Canadian men and women are overweight or obese. Further, the environment to which the offspring are to be exposed perinatally is likely to be similar to that of their parents. Thus, it is important to assess whether the effects of maternal exercise and maternal diet are different from those seen in post-weaning control diet-fed offspring. In Aim 2A I addressed whether there were effects of maternal exercise and maternal diet on insulin tolerance, glucose tolerance, endothelium-dependent vasorelaxation, and adipose tissue distribution of post-weaning western diet-fed mice. Given that there were no biologically-

significant effects of maternal exercise or maternal diet on endothelium-dependent vasorelaxation in post-weaning western diet-fed female offspring this portion of the experiment was stopped prior to its completion to focus on other portions of the study.

5.1 Body Weight in Post-Weaning Western Diet-Fed Female Offspring

Post-weaning western diet-fed female offspring from exercised dams had higher ($p \leq 0.01$) body weights than those from sedentary dams at 5 and 10 weeks post-weaning (**Figure 5.1)**. Further, post-weaning western diet-fed female offspring from exercised dams had higher ($p \leq 0.05$) body weights than those from sedentary dams at 15 and 20 weeks post-weaning (**Figure 5.1)**.

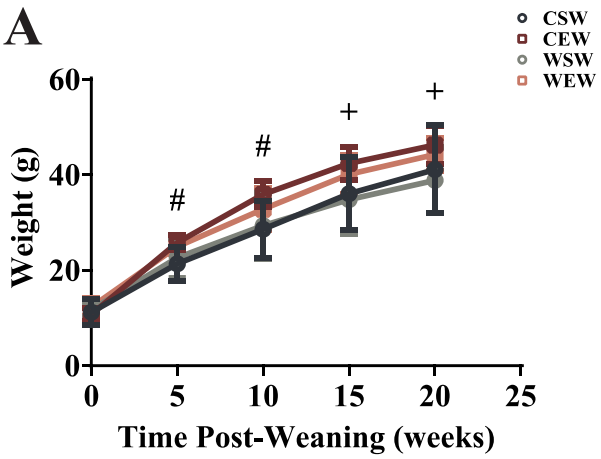

Figure 5.1 Body Weights in Post-Weaning Western Diet-Fed Female Offspring

A. Body weight in post-weaning western diet-fed female offspring. Weights are measured in grams from weaning to 20 weeks post-weaning. Data presented as mean ± standard deviation, n = 6-10 per diet/exercise group, + $p \leq 0.05$ - effect of maternal exercise, # $p \leq 0.01$ - effect of maternal exercise.

5.2 Adiposity in Post-Weaning Western Diet-Fed Female Offspring

Retroperitoneal adipose tissue weight was higher ($p \leq 0.01$) in post-weaning western diet-fed female offspring from exercised dams compared to those from sedentary dams (**Figure 5.2B**). Inguinal adipose tissue weights were higher ($p=0.06$) in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams (**Figure 5.2C**). Interscapular brown adipose tissue weights were higher ($p=0.06$) in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams. There were no significant effects of maternal diet or maternal exercise on gonadal and mesenteric adipose tissue weights (**Figure 5.2A and D**). There were no effects of maternal diet or maternal exercise on interscapular brown adipose tissue weights (**Figure 5.2E**) and total adipose tissue mass (**Figure 5.2F**) in post-weaning western diet-fed female offspring.

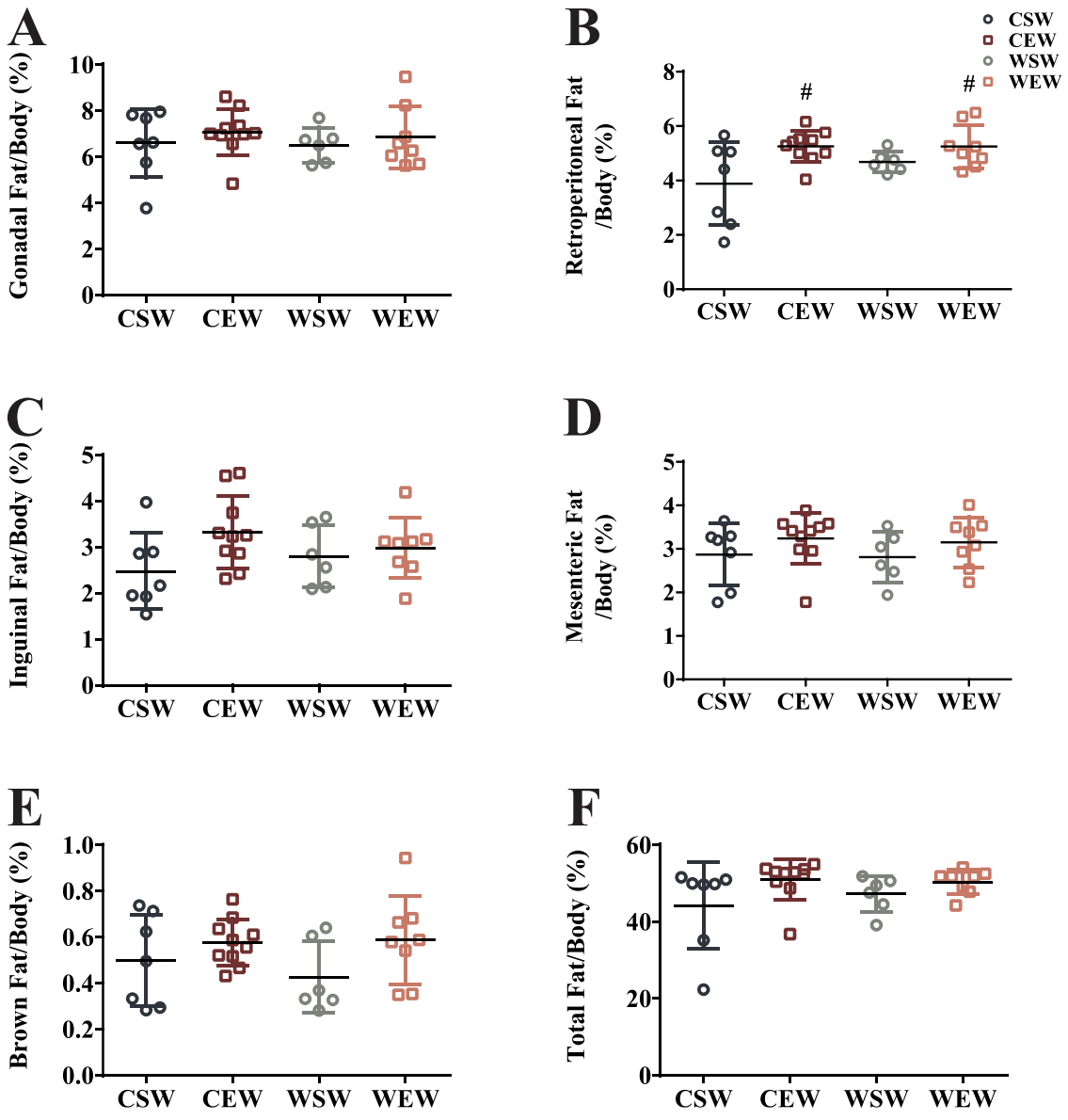

Figure 5.2 Normalized Adipose Tissue Weights in Post-Weaning Western Diet-Fed Female Offspring

A. Gonadal adipose tissue/body weight (%). **B.** Retroperitoneal adipose tissue/body weight (%). **C.** Inguinal adipose tissue/body weight (%). **D.** Mesenteric adipose tissue/body weight (%). **E.** Interscapular brown adipose tissue/body weight (%). **F.** Total fat/body weight (%). Data presented as individual points with mean ± standard deviation, n = 6-10 mice per diet/exercise group, # $p \leq 0.01$ - effect of maternal exercise.

5.3 Lean Mass in Post-Weaning Western Diet-Fed Female Offspring

Liver weights were lower (p=0.07) in post-weaning western diet-fed female offspring from western diet-fed dams compared to control diet-fed dams (**Figure 5.3B**). There were no effects of maternal diet or maternal exercise on heart, liver, kidney, and pancreas weights (**Figure 5.3A-D**). There were no effects of maternal diet or maternal exercise on lean mass (**Figure 5.3E**) in post-weaning western diet-fed female offspring.

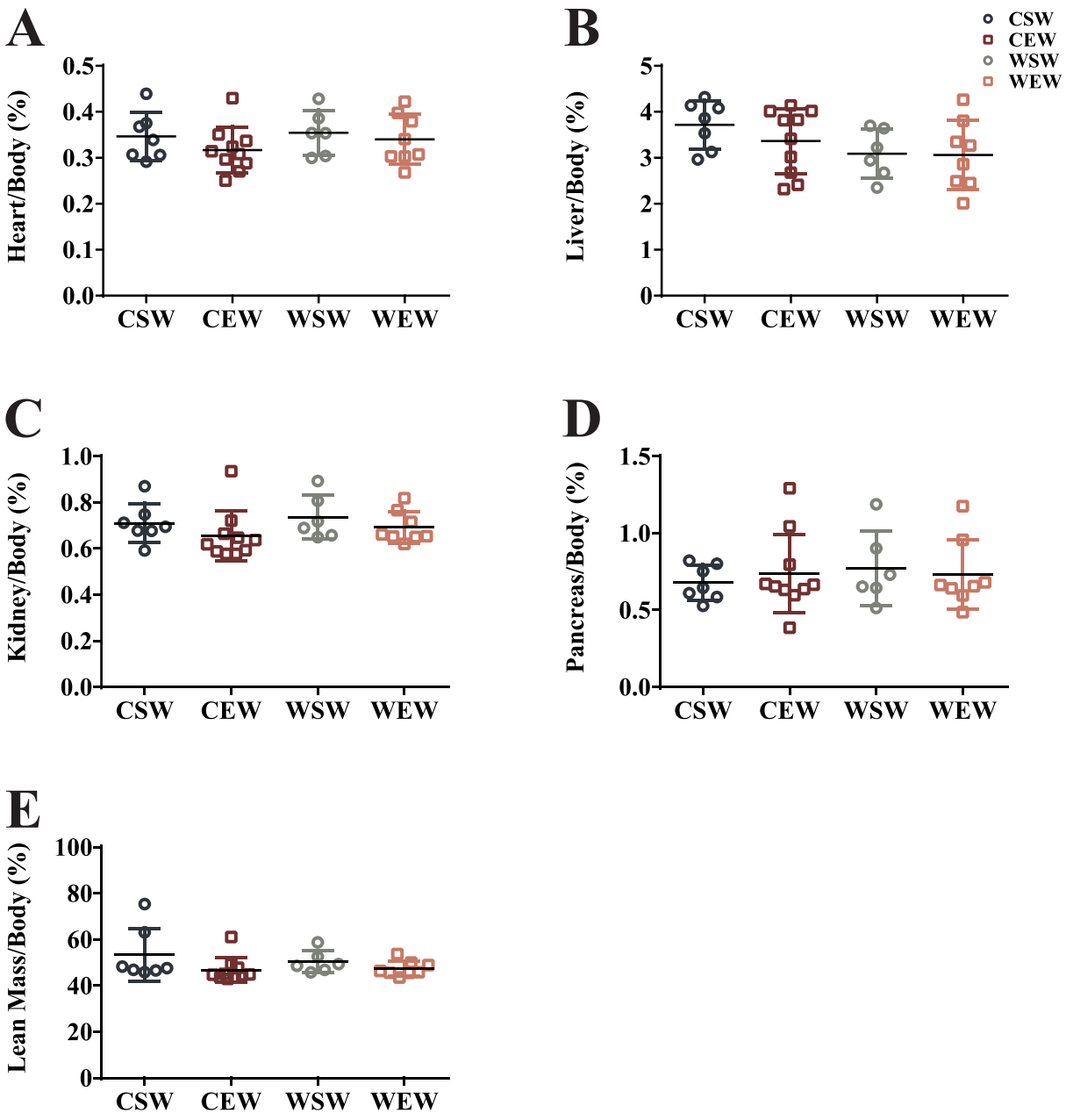

Figure 5.3 Normalized Lean Mass Weights in Post-Weaning Western Diet-Fed Female Offspring

A. Heart/body weight (%). **B.** Liver/body weight (%). **C.** Kidney/body weight (%). **D.** Pancreas/body weight (%). **E.** Lean mass/body weight (%). Data presented as individual points with mean ± standard deviation, n = 6-10 mice per diet/exercise group.

5.4 Insulin Tolerance in Post-Weaning Western Diet-Fed Female Offspring

There were no effects of maternal diet or maternal exercise on blood glucose concentrations at 0 minutes, 15 minutes, 30 minutes, 60 minutes, 90 minutes, or 120 minutes post-administration of insulin solution (**Figure 5.4A**). Further, there were no effects of maternal diet or maternal exercise on IPITT area under the curve calculations (**Figure 5.4B**) and fasting blood glucose concentrations (**Figure 5.4C**) in post-weaning western diet-fed female offspring.

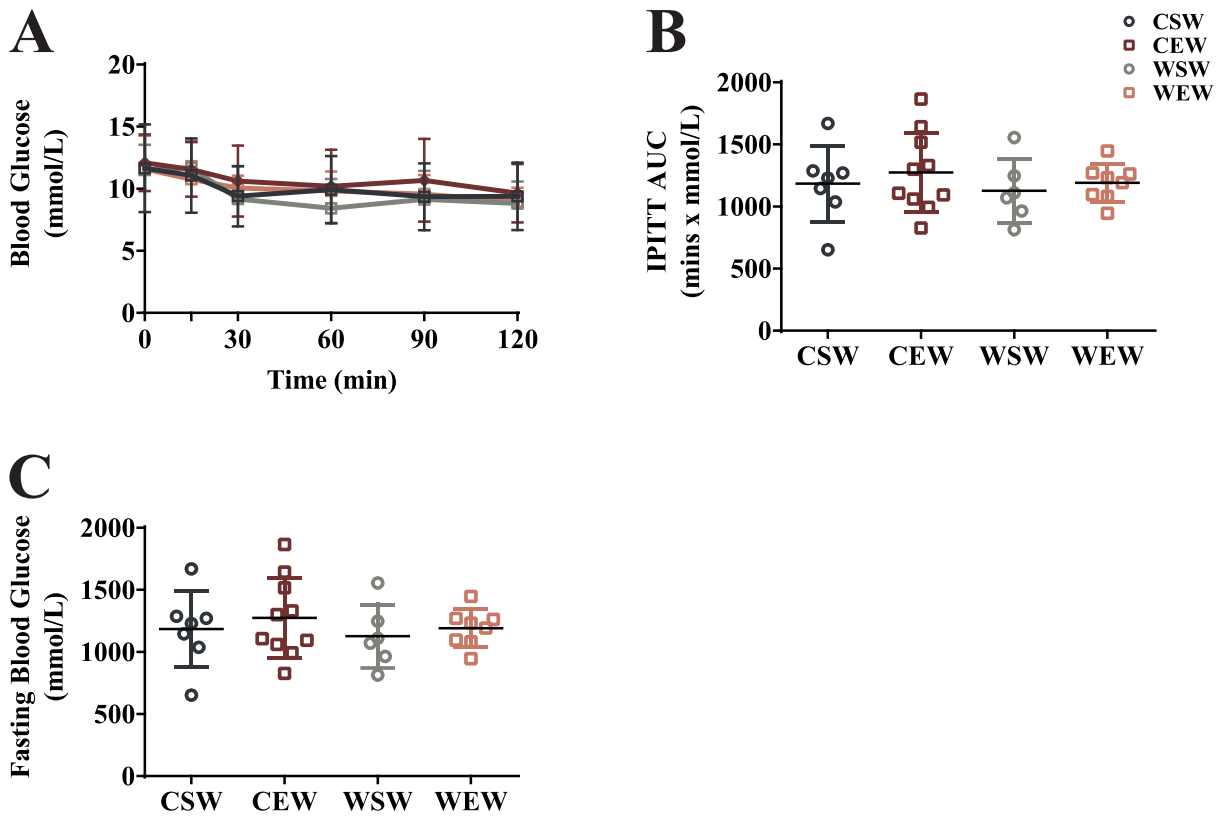

Figure 5.4 Insulin Tolerance in Post-Weaning Western Diet-Fed Female Offspring at 19 Weeks Post-Weaning

A. Blood glucose (mmol/L) versus time post-administration of insulin. Data presented as mean ± standard deviation. **B.** Intraperitoneal insulin tolerance test area under the curve. **C.** Fasting blood glucose (mmol/L) prior to administration of insulin. Data presented as individual points with mean ± standard deviation, n = 6-10 per diet/exercise group.

5.5 Glucose Tolerance in Post-Weaning Western Diet-Fed Female Offspring

Blood glucose concentrations were lower ($p \leq 0.05$) at 90 minutes post-administration of dextrose solution in post-weaning western diet-fed female offspring from exercised dams compared to those from sedentary dams (**Figure 5.5A**). Calculations of IPGTT AUC were lower ($p=0.09$) in post-weaning western diet-fed female offspring from exercised dams compared to those from sedentary dams (**Figure 5.5B**). There were no effects of maternal diet or maternal exercise on blood glucose concentrations at 15 minutes, 30 minutes, 60 minutes, or 120 minutes post-administration of dextrose solution (**Figure 5.5A**). Further, there were no effects of maternal diet or maternal exercise on fasting blood glucose concentrations prior to the administration of dextrose solution in post-weaning western diet-fed female offspring (**Figure 5.5C**).

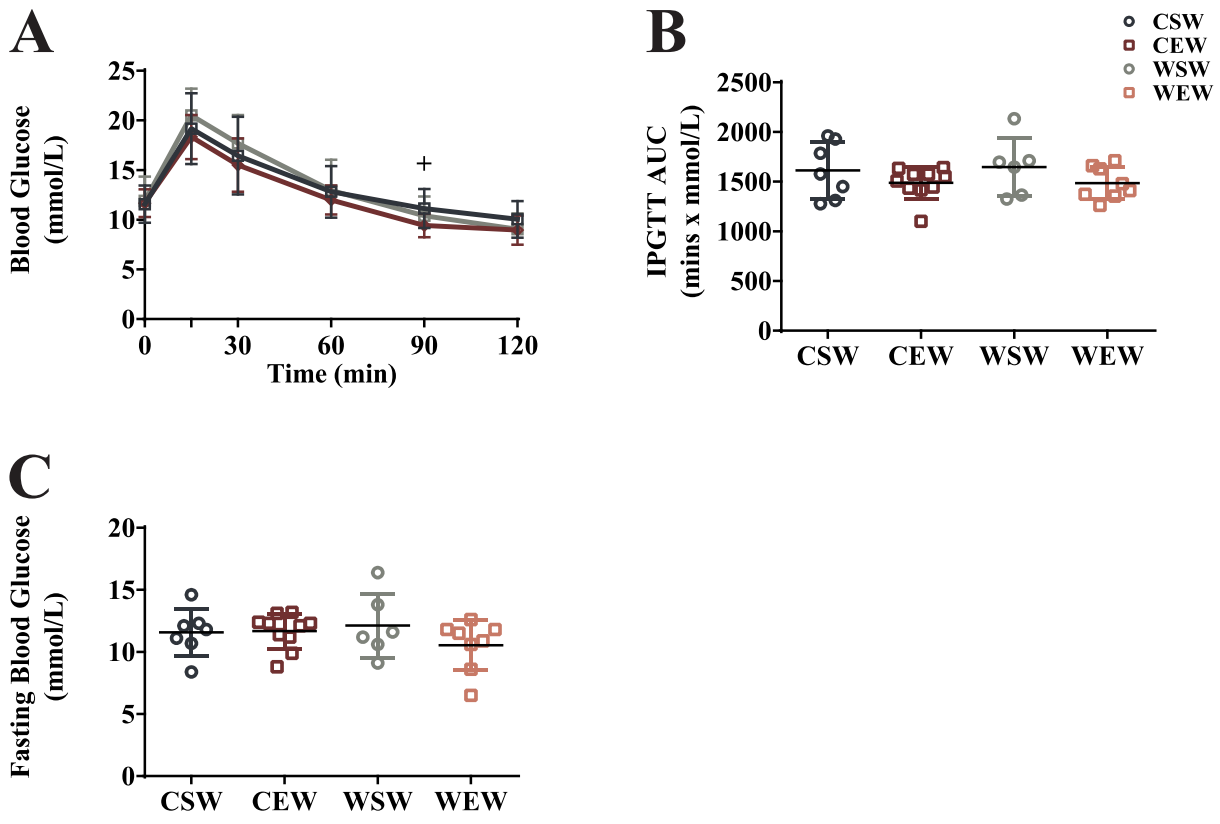

Figure 5.5 Glucose Tolerance in Post-Weaning Western Diet-Fed Female Offspring at 20 Weeks Post-Weaning

A. Blood glucose (mmol/L) versus time post-administration of dextrose solution. Data presented as mean ± standard deviation, + $p \leq 0.05$ - effect of maternal exercise. **B.** Intraperitoneal glucose tolerance test area under the curve. **C.** Fasting blood glucose (mmol/L) prior to administration of dextrose. Data presented as individual points with mean ± standard deviation, n = 6-10 mice per diet/exercise group.

5.6 *Ex Vivo* Functional Assessment of Thoracic Aortae

5.6.1 Acetylcholine-Mediated Vasorelaxation of Thoracic Aortae in Post-Weaning Western-Diet Fed Female Offspring

Acetylcholine dose-response curves were constructed to assess endothelial-dependent vasorelaxation in post-weaning western diet-fed female offspring. Acetylcholine-mediated vasorelaxation of thoracic aortae were lower ($p \leq 0.05$) in response to 10^{-8} M ACh in post-weaning western diet-fed female offspring from exercised dams compared to those from sedentary dams (**Figure 5.6A**). Acetylcholine-mediated vasorelaxation of thoracic aortae tended to be lower ($p=0.07$) in response to 10^{-7} M ACh in post-weaning western diet-fed female offspring from exercised dams compared to those from sedentary dams (**Figure 5.6A**). Acetylcholine dose-response curve $logEC_{50}$ values were higher ($p \leq 0.05$) in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, and higher ($p \leq 0.05$) in post-weaning western diet-fed female offspring from western diet-fed dams compared to control diet-fed dams (**Figure 5.6C**). However, three $logEC_{50}$ values could not be calculated by Graphpad Prism as the 95% confidence intervals were very wide for these specific curves. There were no effects of maternal exercise or maternal diet on ACh-mediated vasorelaxation of thoracic aortae in response to 10^{-10} M ACh, 10^{-9} M ACh, and 10^{-6} M ACh (**Figure 5.6A**). There were no effects of maternal diet or maternal exercise on maximal ACh-mediated vasorelaxation at 10^{-5} M ACh in post-weaning western diet-fed female offspring (**Figure 5.6A**).

5.6.2 Phenylephrine-Mediated Vasoconstriction of Thoracic Aortae in Post-Weaning Control Diet-Fed Female Offspring

Phenylephrine dose-response curves were constructed to assess vasoconstriction in post-weaning western diet-fed female offspring. Phenylephrine-mediated vasoconstrictions of thoracic aortae tended to be lower ($p=0.07$, $p=0.10$, and $p=0.10$) in response to 10^{-7} M PE, 10^{-6} M PE, and 10^{-5} M PE in post-weaning western diet-fed female offspring from western diet-fed dams compared to control diet-fed dams **(Figure 5.6B)**. There were no effects of maternal exercise or maternal diet on PE contraction at 10^{-9} M PE or 10^{-8} M PE in post-weaning western diet-fed female offspring **(Figure 5.6B)**. There were no effects of maternal exercise or maternal diet observed on maximal contraction of aortae due to 10^{-5} M PE in post-weaning western diet-fed female offspring **(Figure 5.6B)**. There were no effects of maternal diet or maternal exercise observed on the $\log EC_{50}$ values calculated from the PE dose-response curves in post-weaning western diet-fed female offspring **(Figure 5.6D)**.

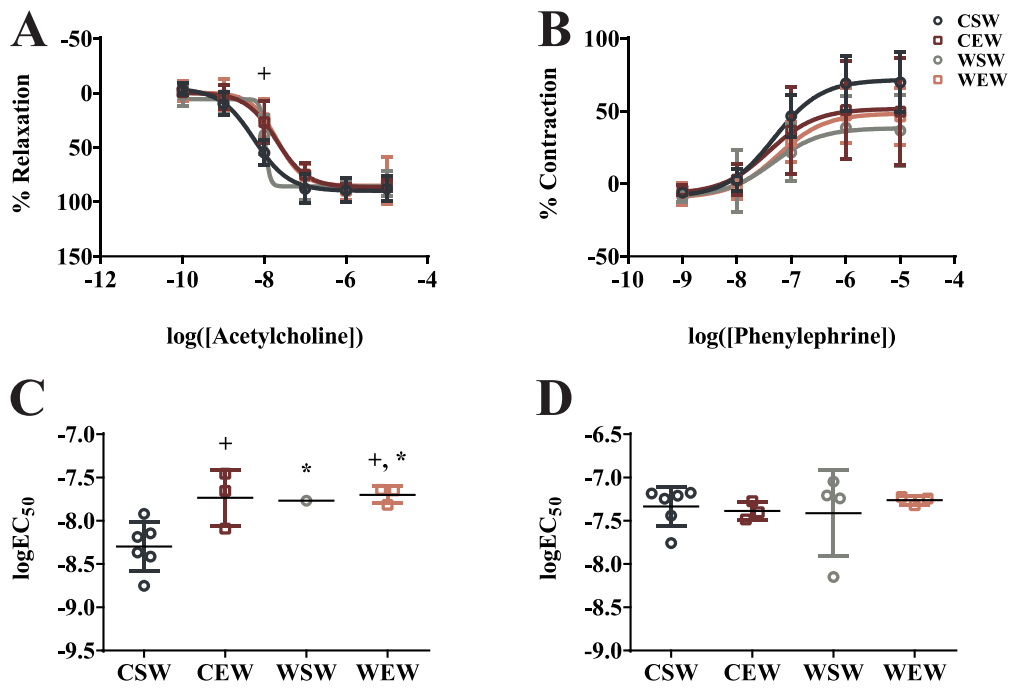

Figure 5.6 Acetylcholine Dose-Response Curves and Phenylephrine Dose-Response Curves in Post-Weaning Western Diet-Fed Female Offspring

A. Acetylcholine dose-response curve of thoracic aortae from post-weaning western diet-fed female offspring. **B.** Phenylephrine dose-response curve of thoracic aortae from post-weaning western diet-fed female offspring. **C.** $logEC_{50}$ calculations of ACh dose-response curves from post-weaning western diet-fed female offspring. **D.** $logEC_{50}$ calculations of PE dose-response curves from post-weaning western diet-fed female offspring. Phenylephrine contraction is measured as % contraction relative to a KCl curve. Acetylcholine-mediated vasorelaxation is measured as % relaxation relative to a PE contraction. Data presented as nonlinear regression with mean ± standard deviation (**A, B)** and individual points with mean ± standard deviation (**C, D)**; n = 3-6 mice per diet/exercise group in **A** and **B**, n = 1-6 mice per diet/exercise group in **C** and n = 3-6 mice per diet/exercise group in **D**, + $p \leq 0.05$, effect of maternal exercise, ***** $p \leq 0.05$, effect of maternal diet.

AIM 2

B. To determine whether these is an association between maternal exercise and maternal obesity on the expression of inflammatory cytokines in the retroperitoneal adipose tissue of adult female offspring fed a post-weaning western diet.

The effects of maternal exercise on the cardiovascular health of their offspring in humans are largely unknown. However, Carter et al. reported in a mouse model that lean mass is higher in male offspring from exercised dams compared to sedentary dams[37]. Further, fat mass was lower in male offspring from exercised dams compared to sedentary dams. These observations were made in addition to improved insulin tolerance and glucose tolerance in the offspring of exercised dams compared to sedentary dams in both sexes. I found in Aim 2A that blood glucose concentrations were lower ($p \leq 0.05$) at 90 minutes post-administration of dextrose solution in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams. Further, I found in Aim 2A that retroperitoneal adipose tissue weights were higher ($p \leq 0.01$) in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams. Following these findings, I hypothesized that although enlarged the retroperitoneal adipose tissue of post-weaning western diet-fed female offspring from exercised dams was of a healthier phenotype compared to those from sedentary dams. Specifically, I hypothesized that the retroperitoneal adipose tissue of post-weaning western diet-fed female offspring form exercised dams would be of an anti-inflammatory phenotype compared to sedentary dams; given the known role of TNF-α on the inhibition of insulin signalling. Thus, in Aim 2B I addressed whether there was an association between maternal exercise and maternal

diet on the expression of inflammatory cytokines in the retroperitoneal adipose tissue of post-weaning western diet-fed female offspring.

5.7 Inflammatory Cytokine Expression in the Retroperitoneal Adipose Tissue of Post-Weaning Western Diet-Fed Female Offspring

Abundance of *Il10* mRNA was higher ($p \leq 0.05$) in the retroperitoneal adipose tissue of post-weaning western diet-fed female offspring from exercised dams compared to those from sedentary dams (**Figure 5.7B**). There was no significant effect of maternal diet or maternal exercise on the mRNA abundance of *Tnf* in the retroperitoneal adipose tissue of post-weaning control diet-fed female offspring (**Figure 5.7A**).

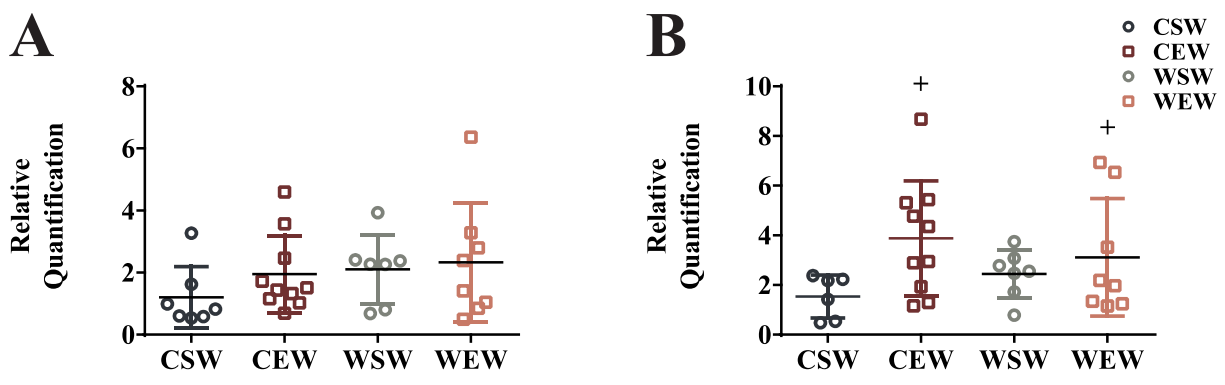

Figure 5.7 Retroperitoneal Adipose Tissue mRNA Abundance of *Tnf* and *Il10* in Post-Weaning Western Diet-Fed Female Offspring

A. Relative mRNA abundance of *Tnf* normalized to abundance of *18S* rRNA and *Ncoa6* mRNA.

B. Relative mRNA abundance of *Il10* normalized to abundance to *18S* rRNA and *Ncoa6* mRNA.

Data presented as individual points with mean ± standard deviation, n = 7-10 mice per diet/exercise group, + $p \leq 0.05$ - effect of maternal exercise.

Chapter 6: General Discussion

My work led to four novel findings: 1) retroperitoneal adipose tissue weights are higher in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, 2) *Il10* mRNA abundance is higher in the retroperitoneal adipose tissue in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, 3) blood glucose concentrations at 90 minutes are lower in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, and 4) kidney weights are higher in post-weaning control diet-fed female offspring from western diet-fed dams compared to control diet-fed dams.

6.1 Tissue Weights and Body Composition in Female Offspring

6.1.1 Tissue Weights and Body Composition in Post-Weaning Control Diet-Fed Female Offspring

Heart and kidney weights were higher in control diet-fed female offspring from western diet-fed dams compared to control diet-fed dams. These results are similar to what has been reported in the literature[29,31,35,36]. For example, it is known that male and female chow-fed Sprague-Dawley rat offspring, from Sprague-Dawley rat dams with DIO exhibit higher adiposity in adulthood[29,31]. Further, chow-fed offspring from C57BL/6 dams with DIO also exhibit higher adiposity[35] along with higher heart mass[36] in adulthood. Interestingly however, gonadal adipose tissue weights were lower in post-weaning control diet-fed female offspring from western diet-fed dams compared to control diet-fed dams. Further, there were no effects of maternal diet or

maternal exercise on body fat percentage as assessed by EchoMRI. Thus, without reproducing the original effect of higher adiposity in the offspring of dams with DIO, it is not possible to state whether exercise can mitigate the effect of maternal obesity on their offspring's health.

At weaning, body weights were higher in female offspring from western diet-fed dams compared to control diet-fed dams as was expected. However, caution must be taken in interpreting that there was no effect of exercise. Female offspring were weaned at three weeks of age and thus, in addition to receiving nutrition from the lactating dam offspring may have consumed the diet designated for the dam in the period leading up to weaning. In agreement with the literature[29,30,35], there was no effect of maternal diet on body weight at 5, 10, 15, or 20 weeks post-weaning. Further, in agreement with the literature there was no effect of maternal exercise on body weight at 5, 10, 15, or 20 weeks post-weaning[37]. However, gonadal adipose tissue weights were lower ($p=0.09$) in post-weaning control diet-fed female offspring from western diet-fed dams compared to those from control diet-fed dams; as opposed to higher visceral adiposity reported by other groups in control diet-fed female offspring from western diet-fed rat dams[29].

The results provided in this study may lend support to the thrifty phenotype hypothesis. For example, lower ($p=0.09$) gonadal adipose tissue weights in post-weaning control diet-fed female offspring from western diet-fed dams may be reflective of the programming of a basal rate of metabolism which is higher in these offspring compared to post-weaning control diet-fed female offspring from control diet-fed dams. However, it is also possible that lower ($p=0.09$) gonadal adipose tissue weights in post-weaning control diet-fed female offspring from western diet-fed dams may also lend support to the efficacy of introducing a control diet, or healthier

diet, to the offspring of western diet-fed dams. However, these two possibilities are not mutually exclusive and warrant further studies.

Additionally, the results provided in this study may lend support to the DOHaD theory. For example, this may be a reflection of programming by maternal excess adiposity on the developing offspring such that the consumption of the maternal diet by offspring is beneficial; that is, they have been programmed for it. This is exhibited by the portion of the study that involved post-weaning western diet-fed female offspring discussed later in this thesis.

Interestingly, human studies have suggested that there is a critical period for adipocyte development that occurs in childhood and adolescence, such that weight loss in adults is less effective in individuals who became obese as children[78,79]. This is because although the volume of adipocytes may change, the number of adipocytes stays constant in lean and obese adults, even after weight loss, due to higher amounts of adipocytes[78,79]. In addition to the possibility of programming a higher basal metabolism in different tissues, such as the brain, liver, and skeletal muscle, it would be interesting to determine whether altered cellularity of adipose tissue may be programmed.

Body composition of post-weaning control diet-fed female offspring was assessed at 19 weeks post-weaning by EchoMRI however there were no effects of maternal diet or maternal exercise on percent body lean mass, percent body fat mass, or percent body total water mass. The finding that there was no effect of maternal exercise on lean mass and fat mass in post-weaning control diet-fed offspring is in agreement with that of Carter et al., one of the few studies that have assessed the effect of maternal exercise on body composition in their offspring[37]. This study demonstrated higher lean mass and lower fat mass in the male offspring of exercised ICR dams 36 weeks post-weaning[37]. However, there was no effect of maternal exercise on lean mass or fat

mass in the female offspring of exercised ICR dams 36 weeks post-weaning[37]. Further, there were no significant effects of maternal diet or maternal exercise on any of the adipose tissue depots individually in post-weaning control diet-fed female offspring; although the gonadal adipose tissue weights tended to be lower in post-weaning control diet-fed female offspring from western diet-fed dams compared to control diet-fed dams.

Heart weights were higher in post-weaning control diet-fed female offspring from western diet-fed dams compared to control diet-fed dams, as expected from the literature[36]. Unexpectedly however, kidney weights were higher in post-weaning control diet-fed female offspring from western diet-fed dams compared to control diet-fed dams. It has been reported that kidney weight, kidney volume, and glomerular number are unchanged in post-weaning control diet-fed offspring from high-fat diet-fed dams compared to control diet-fed dams in Sprague-Dawley rats[33]. However, renal renin activity was higher in post-weaning control diet-fed female offspring from high-fat diet-fed dams compared to control diet-fed dams; although there was no affect of maternal diet on plasma aldosterone concentrations[33].

Again, this may be suggestive of the efficacy of switching the offspring of western diet-fed dams to a control diet. Potentially, higher kidney weight may reflect a compensatory mechanism that is protective against the development of hypertension by increasing filtration capacity. This would also provide context for my finding that there were no effects of maternal exercise or maternal diet on endothelium-dependent vasorelaxation in post-weaning control diet-fed female offspring. Studies have reported programming of nephron number and have suggested that low nephron number is associated with an increased risk of developing hypertension in adult offspring[80,81]; however these studies primarily are concerned with maternal protein restriction, and not the effect of maternal feeding of a western diet or maternal exercise. Nevertheless, it has

also been reported that the number of glomeruli per kidney was lower in subjects with hypertension compared to normotensive controls[82]. Thus, as nephrogenesis occurs *in utero*, it may be useful to consider the effects of maternal diet and maternal exercise on offspring kidney function in regards to the development of hypertension. Whether higher kidney weights in post-weaning control diet-fed female offspring from western diet-fed dams represents a functional change within the kidney that may contribute to metabolic consequences remains to be elucidated.

6.1.2 Tissue Weights and Body Composition in Post-Weaning Western Diet-Fed Female Offspring

Higher retroperitoneal adipose tissue weights were seen in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, whereas I had expected that adipose tissue depots would be of lower weight in offspring from exercised dams. Further, there was no significant effect of maternal diet or maternal exercise on lean mass, fat mass, or total water mass as assessed by EchoMRI at 19 weeks post-weaning when standardized to total body weight.

Potentially, the higher retroperitoneal adipose tissue weights in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams may reflect a redistribution of triglycerides and/or free fatty acids within the body. This is because there was no significant effect of maternal diet or maternal exercise on post-weaning western diet-fed female offspring fat mass as assessed by EchoMRI. Interestingly, inguinal adipose tissue and interscapular brown adipose tissue, adipose tissue depots traditionally thought to reflect a healthier distribution of adipose, tended to be higher in post-weaning western diet-fed female

offspring from exercised dams compared to sedentary dams. Thus, maternal exercise may contribute to improving the overall metabolic health of post-weaning western diet-fed female offspring from exercised dams by allowing for the storage of excess free fatty acids and triglycerides into the retroperitoneal adipose tissue depot, preventing the ectopic deposition of adipose tissue to the liver and skeletal muscle where metabolic consequences could be more severe. Higher retroperitoneal adipose tissue weights in western diet-fed female offspring from exercised dams is a novel finding as to my knowledge, there are no studies currently that address the effect of maternal exercise in the context of western diet-fed offspring. Further, to my knowledge there are no studies currently that address the effect of maternal exercise in the context of maternal diet-induced excess adiposity.

It would be very interesting to continue the study of the retroperitoneal adipose tissue of post-weaning western diet-fed offspring from exercised dams as to my knowledge expansion of visceral adipose tissue in conjunction with higher *Il10* mRNA abundance has not been reported, except by Lumeng et al. wherein the visceral adipose of C-C motif chemokine receptor 2 $(CCR2)^{-/-}$ mice exhibited an anti-inflammatory phenotype when challenged with a high fat-diet[60]. Briefly, this study examined the recruitment of macrophages and the initiation of adipose tissue inflammation in the context of a genetic knockout mouse model challenged with DIO, and is discussed later in this thesis. Thus, it would be interesting to perform RNA sequencing and/or microarrays containing targets enriched for pathways pertaining to inflammation on RNA isolated from the retroperitoneal adipose tissue of western diet-fed female offspring from exercised dams. This would aid in determining whether an anti-inflammatory phenotype is indeed occurring within the retroperitoneal adipose tissue of post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams.

As the brain, liver, and skeletal muscle are the primary sites for glucose disposal it would be interesting to determine whether the triglyceride content within the liver is lower in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams. It would be interesting to determine if the retroperitoneal adipose tissue is expanding to preferentially store lipids in this depot, and in doing so aiding to reduce the amount of free fatty acids within the blood. Potentially, this may be a mechanism for preventing the ectopic deposition of lipids where the consequences on glucose metabolism may be more apparent through disturbances in insulin signalling, and may be informative in the study of non-alcoholic fatty liver disease.

6.2 Glucose Homeostasis in Female Offspring

6.2.1 Glucose Homeostasis in Post-Weaning Control Diet-Fed Female Offspring

There was no significant effect of maternal diet or maternal exercise on insulin tolerance in post-weaning control diet-fed female offspring. This was surprising given that it was reported that insulin tolerance is higher in both male and female offspring of exercised dams compared to sedentary dams[37]. However, differences in the assessment of insulin tolerance in my mice compared to the literature may lie in that I assessed insulin tolerance at 19 weeks of age post-weaning. This is contrast to insulin tolerance being assessed at 33-34 weeks of age in the study performed by Carter et al. that aimed solely to determine the effect of maternal exercise on ICR dams' offspring[37]. Further, the exercise regimen employed by Carter et al. was similar to the one used in this study. However, the ICR mice were given access to an exercise wheel one week prior to the start of breeding.

It is important to note that the time of insulin tolerance assessment and glucose tolerance assessments were chosen based on preliminary studies performed in the Devlin laboratory which showed that mild glucose intolerance could be seen in female C57BL/6 female mice fed a western diet by 20 weeks; which became more pronounced at 30 weeks. Thus, assessments of glucose homeostasis were chosen at the earlier timepoint of 20 weeks to assess whether there are effects of maternal diet and/or maternal exercise, prior to the effect of feeding a western diet itself exerting its negative effect on glucose tolerance.

However, there was no effect of maternal diet or maternal exercise seen in post-weaning control diet-fed female offspring on area under the curve calculations for IPGTTs or fasting blood glucose concentration measurements prior to the administration of dextrose. Further, blood glucose concentrations were lower at 120 minutes post-administration of dextrose solution in post-weaning control diet-fed female offspring from western diet-fed dams compared to those from control diet-fed dams; indicating better glucose tolerance. This was surprising given that it has been shown that the glucose tolerance was worse in the female offspring of western diet-fed C57BL/6 dams compared to control diet-fed dams[35]. However, caution must be taken in interpreting this data as glucose homeostasis was assessed after an overnight fast, protocol which has fallen out of favour as it is more akin to starvation, as mice are nocturnal animals[83].

The finding that there was no effect of maternal exercise was surprising given that it has already been demonstrated by Carter et al. that glucose homeostasis is improved in the offspring of exercised, ICR dams. In addition, blood glucose concentrations were higher at 30 minutes post-administration of dextrose solution in post-weaning control diet-fed offspring from exercised dams compared to sedentary dams; potentially indicating difficulty in returning blood glucose concentrations to baseline levels and/or maintaining glucose homeostasis. Potential

differences between this study and that by Carter et al. may have arisen due to the timing of glucose homeostasis assessment. Potentially, if mice were assessed at a later timepoint such as at the 31-32, 36-37, or 71-72 weeks of age as in the study conducted by Carter et al., differences would be seen between offspring from exercised dams and sedentary dams. Further, there is the possibility that these differences are strain-specific.

6.2.2 Glucose Homeostasis in Post-Weaning Western Diet-Fed Female Offspring

Insulin tolerance and glucose tolerance were assessed in post-weaning western diet-fed female offspring at 19 weeks post-weaning and 20 weeks post-weaning, respectively, to determine whether maternal exercise could mitigate the effect of maternal obesity when offspring were fed a western diet. There was no significant effect observed of maternal diet or maternal exercise on insulin tolerance, IPITT area under the curve calculations, or fasting blood glucose concentrations prior to the start of the IPITT. This was surprising given that it has been shown that there is higher adiposity and insulin resistance in the offspring from dams with DIO. However, many of these findings were made in the context of dams fed a diet high in sucrose[35].

Interestingly, blood glucose concentrations were lower at 90 minutes post-administration of dextrose in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams. This suggests that maternal exercise is beneficial in mitigating the effect of maternal obesity on glucose intolerance in post-weaning western diet-fed female offspring at 20 weeks post-weaning. To my knowledge, this is the first study to assess the effect of maternal exercise in the context of maternal diet-induced excess adiposity on glucose homeostasis in western diet-fed female offspring. In the study conducted by Carter et al., the effect of maternal exercise was assessed in control diet-fed offspring at 31-32, 36-37, and 71-72 weeks of age.

Thus, further studies are warranted to determine whether the improvement of glucose homeostasis in western diet-fed female offspring from exercised dams compared to sedentary dams persists past 20 weeks, or is effectively mitigated through the continued feeding of a western diet.

There was no significant effect of maternal diet or maternal exercise observed on IPGTT area under the curve calculations or fasting blood glucose concentrations prior to the start of the IPGTT. It would be interesting to determine the concentration of insulin within the sera of these animals, both during the insulin secretion tests that were performed as well as during the fasting state; to determine whether maternal exercise may be exerting its effect via differences in the secretion of insulin and to determine whether hyperinsulinemia is present, respectively.

6.3 Endothelium-Dependent Vasorelaxation

Acetylcholine dose-response curve logEC_{50} values were higher in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, and higher in post-weaning western diet-fed female offspring from western diet-fed dams compared to control diet-fed dams. There were no effects of maternal diet or maternal exercise observed on maximal vasoconstriction mediated by PE or maximal vasorelaxation mediated by ACh. Further, there were no significant effects of maternal diet or maternal exercise observed on maximal vasorelaxation due to SNP, as expected (data not shown). Thus, given the null effects of maternal exercise and maternal diet on ACh and PE dose-response curves other aspects of this experiment were focussed on, as exemplified by the low n's in each group. Endothelial dysfunction has been reported to be predictive of one's future risk of a cardiovascular event[44–47]. Further, endothelial dysfunction is one of the earliest biomarkers of vascular disease[40,42,43,84]. Thus, dose-response

curves were constructed to assess the effect of maternal diet and maternal exercise on: vasoconstriction, through the construction of PE dose-response curves; endothelium-dependent vasodilation, through the construction of ACh dose-response curves; and endothelium-independent vasodilation, through the construction of SNP dose-response curves.

However, although ACh dose-response curve $logEC_{50}$ values were higher in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, and higher in post-weaning western diet-fed female offspring from western diet-fed dams compared to control diet-fed dams, these are not likely to be biologically meaningful due to low n's. Further, three curves were excluded from analysis due to very wide confidence intervals in the calculation of $logEC_{50}$ values. Although not directly comparable, this in contrast to what has been reported in the spontaneously-hypertensive Wistar-Kyoto rat, wherein a 6-week exercise regimen restored endothelium-dependent vasorelaxation in abdominal aortae[85]. However, aside from being performed in another rodent model it is important to note that programming was not studied as exercise occurred within the same animal in which vascular function was assessed, in addition to only studying adult male rats.

Presumably due to feasibility of using a larger rodent, much of the literature regarding the assessment of vascular function in regards to exercise has been performed in DIO models using Sprague-Dawley rats. It has been reported that endothelium-dependent vasorelaxation is impaired in abdominal aortae isolated from chow diet-fed offspring from Sprague-Dawley dams fed a chow diet supplemented with animal lard[33]. Further, studies have shown that endothelium-dependent vasorelaxation is impaired in third-order branches of the mesenteric arcade in offspring from Sprague-Dawley dams fed a chow diet supplemented with animal lard[29,30]. In future studies, it would be informative to assess the effect of maternal exercise and maternal diet

on endothelium-dependent vasodilation of resistance arteries as opposed to the thoracic aorta due to the significant amount by which blood flow may be regulated in these vessels.

Exercise has been reported to restore endothelium-dependent vasorelaxation in the femoral artery of hypercholesterolemic rabbits[52]. However, in this study the exercise intervention occurred in the same animal whose vasculature had been assessed *ex vivo*. Nevertheless, it may be informative to determine whether the mice in the current study were hypercholesterolemic; and to examine the aorta for markers of inflammation typically associated with higher lipid deposition. This would be particularly interesting in the context of the finding of higher retroperitoneal adipose tissue weights in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams.

6.4 MCP-1 and Obesity

Monocyte chemoattractant protein 1 (MCP-1) is a major protein involved in the recruitment of monocytes into adipose tissue in obesity[86,87]. Expansion of adipose tissue through the accumulation and storage of lipids has been associated with higher mRNA abundance of *Mcp1*, also known as C-C motif chemokine ligand 2 (*Ccl2*). Likewise, CCR2 is found on a subset of circulating monocytes and mediates monocyte chemotaxis through its interaction with CCL2[61,86]. Further, the recruitment of monocytes predisposed to differentiating into an inflammatory phenotype is higher in the context of obesity[88]. This is due to a higher proportion of monocytes $CCR2^{+}$ monocytes, which are predisposed to differentiating into macrophages with an inflammatory phenotype. This is in comparison to $CCR2^{-}$ monocytes, which are predisposed to differentiating into macrophages with an anti-inflammatory phenotype.

In chow diet-fed C57BL/6 mice, it has been reported that adipose tissue macrophages are primarily derived from monocytes that are $CCR2^{-}$[60,89]. Further, these macrophages exhibit a M2-polarized phenotype, characterized by anti-inflammatory cytokine production. In contrast, mice fed a diet consisting of 45% kcal from fat that adipose tissue macrophages are primarily derived from monocytes that are $CCR2^{+}$[60]; these macrophages exhibit a M1-polarized phenotype, characterized by proinflammatory cytokine production. Further, the expression of Ym1, arginase 1, and Il10 are decreased in adipose tissue macrophages from mice with DIO compared to adipose tissue macrophages isolated from chow diet-fed mice[60]. Due to its composition there is a limited subset of cells within the adipose tissue from which certain anti-inflammatory cytokines may be expressed. These include M2-polarized, or alternatively activated macrophages, and $CD8^{+}$ T regulatory lymphocytes. Importantly, the relative mRNA abundance of these genes is similar when measured in unsorted stromovascular fractions, as compared to macrophages isolated by fluorescence-activated cell sorting[60].

In humans, exercise has been shown to influence the distribution of monocytes predisposed to developing into proinflammatory macrophages or anti-inflammatory macrophages upon leaving the circulation. Monocytes can be classified into two main populations based on their expression of C-X3-C motif chemokine receptor 1 (CX_3CR1)[88]. High expression of CX_3CR1 is associated with a monocyte subset that exhibits CX_3CR1-depedent recruitment to non-inflamed tissues, whereas low expression of CX_3CR1 is associated with a monocyte subset that exhibits active recruitment to inflamed tissues[88]. Further, levels of CX_3CR1 expression have been used to characterize two subsets of human monocytes, $CD14^{+}$ and $CD16^{-}$, and $CD14^{low}$ and $CD16^{+}$, that share the same phenotypic and homing characteristics as those found in mice[88]. It has been reported that a two-fold higher number of circulating inflammatory $CD14^{+}$ and $CD16^{+}$

monocytes can be found in elderly men and women who are sedentary as compared to an age-matched physically active group[69]. Further, the relative proportion of inflammatory to anti-inflammatory monocytes was lower in sedentary elderly men and women following a 12-week intervention consisting of regular exercise[69].

6.5 Expression of Inflammatory Cytokines in the Retroperitoneal Adipose Tissue

There was no significant effect of maternal exercise or maternal diet on the mRNA abundance of *Tnf* or *Il10* in the retroperitoneal adipose tissue of post-weaning control diet-fed female offspring. However, there was higher mRNA abundance of *Il10* in the retroperitoneal adipose tissue of post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams. The relative distribution of inflammatory monocytes to anti-inflammatory monocytes is higher in the context of obesity. Further, it has been demonstrated that this ratio is decreased following a 12-week exercise intervention in previously sedentary elderly men and women[69]. Thus, I assessed the mRNA abundance of *Tnf* and *Il10* in the retroperitoneal adipose tissue of both post-weaning control diet-fed female offspring and post-weaning western diet-fed female offspring.

The retroperitoneal adipose tissue depot was chosen to be analyzed due its counterintuitive expansion in the post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams. Therefore, I hypothesized that the retroperitoneal adipose tissue from post-weaning western diet-fed female offspring from exercised dams was of an anti-inflammatory phenotype compared to sedentary dams; which may be of an inflammatory phenotype. *Tnf* and *Il10* were chosen as mRNA targets to serve as indicators of the proinflammatory or anti-inflammatory phenotype of the adipose tissue. TNF-α and IL10 are

cytokines that are expressed from macrophages that are exhibiting an inflammatory and anti-inflammatory phenotype, respectively.

To my knowledge, this is the first study to assess the effect of maternal exercise in the context of maternal diet-induced excess adiposity on the mRNA abundance of *Tnf* and *Il10* mRNA in the retroperitoneal adipose tissue of control or western diet-fed female offspring. Interestingly however, my findings may be informed by the findings of Lumeng et al. who reported that adipose tissue weight was higher in high-fat diet-fed CCR2$^{-/-}$ mice compared to wild-type controls. It is known that high-fat diet-fed mice have higher adipose tissue weights. Further, both high-fat diet-fed CCR2$^{-/-}$ mice and high-fat diet-fed wild-type mice have higher adipose tissue weights compared to control. However, there is a lower amount of inflammatory macrophages within the adipose tissue of CCR2$^{-/-}$ mice compared to obese controls. Further, the adipose tissue macrophages that are present within the adipose tissue of CCR2$^{-/-}$ mice have lower mRNA abundance of markers of the M1 phenotype[60,61,90] and higher mRNA abundance of markers of the M2 phenotype[60].

It would be interesting to determine whether in conjunction with the higher *Il10* mRNA abundance seen in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, if the phenotype of the retroperitoneal adipose tissue is similar to that in the high-fat diet-fed CCR2$^{-/-}$ mice; whose adipose tissue weights were higher, albeit with an anti-inflammatory phenotype[60]. Therefore, while higher mRNA abundance of *Il10* is suggestive of an anti-inflammatory phenotype in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, it may be informative to measure the mRNA and protein abundance of *Ccl2* and MCP-1 to normalize the mRNA abundance of *Il10* and *Tnf* to the macrophage content of the adipose tissue; to gain information on the phenotype of the

macrophages present. Potentially, it would be interesting to extend to studies involving the use of next-generation sequencing strategies to determine whether the tissue exhibits a mRNA abundance profile characteristic of a M2, anti-inflammatory phenotype.

6.6 Beta Cell Death Assay

The pyrosequencing assay that was developed displayed linearity with respect to mixtures containing known amounts of liver to MIN6 DNA. However, as this was a pilot study, the n's for the animal portion of the experiment are low. Thus, to determine whether these findings align with those of Akirav et al. further development and verification of the assay is warranted[77]. To ensure accuracy and consistency with the ratio at which liver and MIN6 DNA were mixed, mixtures were prepared prior to bisulfite conversion; to minimize error associated with differences in elution. This is especially important given the difficulty of quantifying bisulfite-converted DNA, as well as the low amount of DNA used in this assay. However, spikes downward in the percent gene methylation observed at CpG +177 of *Ins1*, potentially reflective of DNA coming from beta cell death, are promising as these occurred prior to changes in blood glucose were seen; potentially increasing the amount of interventions available.

It would be interesting to analyze the serum samples collected at earlier timepoints to determine the earliest time at which a statistically significant, and biologically-meaningful deviation from baseline may be detected. Further, it is important to note that not all NOD mice progress to autoimmune diabetes. Thus, these findings suggest that this assay may be useful as an indicator of beta cell death. However, further studies such as verification in a mouse model involving the administration of a beta cell toxin, such as STZ, as well as higher n's would be informative.

6.7 Conclusions

6.7.1 Post-Weaning Control Diet-Fed Female Offspring

1. Maternal consumption of a western diet and maternal exercise do not affect post-weaning control diet-fed female offspring weights from weaning through to 20 weeks post-weaning.
2. Maternal consumption of a western diet results in higher heart and kidney weights in post-weaning control diet-fed female offspring.
3. Maternal consumption of a western diet and maternal exercise likely do not affect endothelium-dependent vasorelaxation of the thoracic aorta in post-weaning control diet-fed female offspring.
4. Maternal consumption of a western diet and maternal exercise do not affect insulin tolerance in post-weaning control diet-fed female offspring at 19 weeks post-weaning.
5. Maternal consumption of a western diet results in improved glucose tolerance in post-weaning control diet-fed female offspring at 20 weeks post-weaning.
6. Maternal consumption of a western diet and maternal exercise do not affect *Tnf* or *Il10* mRNA abundance in the retroperitoneal adipose tissue of post-weaning control diet-fed female offspring.

6.7.2 Post-Weaning Western Diet-Fed Female Offspring

1. Maternal exercise results in higher post-weaning western diet-fed female offspring weights from weaning through to 20 weeks post-weaning.
2. Maternal consumption of a western diet and maternal exercise likely do not affect endothelium-dependent vasorelaxation of the thoracic aorta in post-weaning western diet-fed female offspring.
3. Maternal consumption of a western diet and maternal exercise do not affect insulin tolerance in post-weaning western diet-fed female offspring at 19 weeks post-weaning.
4. Maternal exercise is beneficial in improving glucose tolerance in post-weaning western diet-fed female offspring at 20 weeks post-weaning.
5. Maternal exercise results in higher retroperitoneal adipose tissue weight in post-weaning western diet-fed female offspring.
6. Maternal consumption of a western diet and maternal exercise do not affect *Tnf* mRNA abundance in the retroperitoneal adipose tissue of post-weaning western diet-fed female offspring.
7. Maternal exercise results in higher *Il10* mRNA abundance in the retroperitoneal adipose tissue of post-weaning western diet-fed female offspring.

6.7.3 Overall Conclusions

1. The efficacy of maternal exercise in mitigating the adverse effects of maternal obesity could not be evaluated in post-weaning control diet-fed female offspring as the adverse effects of maternal diet-induced excess adiposity could not be reproduced.
2. Maternal exercise results in improved glucose homeostasis, higher retroperitoneal adipose weight, and higher retroperitoneal adipose tissue *Il10* mRNA abundance in post-weaning western diet-fed female offspring.

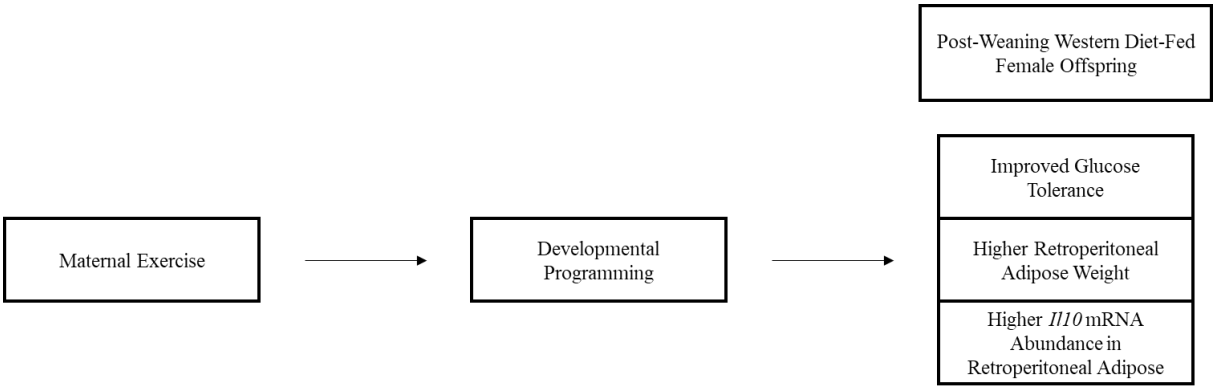

Figure 6.1 Overarching Summary of Thesis

6.8 Future Directions

Current guidelines recommend moderate exercise for all pregnant women in promoting a healthy lifestyle[27]. However, my findings in Aim 2 from the study of maternal exercise and maternal diet in post-weaning western diet-fed female offspring warrant further study into whether maternal exercise is beneficial for their female offspring. Specifically, it was an unexpected finding to determine that retroperitoneal adipose tissue weights were higher in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams.

Additionally, although it was determined that *Il10* mRNA abundance was higher in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, which may indicate an anti-inflammatory phenotype of the adipose tissue, further studies are warranted to gain a more holistic view of the phenotype of the adipose tissue of these mice. As mentioned previously, it would also be interesting to determine whether the higher retroperitoneal adipose tissue weights in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams reflect a redistribution of triglycerides within the body; preventing the ectopic deposition of lipid into the liver or skeletal muscle, where the consequences on metabolism could be more severe.

Preliminary studies conducted prior to this study in the Devlin laboratory determined that mild glucose intolerance can be seen in western diet-fed female C57BL/6 mice at 20 weeks post-weaning, with more pronounced glucose intolerance occurring at 30 weeks post-weaning. Although it was determined that glucose tolerance was improved in post-weaning western diet-fed female offspring from exercised dams compared to sedentary dams, it is important to determine whether this modest improvement in glucose tolerance can be sustained at later timepoints.

6.9 Limitations

A caveat of this work is that there is variability in what constitutes the exercise treatment. Metabolic cages were not used in this study as they are extremely stressful, so much so that pregnant dams are not allowed in them. Further, in rats glucocorticoid exposure in late gestation has been shown to program glucose intolerance and hypertension in adult offspring[91,92], precluding the use of metabolic cages in this study. Thus, it is difficult to ascertain the homogeneity as well as effect size of the exercise treatment in comparing the dams given access to a running wheel for voluntary exercise versus sedentary dams. This is because although exercise was facilitated through the inclusion of an exercise wheel, dams termed sedentary may have achieved a biologically-meaningful amount of exercise without this enrichment.

However, data on how much the dams ran was collected by a PhD student within the lab (Nicha Boonpattrawong). There were no differences in the distance run on the exercise wheel between exercised western diet-fed dams and exercised control diet-fed dams. Further, a citrate synthase activity assay was used by Nicha to confirm that there was a significant effect of exercise. There were no differences in citrate synthase assay activity between exercised western diet-fed dams and exercised control diet-fed dams.

The significant effect of exercise on citrate synthase assay activity was expected given that enrichment was provided through the exercise wheel. However, it was interesting to see that there were no differences in both the citrate synthase activity assay and distance run on the exercise wheel between exercised western diet-fed dams and exercise control diet-fed dams. It was expected that the excess adiposity imparted onto western diet-fed dams may preclude their mobility, which would be reflected in the distance run on the exercise wheel.

Secondly, there are differences in the number of offspring produced from dams versus a human mother. It would be interesting to determine whether the amount of nutrients as well as potentially, inflammatory factors to which an individual offspring is exposed *in utero* in the context of maternal obesity may be diluted across littermates. In this study, there was no effect of maternal exercise or maternal diet on litter size.

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