ABSTRACT

Title of Document: **EXAMINING THE EFFECTS OF EXERCISE ANCESTRY ON TWO GENERATIONS OF MOUSE OFFSPRING.**

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This dissertation research is comprised of three projects examining the effect of voluntary parental exercise on health-related phenotypes in two generations of mouse offspring. We developed a novel model of exercise ancestry where C57BL/6 mice (F0) were exposed to voluntary exercise (EX) or a sedentary (SED) lifestyle and were bred with like-exposed mates to produce first-generation (F1) offspring; F1 offspring were bred with like-exposed offspring to produce second-generation offspring (F2). F0 mice exercised before breeding and continuously through gestation and lactation; all offspring remained sedentary after weaning, thus F0 exercise exposure was the only distinguishing factor between offspring. The first project examined whole body and tissue masses, glucose tolerance, and skeletal muscle gene expression in two generations of 8-week old offspring of exercised vs. sedentary parents. F1 EX females were lighter with less fat mass compared to F1 SED females. F2 EX females had lower baseline blood glucose and impaired glucose tolerance.

Further, skeletal muscle lipogenic gene expression was downregulated in females with exercise ancestry, while it was upregulated in males with exercise ancestry. The second study further examined these phenotypes in two generations of adult (28 week) offspring. Parental exercise did not influence offspring body mass or glucose tolerance in 28 week-old offspring, though F1 EX females had higher baseline glucose. Additionally, while some differences in skeletal muscle gene expression were observed, the effect of parental exercise on offspring was blunted at 28 compared to 8 weeks of age. The third study further examined the effects of parental exercise in skeletal muscle as well as adipose and hepatic tissue with regards to metabolite content and gene expression. Exercise ancestry did not affect offspring skeletal muscle or liver triglyceride or glycogen content. Further, there were no effects of exercise ancestry on gene expression levels of glycogen- or triglyceride-related enzymes in skeletal muscle, liver, or adipose tissue. Overall, these studies suggest no adverse effects of parental exercise on metabolic health in multiple generations of mouse offspring.

EXAMINING THE EFFECTS OF EXERCISE ANCESTRY ON TWO GENERATIONS OF MOUSE OFFSPRING.

By

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Chapter 1: Introduction and Specific Aims

Noncommunicable diseases such as cardiovascular disease, diabetes, and cancers make up 87% of all deaths in the United States (5). It is well established that preventable lifestyle factors (i.e., diet, physical activity, exposure to toxins) play a critical role in the development of many of these diseases; however, increasing evidence suggests environmental stimuli experienced during development or early-life can also alter adult disease risk. During development, an organism experiences critical windows of plasticity, especially during times of rapid cell proliferation or differentiation, where it is particularly sensitive to the environment. The environment during these stages is therefore critical for the proper development of tissues and major physiologic processes (12). Though this plasticity can be advantageous, allowing the organism to fine-tune gene expression based on the environment to meet current needs, improper development of organ systems can alter the organism's control of physiology and homeostasis throughout life (117).

Both epidemiological and animal studies support this notion that alterations in environmental factors during development can affect the adult phenotype of the organism. This phenomenon has been studied primarily with regards to nutritional imbalances (under- or over-nutrition and exposure to toxins). The impact of these various interventions have been similar with offspring displaying phenotypes related to the metabolic syndrome such as obesity and impaired glucose metabolism (56), suggesting common mechanisms may play a role. In mammals, the "environment" is transmitted to the developing organism through the maternal environment and,

specifically, the placenta. Studies of paternal-specific stimuli altering offspring phenotypes suggest additional mechanisms are also involved.

Parental physical exercise represents yet another environmental stimulus that could influence offspring development. Physical exercise affects many organ systems throughout the body, including the metabolic pathways that respond to changes in nutrition. Acute responses or chronic metabolic adaptations to exercise could directly (e.g., maternal exposure during pregnancy or lactation) or indirectly (e.g., maternal or paternal exposures prior to conception) affect a developing organism. The benefits of exercise on the individual are well established, but comparatively little is known about the effects of maternal or paternal exercise on the developing fetus.

One of the mechanisms by which environmental factors experienced during development are thought to alter adult phenotypes is through the control of gene expression without alterations in the DNA sequence of the genes themselves (21). For example, small changes in the expression levels of key metabolic genes could have a large impact on the subsequent protein abundance and enzyme activity. If the DNA contained in the germ cells is affected, these modifications could even be passed through multiple generations.

Overall Aim

The overall aim of this dissertation research was to investigate the effects of parental exercise on mouse offspring phenotypes related to metabolism. To comprehensively characterize these effects, a wide range of phenotypes was investigated from the whole body to the transcriptional level.

Specific Aim 1

To determine the impact of exercise ancestry on body morphology, metabolic phenotypes and skeletal muscle gene expression in two generations of mouse offspring.

To achieve this aim, we developed a novel model of exercise ancestry where C57BL/6 mice were exposed to voluntary exercise (EX) or sedentary lifestyle (SED) and bred with like-exposed mates to produce an F1 generation. F1 mice of both ancestries remained sedentary and were either sacrificed at 8 wk or bred with littermates to produce an F2 generation, which also remained sedentary and was sacrificed at 8 wk.

First, we examined body, fat, organ and skeletal muscle mass differences. We found F1 EX females were lighter than F1 SED females and that F1 SED females had higher tibialis anterior and omental fat masses. Second, we examined metabolic phenotypes such as glucose tolerance and circulating insulin and lipids. We found lower serum insulin in F1 SED females compared to F1 EX females. Also, F2 EX females had impaired glucose tolerance compared to F2 SED females. Lastly, we examined gene expression differences by skeletal muscle microarray and targeted gene expression analyses. The microarray analysis revealed many generation-specific up- and down-regulated transcripts between EX and SED offspring. Three of the transcripts (*Adipoq*, *Cidec*, and *Scd1)* have been previously linked to lipogenesis. They were all upregulated in F1 SED males, with a similar pattern observed in F0 and F2 males but all downregulated in females, indicating the possibility of a sex-specific alteration in lipogenesis based on EX ancestry. The targeted analysis of gene

expression also revealed several generation- and sex-specific differences in mRNA expression of multiple genes related to metabolism, though no striking overall pattern was observed.

We concluded that EX ancestry can affect whole-body and transcription-level offspring phenotypes across two generations, but in a generation- and sex-dependent manner. Together, our results reflect a small, but broad impact of EX ancestry. The manuscript resulting from the experiments of Specific Aim 1 was published in *Experimental Physiology* and comprises Chapter 3 of this dissertation.

Specific Aim 2

To determine the effect of exercise ancestry on body morphology, metabolic phenotypes and skeletal muscle gene expression in two generations of mature mouse offspring.

The purpose for this study was two-fold. First, we aimed to extend our findings from Study 1 while exerting tighter control over some of our techniques related to breeding and whole-body phenotype measurements. Second, based on the fact that we found relatively small differences in phenotypes between EX and SED offspring in Study 1, we wanted to further stress the offspring. We chose aging as our additional metabolic stressor and examined the same offspring phenotypes as in Study 1, but at 28 weeks of age. We hypothesized that larger differences between EX and SED offspring would be observed at 28 weeks of age.

In 8-week old offspring, we found that body mass tended to be higher in F1 EX offspring. Liver mass was higher in F1 EX males and tended to be higher in F1 EX females. In the F2 generation, average plantaris mass tended to be lower in SED

males. F2 female mice with EX ancestry had lower baseline blood glucose and tended to have lower IPGTT area under the curve (AUC). We found higher *Cytc* and tendencies towards higher *Pparg*, and *Scd1* mRNA expression in F1 EX females compared to F1 SED females and lower *Cidec*, and *Scd1* and a tendency for higher *Adipoq* mRNA expression in F2 EX males compared to F2 SED males. Though our sample size and thus statistical power was smaller in Study 2 for the 8-week offspring, our gene expression results, particularly our qRT-PCR targets, follow some similar trends to those observed in Study 1, though not all of our results were supported.

In 28-week old offspring, we found lower liver and soleus mass in F1 and F2 EX males compared to F1 and F2 SED males. F1 EX females had higher baseline glucose. *Pgc1a* mRNA expression was higher in F1 EX males than F1 SED males and *Cox1* mRNA expression was lower in F2 EX males compared to F2 SED males. No other differences were observed. Interestingly the difference in male offspring liver mass was in the opposite direction at 8 compared to 28 weeks of age. Similarly, 28 week old EX F2 females had better glucose tolerance compared to worse glucose tolerance at 8 weeks. Our hypothesis that aging the offspring to 28 weeks would increase the phenotype separation between EX and SED offspring was not supported. It is possible that 28 weeks is not a sufficient duration of aging to see this separation, as other researchers have not seen differences in whole body and glycemia-related phenotypes until even older ages (33, 34).

Overall, as a result of this study we maintain our prior conclusion that EX ancestry can affect whole-body and transcription-level offspring phenotypes across

two generations, but that these effects are not only generation- and sex-dependent, but also strongly impacted by offspring age. The manuscript resulting from the experiments for Specific Aim 2 comprises Chapter 4 of this dissertation.

Specific Aim 3

To determine the effect of exercise ancestry on metabolic phenotypes in skeletal muscle, adipose tissue, liver, and serum in two generations of mature mouse offspring.

Our focus on skeletal muscle phenotypes through the first two aims was based on the clear impact of exercise on skeletal muscle. However, it is possible that other tissues may be more sensitive to developmental programming. Due to the hallmark obesity and insulin resistance observed in many models of altered maternal nutrition, offspring adipose and hepatic tissue are frequently studied.

Carter et al. (33) used a similar study design to ours (perinatal exercise exposure) and observed enhanced glucose disposal in response to a glucose or insulin challenge in the offspring of exercised mice. This difference in glucose disposal was mimicked by enhanced glucose uptake in response to insulin by soleus and adipose tissue from the offspring of exercised mice. This effect was particularly dramatic in the adipose tissue, suggesting adipose tissue insulin sensitivity is contributing to the enhancement of whole-body glucose disposal in the offspring of exercised mice. These phenotypes, however, were not observable until the offspring reached 7 months of age. Thus, examining the control of glucose as well as lipid homeostasis prior to the development of overt insulin resistance may help uncover the underlying dysfunction preceding glucose intolerance.

Though limited information exists regarding the effects of maternal/paternal exercise on offspring liver, both adipose and hepatic tissue work in concert with skeletal muscle to maintain metabolic homeostasis. Defects in the normal metabolic function of one or more of these tissues are likely to precede overt pathology; we therefore examined basic metabolite stores (glycogen and TAG in skeletal muscle and liver) in these tissues and the gene expression of the key enzymes regulating the storage and breakdown of these metabolites in skeletal muscle, liver, and adipose tissue. We hypothesized that mature mouse offspring with an exercise ancestry would store less TAG and more glycogen in skeletal muscle and liver compared to offspring of sedentary parents. We also hypothesized that these differences would be associated with more glycogenic/less glycogenolytic and less lipogenic/more lipolytic gene expression patterns in metabolically active tissues of EX ancestry offspring. Contrary to our hypotheses, we did not observe any differences in TAG or glycogen storage or associated gene expression in skeletal muscle, liver, or adipose tissue. The manuscript resulting from the experiments for Specific Aim 3 comprises Chapter 5 of this dissertation.

Chapter 2: Review of Literature

Overview

The first section of this literature review will describe basic the metabolic processes required for the storage and breakdown of carbohydrate and lipid. The remainder of this review will discuss the existing developmental programming literature with a focus on the effect of parental environment on offspring metabolic health; this section will include epidemiological and experimental evidence of developmental programming, a detailed review of parental exercise on offspring outcomes in humans and animal models, a discussion of proposed mechanisms of developmental programming (including mechanisms specific to maternal exercise), and a review of multigenerational inheritance of offspring phenotypes.

Metabolism

Humans and animals gain energy from consuming and breaking down carbohydrates, fats, and proteins. The energy released from breaking down these carbon-containing compounds is used to generate ATP, which can then be used to power energy-requiring processes such as movement and growth. Only a limited amount of ATP can be stored, and animals have thus evolved to store excess nutrients (glucose and fatty acids) to act as reserves for periods of fasting. Specialized cells contribute to this storage process; most excess glucose is stored as glycogen in the skeletal muscle and liver and most excess lipid is stored in adipocytes. Thus energy balance requires complex coordination between energy-storing and energy-utilizing tissues as well as organs involved in metabolic regulation; these tissues communicate

via secreted hormones and cytokines, which affect gene transcription and/or induce protein-signaling cascades. The purpose of this section of the literature review is to briefly review the metabolic processes controlling carbohydrate and/or lipid storage and release, highlighting the unique aspects of metabolism in each of these specialized tissues (muscle, liver, and adipose). A schematic overview of the metabolic processes reviewed here is provided in Figure 2.1.

Carbohydrate Metabolism

Glycogen is a highly-branched polysaccharide made up of hundreds of glucose molecules joined by glycosidic bonds. Storing glucose as glycogen is advantageous, as it reduces the burden of glucose molecules on cell osmotic pressure (196). Skeletal muscle is the major site of glycogen storage in the body, though the liver stores more glycogen per gram of tissue (196). Muscle takes up glucose from the blood via the insulin-sensitive GLUT4 glucose transporter. GLUT4 is generally sequestered in storage vesicles, but translocates to the plasma membrane in response to insulin signaling or muscular contraction via parallel but distinct signaling pathways. In contrast to skeletal muscle, the liver takes up glucose when the circulating blood glucose level exceeds the euglycemic threshold, independent of insulin stimulation. Glucose uptake in the liver occurs via the hepatic glucose transporter GLUT2, which maintains intracellular and extracellular glucose concentrations in equilibrium (161).

Glucose must be phosphorylated to glucose-6-phosphate by hexokinase 2 (Hk2, in muscle) or glucokinase (GK, in liver) upon entry to the cell to maintain a concentration gradient of glucose promoting continued glucose uptake (82). The

accumulation of glucose-6-phosphate activates the glycogen synthase (GS) enzyme (24). Glycogen synthase is the rate-limiting enzyme of glycogenesis and is regulated by phosphorylation-induced inactivation in addition to allosteric activation by glucose-6-phosphate (119). Insulin activates GS by promoting dephosphorylation of the enzyme via activation of protein phosphatase-1 as well as the inactivation of upstream kinases, such as glycogen synthase kinase 3, among others (141). To synthesize glycogen, glucose-6-phosphate must be converted to glucose-1-phosphate by phosphoglucomatase. Glucose-1-phosphate then reacts with uridine triphosphate to form UDP-glucose, the immediate precursor to glycogen. Lastly glycogen synthase adds glucose units from UDP-glucose to the non-reducing end of an existing glycogen molecule, releasing UDP in the process (196).

Glycogenolysis is the cleavage of glucose units from the glycogen molecule through hydrolysis. The glycogen phosphorylase enzyme catalyzes the cleavage of the terminal glycosidic linkages of glycogen, releasing glucose-1-phosphate. Glycogenolysis is regulated by the activity of phosphorylase. In muscle, phosphorylase activity is increased by calcium and epinephrine; it can also be regulated by the concentrations of glycogen and inorganic phosphate or by allosteric effectors (ATP, ADP, AMP, IMP) (196). In liver, phosphorylase is primarily regulated by glucagon (activating) and insulin (inactivating). Glucose-1-phosphate is converted into glucose-6-phosphate by phosphoglucomutase (196). Muscle does not express glucose-6-phosphatase, so it cannot release glucose back into the bloodstream. Therefore the glucose-6-phosphate released from muscle glycogen is primarily directed towards glycolysis and used to meet the energy demands of the

muscle cell. In contrast, the majority of the glucose liberated from liver glycogen enters the circulation to maintain blood glucose levels. This is made possible by the expression of glucose-6-phosphatase by the liver, which dephosphorylates glucose-6-phosphate into free glucose, which can be released into the bloodstream (203).

In addition to its ability to release glucose liberated from glycogen, the liver can also synthesize glucose from noncarbohydrate precursors through gluconeogenesis. Essentially, gluconeogenesis is the reversal of the glycolytic pathway, with alternate reactions working around the enzymatically irreversible steps of glycolysis. When lactate is the precursor, it is taken up and oxidized into pyruvate. Next, pyruvate is converted to oxaloacetate via pyruvate carboxylase (many amino acids, when deaminated, can also be transformed into oxaloacetate). Next, phosphoenolpyruvate carboxykinase (Pepck) catalyzes the reaction converting oxaloacetate to phosphoenolpyruvate. The next several steps reversing the glycolytic pathway are reversible, so glycolysis can run backward until fructose-1,6-bisphosphate is formed. Here, fructose-1,6-bisphosphatase removes a phosphate group, producing fructose-6-phosphate, which is reversibly converted to glucose-6-phosphate. Glucose-6-phosphatase removes the phosphate group, resulting in free glucose. The rate of gluconeogenesis is primarily determined by the activities of Pepck, fructose-1-6, bisphosphatase, and glucose-6-phosphatase (G6P). Pepck catalyzes one of the rate-limiting steps of gluconeogenesis and G6P catalyzes the final step, converting glucose-6-phosphate to free glucose. Glycerol is another precursor for glycolysis; it is phosphorylated by glycerol kinase to glycerol-3-P, which is oxidized to produce dihydroxyacetone phosphate by glycerol 3-P

dehydrogenase. These genes are also controlled at the transcriptional level by hormones; transcription of *G6pc* and *Pepck* is stimulated by glucocorticoids and glucagon (released during fasting) and repressed by insulin (released after carbohydrate feeding) (95).

Lipid Metabolism

Excess lipid is predominantly stored in adipocytes (fat cells) in white adipose tissue, though small amounts of lipid can be found in many other tissues. The primary metabolic functions of white adipose tissue are to store fatty acids (in the form of triacylglycerols, abbreviated TAG) and to mobilize those fatty acids (along with glycerol) in response to energetic demands. Most of an adipocyte's volume is comprised of TAG within a lipid droplet. These lipid droplets consist of a neutral lipid core surrounded by a phospholipid monolayer. Perilipin proteins, residing within this monolayer, promote TAG storage by protecting the TAG from lipases (25).

Fatty acids are either taken up from the bloodstream or produced *de novo*. Adipose tissue, liver, and skeletal muscle can all synthesize fatty acids from non-lipid precursors, though of these tissues only the liver contributes substantially. Long-term regulation of fatty acid and triglyceride synthesis in the liver is controlled transcriptionally through a number of genes encoding proteins collectively termed "lipogenic enzymes." These enzymes are upregulated with carbohydrate feeding, either by insulin or glucose (163), and lead to the conversion of excess carbohydrate into triglycerides. The primary source of TAG storage in adipose tissue is from the uptake of circulating fatty acids from circulating TAG contained in chylomicrons or

very-low-density lipoproteins. Lipoprotein lipase (produced by the adipocytes) liberates the fatty acids from these TAG molecules. Once fatty acids are liberated and enter the adipocyte, they are attached to coenzyme A via acyl CoA synthetase. In skeletal muscle, circulating fatty acids are taken up by a combination of passive diffusion and protein-mediated transport, where they can be oxidized or stored as TAG or other lipid types. The fate of fatty acids in skeletal muscle is determined by a variety of factors including the muscle fiber type, hormonal milieu, and the current muscle energy requirements (210). Regardless of the tissue, TAG is synthesized by the sequential addition of fatty acids to a glycerol backbone. In the remodeling pathway, two fatty acids are added to a monoacylglycerol by the monoglycerol acyltransferase and diacylglycerol transferase (DGAT) enzymes, respectively. In *de novo* lipogenesis, glycerol-3-phosphate is sequentially acylated by glycerol phosphate acyltransferase and lysophosphatidic acid acyltransferase and then dephosphorylated by phosphatidic acid phosphatase, resulting in diacylglycerol. The diacylglycerol produced by the *de novo* is also converted to TAG by DGAT, making DGAT the common link between these two TAG formation pathways (183). Two isoforms of DGAT exist, DGAT1 and DGAT2. DGAT2 is more critical to TAG synthesis based on knockout studies, where the absence of DGAT1 led to reduced adiposity while the absence of DGAT2 led to lethal lipopenia (183). DGAT2 overexpression in skeletal muscle led to higher TAG content in young adult mice (123); similarly, DGAT2 overexpression in liver led to increased liver TAG content and steatosis (145).

Lipolysis occurs in response to increases in energy demand. TAG stored in adipose tissue is broken down into fatty acids and glycerol and primarily released into

the bloodstream, whereas in the muscle, the fatty acids liberated from TAG hydrolysis are oxidized to meet the energetic demands of the muscle cell. Regardless TAG must be broken down into fatty acids before it can be oxidized or released to blood stream. Three lipases remove the fatty acids: adipose tissue triglyceride lipase (ATGL), hormone-sensitive lipase (HSL) and monoacylglycerol lipase. ATGL specifically catalyzes the hydrolysis of TAG, resulting in diacylglycerol and a fatty acid. HSL can catalyze the hydrolysis of both TAG and diacylglcerol. ATGL and HSL combined are responsible for about 95% of TG hydrolysis in adipocytes (179). When ATGL associates with the protein CGI-58, the catalytic activity of ATGL is increased (87). HSL is activated via phosphorylation in response to epinephrine/norepinephrine, or calcium stimulation, allowing HSL to translocate to the surface of the lipid droplet. Phosphorylation events also prevent the perilipins coating the lipid droplet from preventing lipolysis. The final lipolytic step is catalyzed by monoacylglycerol lipase (196).

The proper function and regulation of these basic metabolite processes are critical for the maintenance of metabolic health in humans as alterations in metabolic function are associated with the development of metabolic disease. Over the last 25 years, evidence supporting a developmental origin of adult metabolic dysfunction has accumulated; the following section will review this literature.

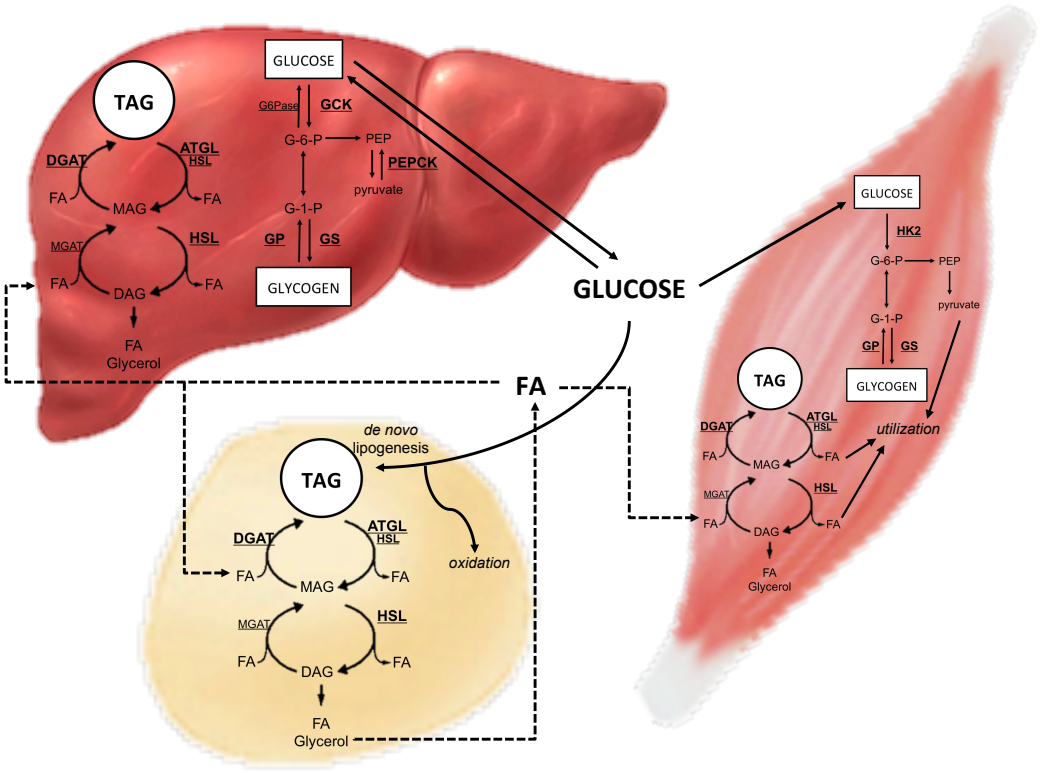

Figure 2.1. Overview of glycogen and triacylglycerol storage and breakdown in skeletal muscle, liver, and adipose tissue. This figure is focused on the metabolic processes relative to this dissertation work and is not intended to provide a comprehensive overview of metabolic processes in these tissues. TAG: triacylglycerol, DAG: diacylglycerol, MAG: monoacycl glycerol, FA: fatty acid, DGAT: diacyclglyceroltransferase, MGAT: monoacylglycerol transferase, ATGL: adipose triglyceride lipase, HSL: hormone-sensitive lipase, G-6-P: glucose-6-phosphate, G-1-P: glucose-1-phosphate, G6Pase: glucose-6-phosphatase, GCK: glucokinase, PEP: phosphoenolpyruvate, PEPCK: phosoenolpyruvate carboxykinase, GP: glycogen phosphorylase, GS: glycogen synthase, HK2: hexokinase 2

Developmental Programming

In the late 1980s David Barker and his colleagues (11) observed a correlation between low birth weight and cardiovascular mortality; they subsequently observed a similar correlation of low birth weight with poor glucose tolerance and insulin resistance (93) in adult men. These studies gave rise to Barker's "thrifty phenotype" hypothesis (94), which proposes exposure to undernutrition during fetal or early life

alters fundamental metabolic processes controlling substrate metabolism. While these alterations may be advantageous in times of low nutrient availability, in times of adequate or excess nutrient availability these changes become deleterious and result in disease states such as obesity and impaired glucose tolerance. The "thrifty phenotype" hypothesis gave way to the paradigm now known as the Developmental Origins of Health and Disease hypothesis, which has become a scientific field in its own right.

Some of the most substantial support for these hypotheses has come from a series of studies on the long-term health of individuals who were exposed during fetal or early life to the Dutch Famine of 1944-45 (192). From October, 1944 to through May, 1945 a portion of the Netherlands with a population of 4.3 million people was blocked from food shipment supplies. As a result, food rations quickly fell from 1600 kilocalories per day to below 1,000 and as low as 500 kilocalories per day (202). Prenatal famine exposure was associated with increased body weight, BMI, and waist circumference (167, 191) as well as a higher prevalence of impaired glucose tolerance (57). Since the publication of these findings, many other studies of adult health outcomes related to prenatal or early exposure to other temporary famines have been conducted (202), early life nutrition and adult health and development) with similar findings.

Experimental Models of Developmental Programming

Several models of maternal undernutrition have been developed to experimentally test the observations from epidemiological studies like the Dutch

Hunger Winter studies; these include maternal caloric restriction, macro- and micro-nutrient restriction, and uterine artery ligation. More recently, models of maternal overnutrition and obesity have been investigated. Interestingly, both maternal under- and over-nutrition yield consistent offspring phenotypes, including obesity, perturbed glucose homeostasis, and insulin resistance (118). Greater adiposity, for example, has been observed in the offspring of animals exposed to maternal protein restriction, high fat diet, and obesity (15, 17, 150). Similarly, impaired offspring glucose tolerance has been observed in the offspring of both protein-restricted (151) and high fat diet-fed rodents (182). Higher serum insulin levels have also been observed in the offspring of both protein-restricted (136) and high fat diet fed dams (8, 18, 182).

Animal models of developmental programming have also demonstrated effects of a variety of maternal environments on the function of basic metabolite storage systems (TAG and glycogen storage) in skeletal muscle, adipose tissue, and liver. Maternal consumption of a high fat hypercaloric cafeteria diet "composed of pâté, cheese, bacon, potato chips, biscuits and chocolate" was associated with greater adipose tissue mass and elevated serum and adipose tissue TAG content in offspring (18). Elevated serum TAG content has also been observed in the offspring of protein-restricted (136) and stressed (27) dams. Conversely, another study of maternal protein restriction in rats found exposed offspring were lighter and had less visceral and total fat along with lower circulating insulin, glucose, triglycerides and cholesterol (72). Higher offspring liver TAG content has been observed in a number of models of maternal dietary programming, including maternal caloric restriction in sheep (81, 104) and rats (135), as well as maternal high-fat feeding (8). Both maternal caloric

restriction (216) and overfeeding (211) in sheep led to greater accumulation of intramuscular triglyceride in offspring. Higher liver glycogen has also been detected in offspring exposed to maternal caloric restriction (81), protein restriction (72, 86), and stress (77). Offspring muscle glycogen was also elevated in female offspring in response to maternal protein restriction (215).

While most of the available research on developmental programming has focused on the effects of maternal interventions on offspring health, the effects of the paternal environment have also been investigated. Pre-mating fasting of males resulted in lower offspring serum glucose concentration compared to the offspring of males who were not fasted prior to mating (7). Male rats that were chronically exposed to a high fat diet had female offspring with impaired insulin secretion and glucose tolerance compared to the offspring of control fathers (148). Protein-restriction has also been studied in male rats (31); transcriptional profiling demonstrated elevated expression of lipid and cholesterol biosynthesis-related genes in the livers of offspring whose fathers were protein-restricted. Further analysis of hepatic lipid content indicated cholesterol depletion along with higher levels of free fatty acids and triglycerides in offspring of protein-restricted fathers.

Effects of Parental Exercise on Offspring Health

Parental physical exercise represents another environmental stimulus that could, like parental nutrition, program offspring metabolic health. Exercise affects many organ systems throughout the body, including the same metabolic pathways that respond to nutritional interventions, but little is known about the effects of

maternal or paternal exercise on the developing fetus. Here we review the existing literature related to the influence of parental exercise on offspring health.

Parental Exercise and Offspring Outcomes in Humans

Most of the available research on parental exercise in humans is related to maternal exercise and/or fitness levels. Further, these studies are thus far limited to neonatal and childhood outcomes. Women who exercised before and during pregnancy had infants with lower (but still normal) birth weights compared to women who did not exercise; these lower birth weights were associated with lower infant fat mass with no difference in lean mass (38, 44). In one study, women who started exercising during pregnancy had infants with higher birth weight as a result of increased lean mass with no change in fat mass (40); these observations were associated with greater placenta growth and volume. Conversely, another study of women who began exercising in mid-gestation found lower birth weights, but no differences in body composition in the offspring of exercising women (100); lower cord blood concentrations of insulin-like growth factors I and II were also observed in the offspring of exercising mothers. Cessation of exercise training during pregnancy is associated with higher infant birth weights due to greater infant fat mass compared to infants of mothers who were sedentary throughout their pregnancy (42). Several other studies of exercise during pregnancy have yielded no effects on birth weight (16, 46, 48, 112).

At one year of age, the offspring of exercising mothers (who had lower birth weight and fat mass) were comparable in weight and body composition to the

offspring of less active mothers (43). Interestingly, at five years of age, the children of mothers who exercised weighed less and had less fat mass than the children of less active mothers (45). It is important to note that these two follow-up studies were performed on different birth cohorts. While both cohorts were lighter and leaner at birth, the absence of a persistent effect in the cohort followed up on at one year of age could be explained by the less intensive exercise program undergone during exercise in the mothers of these children, suggesting maternal exercise intensity and volume may be important to long-term offspring health outcomes. A recent prospective cohort study of 4665 maternal-offspring pairs studied the association of self-reported maternal physical activity in mid-gestation with cardiovascular risk factors in offspring at 15 years of age (143). Higher levels of maternal physical activity were associated with lower BMI, waist circumference, glucose, and insulin prior to adjusting for confounders, but all associations were null after adjustment. Similarly, another smaller (439 offspring) prospective study found no protective associations of maternal physical activity on markers of metabolic syndrome; in fact, subtle adverse associations of higher levels of physical activity with body mass index, HDL cholesterol, and diastolic blood pressure were detected (52).

Beyond morphological traits, infants born to exercising mothers performed better on orientation and state regulation at five days after birth compared to infants born to less active mothers, suggesting improved neurological development in the offspring of exercising women (42). At one year of age, neurodevelopmental outcomes were did not differ between the children of exercising and sedentary mothers (43); however, at five years of age the children of mothers who exercised

during pregnancy performed better on oral language skill and intelligence tests (45). It is important to consider when interpreting these results that some more recent large epidemiological studies have suggested that the independent effect of exercise during pregnancy is minor and may be largely explained by the confounding effects of maternal body composition (74, 108).

Parental Exercise and Offspring Outcomes in Animal Models

The use of animal models to study the effects of parental exercise on offspring health is critical, as animal models allow for more careful control of experimental variables and offer more flexibility in the tissues to be studied. Further, the short gestation and lifespan of rodents in particular allows researchers to study the more long-term effects of parental exercise on offspring health.

Maternal exercise in rodents has been associated with smaller litters (199) and birth weights (60, 102, 162) in some studies, but these findings are not consistent (33, 34, 146, 171). In these studies, higher exercise intensities were more often associated with lower offspring birth weights.

Much of the available literature regarding maternal exercise and offspring outcomes has focused on neurological phenotypes. Briefly, maternal swimming during gestation was associated with greater brain-derived neurotrophic factor expression, more hippocampal neurogenesis, and better short-term memory (120). Offspring of dams exposed to treadmill exercise before and during pregnancy performed better in an open field anxiety test compared to the offspring of control dams; this was associated with greater prefrontal cortex expression of brain-derived

neurotrophic factor and vascular endothelial growth factor expression (2). Better spatial memory has also been observed in the offspring of exercised compared to control dams, an effect that was associated with greater hippocampal neurogenesis (54).

More recently, researchers have begun to study the physiological effects of maternal exercise. Vega et al. (205) observed lower glucose values in the 36-day old offspring of rats exposed to controlled wheel running. In this study, dams exercised on a wheel for two 15-minute sessions per day for 30 days prior to breeding and for one 15-minute session per day during gestation. No differences in fat pad mass, serum triglycerides, insulin, or leptin were detected in the offspring.

Carter et al. (33, 34) were the first to study the effect of maternal exercise on the metabolic health of mature rodent offspring; they observed improved mature offspring insulin sensitivity and glucose regulation in both mouse and rat models of maternal exercise. In the mouse model, dams were exposed to voluntary wheel running exercise one week before and during pregnancy and through two weeks of lactation (33). The offspring of exercised dams had better glucose tolerance from 7 to 16 months of age (when the study was terminated) compared to the offspring of sedentary dams. Further, maternal exercise was associated with enhanced *in vivo* and *ex vivo* insulin-stimulated glucose uptake into adipose and skeletal muscle compared to the offspring of control dams, indicating better insulin sensitivity. Lastly, male offspring from exercised dams had lower fat and higher lean body mass by nine months of age compared to male offspring of sedentary dams. In a study using the same intervention model in rats, glucose disposal was enhanced in female offspring

of exercised dams by 10 months of age; by 17 months of age, female offspring of exercise dams had enhanced insulin sensitivity as measured by hyperinsulinemic euglycemic clamp (34). The researchers also noted lower fasting insulin and fat pad mass, but no difference in glucose tolerance in the exercising F0 females mid-gestation.

Another series of recent studies has investigated the effect of maternal bipedal stance exercise in rats; rats are required to "stand" on their hindlimbs in order to reach their food and must maintain that position to eat or drink (212). Fetuses exposed to this type of maternal exercise were heavier, longer, and had greater placental mass compared to fetuses from control rats (172) but did not differ in body mass at birth or weaning (171), or through adulthood (170). However, in stark contrast to the findings of Carter (33), the mature male offspring of exercised rats had higher body fat and less lean mass compared to the offspring of control rats (170). Significantly higher serum undercarboxylated osteocalcin (uOC) concentrations were also observed in the male offspring of exercised dams. Interestingly, uOC may regulate metabolism; uOC injections reduced glucose and improved insulin sensitivity in normal mice (105). No effects of maternal exercise on offspring food intake or efficiency, blood glucose, or grip strength were detected in this model (170), but this study highlights a potential endocrine role of bone in mediating the metabolic effects of parental exercise on offspring.

Proposed Mechanisms of Developmental Programming

Developmental origins researchers have proposed numerous potential

mechanisms linking parental environment to offspring health outcomes. These include (but are not limited to) structural differences in organ development, epigenetic modifications, changes in glucocorticoid exposure, alterations in appetite regulation, and oxidative stress.

Structural changes to organs

Tissue and organ structure is, by necessity, very plastic during development. Any alterations in offspring environment that disrupt the processes of cell proliferation and/or differentiation could have permanent effects on tissue size or the relative content of different cell types within tissues. Maternal protein restriction in rats reduces pancreatic mass in offspring along with islet cell mass and the relative concentration of β-cells (the insulin-producing cells of the pancreas) within islets (158, 188). In skeletal muscle, maternal undernutrition alters the number of secondary muscle fibers formed during development (66) as well as fiber type composition (51, 73), and intramuscular fat content (51). The livers of rat offspring exposed to maternal protein restriction have fewer, but larger lobules (29). In adipose tissue, maternal undernutrition is associated with a greater proportion of large fat cells in offspring visceral fat (149).

Epigenetic Modifications

Epigenetics is the study of heritable changes in gene expression that are independent of changes in DNA sequence. DNA methylation and histone modification are the two primary types of epigenetic marks, though various

noncoding RNAs and prions are also included in some definitions. DNA methylation in promoter regions generally suppresses gene expression through directly blocking the access of transcriptional machinery or interfering with transcription factor binding. Conversely, histone acetylation generally promotes gene expression (20).

In animals, the majority of methylated DNA in the genome is demethylated following fertilization and then remethylated *de novo* in a developmental stage- and tissue-specific manner. Epigenetic marks are thought to be stably inherited, which allows marks to be passed from cell to cell during division and may even allow phenotypic traits induced by developmental programming to be passed on to subsequent offspring where germ cells are affected (187). Epigenetic modifications are particularly sensitive to environmental factors during development because this is the time when these marks are already undergoing modification (88); fully developed tissues, while still somewhat plastic, are likely more resistant to environmental stimuli. This mechanism could explain why the expression of genes and proteins remain altered in adult offspring, long after the environmental signal is withdrawn.

In humans, a 5% reduction in methylation of the insulin-like growth factor gene was observed in adults who were exposed to famine in early gestation when compared to their unexposed siblings (97). Similar differences in the methylation status of several other genes related to growth and metabolic disease have since been associated in adults prenatally exposed to famine (197). Godfrey et al. (85) correlated umbilical cord DNA methylation of the retinoid X-receptor-α gene with both maternal diet composition and childhood adiposity at 9 years of age. While not a

causative finding, this study implicates a potential role of perinatal epigenetic status on later health in humans.

In animals, Lillycrop et al. (126) observed, for the first time, that alterations in epigenetic regulation of specific genes in offspring could be induced by alterations in maternal nutrition. Specifically, differential methylation of the glucocorticoid receptor and peroxisome proliferator activated receptor α (*Pparα*) genes was observed in the livers of offspring exposed to maternal protein restriction. DNA methylation was lower while mRNA expression was dramatically higher in the offspring of protein-restricted dams for both the glucocorticoid receptor gene and *Pparα* (126). A subsequent study by the same group observed the opposite effect, with hypermethylation and lower expression of both *Pparα* and the glucocorticoid receptor in the livers of offspring of calorie-restricted mothers (84); these findings were associated with obesity and impaired glucose homeostasis in offspring (28). Similarly, paternal protein restriction substantially (30%) upregulated DNA methylation at a predicted enhancer of *Pparα* in offspring liver; this upregulation was associated with a downregulation of *Pparα* gene expression in the same tissue. Further, the expression levels of many lipid synthesis genes were upregulated in the same tissue, consistent with the role of *Pparα* in the regulation of lipid metabolism (31).

Epigenetic modifications could alter the expression of a wide range of gene targets. Even small, temporary changes in gene expression during development could have a large impact on the morphology of the tissues developing at the time of the

insult (e.g., more or less proliferation; alterations in genes promoting alternative lineages of progenitor cells).

Glucocorticoids

Another hypothesized mechanism by which developmental programming occurs is via glucocorticoids. 11β-hydroxysteroid dehydrogenase Type 2 (11βHSD2) is a placental enzyme responsible for maintaining the glucocorticoid gradient between mother and fetus (180). Lower placental mRNA expression and activity of 11βHSD2 has been observed in undernourished pregnancies (22, 116). Offspring of protein-restricted rodents have increased glucocorticoid receptor expression and higher activities of glucocorticoid sensitive enzymes (115). The resultant excessive glucocorticoid exposure results in persistent alterations in hypothalamic-pituitary-adrenal axis activity. This proposed mechanism is most closely related to alterations in offspring blood pressure (159). Glucocorticoids also help regulate tissue proliferation and differentiation during development (76), thus overexposure to glucocorticoids may also be linked to the alterations in tissue structure discussed above.

The hypothesized glucocorticoid mechanism has also been linked with epigenetics. Offspring of protein restricted rat dams had dramatically upregulated liver glucocorticoid receptor expression, which was associated with reduced binding of DNA methyltransferase to and hypomethylation of the promoter of GR110 (127). This suggests maternal undernutrition established an epigenetic mark promoting increased glucocorticoid receptor expression and thus glucocorticoid hypersensitivity.

Appetite Regulation

The hypothalamus is responsible for regulating appetite. In rodents, alterations in maternal nutrition induce changes in the hypothalamic circuitry as well as the expression and action of neuropeptides such as insulin, leptin, ghrelin, and neuropeptide Y, among others (142). Offspring exposed to maternal overnutrition *in utero* or during lactation had altered hypothalamic circuitry along with greater fat mass and glucose intolerance (63).

Oxidative Stress

Oxidative stress is an imbalance between reactive oxygen species production and antioxidant defense capacity. Rodent pups that were growth-restricted *in utero* have higher levels of oxidative stress and impaired mitochondrial function in the pancreatic β-cell, liver, and skeletal muscle compared to control pups (157, 181, 184). The authors propose the downstream effects of mitochondrial dysfunction across these tissues contributed to the development of Type 2 diabetes. Specifically, low liver mitochondrial function in liver was associated with suppressed pyruvate oxidation, enhancing hepatic gluconeogenesis and glucose output (157), while the chronic reduction of ATP production in muscle reduced the need for glucose transport into the muscle, further contributing to hyperglycemia (181).

Oxidative stress may further affect genomic DNA (37); telomeres, in particular, are sensitive to oxidative damage and increased oxidative damage at these regions can induce telomere shortening and accelerated aging (207). This hypothesis

is supported by the fact that many of the phenotypes programmed by prenatal or early-life environment are classically associated with aging (e.g., obesity, glucose intolerance, cardiovascular disease risk).

Sex-Specific Offspring Outcomes

Sexually dimorphic effects of developmental programming are evident across many models of developmental programming in both humans and animals (1); these effects have been observed for differences in body mass, body composition, and glucose tolerance in addition to many other phenotypes. The mechanisms responsible for sex-specific offspring effects are, at present, largely speculative. The placentas of female offspring may be more readily affected by prenatal insults (78, 137), though conversely, the adaptability of the female placenta may actually protect female offspring from environmental insult (190). Male and female offspring develop at different rates *in utero* as well as postnatally through sexual maturity, thus male and female offspring may be in a slightly different stage of development when exposed to an acute maternal influence. Male and female fetuses are also exposed to varying levels of sex-steroids *in utero* (13) and sex-steroid exposure can be further altered by intrauterine position in polytocous species such as mice (175). Lastly, epigenetic regulation varies between male and female embryos (70), which could result in altered epigenetic programming in offspring between sexes.

Exercise-Specific Potential Mechanisms

During pregnancy, skeletal muscle and adipose tissue become more insulin resistant (174), which increases maternal fat deposition and decreases maternal glucose uptake and utilization; these alterations maintain sufficient glucose availability for the developing fetus. Conversely, exercise decreases adipose tissue mass (195) and enhances insulin sensitivity in skeletal muscle (98). While greater insulin sensitivity is generally advantageous for health, elevated maternal insulin sensitivity during pregnancy has been associated with fetal growth restriction (35).

In a study of moderate-intensity (65% of predicated VO_2max) exercise training in pregnant women, however, neither maternal insulin sensitivity nor any other parameters of glucose regulation were affected despite a sufficient training stimulus evidenced by improvements in submaximal exercise performance (100). These findings suggest the physiological response of insulin sensitivity to pregnancy supersedes the physiological response to exercise training observed in nonpregnant individuals, prioritizing fetal growth. It is important to note that the absence of a chronic effect on insulin sensitivity does not exclude the possibility of exercise-induced acute alterations in maternal insulin action and any subsequent effect on fetal growth or metabolism.

In humans, chronic exercise increases placental growth, size, and blood flow in early pregnancy (40, 41, 44). While greater placenta size and blood flow could improve glucose and oxygen delivery to the fetus, acute exercise bouts may cause intermittent reductions in blood flow to the placenta in favor of directing blood to maternal muscle and skin (40, 173). Fetal glucose uptake, however, was not affected

despite dramatically reduced placental perfusion in response to acute maternal hypoxia (194). In addition to the effect of exercise on placental blood flow, however, single exercise bouts have been shown to temporarily reduce maternal circulating glucose and insulin (23, 39) and increase insulin sensitivity (213) following an exercise session in pregnant females. The combined effect of reduced placental blood flow along with lower maternal circulating glucose and insulin result in acute reductions in glucose delivery to the fetus; these reductions could impact the fetus, as maternal glucose levels signal the fetal pancreas to release insulin. Animal studies indicate that acute increases in glucose concentration stimulate fetal insulin secretion while chronic overexposure to glucose blunt glucose-stimulated fetal insulin secretion (36). Further, maternal glucose can modify fetal expression of glucose transporters; acute maternal hyperglycemia downregulated GLUT4 expression in fetal skeletal muscle and adipose tissue and was associated with insulin resistance in the offspring (3, 53). Intermittent reductions in nutrient delivery to the fetus may lead to lower average fetal insulin concentrations, which could downregulate fetoplacental growth (47). The lower expression levels of insulin-like growth factor I and II observed in cord blood from offspring of exercising women support this hypothesis (100).

In addition to the many metabolic effects of exercise training, exercise is also associated with benefits related to cognition, mood, and anxiety (134). Similar to the cognitive effects of exercise in the general population, women who exercise report lower anxiety and more stable moods compared to sedentary women (49, 164). Lower circulating corticosterone levels have also been observed in pregnant rats exposed to wheel running compared to sedentary rats (205). Given the established negative

effects of maternal stress on human and animal offspring (139), reduced maternal stress through maternal exercise may also play a role in improving offspring health outcomes.

Exercise can also influence breast milk composition. In humans, moderate to intense exercise training during lactation does not affect lipid or lactose concentrations, but does increase protein content of breast milk (61) and milk volume (130). In rats, swim exercise did not alter milk protein or lipid content but did lower lactose concentrations (200). The use of animal models to study the effects of maternal exercise on postnatal nutrition, specifically, is limited given the immature stage of development at which rodent pups are born and begin nursing relative to humans.

Multigenerational Inheritance of Offspring Phenotypes

Evidence from both human and animal studies supports the notion that the effects of developmental programming are not necessarily limited to the directly exposed offspring, but that they can be transmitted to subsequent generations. A birth cohort study from Sweden observed a link between paternal grandparents' food availability during their slow growth study and their grandchildren's subsequent longevity and risk of cardiovascular and metabolic disease (109, 110). Paternal grandfathers' food supply was linked with their grandsons' longevity and paternal grandmothers' food supply was linked with their granddaughters's longevity (155). Additionally, adult females who were exposed to the Dutch Hunger Winter *in utero* bore offspring who were more than twice as likely to develop cardiometabolic disease than the offspring of unexposed controls (152). In animal models, F0 maternal

undernutrition led to insulin insensitivity and glucose intolerance in F1 and F2 offspring (107, 138, 214). Maternal overnutrition in the F0 generation also influenced insulin sensitivity in both F1 and F2 offspring (68). Only one study beyond those comprising this dissertation has examined the effect of maternal exercise over multiple generations of offspring. Pinto & Shetty (162) found maternal swimming led to low-birth-weight pups in F1 and F2 offspring; this observation was independent of whether the F1 dams were also exposed to exercise.

Three potential mechanisms have been postulated to explain multigenerational observations (64). First, the phenotype may be the result of an environmental stimulus that persists for multiple generations, programming the same phenotypes in each successive generation. An example of a persistent phenotype could be the chronic food insecurity or stress in some socioeconomic populations. Such a pattern appears heritable, but may be largely developmental within each generation of offspring (114). Unsurprisingly, rodent models of persistent maternal overnutrition (F0 and F1 mothers fed the same high fat diet) produce F2 offspring that are particularly susceptible to obesity and hepatic steatosis (124).

The second proposed mechanism for multigenerational effects is that a single exposure in the F0 generation produces a multigenerational phenotype where the programming of the F1 fetus affects the adult F1 female's physiology in such a way that the intrauterine environment she provides programs the F2 fetus. This process can lead to a similar phenotype with a potentially different cause. For example, F1 offspring of a protein-restricted F0 dam were insulin sensitive but lacked sufficient insulin secretory capacity, resulting in a glucose intolerant phenotype (50). The

offspring of these F1 females were insulin-resistant and hyperinsulinemic, though the resultant phenotype of glucose intolerance was the same (138). These findings suggest unique developmental programming events in glucose/insulin metabolism in each generation. It is also important to remember that the primordial germ cells that will eventually give rise to the F2 generation are simultaneously exposed to any environmental insult to which the F0 female is exposed, so a direct effect of the F0 environment on the F2 offspring cannot be ruled out.

According to Skinner (185), information about the parental exposure must be transmitted through the germline for true "transgenerational" inheritance. A transgenerational effect, therefore cannot be observed until F3 and beyond (though one may be present in earlier generations, particularly when only the father is exposed). It is also important to note that not all F3 effects are necessarily due to germline transmission, however; an F2 offspring expressing a disease phenotype as discussed in the previous mechanism above may influence her offspring outside of a germline effect. Until recently, developmental biologists believed that all epigenetic marks were completely reset in primordial germ cells and then reestablished during development, clouding the potential for an epigenetic mechanism of transgenerational inheritance; however, new research indicates that some (rare) DNA methylation marks escape the resetting process (92). Further, it is now known that approximately one percent of histone marks (71) as well as a number of RNA species are retained in differentiated mouse sperm (6). Together these retained features allow for the transmission of paternal epigenetic information to offspring DNA.

In rats, F0 maternal protein restriction led to lower insulin secretory capacity in F1 offspring and insulin resistance in F2 offspring as well as in F3 males, though the severity of insulin resistance was diminished in the F3 generation (19). In another rodent study, a high fat F0 maternal diet resulted in increased body length and insulin resistance through the F1 and F2 generations of both maternal and paternal lines. While the F3 offspring were no longer insulin resistant, paternal line females still inherited greater body length (69). It is important to note, however, that other animal studies, despite demonstrating an F2 effect, have not found evidence of transmission to F3 offspring (65, 96).

Summary

It is clear from the existing literature that alterations in parental environment can induce substantial effects on offspring metabolic health outcomes, though the specific mechanism(s) responsible for transmitting information about the parental environment into offspring phenotypes remain unclear. The influence of parental exercise as an environmental stimulus affecting offspring health is understudied relative to the wealth of published literature on the influence of parental nutritional interventions. Further, those studies that have examined the effect of parental exercise on offspring metabolic health lack support for the potential underlying mechanisms responsible for their observed alterations in metabolic phenotypes. The studies comprising this dissertation will further explore the influence of parental exercise on offspring metabolic health while investigating a potential role of altered basal metabolic gene expression in the development of offspring phenotypes.

Chapter 3: Sex-Specific Effects of Exercise Ancestry on Metabolic, Morphological, and Gene Expression Phenotypes in Multiple Generations of Mouse Offspring

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Title: Sex-Specific Effects of Exercise Ancestry on Metabolic, Morphological, and Gene Expression Phenotypes in Multiple Generations of Mouse Offspring

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Abstract

Early life and pre-conception environmental stimuli can affect adult health-related phenotypes. Exercise training is an environmental stimulus affecting many systems throughout the body and appears to alter offspring phenotypes. The aim of this study was to examine the influence of parental exercise training, or "exercise ancestry," on morphological and metabolic phenotypes in two generations of mouse offspring. F0 C57BL/6 mice were exposed to voluntary exercise or sedentary lifestyle and bred with like-exposed mates to produce an F1 generation. F1 mice of both ancestries were sedentary and sacrificed at 8 wk or bred with littermates to produce an F2 generation, which was also sedentary and sacrificed at 8 wk. Small, but broad generation- and sex-specific effects of exercise ancestry were observed for body mass, fat and muscle mass, serum insulin, glucose tolerance, and muscle gene expression. F1 EX females were lighter than F1 SED females, and had lower absolute tibialis anterior and omental fat masses. Serum insulin was higher in F1 EX females compared to F1 SED females. F2 EX females had impaired glucose tolerance compared to F2 SED females. Analysis of skeletal muscle mRNA levels revealed several generation- and sex-specific differences in mRNA levels for multiple genes, especially those related to metabolic genes (e.g., F1 EX males had lower mRNA levels of *Hk2*, *Ppard*, *Ppargc1α*, *Adipoq*, and *Scd1* than F1 SED males). These results provide preliminary evidence that parental exercise training can influence health-related phenotypes in mouse offspring.

Introduction

The beneficial effects of exercise training on metabolic health are well characterized. Among other outcomes, exercise training leads to increased mitochondrial oxidative capacity, which may be protective against a variety of chronic diseases (201). In addition, exercise training improves glucose homeostasis and fat oxidation (75, 101). In contrast, lack of exercise (or sedentary lifestyle) is associated with chronic disease development and all-cause mortality (144).

Mounting evidence suggests early life (even pre-conception) events can affect adult health-related phenotypes, such as disease risk; this is referred to as the developmental origins of health and disease hypothesis (83). Maternal protein restriction results in offspring with lower birth weights and adult metabolic dysfunction (154). Excess maternal caloric intake also affects offspring health, leading to excess adiposity (15) as well as reduced muscle force production (14) in exposed offspring compared to mothers consuming a normal diet. The existing literature is primarily focused on maternal factors, however there is emerging evidence that dietary and other environmental factors can also influence offspring health through the paternal line (148).

Exercise training is an environmental stimulus affecting many systems throughout the body, and it may be capable of inducing transgenerational modifications similar to these more commonly studied nutritional interventions. The clinical literature concerning the effect of maternal exercise on maternal, fetal, and child health has been reviewed elsewhere (111). In summary, exercise during pregnancy appears to have beneficial effects in the mother and fetus. Child health

outcomes are less clear, with studies reporting greater, lower, or no difference in body weight between neonates whose mothers did or did not exercise during pregnancy (111). In those studies that observed decreased body weight, the difference appeared to be due to a lower percent body fat (38). The impact of maternal exercise on adult health outcomes or the impact on multiple generations has not been studied in humans. Likewise in rodents, varying effects of maternal exercise have been observed. Maternal treadmill running has been linked to smaller litters in some (199) though not all (146) studies, but has not been associated with fetal or offspring body mass or length (146, 199). Similarly, maternal swimming has been associated with lower birth weight offspring in one (162) but not all studies (200). Two recent studies documented beneficial adaptations in body composition and glucose and insulin dynamics in the mature offspring of dams who had access to a voluntary running wheel during the perinatal period (33, 34). To the best of our knowledge, only one study has examined the impact of maternal exercise over multiple generations of offspring; there, maternal swimming led to low-birth-weight pups in the first generation. This growth retardation was also observed in a second generation of rat pups born to the offspring of exercised dams, regardless of whether the first generation offspring were also exposed to exercise (162). Whether these observed changes in body size and/or composition are associated with metabolic health or function has not been elucidated. Additionally, the potential impact of paternal exercise has not been examined.

Thus, we sought to determine how body morphology, metabolic phenotypes and skeletal muscle gene expression are affected by exercise ancestry in multiple

generations of mouse offspring. Our aims were 1: determine body, fat, organ and skeletal muscle mass differences, 2: examine metabolic phenotypes such as glucose tolerance and circulating insulin and lipids, and 3: determine gene expression differences by skeletal muscle microarray and targeted gene expression analyses. We hypothesized that multiple generations of offspring from exercise ancestry would exhibit advantageous morphological, metabolic and gene expression phenotypes compared to offspring from a sedentary ancestry.

Methods

Ethical Approval

All animal procedures were performed in accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the University of Maryland (Appendix A).

Animal Procedures

We performed a breeding experiment including three generations of animals that were either exposed to exercise or kept sedentary in the F0 generation. An overview of our experimental design and timeline is provided in Figure 3.1. A standard diet (Purina Prolab RMH 3000, 60% carbohydrate, 14% fat, 26% protein) and water were provided *ad libitum* for all generations in all conditions. 20 male and 20 virgin female 5-wk old C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME); these mice made up the F0 generation. C57BL/6 is an inbred strain of mouse that was chosen based on their propensity to perform voluntary wheel

running, their common use in research studies of exercise, nutrition, metabolism, and gene expression, and their fecundity. Until 8 weeks of age, these animals were kept sedentary (i.e., unexposed to voluntary running wheel; standard cage conditions) and group-housed with same-sex mice.

At 8 weeks of age, each sex was randomly split into two condition groups: exercise (EX) and sedentary (SED) (n=10 per group). F0 animals were placed into standard cages (F0 SED) or in cages with computer-monitored voluntary running wheels (F0 EX). F0 mice were housed individually in their respective cages for 10 weeks. Following this intervention males and females from like conditions (EX or SED) were randomly paired for mating. The F0 EX breeding pairs had continued access to the running wheel throughout the breeding period, however running activity could not be monitored during the breeding period as there were two mice in each cage. Males were removed after pregnancy was visually confirmed by abdominal distention, vaginal plug and/or and body weight changes. After removal, F0 males had continued access to the running wheel (EX) or SED condition until sacrifice at 20 weeks (a total of 12 weeks of wheel access). F0 females had continued access to the running wheel (EX) or SED condition during pregnancy and lactation and were sacrificed at 25 weeks after the F1 mice were weaned (a total of 17 weeks of wheel access). Eight of ten breeding pairs in each condition produced viable litters. All of the F0 animals that underwent the intervention period (EX or SED condition) were included in the F0 analyses. The pups resulting from this original mating were designated as F1. A random group of 10 male and 10 female F1 pups from each condition (EX and SED) were designated as F1 breeders and were mated at 8 weeks

with F1 littermates to produce the F2 generation. Of the 10 breeding pairs per condition, 7 F1 EX and 9 F1 SED pairs produced viable litters. The remaining F1 pups were glucose tolerance tested and sacrificed at 8 weeks. F2 pups from each condition were glucose tolerance tested and sacrificed at 8 weeks. Body weight was monitored weekly for all animals. F1 and F2 offspring remained sedentary throughout the experiment. Following weaning at 21-25 days, all F1 and F2 offspring were group-housed with same-sex littermates. We used 1-7 offspring per litter for the 8-week analyses.

Intraperitoneal Glucose Tolerance Test (IPGTT)

Glucose tolerance tests were performed on all F0, F1, and F2 mice at 8 (F1 and F2 generations), 20 (F0 males), or 25 (F0 females) weeks. For F0 EX mice, the voluntary running wheels were locked 36 hours prior to the glucose tolerance test to limit the effect of acute exercise. All animals were fasted overnight (12 hr) prior to glucose tolerance testing. Baseline blood glucose measurements were made and then each mouse was injected intraperitoneally with 2.0 mg of D-glucose (Sigma-Aldrich, St. Louis, MO) per gram of body mass. Blood glucose was measured 30, 60, 90, and 120 minutes after injection in all animals; blood glucose was also measured 15 min after injection in F2 males and females. Area under the curve for concentration vs. time was calculated using the linear trapezoidal rule. Blood glucose measurements were made using a rodent-specific glucometer (AlphaTRAK; Abbott Laboratories, Abbott Park, IL) on blood removed from a tail snip. Following the glucose tolerance test, wheels were unlocked and animals were returned to *ad libitum* food and water.

Tissue & Serum Collection

Animals were euthanized 2-5 days following the glucose tolerance test. To limit the effects of acute exercise and feeding, running wheels were locked 24 hours and animals were fasted for 4 hours prior to euthanasia. Euthanasia was performed under isoflurance anesthesia; the method of euthanasia was exsanguination by cardiac puncture followed by removal of the heart. Heart, liver, omental fat, cerebellum, tibialis anterior (TA), extensor digitorum longus (EDL), soleus, plantaris, gastrocnemius, and quadriceps muscles were dissected, weighed, and flash frozen in liquid nitrogen and then stored at -80°C until analysis. Approximately 1 mL of blood was obtained from a cardiac puncture and allowed to coagulate. The coagulated blood was centrifuged at 1750 x g for 15 minutes to obtain serum. Serum was removed to a fresh tube and stored at -80°C until analysis.

Serum Measures

Serum triglyceride and glycerol were measured using the Serum Triglyceride Determination Kit (TR0100; Sigma-Aldrich, St. Louis, MO). Serum insulin was measured following a 4-hour fast using a mouse-specific ELISA kit (80-INSMS-E01, ALPCO Diagnostics, Salem, NH).

Tissue Preparation

The gastrocnemius muscle was chosen for analysis because it is a mixed-fiber type muscle (9) and therefore may be more representative of the average mouse skeletal muscle than a predominantly fast or slow muscle . Whole gastrocnemius

muscle was powdered in liquid nitrogen. Total RNA was isolated with Trizol reagent (15596-026, Life Technologies, Grand Island, NY), DNase-treated, and quantified with the NanoDrop (Bio-Rad, Hercules, CA) spectrophotometer. Reverse transcription was performed with 1μg of total RNA with the High-Capacity cDNA RT kit (4368813, Life Technologies, Grand Island, NY).

Microarray

Genome-wide analyses of gene expression were performed on subsets of F1 and F2 males. Total RNA was extracted from powdered gastrocnemius muscle using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Germantown, MD). All samples were diluted to 98 ng/μl and pooled by generation and condition (5 pooled samples per array). A total of 8 arrays were performed (2 arrays each for F1 EX males, F1 SED males, F2 EX males, and F2 SED males). Microarray experiments were performed as two-color experiments using GeneChip Mouse Exon 1.0 ST Array chips (Affymetrix, Santa Clara, CA). The microarray gene expression data were imported from the probe cell intensity CEL files and the Affymetrix Expression Console software was used to calculate the summary measure of the probe level data. These microarray data have been deposited in the NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) website and can be accessed through GEO Series accession number GSE40469.

Gene Expression

Real-time quantitative PCR was used to assess the gene expression level of adiponectin (*Adipoq)*, cell death-inducing DFFA-like effector c (*Cidec)*, and stearoyl-Coenzyme A desaturase 1 (*Scd1)* (n=10-20 per group). Primer and probe sequences were designed for each gene's mRNA sequence using PrimeTime qPCR Assay designer (IDT). *18S* rRNA was used as an expression control for both real-time and gel-based PCR and did not differ between treatment groups. RT-PCR was used to measure the expression of metabolic genes (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha, *Ppargc1a*; pyruvate dehydrogenase kinase, isozyme 4, *Pdk4*; peroxisome proliferator activated receptor alpha, delta, and gamma, *Ppara, Ppard*, and *Pparg*, aminolevulinate, delta-, synthase 1, *Alas1*; hexokinase 2, *Hk2*; cytochrome c, somatic, *Cycs*; citrate synthase, *Cs*; and cytochrome c oxidase subunit I, *Cox1*) (n=7-10 per group). Primer and probe sequences and PCR conditions are available upon request. Products were visualized on 1.5% agarose gels using ethidium bromide. qPCR data were normalized to *18S* using the -ΔCt method (176) and expressed as fold induction ($2^{-\Delta Ct}$) of mRNA expression compared to the corresponding EX group (1.0-fold induction). Relative band intensities from PCR gels were be analyzed with NIH ImageJ software and normalized to *18s*. Values are shown as means ± standard error.

Statistics

Two-tailed t-tests were used to compare body and tissue masses, serum measures, and IPGTT AUC between EX and SED groups within each sex and

generation. A repeated-measures ANOVA was used to compare the IPGTT response between EX and SED groups within each sex and generation. One- (F1 male RT-qPCR analysis) and two- (all other analyses) tailed t-tests were used to compare gene expression between EX and SED groups within each sex and generation using SPSS version 18. Statistical significance was accepted at $p < 0.05$. A 2-stage ANOVA procedure was carried out on the 4 arrays of the F1 generation and F2 generations, respectively. Details about this procedure can be found in Lee (121). Briefly, in the first-stage ANOVA, a one-way ANOVA model for EX vs. SED ancestry was fitted on the entire dataset, resulting in normalized estimates of gene expression centered by the ancestry factor. These normalized values were used in a second-stage ANOVA to identify which probe sets were significantly differentially expressed between EX and SED ancestries.

Results

During the pre-breeding intervention period, F0 EX males ran an average of 6558 ± 503 m x 24 hr^{-1} while F0 EX females ran a significantly greater ($p < 0.05$) average of 8378 ± 533 m x 24 hr^{-1}. Running behavior was only measured prior to mating, though the F0 EX mice had continued access to the voluntary running wheel throughout breeding, pregnancy and lactation. To examine the effects of parental exercise, we characterized the body mass, various tissue masses, glucose tolerance, and serum insulin, glycerol, and triglyceride levels in the original parent generation (F0) and F1 and F2 offspring from EX and SED ancestries.

F0 generation

Males: After the intervention period, F0 EX males weighed significantly less than F0 SED males ($p=0.024$; Table 3.1). F0 EX males also had significantly less omental fat mass compared to SED males ($p=0.003$; Table 3.1), a difference that was maintained after normalizing to body mass ($p=0.009$; Table 3.2). Additionally, when normalized to body mass, F0 EX males had greater EDL mass and lower omental fat mass compared to F0 SED males ($p=0.021$ and $p=0.001$, respectively; Table 3.2). There were no other differences in organ or tissue mass in F0 males. Baseline blood glucose and glucose tolerance was not affected by the EX intervention in F0 males (Figure 3.2A).

Females: F0 female body mass was not affected by the EX intervention, but EX females had significantly greater soleus mass and normalized soleus mass than SED females ($p=0.001$ for both; Tables 3.1 & 3.2). Baseline blood glucose concentration was not affected by the EX intervention in females. However, glucose tolerance tended to be better ($p=0.051$, 16.4%) in EX compared to SED females as indicated by AUC and EX females had significantly lower ($p=0.001$) blood glucose 60 minutes following the glucose injection (Figure 3.2B).

F1 generation

The F1 offspring sacrificed at 8 wk of age for analysis was comprised of 20 EX males, 17 SED males, 12 EX females, and 18 SED females. F1 litter size ranged from 3-9 (EX) and 4-9 (SED). The average litter size was not affected by EX (6.5 offspring/litter for F1 EX and 6.9 offspring/litter for F1 SED, $p>0.05$).

Males: There were no differences in body mass in F1 male offspring at 8 weeks. F1 males from EX parents had lower soleus, EDL, and quadriceps masses (all $p < 0.05$; Table 3.1) than F1 males from SED parents, though only EDL mass remained significantly lower in F1 EX males after normalizing to body mass ($p=0.015$, Table 3.2). When normalized to body mass, F1 EX males had greater heart mass compared to F1 SED males ($p=0.01$, Table 3.2). No differences were observed in baseline blood glucose concentration, glucose tolerance (Figure 3.2C) or serum insulin, free glycerol, and triglyceride (Table 3.3) between EX and SED F1 males.

Females: F1 female offspring from EX parents were significantly lighter (5%) at 8 weeks ($p=0.001$; Table 3.1) compared to offspring from SED parents. F1 females from EX parents also had lower TA and omental fat masses compared to F1 females from SED parents ($p < 0.05$; Table 3.1). After normalizing to body mass, the difference in TA mass was no longer significant, but omental fat mass remained lower in F1 EX females ($p=0.017$; Table 3.2). No differences in baseline blood glucose concentration or glucose tolerance were observed between EX and SED in F1 females (Figure 3.2D), but serum insulin was significantly higher (55%) in F1 EX females compared to SED females ($p=0.008$; Table 3.3). Serum free glycerol and total triglyceride concentration were not affected by EX ancestry in F1 females (Table 3.3).

F2 generation

The F2 generation was comprised of 18 EX males, 18 SED males, 14 EX females, and 23 SED females. F2 litter size ranged from 3-7 (EX) and from 1-7

(SED). The average litter size was not affected by EX ancestry (4.6 F2 offspring for both F2 EX and F2 SED, $p>0.05$).

Males: At 8 weeks, no differences in absolute or normalized body, organ, or muscle weights were observed between F2 male offspring with EX and SED ancestries (Tables 3.1 & 3.2). No differences in baseline blood glucose concentration or glucose tolerance were observed in F2 male offspring between EX and SED ancestries (Figure 3.2E). No differences in serum insulin, free glycerol or triglycerides were observed between EX and SED F2 male offspring (Table 3.3)

Females: There were no differences in body, organ, or muscle weights between F2 EX and SED female offspring (Table 3.1), however after normalizing to body mass, F2 EX females had lower soleus, EDL, and omental fat mass ($p=0.003$, 0.015, and 0.048, respectively; Table 3.2). Baseline blood glucose concentration did not vary between EX and SED F2 female offspring, but EX offspring exhibited slightly impaired glucose tolerance compared to SED offspring as indicated by a larger (14%) AUC ($P=0.015$; Figure 3.2F). Additionally, EX female offspring had significantly higher blood glucose concentration at both 60 and 120 minutes after the glucose injection ($p=0.049$ and <0.001, respectively). There were no differences in serum insulin, free glycerol, or triglyceride between EX and SED F2 offspring (Table 3.3).

Gene Expression Analyses

In order to broadly assess the impact of EX ancestry on muscle gene expression, we performed a genome-wide gene expression microarray on

gastrocnemius muscle of F1 and F2 males. In F1 males, 86 targets were upregulated and 23 targets were downregulated with EX ancestry (see Supplementary Figure 3.1, Supplementary Table 3.1). In F2 males, 142 targets were upregulated and 35 targets were downregulated with EX ancestry (see Supplementary Figure 3.2, Supplementary Table 3.2). Examination of the differentially regulated genes revealed three genes that have been linked to lipid metabolism: *Adipoq*, *Cidec*, and *Scd1* were all downregulated in F1 EX males. We used real-time qPCR analysis of these genes to validate the microarray findings and further examine the expression of these genes in our remaining groups.

Real-time qPCR analysis confirmed the lower gastrocnemius expression of *Adipoq* and *Scd1* in F1 EX males ($p < 0.05$, Figure 3.4C) and demonstrated a tendency for lower expression of *Cidec* ($p = 0.06$, Figure 3.4C). We also examined the expression level of these genes in F0 and F2 males and found lower expression of *Scd1* in F2 males with EX ancestry compared to F2 males of SED ancestry ($p=0.07$; Figure 3.4E), but no other differences. We also measured the gastrocnemius expression levels of these genes in all three generations of females. Interestingly, many of the patterns observed in the males were reversed in females. In F0 females, there was a tendency for higher expression of *Adipoq* and *Cidec* in the EX group ($p=0.06$-0.09; Figure 3.4B). *Adipoq* expression was significantly higher in F1 EX females and *Scd1* expression was significantly higher in F2 EX females (Figure 3.4F).

To further investigate processes that might contribute to some of the observed body and tissue mass and glucose metabolism differences, we also performed targeted gene expression analyses in gastrocnemius muscle. We examined a number of genes

that have been previously associated with acute exercise, exercise training responses or other metabolic health outcomes (122, 140). These included *Alas1*, *Cox1*, *Cycs*, *Cs*, *Hk2*, *Pdk4*, *Ppara*, *Ppard*, *Pparg*, and *Ppargc1a*. No significant differences in expression of any of these genes were observed in F0 males. In F0 females, *Pdk4* expression was significantly lower in exercised animals (Figure 3.3A). There was a tendency for *Pparg* expression to be higher in exercised females, but this difference did not reach statistical significance ($p=0.07$; Figure 3.3A). No expression differences were observed for any of these genes in F1 females. F1 males with EX ancestry had significantly lower expression levels of *Hk2*, *Ppard*, and *Ppargc1a* (all $p<0.05$; Figure 3.3B). Additionally, F1 males with EX ancestry tended to have lower expression levels of *Cs*, *Ppara*, and *Alas1*, though these differences did not reach statistical significance ($p=0.06$-0.09; Figure 3.3B). F2 males with EX ancestry had significantly higher expression levels of *Cycs* and significantly lower expression levels of *Cox1* and *Pparg* (all $p<0.05$; Figure 3.2C). F2 EX females had significantly lower expression levels of *Pparγ* and significantly higher expression levels of *Ppargc1a* (all $p<0.05$; Figure 3.2D). In addition, F2 females with EX ancestry tended to have higher expression levels of *Alas1*, *Hk2*, and *Ppard*, though these differences did not reach statistical significance ($p=0.06$-0.09; Figure 3.3D).

Discussion

In this study, we sought to determine the effect of EX ancestry on the morphological and metabolic phenotypes of two generations of offspring. This study investigated the influence of maternal and paternal EX ancestry on anatomical

characteristics and metabolic phenotypes, including skeletal muscle gene expression. Our results indicate broad effects of EX ancestry on various offspring phenotypes, including body mass, fat and muscle mass, fasting serum insulin, and glucose tolerance. Further, we observed effects of EX ancestry on the expression of several gastrocnemius muscle mRNAs through two generations of offspring.

Following the EX training period, F0 EX males were lighter with less omental fat mass and greater normalized muscle mass than SED males. F0 EX females had greater soleus mass than SED females but did not differ in fat or body mass. Other researchers have also observed lower body mass in male, but not female mice after a period of voluntary exercise (55), though this is not universal as some have observed decreased body mass in both sexes (193) or no differences in either sex (4). There were no differences in glucose tolerance in males following the exercise intervention and a tendency for improved glucose tolerance in exercised females. Though forced exercise can induce improvements in glucose tolerance in mice (106), no other studies that we are aware of have observed an effect of voluntary exercise on glucose tolerance in non-obese mice on standard chow. Our voluntary wheel running approach alleviates the induction of systemic handling stress seen with forced running or swimming, however it does not allow us to control exercise volume or intensity. We anticipated that voluntary running wheel would be sufficient to produce significant adaptations as measurable changes in muscle fiber type content have been observed in comparable studies in the gastrocnemius muscle in the same mouse strain as used here (4).

F1 females with EX ancestry were lighter than those with SED ancestry at 8 weeks of age; no other differences in offspring body mass were observed. Maternal exercise training has previously led to offspring with lower (162) or not different (33) body mass. Our study does not allow us to identify a specific mechanism leading to lower body mass in F1 EX females because F0 dams were exposed to exercise from pre-conception through gestation and lactation (e.g. epigenetic, in utereo, or lactational mechanisms). There does not appear to be a transgenerational effect (e.g., F2 offspring were unaffected) of parental exercise on body mass at 8 weeks of age in our study. Only one other study we are aware of has examined the effect of maternal exercise on offspring mass over multiple generations. Pinto and Shetty (162) found EX ancestry led to decreased body mass in both F1 and F2 offspring; however, the exercise intervention in that study was forced swimming, which is often questioned as a form of exercise in a rodent (10) and may have induced an additional maternal stress independent of the exercise stimulus. Maternal stress alone has induced both higher (178) and lower (153) offspring body mass in other studies.

Although we did not measure body composition, we examined individual tissue masses to identify potential differences in organ mass in the offspring. F1 males with EX parents had lower muscle masses than F1 males from SED parents, and F1 females from EX parents had lower TA and omental fat mass compared to F1 females from SED parents. Recent evidence suggests that the maternal environment can have significant effects on developmental partitioning of muscle, adipose tissue, and connective tissue precursors (67); however, we are unable to confirm if this would explain our differences. Interestingly, F1 EX males had lower muscle masses

compared to F1 SED males, however, there were no corresponding differences in omental fat pad mass. Carter *et al.* (33) observed lower fat mass and higher lean mass percentages in mature (39 wk) male, but not female, offspring of exercised dams, while in a similar study, no body composition differences were observed (34). Though these results are not directly comparable due to the difference in offspring age and method of body composition assessment, it is interesting that we observed a contrasting effect, with lower fat mass in only female offspring. In the F2 offspring, EX females had lower relative muscle and omental fat pad mass compared to F2 SED females. The differential effects of EX ancestry on offspring body and tissue mass between sexes and generations suggest the observed differences may be induced through sex- and/or generation-specific mechanisms.

Glucose tolerance was affected only in F2 female offspring where those with EX ancestry had slightly reduced glucose tolerance than those with SED ancestry. It is possible that the greater fasting insulin concentration observed in F1 EX females could have led to impaired glucose disposal and thus hyperglycemia during pregnancy if insulin resistance developed in the F1 EX females. Mild maternal hyperglycemia during pregnancy has been linked with reduced glucose tolerance in rat offspring (79, 80) Two recent studies have examined the influence of maternal exercise on glucose tolerance in healthy animals. In both, offspring from exercised dams had improved glucose tolerance as well as insulin sensitivity (33, 34). It is critical to note, however, that these improvements were not observed until offspring were 31-32 weeks (33) or 10 months of age (34). Together with our results this

indicates that while the positive effects of exercise training on glucose tolerance may be transmittable to offspring, they are not yet apparent at a young age.

The effects of EX and EX ancestry on gene expression were examined by a combination of global mRNA profiling and targeted gene expression analysis of gastrocnemius muscle. Our study is the first to examine the effect of maternal exercise or exercise ancestry on skeletal muscle gene expression. Our microarray approach revealed a number of mRNA transcripts were differentially expressed as a function of EX ancestry in both generations, with a greater number of transcripts affected in the F2 compared to the F1 generation. Surprisingly, there was no overlap in differentially expressed transcripts between F1 and F2 offspring, again highlighting the difference between *in utero* versus transgenerational effects. Three of the differentially regulated transcripts (*Adipoq*, *Cidec*, and *Scd1*) have previously been associated with lipid metabolism and specifically intramuscular lipogenesis. *Adipoq* codes for the adipokine adiponectin, that when secreted from adipocytes promotes fatty acid oxidation and enhances insulin sensitivity (198) in muscle. *Adipoq* mRNA is expressed in muscle (58, 128), but it is unclear whether the resultant protein has similar effects to circulating adiponectin. *Adipoq* mRNA in muscle is associated with greater intramuscular fat (208) and lipotoxicity (59), but also enhanced insulin sensitivity (128). *Cidec* codes for cell death-inducing DFFA-like effector c, which promotes apoptosis (125) and is upregulated in adipose tissue during adipogenesis (166). In muscle, *Cidec* mRNA expression is associated with de novo lipogenesis (209). *Scd1* codes for steroyl-CoA desaturase-1, which enzymatically regulates the formation of monounsaturated fatty acids within the cell (113). *Scd1* mRNA

expression in muscle is associated with the increased intramuscular triglyceride concentrations observed with both obesity (103) and exercise training (62). These lipogenic transcripts were downregulated in F1 EX male offspring, with a similar pattern observed in the F0 and F2 generations. In females, we found the opposite result, an overall tendency towards increased expression of *Adipoq*, *Cidec*, and *Scd1* in EX / EX ancestry females. Recalling that F1 EX females had less omental fat than F1 SED ancestry females while there were no differences in fat mass in male offspring, we speculate that EX ancestry females preferentially decreased adipose tissue lipid storage compared to SED ancestry females while EX ancestry males preferentially decreased ectopic (including muscle) lipid storage.

In addition to the targets identified by the microarray, we examined a number of *a priori* metabolic gene expression targets. Direct exposure to EX did not lead to differences in basal expression of the *a priori* metabolic genes in F0 males and only *Pdk4* expression was significantly lower in EX females. Although altered expression levels of the selected genes have been observed following exercise in previous studies (165, 177), the lack of expression differences in our study is not surprising because sacrifice occurred 24 hours after the last bout of wheel running and in humans, the exercise-induced increase in transcription of some metabolic genes is transient and expression returns to baseline by 24 hours post-exercise (160). In F1 offpring, EX males tended to have lower expression levels of *Cs*, *Ppara*, and *Alas1*. If these gene expression differences reflect a more extensive effect of EX ancestry on skeletal muscle gene expression then perhaps the morphological differences seen in the F1 EX males (smaller soleus, EDL, and quadriceps muscle mass) can be partially explained

by an impact of EX ancestry on skeletal muscle gene expression and tissue development. Interestingly, there were more significant effects of EX ancestry on metabolic gene expression in F2 offspring than F1 offspring. The magnitude of the difference between EX and SED ancestries, however, was smaller, which may explain why the differences in gene expression were not associated with gross morphological changes in the F2 generation. Alternatively, EX may uniquely affect skeletal muscle gene expression in the F2 offspring through the germ line whereas the gross morphological effects observed in the F1 generation may be related to a direct influence of exercise during the F1 *in utero* period.

Altered maternal nutrition and other stressors have been associated with epigenetic changes in a number of genes and tissues, however in many of these studies the associated physiological effects of these gene changes do not present until later in life or following a metabolic challenge such as a high fat diet (206). Similarly, the lack of overt physiological phenotype observed in the present study may be related to the young age (8 wk) of our animals and/or their exposure to only typical cage conditions and diet. The body composition differences between offspring of sedentary and exercised dams observed by Carter *et al.* (33) were observed at 39-40 weeks of age. Further, the authors note that the observed differences in glucose tolerance were not detectable until 7 months of age (33). Thus we believe future studies should investigate the effects of EX ancestry on metabolic phenotypes such as glucose tolerance in animals following aging or a metabolic challenge.

We observed differences in gene expression patterns between males and females in multiple offspring groups. For several genes, the expression pattern was

inverted between F1 males and F2 females, with *Alas1*, *Hk2*, *Ppard*, and *Ppargc1a* all lower in EX F1 male compared to SED while they were higher in F2 EX females compared to SED. Overall, EX ancestry led to primarily greater mRNA expression in female offspring and lower mRNA expression in male offspring. These patterns along with the body and tissue weight results demonstrate an apparent sex-specific effect of EX ancestry on offspring outcomes. Sex-specific environmental influences have been observed previously for offspring phenotypes in both humans (133, 191) and rodents (33, 68, 204). In humans, prenatal exposure to famine was associated with increased BMI (191) and blood lipids (133) in women, but not men. In mice, *in utero* protein restriction influenced the development of metabolic dysfunction in female, but not male offspring (204). Additionally, maternal high fat diet induced greater body length in both sexes of second-generation offspring, but higher Igf1 levels in females only (68). With regard to exercise, voluntary maternal wheel running in the perinatal period led to higher lean and lower fat mass percentages in mature male, but not female offspring (32, 33). One proposed mechanism for these sex-dependent observations is a difference in placental function between male and female fetuses. Chronic maternal stress in mice led to sex-specific differences in placenta gene expression where male placentas had greater expression levels of genes important to growth while female placentas were not affected (147). Several possible mechanisms could lead to this sex-specificity, such as an effect of the environmental stimulus on early development (gametogenesis or embryonic sexual differentiation). Alternately, the environment could interact with the offspring's own sex chromosomes or hormones later in development.

A critical difference between our study and the other studies of maternal exercise and offspring phenotypes is the inbred status of the mice used in our study. The rats used in the Pinto and Shetty (162) study and the mice and rats used in the Carter *et al.* (33, 34) studies were both from outbred lines, while we studied C57Bl/6 mice, an inbred strain. Though there are additional confounding factors, the effect of maternal exercise in outbred lines appears to be more dramatic than the effect of exercise ancestry observed in the present study. Interestingly, other environmentally-induced transgenerational effects on offspring health have also been shown to be dependent on the inbred vs. outbred status of the rodent line used for the experiment (89, 186). Guerrero-Bosagna et al. (89) found maternal vinclozolin treatment led to increased adult onset disease in an outbred, but not an inbred mouse model. This is the only study we are aware of to directly compare transgenerational phenotypes in an inbred and outbred mouse strain within the same study. The mechanisms contributing to this differential response between inbred and outbred lines of rodents have not yet been identified. However, we speculate that the stress of continued inbreeding may modify the epigenetic mechanisms responsible for manifestation of environmentally induced phenotypic changes, thus making the inbred organism less susceptible to epigenetic reprogramming of the germ line in response to environmental factors. Together these studies suggest a critical role of rodent strain when examining the role of the environment in modifying transgenerational phenotypes.

We would like to acknowledge some limitations to our study related to the breeding and pre-weaning methodology. The litter sizes reported reflect those offspring that were weaned from each breeding pair. We did not collect information

on litter size, survival or body mass prior to weaning. Additionally, we recognize that the variability in litter size and weaning time could have affected early offspring nutrition. Overnutrition induced by culling litters during lactation leads to neonatal overgrowth and can induce the development of obesity and glucose intolerance (156) as well as a number of other cardiometabolic risk factors (91). As noted previously, 1-7 offspring per litter were used for analysis in this study. The use of multiple offspring per litter was unavoidable due to sample size limitations. However, it is important to note that the use of multiple offspring per litter could confound the findings due to the "litter effect", where pups within litters are more similar to one another than pups between litters (99).

In summary, EX ancestry affects various offspring phenotypes across two generations, but in a generation- and sex-dependent manner. We have observed effects at both the whole-body (body and tissue mass and glucose tolerance) and skeletal muscle gene expression levels, which together reflect a broad impact of EX ancestry. This study is the first to examine the effect of exercise ancestry on a broad range of metabolic phenotypes, however this study is descriptive in nature and the specific mechanisms responsible for the observed phenotypes were not elucidated. Future studies should examine the potential influences of maternal vs. paternal exercise, and their cooperative effects. Similarly, additional work should isolate the EX exposure to discrete phases (i.e. pre-conception, gestation, lactation) to provide further insight into potential mechanisms.

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Disclosures

No disclosures or conflicts of interest.

Tables

Table 3.1. Mean body, organ, and muscle mass in exercise and sedentary ancestry groups for males and females of each generation (F0, F1, and F2).

	Males		Females	
F_0 generation	**EX (N=10)**	**SED (N=10)**	**EX (N=10)**	**SED (N=10)**
Body Mass (g)	29.1 ± 0.1	31.1 ± 0.7*	27.4 ± 0.7	26.7 ± 0.5
Heart (mg)	143.6 ± 4.0	145.7± 4.3	157.7 ± 9.2	155.6 ± 6.3
Cerebellum (mg)	51.9 ± 3.8	53.2 ± 2.7	64.5 ± 3.7	63.2 ± 3.3
Gastrocnemius (mg)	144.7 ± 3.6	140.6 ± 2.9	117.1 ± 3.4	111.1 ± 3.9
Soleus (mg)	11.6 ± 0.4	10.9 ±0.7	9.5 ± 0.3	7.1 ± 0.4*
EDL (mg)	16.6 ± 0.7	14.3 ± 0.9	7.5 ± 0.8	8.5 ± 0.4
Plantaris (mg)	21.5 ± 0.6	21.2 ± 1.1	16.2 ± 1.0	17.2 ± 1.8
TA (mg)	55.8 ± 1.8	55.3 ± 1.2	39.3 ± 1.2	39.2 ± 1.8
Quadriceps (mg)	216.8 ± 7.6	212.2 ± 3.5	124.6 ± 6.3	116.9 ± 36.2
Omental Fat (mg)	608.9 ± 34.7	929.8 ± 78.0*	387.1 ± 49.9	346.6 ± 44.1
Liver (mg)	1042.9 ± 34.8	1274.9 ± 52.5	1238.8 ± 33.1	1241.9 ± 76.1
F_1 generation	**EX (N=20)**	**SED (N=17)**	**EX (N=12)**	**SED (N=16)**
Body Mass (g)	22.3 ± 0.2	22.5 ± 0.3	17.5 ± 0.2	19.0 ± 0.3*
Heart (mg)	107.6 ± 1.8	103.3 ± 1.7	92.2 ± 1.4	96.0 ± 2.6
Cerebellum (mg)	51.4 ± 2.0	51.5 ± 2.9	51.0 ± 2.8	53.4 ± 1.7
Gastrocnemius (mg)	108.2 ± 1.8	107.8 ± 1.4	77.7 ± 2.4	82.5 ± 2.3
Soleus (mg)	6.6 ± 0.2	7.3 ± 0.3*	5.9 ± 0.5	6.0 ± 0.2
EDL (mg)	8.9 ± 0.4	12.4 ± 1.4*	10.7 ± 2.5	9.5 ± 1.0
Plantaris (mg)	14.3 ± 0.4	14.2 ± 0.05	10.3 ± 0.5	11.4 ± 0.5
TA (mg)	39.0 ± 0.8	41.2 ± 0.9	29.8 ± 0.5	33.0 ± 0.8*
Quadriceps (mg)	112.1 ± 6.8	135.4 ± 8.8*	98.3 ± 6.3	98.5 ± 6.2
Omental Fat (mg)	257.6 ± 8.3	274.9 ± 15.6	111.3 ± 7.6	154.7 ± 11.2*
Liver (mg)	965.5 ± 17.8	973.3 ± 32.3	770.0 ± 22.0	799.3 ± 24.6
F_2 generation	**EX (N=18)**	**SED (N=18)**	**EX (N=14)**	**SED (N=23)**
Body Mass (g)	22.1 ± 0.2	22.5 ± 0.2	18.6 ± 0.3	18.2 ± 0.2
Heart (mg)	119.2 ± 4.3	113.1 ± 2.4	103.6 ± 3.0	96.0 ± 2.4
Cerebellum (mg)	47.3 ± 2.4	54.0 ± 3.3	43.8 ± 2.8	49.4 ± 3.0
Gastrocnemius (mg)	92.1 ± 2.7	97.0 ± 3.3	71.8 ± 1.5	74.3 ± 1.3
Soleus (mg)	6.9 ± 0.3	7.1 ± 0.4	5.3 ± 0.2	5.7 ± 0.1
EDL (mg)	7.7 ± 0.3	7.9 ± 0.6	5.8 ± 0.2	5.5 ± 0.3
Plantaris (mg)	11.6 ± 0.6	12.4 ± 0.5	8.8 ± 0.3	9.3 ± 0.3
TA (mg)	36.9 ± 0.7	36.8 ± 15.7	28.7 ± 0.6	29.4 ± 0.8
Quadriceps (mg)	97.5 ± 27.2	101.4 ± 25.6	81.2 ± 2.7	75 ± 2.1
Omental Fat (mg)	253.3 ± 15.7	279.0 ± 8.9	129.7 ± 35.4	147.0 ± 61.7
Liver (mg)	866.6 ± 29.2	973.3 ± 45.8	736.3 ± 35.4	727.7 ± 21.2

Values are means ± SEM.
*significantly different from EX within sex and generation ($p < 0.05$)

Table 3.2. Organ and muscle masses relative to body mass in exercise and sedentary ancestry groups for males and females of each generation (F0, F1, and F2).

	Males		Females	
F_0 generation	**EX (N=10)**	**SED (N=10)**	**EX (N=10)**	**SED (N=10)**
Heart	4.94 ± 0.10	4.70 ± 0.15	5.76 ± 0.28	5.84 ± 0.25
Cerebellum	1.78 ± 0.13	1.71 ± 0.09	2.36 ± 0.13	2.48 ± 0.14
Gastrocnemius	4.98 ± 0.08	4.54 ± 0.12*	4.29 ± 0.11	4.16 ± 0.13
Soleus	0.40 ± 0.01	0.35 ± 0.03	0.35 ± 0.01	0.27 ± 0.01*
EDL	0.57 ± 0.03	0.47 ± 0.03*	0.27 ± 0.03	0.32 ± 0.02
Plantaris	0.74 ± 0.02	0.68 ± 0.03	0.59 ± 0.04	0.64 ± 0.05
TA	1.92 ± 0.07	1.78 ± 0.04	1.44 ± 0.04	1.46 ± 0.06
Quadriceps	7.46 ± 0.26	6.87 ± 0.24	4.58 ± 0.30	4.39 ± 0.16
Omental Fat	20.89 ± 1.06	29.60 ± 2.01*	14.04 ± 1.76	12.84 ± 1.50
Liver	45.50 ± 0.79	41.06 ± 1.58	45.64 ± 1.80	46.50 ± 2.75
F_1 generation	**EX (N=20)**	**SED (N=17)**	**EX (N=12)**	**SED (N=16)**
Heart	4.83 ± 0.07	4.58 ± 0.06*	5.28 ± 0.10	5.07 ± 0.10
Cerebellum	2.20 ± 0.15	2.28 ± 0.12	2.92 ± 0.16	2.95 ± 0.09
Gastrocnemius	4.86 ± 0.07	4.52 ± 0.29	4.44 ± 0.13	4.46 ± 0.05
Soleus	0.30 ± 0.01	0.33 ± 0.01	0.34 ± 0.03	0.32 ± 0.01
EDL	0.40 ± 0.02	0.55 ± 0.06*	0.61 ± 0.14	0.53 ± 0.06
Plantaris	0.64 ± 0.01	0.63 ± 0.02	0.59 ± 0.03	0.61 ± 0.02
TA	1.75 ± 0.03	1.83 ± 0.03	1.71 ± 0.03	1.76 ± 0.03
Quadriceps	5.05 ± 0.32	6.00 ± 0.37	5.66 ± 0.40	5.42 ± 0.32
Omental Fat	11.57 ± 0.35	12.12 ± 0.55	6.36 ± 0.42	8.29 ± 0.60*
Liver	43.44 ± 0.84	43.13 ± 1.16	43.97 ± 1.00	43.16 ± 0.95
F_2 generation	**EX (N=18)**	**SED (N=18)**	**EX (N=14)**	**SED (N=23)**
Heart	5.40 ± 0.19	5.04 ± 0.12	5.58 ± 0.14	5.28 ± 0.95
Cerebellum	2.15 ± 0.12	2.42 ± 0.16	2.35 ± 1.50	2.73 ± 1.74
Gastrocnemius	4.17 ± 0.11	4.10 ± 0.28	3.86 ± 0.06	4.09 ± 0.44*
Soleus	0.31 ± 0.01	0.32 ± 0.02	0.28 ± 0.01	0.32 ± 0.01*
EDL	0.35 ± 0.01	0.35 ± 0.03	0.32 ± 0.01	0.30 ± 0.02
Plantaris	0.53 ± 0.02	0.55 ± 0.02	0.47 ± 0.01	0.51 ± 0.02
TA	1.67 ± 0.03	1.65 ± 0.07	1.54 ± 0.03	1.62 ± 0.04
Quadriceps	4.42 ± 0.12	4.53 ± 0.13	4.38 ± 0.15	4.13 ± 0.10
Omental Fat	11.49 ± 0.68	12.42 ± 0.37	6.95 ± 0.47	8.10 ± 0.33*
Liver	39.33 ± 1.38	43.45 ± 1.99	39.60 ± 1.64	40.17 ± 1.23

Values are means ± SEM, all units are mg g^{-1} body mass.
*significantly different from EX within sex and generation ($p < 0.05$)

Table 3.3 Serum insulin, glycerol, and triglyceride levels in exercise and sedentary ancestry groups for male and female offspring (F1 and F2).

	Males		Females	
F_1 generation	**EX (N=9)**	**SED (N=8)**	**EX (N=12)**	**SED (N=16)**
Insulin (ng mL^{-1})	0.30 ± 0.04	0.26 ± 0.06	0.33 ± 0.05	0.15 ± 0.02 *
Free Glycerol (mg dL^{-1})	43.0 ± 4.0	58.2 ± 6.6	51.3 ± 8.0	54.0 ± 5.6
Total Triglyceride (mg dL^{-1})	94.9 ± 8.8	109.8 ± 10.8	80.8 ± 6.9	80.8 ± 5.6
F_2 generation	**EX (N=18)**	**SED (N=18)**	**EX (N=14)**	**SED (N=23)**
Insulin (ng mL^{-1})	0.28 ± 0.04	0.25 ± 0.03	0.23 ± 0.04	0.22 ± 0.04
Free Glycerol (mg dL^{-1})	31.9 ± 4.3	36.5 ± 7.6	34.0 ± 7.7	46.7 ± 7.7
Total Triglyceride (mg dL^{-1})	100.4 ± 3.3	94.0 ± 11.8	67.6 ± 6.1	77.8 ± 7.4

*significantly different from EX within sex and generation ($p < 0.05$)

Figures

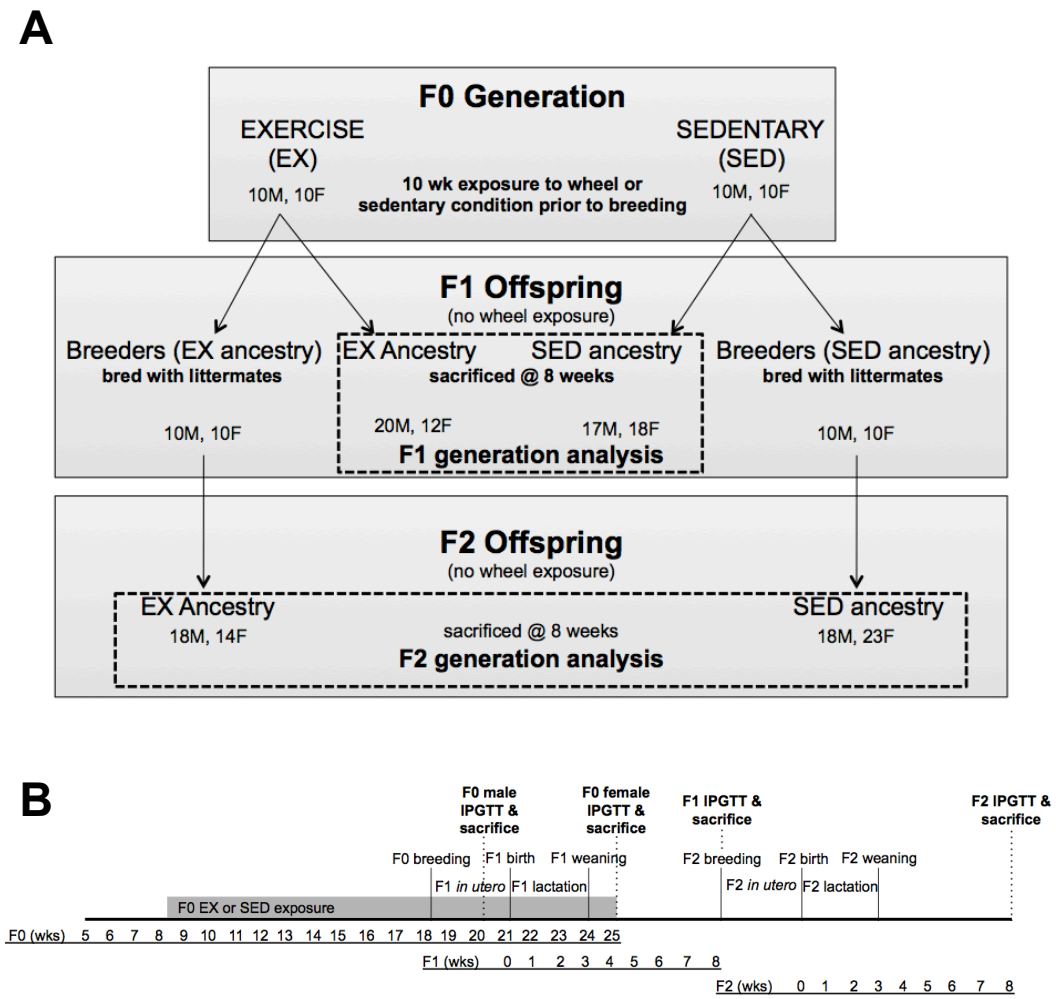

Figure 3.1 Experimental design. A, overview. B, time line.

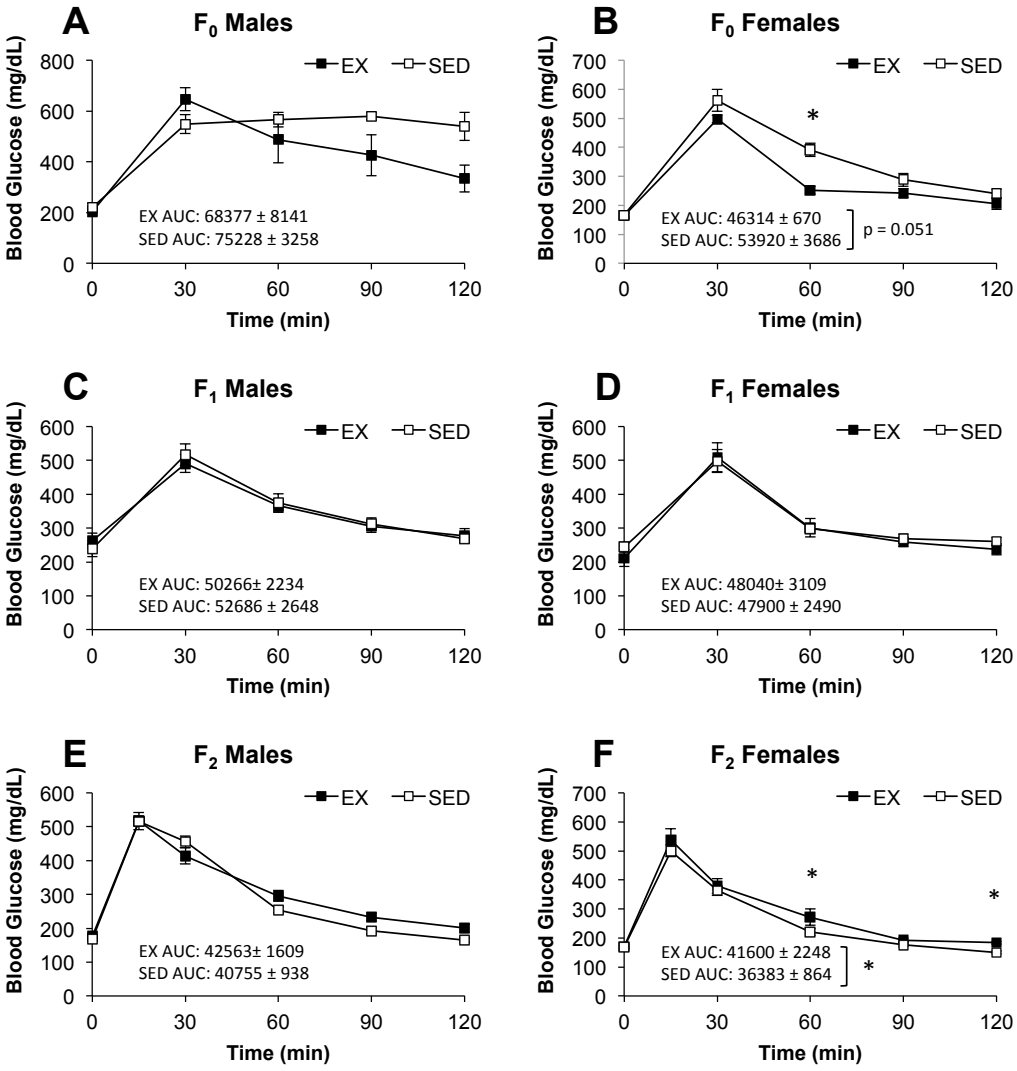

Figure 3.2. Blood glucose concentration during an intraperitoneal glucose tolerance test in: (A) F0 males, (B) F0 females, (C) F1 males, (D) F1 females, (E) F2 males, and (F) F2 females. Mice were fasted 12 hours and given a 2 g/kg body mass glucose load at time 0. Glucose levels were determined before and 30, 60, 90, and 120 min after injection. Blood glucose was also measured 15 min after injection in F2 males and females. Area under the curve for concentration vs. time was calculated using the linear trapezoidal rule. Values are means ± SE. *significant difference in AUC between EX and SED within sex and generation ($p < 0.05$)

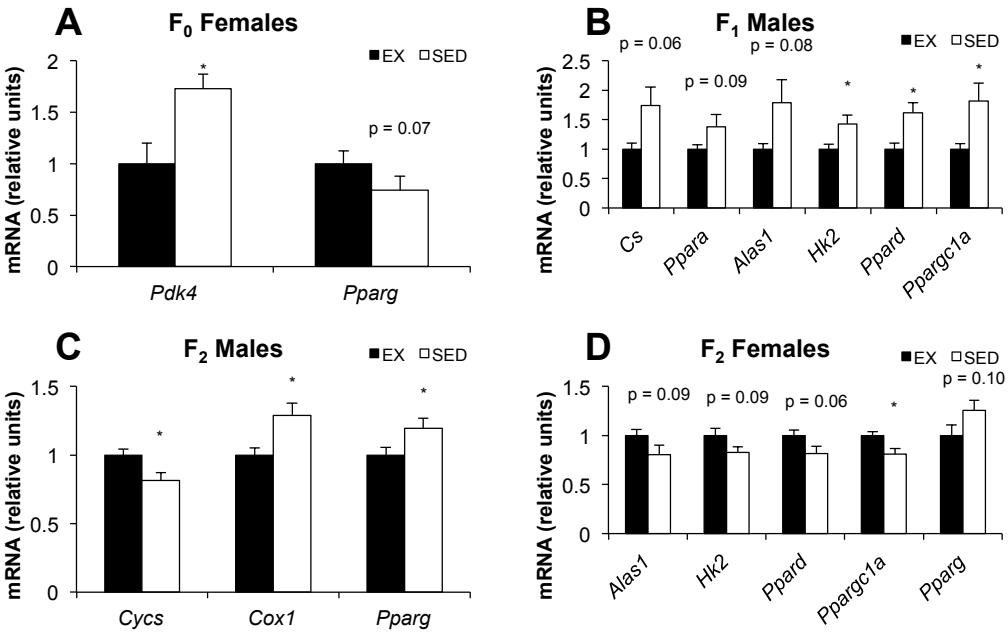

Figure 3.3. Relative gastrocnemius muscle mRNA levels of *Alas1*, *Cox1*, *Cycs*, *Cs*, *Hk2*, *Pdk4*, *Ppara*, *Ppard*, *Pparg*, and *Ppargc1a* determined by RT-PCR in: (A) F0 females, (B) F1 males, (C) F2 males, and (D) F2 females. For each offspring group, only mRNA targets with $p \leq 0.10$ between EX and SED ancestry are shown in the figure; no mRNA targets met this threshold for F0 males or F1 females. Average expression level in EX was set to 1.0. Sample sizes were n=7-10. *significantly different from EX within sex and generation ($p < 0.05$)

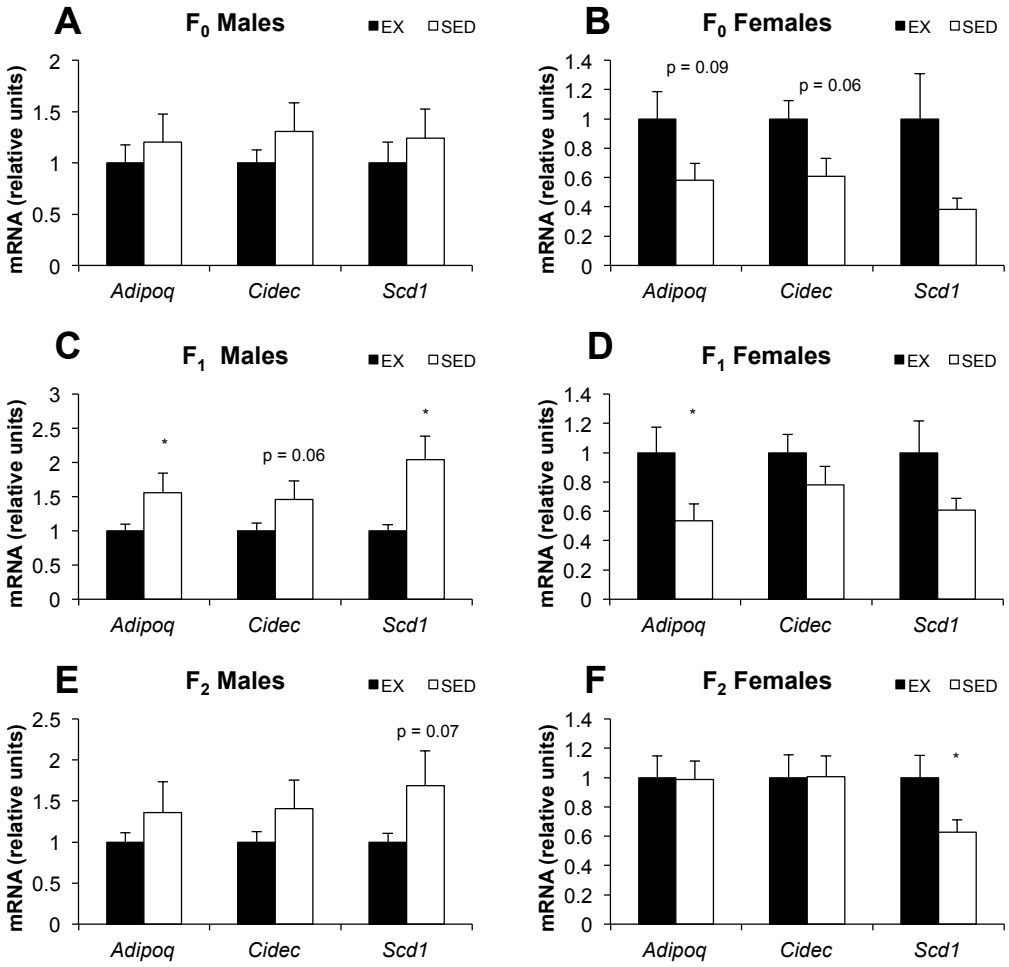

Figure 3.4. Relative gastrocnemius muscle mRNA levels of *Adipoq*, *Cidec*, and *Scd1* determined by RT-qPCR in: (A) F0 males, (B) F0 females, (C) F1 males, (D) F1 females, (E) F2 males, and (F) F2 females. Average expression level in EX was set to 1.0. Sample sizes were n=10-20. *significantly different from EX within sex and generation ($p < 0.05$)

Supplementary Material

Supplementary Table 3.1. List of transcripts significantly affected by exercise compared to sedentary ancestry in F1 males.

Upregulated Transcripts			
Probe ID	**Gene**	**mRNA Accession Number**	**Fold Change**
10582882	---	---	7.08
10368222	4930444G20Rik	NM_053264	6.33
10608646	---	NM_001013828.1	5.25
10455954	Gm4951	NM_001033767	4.52
10462390	Cd274	NM_021893	4.46
10399823	---	ENSMUST00000083266	3.48
10562592	Gm5114	NM_177890	3.02
10582888	---	ENSMUST00000099042	2.79
10582899	---	ENSMUST00000099035	2.78
10598794	---	---	2.70
10561166	---	---	2.23
10554162	---	---	2.15
10442270	1300003B13Rik	BC025651	2.03
10597513	---	ENSMUST00000083215	1.96
10474687	---	ENSMUST00000083911	1.87
10494753	---	ENSMUST00000121385	1.78
10532019	EG634650	NM_001039647	1.77
10368370	Gm8681	XR_032130	1.68
10574427	Impdh2	NM_011830	1.63
10589790	---	---	1.58
10439889	---	---	1.34
10585976	Myo9a	NM_173018	1.33
10598041	---	NC_005089	1.23
10531407	Cxcl9	NM_008599	1.16
10385507	OTTMUSG00000005523	NM_001045540	1.08
10376324	Gm12250	NM_001135115	0.81
10507431	---	---	0.69
10582896	---	---	0.62
10608693	---	M31319.1	0.55
10409259	---	---	0.49
10424347	Gm7691	XM_001473789	0.46
10582916	---	---	0.19
10414706	---	AJ311366	0.13
10582890	---	ENSMUST00000099042	0.06
10431635	---	ENSMUST00000082986	0.06
10423803	Gm5213	XR_032412	0.03

Downregulated Targets			
Probe ID	**Gene**	**mRNA Accession Number**	**Fold Change**
10385518	Tgtp	NM_011579	-30.05

10434747	Adipoq	NM_009605	-12.52
10399820	Acp1	NM_001110239	-11.52
10455961	Iigp1	NM_001146275	-8.84
10385533	Tgtp	NM_011579	-7.12
10346069	Gm8304	XM_001477007	-6.10
10415642	Sap18	NM_009119	-4.50
10598236	Nudt11	NM_021431	-4.44
10458052	Epb4.1l4a	NM_013512	-3.62
10539574	Npm3-ps1	NR_002702	-3.56
10409876	Ctla2a	NM_007796	-3.18
10376326	Irgm2	NM_019440	-2.86
10496592	Gbp2	NM_010260	-2.82
10546929	Cidec	NM_178373	-2.81
10605355	---	ENSMUST00000083925	-2.72
10562657	Gm5595	NM_001008427	-2.64
10374117	---	ENSMUST00000117212	-2.54
10360324	LOC100045972	XR_030924	-2.53
10471550	Rpl12	BC075731	-2.47
10388898	Fam58b	BC027022	-2.43
10385500	Irgm1	NM_008326	-2.42
10424555	---	ENSMUST00000083234	-2.24
10531994	Mpa2l	NM_194336	-2.23
10344835	---	GENSCAN00000012277	-2.21
10524312	Ttc28	ENSMUST00000100894	-2.10
10596257	Dnajc13	NM_001163026	-2.08
10459066	LOC100044195	BC150900	-2.03
10482073	---	ENSMUST00000121664	-1.99
10367960	Pex3	NM_019961	-1.98
10583316	Taf1d	BC056964	-1.83
10346191	Stat1	NM_009283	-1.77
10456005	Cd74	NM_001042605	-1.70
10556167	---	GENSCAN00000037385	-1.69
10346722	Nbeal1	NM_173444	-1.66
10479228	Etohi1	ENSMUST00000098999	-1.61
10362424	Trdn	NM_029726	-1.56
10537227	Tmem140	NM_197986	-1.56
10549592	---	ENSMUST00000097237	-1.55
10459375	Txnl1	NM_016792	-1.53
10603911	---	---	-1.51
10480345	---	ENSMUST00000117515	-1.51
10500133	Prune	NM_173347	-1.51
10440909	---	ENSMUST00000103851	-1.50
10583806	Gm6581	XR_001828	-1.49
10353773	---	ENSMUST00000044356	-1.49
10457250	Arhgap12	NM_001039692	-1.49
10389373	Appbp2	NM_025825	-1.48

10484512	Olfr992	NM_146865	-1.48
10485357	---	ENSMUST00000099683	-1.47
10455970	BC023105 // BC023105	BC023105	-1.45
10415873	---	mmu-mir-598	-1.43
10587627	Cyb5r4	NM_024195	-1.43
10533085	Pebp1	NM_018858	-1.43
10608681	---	NM_001034859.1	-1.42
10498160	Ufm1	NM_026435	-1.38
10558295	Zranb1	ENSMUST00000106157	-1.34
10496569	Gbp6	NM_145545	-1.31
10497265	Fabp4	NM_024406	-1.31
10462363	Jak2	NM_008413	-1.28
10392087	Ccdc47	NM_026009	-1.28
10548661	---	---	-1.27
10569017	Ifitm3	NM_025378	-1.25
10467979	Scd1	NM_009127	-1.25
10444830	H2-Q7	NM_010394	-1.24
10418927	Bmpr1a	NM_009758	-1.24
10362462	Trdn	NM_029726	-1.24
10529873	Rab2a	NM_021518	-1.24
10362428	Trdn	NM_029726	-1.22
10362422	Trdn	NM_029726	-1.21
10497483	Hmgb1	NM_010439	-1.18
10475414	B2m	NM_009735	-1.17
10584777	Ddx6	NM_001110826	-1.15
10362442	Trdn	NM_029726	-1.14

Supplementary Table 3.2. List of transcripts significantly affected by exercise compared to sedentary ancestry in F2 males.

Upregulated Transcripts

Probe ID	Gene	mRNA Accession Number	Fold Change
10473107	---	---	87.46
10584578	Hspa8	M13967	15.01
10378445	E130309D14Rik	BC150886	13.13
10584576	Hspa8	M13967	10.77
10413012	Fut11	AK034234	10.69
10414932	---	ENSMUST00000103643	10.43
10606016	Il2rg	NM_013563	8.92
10574598	Es31	BC057188	8.86
10548207	Pzp	NM_007376	7.55
10547655	---	---	7.36
10546086	---	ENSMUST00000090438	6.92
10592727	Rnf26	ENSMUST00000065379	6.19
10570513	2900016B01Rik	ENSMUST00000058886	5.15
10392142	Cd79b	NM_008339	5.12
10362939	---	ENSMUST00000095717	4.85
10434689	Ahsg	NM_013465	4.29
10433887	Pkp2	NM_026163	4.22
10345704	---	ENSMUST00000083695	4.03
10414744	---	DQ340292	4.01
10513521	Mup20	NM_001012323	3.86
10538868	---	ENSMUST00000103302	3.74
10468448	---	---	3.70
10604661	---	ENSMUST00000054770	3.66
10419726	---	---	3.55
10544648	Dfna5	NM_018769	3.47
10550451	---	ENSMUST00000081336	3.31
10425333	Apobec3	ENSMUST00000100423	3.19
10526098	Scand3	NM_183088	3.14
10514645	---	ENSMUST00000119478	3.06
10414736	---	ENSMUST00000103580	2.98
10435489	Ccdc58	NM_198645	2.81
10560089	---	ENSMUST00000094828	2.48
10531149	Gc	NM_008096	2.48
10517165	Cd52	NM_013706	2.48
10350689	Ncf2	NM_010877	2.45
10397780	A630072L19Rik	AK153749	2.42
10573210	Ptger1	NM_013641	2.42
10460146	---	BC028925	2.41
10511429	Car8	NM_007592	2.31
10537880	---	---	2.28
10545208	Gm189	ENSMUST00000103357	2.25
10409259	---	---	2.23

10558454	Glrx3	NM_023140	2.22
10361129	---	ENSMUST00000083887	2.21
10607398	---	ENSMUST00000122032	2.15
10433172	Glycam1	NM_008134	2.09
10526339	Trim50	NM_178240	2.06
10351197	Sell	NM_011346	2.06
10422393	---	GENSCAN00000028428	2.05
10408848	---	GENSCAN00000041460	2.03
10450189	Btnl5	NR_004051	1.95
10541480	Mug1	NM_008645	1.88
10362426	Trdn	NM_029726	1.83
10529299	Slbp	NM_009193	1.77
10502230	---	---	1.73
10478421	Kcnk15	BC147256	1.68
10546450	Adamts9	NM_175314	1.65
10422009	---	NM_028615	1.64
10513512	Mup1	NM_001163011	1.63
10381930	---	---	1.63
10355264	Gm10072	ENSMUST00000076473	1.62
10436200	Gm8824	XR_034572	1.61
10593015	Cd3g	NM_009850	1.61
10551025	Cd79a	NM_007655	1.60
10428449	---	ENSMUST00000083378	1.55
10369481	H2afy2	NM_207000	1.51
10454059	---	ENSMUST00000069552	1.50
10566205	Dub2a	NM_001001559	1.49
10603706	Med4	NM_026119	1.44
10355528	Tns1	NM_027884	1.34
10533869	Ccdc92	NM_144819	1.33
10465838	Mta2	NM_011842	1.29
10529457	Cpz	NM_153107	1.29
10507431	---	---	1.22
10598085	ATP6	ENSMUST00000119235	1.22
10513437	Mup2	NM_008647	1.20
10513497	Mup2	NM_001045550	1.20
10523062	Alb	NM_009654	1.19
10513504	Mup2	NM_001045550	1.19
10513428	Mup2	NM_001045550	1.19
10513455	Mup2	NM_008647	1.18
10513472	Mup2	NM_008647	1.18
10513420	Mup7	NM_001134675	1.17
10450920	AY036118	AY036118	1.12
10575955	Klhl36	NM_146219	0.97
10435185	---	ENSMUST00000093713	0.94
10446425	---	ENSMUST00000083372	0.88
10430818	Tnfrsf13c	NM_028075	0.80

10373610	Olfr767	NM_146318	0.80
10437205	Pcp4	NM_008791	0.66
10520948	Plb1	NM_001081407	0.59
10569303	---	---	0.53
10414706	---	AJ311366	0.53
10582888	---	ENSMUST00000099042	0.47
10598771	Maoa	NM_173740	0.42
10515974	Nfyc	NM_008692	0.35
10381416	Rnd2	NM_009708	0.33
10379223	1810012P15Rik	NM_001076681	0.33
10517419	---	ENSMUST00000119054	0.33
10379153	Aldoc	NM_009657	0.15
10358224	Ptprc	NM_001111316	0.14
10558866	Drd4	NM_007878	0.13
10499703	---	ENSMUST00000083111	0.05

Downregulated Transcripts

Probe ID	Gene	mRNA Accession Number	Fold Change
10536593	Tsen15	NM_025677	-14.93
10576896	Gm9457	XM_974415	-12.43
10584252	Gm9513	EU703629	-7.71
10598075	---	NC_005089	-6.77
10579987	Scoc	NM_001039137	-6.76
10507589	---	ENSMUST00000122207	-6.60
10362271	---	GENSCAN00000041810	-5.53
10442081	---	mmu-mir-99b	-4.39
10385297	Gabra1	NM_010250	-3.93
10608675	---	NM_011458.1	-3.91
10513912	Aldoart1	EF662061	-3.67
10538965	Fabp1	NM_017399	-3.04
10458762	---	ENSMUST00000097581	-2.95
10574478	Dync1li2	NM_001013380	-2.91
10430372	Rac2	NM_009008	-2.64
10383358	---	ENSMUST00000076255	-2.57
10532180	Cplx1	NM_007756	-2.37
10362454	Trdn	NM_029726	-2.26
10441753	Plg	NM_008877	-2.17
10538352	---	ENSMUST00000083282	-2.13
10434719	Kng1	NM_001102411	-2.13
10580624	Es1	NM_007954	-2.12
10390961	Krtap17-1	NM_001099774	-2.10
10568731	---	---	-2.08
10548857	Hist4h4	NM_175652	-1.96
10365134	---	ENSMUST00000102273	-1.90
10362416	Trdn	NM_029726	-1.90
10437149	---	---	-1.87

10373651	---	ENSMUST00000102219	-1.73
10450224	---	GENSCAN00000027119	-1.70
10589846	---	ENSMUST00000122685	-1.68
10597513	---	ENSMUST00000083215	-1.67
10541410	Mug1	NM_008645	-1.65
10466528	Rfk	NM_019437	-1.65
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10582584	---	ENSMUST00000101826	-1.63
10539581	---	ENSMUST00000089584	-1.61
10514532	Cyp2j5	NM_010007	-1.53
10412657	---	---	-1.53
10564161	Snord116	NR_002895	-1.43
10564163	Snord116	NR_002895	-1.41
10564167	Snord116	NR_002895	-1.41
10564171	Snord116	NR_002895	-1.41
10564173	Snord116	NR_002895	-1.41
10564175	Snord116	NR_002895	-1.41
10564179	Snord116	NR_002895	-1.41
10564181	Snord116	NR_002895	-1.41
10564185	Snord116	NR_002895	-1.41
10564187	Snord116	NR_002895	-1.41
10564189	Snord116	NR_002895	-1.41
10564191	Snord116	NR_002895	-1.41
10564193	Snord116	NR_002895	-1.41
10564195	Snord116	NR_002895	-1.41
10564197	Snord116	NR_002895	-1.41
10564199	Snord116	NR_002895	-1.41
10564201	Snord116	AF241256	-1.41
10564205	Snord116	AF241256	-1.41
10564207	Snord116	AF241256	-1.41
10521698	---	AK149205	-1.37
10365204	Gm10777	ENSMUST00000099432	-1.37
10555233	---	ENSMUST00000083377	-1.33
10430572	---	---	-1.31
10497644	Sec62	NM_027016	-1.29
10408121	---	ENSMUST00000102282	-1.28
10394936	---	ENSMUST00000101540	-1.28
10356170	---	ENSMUST00000116749	-1.24
10434283	---	ENSMUST00000102219	-1.24
10394938	---	ENSMUST00000116749	-1.23
10399657	---	ENSMUST00000116749	-1.23
10471909	---	ENSMUST00000118928	-1.21
10400708	---	ENSMUST00000102219	-1.19
10362672	---	ENSMUST00000099956	-1.17
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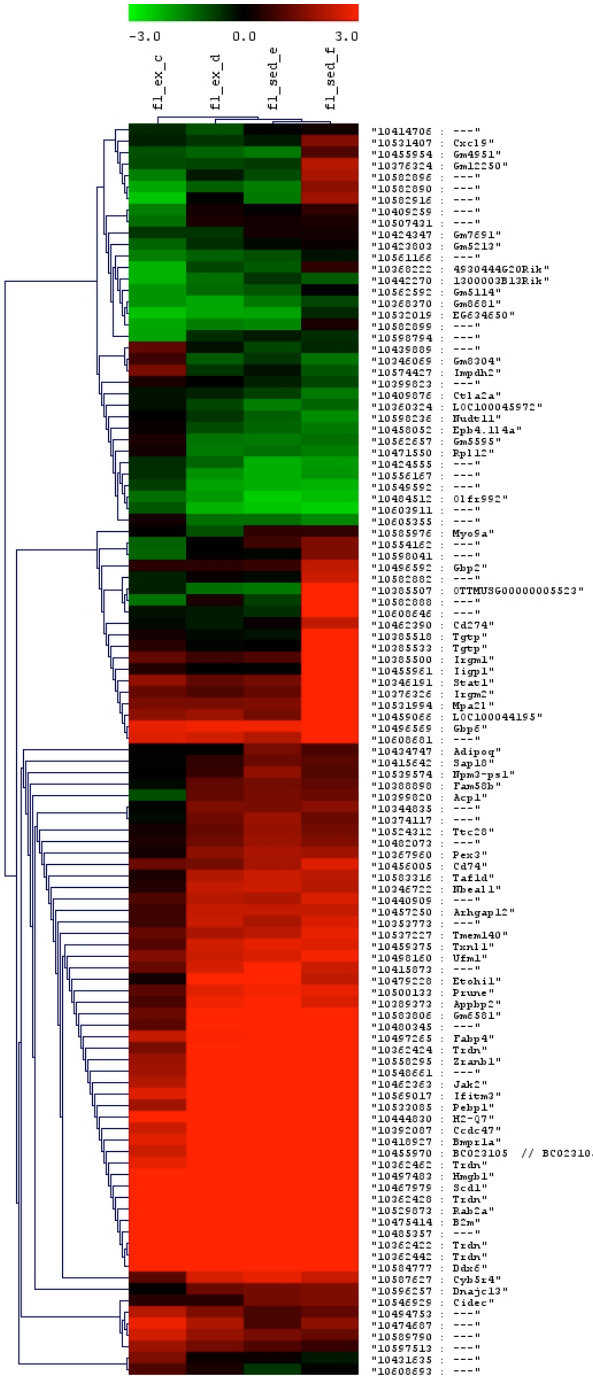
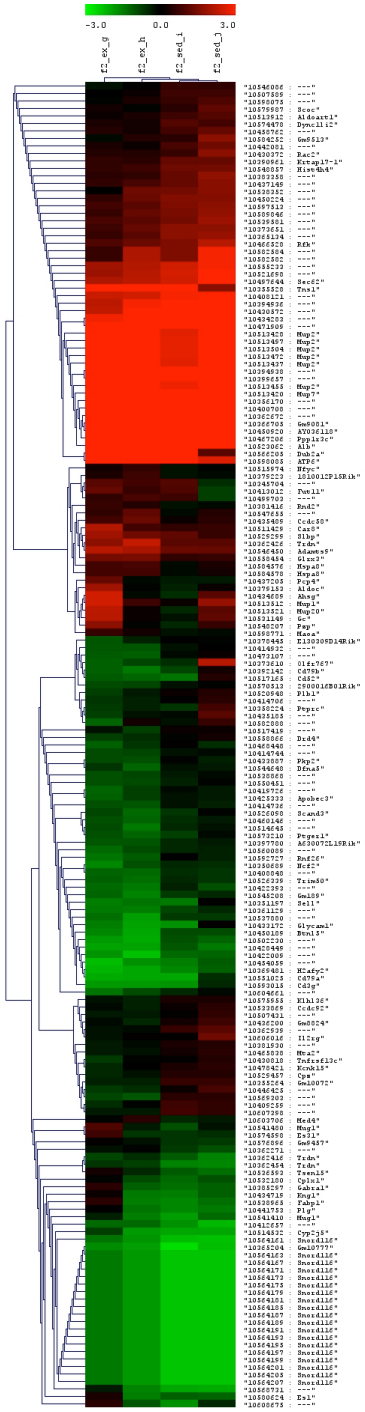
10366705	Gm9081	XR_030920	-1.11



Supplementary Figure 3.1. Heatmap of microarray data showing mRNA targets differentially expressed in gastrocnemius muscle between offspring of exercise compared to sedentary ancestry in F1 males, color-coded and clustered according to normalized expression pattern. Each letter indicates one array performed on mRNA from five pooled offspring of the same condition.



Supplementary Figure 3.2. Heatmap of microarray data showing mRNA targets differentially expressed in gastrocnemius muscle between offspring of exercise compared to sedentary ancestry in F2 males, color-coded and clustered according to normalized expression pattern. Each letter indicates one array performed on mRNA from five pooled offspring of the same condition.

Chapter 4: Effects of Exercise Ancestry on Metabolic, Morphological, and Gene Expression Phenotypes in Multiple Generations of Mature Mouse Offspring

Title: Effects of Exercise Ancestry on Metabolic, Morphological, and Gene Expression Phenotypes in Multiple Generations of Mature Mouse Offspring

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Abstract

Recent evidence indicates that parental exercise prior to breeding or during offspring development can alter body mass and gene expression in young mouse offspring. The current study aimed to examine these phenotypes in mature mouse offspring exposed to parental exercise. First-generation (F0) C57BL/6 mice were exposed to voluntary exercise (EX) or sedentary (SED) lifestyle and bred with like-exposed mice to produce an F1 generation. A subset of F1 mice was bred with like-exposed F1 offspring to produce an F2 generation. Body and tissue anthropometry, glucose tolerance tests and skeletal muscle mRNA expression were analyzed in offspring at 8 and 28 weeks. At 8 weeks, liver mass was higher in F1 EX males. F2 EX female offspring tended to have lower baseline blood glucose. F1 EX female offspring had higher muscle *Cytc* expression compared to F1 SED female offspring. F2 EX males had lower *Cidec* and *Scd1* expression compared to F2 SED males. At 28 weeks, liver and soleus masses were lower in EX males compared to SED males. F1 EX females had higher baseline glucose than F1 SED females. *Pgc1a* mRNA expression was higher in F1 EX males than F1 SED males and *Cox1* mRNA expression was lower in F2 EX males compared to F2 SED males. Overall, aging the offspring to 28 weeks did not increase phenotype separation between EX and SED offspring. We maintain EX ancestry may affect whole-body and transcription-level offspring phenotypes across two generations, but that these effects are not only generation- and sex-dependent, but also impacted by offspring age.

Introduction

Early life events, potentially even those occurring prior to conception, can impact adult physiology. Various manipulations of parental status (i.e. protein restriction, high fat diet, induced stress, etc.) can alter offspring physiology at birth and into adulthood. These physiological alterations induced during development can then manifest as disease in adulthood.

Exercise training leads to many beneficial metabolic health outcomes, including improved glucose homeostasis and lipid oxidation (75, 101), while a sedentary lifestyle is associated with increased incidence of chronic disease (144). Exercise is also beneficial for pregnant women (111), though the effects of maternal and/or paternal exercise on the developing offspring remain less clear, as few studies have examined the effect of voluntary parental exercise. Carter et al. (33, 34) described beneficial alterations in body composition and glucose and insulin dynamics in the mature mouse offspring of dams who had access to a voluntary running wheel during the perinatal period.

Recently, we investigated the potential of parental (both maternal and paternal) voluntary exercise on two generations of young mouse offspring (90). We exposed mice (F0) to voluntary wheel running prior to breeding and through gestation and lactation (in dams). The resultant first generation (F1) female offspring from exercised parents weighed less and had less omental fat mass at 8 weeks compared to females from sedentary parents. Interestingly, this finding was not observed in males or in either sex of second-generation (F2) offspring. We identified differences in basal gene expression in both generations and sexes; these differences were both

generation- and sex-specific. Overall, we determined that parental exercise could broadly alter offspring phenotypes, though these effects were small and specific.

In the current study, we sought to extend our investigation to include adult offspring. Our aims were to compare body and tissue masses, glucose tolerance, and metabolic gene expression patterns between offspring from exercised versus sedentary parents. We again hypothesized that exercise ancestry would alter offspring phenotypes through two generations and that the differences between exercise- and sedentary-ancestry offspring would be larger in adult offspring than in young offspring.

Methods

All animal procedures were performed in accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the University of Maryland (Appendix A). A standard diet (Purina Prolab RMH 3000, 60% carbohydrate, 14% fat, 26% protein) and water were provided ad libitum for all generations in all conditions.

We used a breeding paradigm to produce two generations of offspring (F1 and F2) from an initial F0 generation that was either exposed to exercise or kept sedentary. 20 male and 20 virgin female 5-wk old C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME); these mice made up the F0 generation. C57BL/6 is an inbred strain of mouse that was chosen based on their propensity to perform voluntary wheel running, their common use in research studies of exercise, nutrition, metabolism, and gene expression, and their fecundity. Until 8 weeks of age,

these animals were kept sedentary (i.e., unexposed to voluntary running wheel; standard cage conditions) and group-housed with same-sex mice.

At 8 weeks of age, each sex was randomly split into two condition groups: exercise (EX) and sedentary (SED) (n=10 per group). Mice in the F0 SED group were housed individually in standard cages without access to running wheels. Mice in the F0 EX group were housed individually in cages with computer-monitored voluntary running wheels. After group assignment, F0 mice were exposed to their group condition for 12 weeks. Following this intervention males and females from like conditions (EX or SED) were randomly paired for mating. The F0 EX breeding pairs had continued access to the running wheel throughout mating, pregnancy, and lactation; however running activity could not be accurately measured during the breeding period as there were two mice in each cage. Males were removed from female cages after two weeks. The pups resulting from this original mating were designated as F1. A random group of 10 male and 10 female F1 pups from each condition (EX and SED) were designated as F1 breeders and were mated at 8 weeks with like-condition (EX or SED) F1 offspring to produce the F2 generation. The remaining F1 pups were glucose tolerance tested and sacrificed at 8 weeks. F2 pups from each condition were glucose tolerance tested and sacrificed at 8 weeks or 28 weeks. Following weaning at 21 days, all F1 and F2 offspring were group-housed with same-sex littermates. Body weight was monitored weekly for all animals. F1 and F2 offspring remained sedentary throughout the experiment. Only litters 8 or fewer offspring were used for analysis and no more than 3 offspring per sex per litter were studied per age group.

Intraperitoneal Glucose Tolerance Test (IPGTT)

Glucose tolerance tests were performed on all F1 and F2 offspring at 8 and 28 weeks. All animals were fasted for 6 hours prior to glucose tolerance testing. Baseline blood glucose measurements were made and then each mouse was injected intraperitoneally with 2.0 mg of D-glucose (Sigma-Aldrich, St. Louis, MO) per gram of body mass. Blood glucose was measured 15, 30, 60, 90, and 120 minutes after injection in all animals. Area under the curve for concentration vs. time was calculated using the linear trapezoidal rule. Blood glucose measurements were made using a rodent-specific glucometer (AlphaTRAK; Abbott Laboratories, Abbott Park, IL) on blood removed from a tail snip. Following the glucose tolerance test animals were returned to ad libitum food and water access.

Tissue & Serum Collection

Animals were euthanized 24 hours after the glucose tolerance test; animals were fasted for 6 hours prior to euthanasia. Euthanasia was performed under isoflurane anesthesia; the method of euthanasia was exsanguination by cardiac puncture followed by removal of the heart. Heart, liver, omental fat, cerebellum, tibialis anterior (TA), extensor digitorum longus (EDL), soleus, plantaris, gastrocnemius, and quadriceps muscles were dissected, weighed, and flash frozen in liquid nitrogen and then stored at -80°C until analysis. Approximately 1 mL of blood was obtained from a cardiac puncture and allowed to coagulate. The coagulated blood was centrifuged at 1750 x g for 15 minutes to obtain serum. Serum was removed to a fresh tube and stored at -80°C until analysis.

Gene Expression

Total RNA was isolated from frozen powdered gastrocnemius muscle with Trizol reagent (15596-026, Life Technologies, Grand Island, NY), DNase-treated, and quantified by spectrophotometer. Reverse transcription was performed with 1μg of total RNA with the High-Capacity cDNA RT kit (4368813, Life Technologies, Grand Island, NY).

Real-time quantitative PCR was used to assess the gene expression level of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*Ppargc1a*), adiponectin (*Adipoq*), cell death-inducing DFFA-like effector c (*Cidec*), and stearoyl-Coenzyme A desaturase 1 (*Scd1*) (n=3-11) per group). Primer and probe sequences were designed for each gene's mRNA sequence using PrimeTime qPCR Assay designer (IDT). 18s rRNA was used as an expression control for both real-time and gel-based PCR and did not differ between treatment groups. RT-PCR was used to measure the expression of metabolic genes (peroxisome proliferator activated receptor alpha, delta, and gamma, (*Ppara*, *Ppard*, and *Pparg*); hexokinase 2, *Hk2*; cytochrome c, somatic, *Cycs*; citrate synthase, *Cs*; and cytochrome c oxidase subunit I, *Cox1*) (n=3-11 per group). Products were visualized on 1.5% agarose gels using ethidium bromide. Primer and probe sequences and PCR conditions are available upon request. qPCR data were normalized to 18s using the -ΔCt method (176) and expressed as fold induction (2-ΔCt) of mRNA expression compared to the corresponding EX group (1.0-fold induction). Relative band intensities from PCR gels were be analyzed with NIH ImageJ software and normalized to 18s. Values are shown as means ± standard error.

Statistics

Two-tailed t-tests were used to compare body and tissue masses and IPGTT AUC between EX and SED groups within each sex and generation. A repeated-measures ANOVA was used to compare the IPGTT response between EX and SED groups within each sex and generation. Two-tailed t-tests were used to compare gene expression between EX and SED groups within each sex and generation. Statistical analyses were performed using SPSS version 18, and statistical significance was accepted at $p < 0.05$.

Results

The final offspring numbers for analysis are shown in Table 4.1. Two F0 EX breeding pairs did not produce viable F1 pups, and three F1 breeding pairs each from EX and SED ancestries did not produce viable F2 pups. Average litter size for F1 offspring was 4.3 ± 0.4 EX and 4.6 ± 0.3 SED offspring/litter, while average litter size for F2 offspring was 5.0 ± 1.1 EX and 4.7 ± 0.7 SED offspring/litter. There were no significant differences in litter size between groups or significant differences in sex ratio between groups (data not shown).

Young Offspring (8 weeks)

Body and tissue mass are shown in Table 4.2. In 8-week old offspring, we found that body mass tended to be higher in F1 EX offspring, regardless of sex (males, $p = 0.05$; females, $p = 0.06$). Liver mass was higher in F1 EX offspring, regardless of sex ($p < 0.05$). In the F2 generation, average plantaris mass tended to be lower in SED males compared to EX males ($p = 0.07$). No other differences in tissue

mass between offspring of EX and SED ancestry were detected in these young offspring. F2 female mice with EX ancestry tended to have lower baseline blood glucose following a 6-hour fast ($p = 0.051$, Figure 4.2A&B). No differences in area under the curve were detected during the glucose tolerance test.

F1 EX females had higher skeletal muscle *Cytc* ($p < 0.05$) and tended to have higher *Pparg*, ($p = 0.05$) and *Scd1* ($p = 0.08$) mRNA expression compared to F1 SED females (Figure 4.3B). F2 EX males had lower *Cidec* and *Scd1* ($p < 0.05$) and a tendency for higher *Adipoq* ($p = 0.10$) mRNA expression compared to F2 SED males (Figure 4.3C). No other differences in gene expression were detected.

Mature Offspring (28 weeks)

There was no effect of exercise ancestry on body mass in 28-week old offspring (Table 4.3). F1 EX males had lower liver mass ($p < 0.05$) and F1 and F2 EX males had lower liver and soleus mass (all $p < 0.05$) than SED males within the same generation. F2 EX males also had lower gastrocnemius mass ($p < 0.05$) and tended to have lower heart mass ($p = 0.06$). No other differences in tissue masses were detected in these mature offspring. Baseline blood glucose was higher in F1 EX females compared to F1 SED females ($p < 0.05$, Figure 4.2D), but glucose tolerance was not affected by exercise ancestry in either sex or generation of offspring (Figure 4.2C&D).

Skeletal muscle *Pgc1a* mRNA expression was higher in F1 EX males compared to F1 SED males ($p < 0.05$, Figure 4.4A) and *Cox1* mRNA expression was lower in F2 EX males compared to F2 SED males ($p < 0.05$, Figure 4.4C). No other differences in mRNA expression were detected.

Discussion

This is the first study to investigate the effects of parental exercise on health-related phenotypes in two generations of mouse offspring at both young and mature age points. We observed generation-, sex-, and age-specific effects of exercise ancestry in the offspring studied; however, contrary to our hypothesis, we did not observe more dramatic differences in mature (28-week) compared to 8-week old offspring.

In 8-week old offspring in the current study, body mass tended to be higher in F1 EX offspring, regardless of sex. We previously observed the opposite response, with significantly higher body mass in 8-week old F1 SED female offspring, but no difference in male offspring (90). Although variability in the effect of maternal exercise on offspring body mass has been reported (33, 162), this is the first observation of higher body mass in the offspring of exercised dams. Liver mass was higher in F1 EX males and tended to be higher in F1 EX females, while no differences in liver mass were observed in our previous study(90).

In the current study, 8-week old F2 female mice with EX ancestry tended to have lower baseline blood glucose. In our previous study, there were no differences in baseline blood glucose between offspring with EX compared to SED ancestry but F2 EX females had slightly higher IPGTT area under the curve (90).

We also examined skeletal muscle gene expression differences between offspring of EX and SED ancestry at 8 weeks of age. We found higher *Cytc* expression and tendencies towards higher *Pparg*, and *Scd1* mRNA expression in F1 EX females compared to F1 SED females; none of these differences were observed in

our previous study (90). In F2 males, we found lower *Cidec* and *Scd1* expression and a tendency for higher *Adipoq* mRNA expression in EX offspring; we also observed a tendency towards lower *Scd1* expression in F2 EX males in our previous study (90). To our knowledge, ours are the only investigations of parental exercise interventions to include gene expression analyses.

Overall, our present findings in 8-week old offspring were not consistent with our previous study. There are several potential contributors to this inconsistency. First, though we maintained a similar experimental design, we made small modifications to our procedures relative to our previous study (90). These included an extension of the pre-breeding exercise intervention to 12 instead of 10 weeks, breeding F1 non-littermates instead of littermates, including only offspring born in litter sizes of 4-7, and fasting animals for 6 hours prior to IPGTT and sacrifice instead of 12 and 4 hours, respectively. Lastly, as the primary aim of the current study was to investigate the effects of exercise ancestry on mature offspring, our sample size for 8-week old offspring was very limited relative to our previous study. This reduced our statistical power to detect differences in 8-week old offspring in the current study.

There were no differences in body mass in 28-week old offspring, demonstrating a normalization of the tendency toward higher body mass in F1 EX ancestry offspring observed at 8 weeks of age. Liver and soleus muscle mass were lower in F1 EX males relative to F1 SED males. Interestingly, this represents an opposite effect of exercise ancestry on liver mass to that observed at 8 weeks of age. F1 EX females developed higher baseline blood glucose compared to F1 SED females at 28 weeks of age while the lower blood glucose vales in F2 EX females

observed at 8 weeks were no longer apparent. No differences in glucose tolerance were observed at 28 weeks, in contrast to the findings of Carter et al. (33), who reported improved glucose tolerance in the offspring of exercised dams compared to the offspring of sedentary dams at 7 months of age.

Few gene expression differences were detected in 28-week old offspring (higher *Pgc1a* expression in F1 EX compared to SED males and lower *Cox1* expression in F2 EX compared to SED males). None of the gene expression differences observed at 8 weeks of age were apparent at 28 weeks of age. This suggests that transcriptional differences stimulated by exercise ancestry during development may "fade" over time or be further modified by the current environment. In our study, offspring environment was identical from 3 to 28 weeks of age for all offspring.

Overall, our hypothesis that aging the offspring to 28 weeks would increase the phenotype separation between EX and SED offspring was not supported. It is possible that 28 weeks is still not a sufficient duration of aging to see this separation, as other researchers have not seen differences in whole body and glycemia-related phenotypes until even older ages (33, 34). It is important to note that outbred strains of rodents have been used in other studies, while an inbred strain was used in the current study. There is evidence to suggest inbred vs. outbred status can influence environmentally-induced transgenerational effects on offspring health phenotypes (89, 186).

Overall, as a result of this study we maintain our prior conclusion that EX ancestry may affect whole-body and transcription-level offspring phenotypes across

two generations, but are cognizant that the observed effects are small and variable. Further, we conclude that the physiological effects of exercise ancestry are not only generation- and sex-dependent, but also strongly impacted by offspring age. Future studies should evaluate offspring phenotypes as they continue to age beyond 28 weeks. Additional metabolic stressors, such as subjecting the offspring to a high-fat diet, should also be considered.

Tables

Table 4.1. Final Offspring Numbers for Analyses

Generation	Age	Exercise (EX)		Sedentary (SED)	
		Male	Female	Male	Female
F1 Offspring	8 weeks	10	3	8	10
	28 weeks	10	10	10	9
F2 Offspring	8 weeks	10	7	4	10
	28 weeks	11	8	10	8

Table 4.2. Body and Tissue Mass in 8-week old Offspring.

	Males				Females			
F1 generation	**EX**		**SED**		**EX**		**SED**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Body Mass (g)	24.20	0.49	22.92	0.31	20.49	0.20	19.01	0.38
Heart (mg)	123.90	3.60	116.40	3.21	108.40	3.00	100.50	2.56
Liver (g)	1.25	0.06	1.09*	0.03	1.07	0.01	0.98*	0.03
Omental Fat (g)	237.70	8.78	255.00	11.90	146.20	15.30	155.30	8.41
Testis (mg)	92.40	2.02	90.10	1.34				
T. Anterior (mg)	46.90	1.23	45.50	1.21	38.10	0.72	36.10	2.12
EDL (mg)	10.00	0.74	9.28	0.34	8.02	0.89	7.86	0.64
Soleus (mg)	8.50	0.56	8.88	0.48	7.65	0.58	6.62	0.36
Plantaris (mg)	16.00	0.74	15.20	0.93	15.96	0.74	15.24	0.93
Gastrocnemius (mg)	112.40	5.13	110.60	7.02	87.70	2.20	82.40	4.09
F2 generation	**EX**		**SED**		**EX**		**SED**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Body Mass (g)	22.73	0.46	23.25	0.50	18.92	0.26	19.42	0.18
Heart (mg)	119.20	3.07	123.80	7.23	103.60	4.28	107.40	3.08
Liver (g)	1.08	0.03	1.11	0.01	0.82	0.04	0.87	0.02
Omental Fat (mg)	225.10	14.70	243.60	25.70	163.90	13.40	172.00	7.06
Testis (mg)	87.50	1.21	87.40	1.49				
T. Anterior (mg)	45.40	1.05	44.60	1.87	37.20	1.00	37.30	0.67
EDL (mg)	9.18	0.63	9.29	0.43	8.66	0.22	7.97	0.52
Soleus (mg)	8.47	0.28	8.75	0.55	7.34	0.45	7.42	0.14
Plantaris (mg)	16.30	0.47	14.40	1.02	12.50	0.52	12.90	0.24
Gastrocnemius (mg)	117.10	3.34	112.80	3.51	90.40	2.02	92.30	2.27

* significantly different from EX within sex and generation ($p < 0.05$)

Table 4.3 Body and Tissue Mass in 28-week old Offspring.

	Males				Females			
F1 generation	**EX**		**SED**		**EX**		**SED**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Body Mass (g)	28.63	0.34	29.46	0.52	25.45	0.25	25.87	0.35
Heart (mg)	141.20	5.75	143.60	5.30	136.10	3.83	136.00	4.25
Liver (g)	1.21	0.04	1.31*	0.02	1.13	0.05	1.05	0.06
Omental Fat (mg)	501.20	31.00	585.10	75.50	330.90	34.60	399.10	54.30
Testis (mg)	100.30	2.42	93.10	3.57				
T. Anterior (mg)	50.90	0.79	52.10	0.84	43.40	0.95	45.60	0.85
EDL (mg)	10.40	0.32	10.20	0.43	10.36	0.32	10.95	0.43
Soleus (mg)	9.14	0.41	10.20*	0.27	8.23	0.27	8.79	0.30
Plantaris (mg)	18.00	0.82	17.60	0.81	14.60	0.41	15.20	0.59
Gastrocnemius (mg)	140.40	3.78	137.40	1.34	116.80	2.99	118.20	3.14
F2 generation	**EX**		**SED**		**EX**		**SED**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Body Mass (g)	28.19	0.58	28.87	0.60	23.85	0.40	24.11	0.58
Heart (mg)	126.80	2.75	137.50	4.82	120.60	3.79	117.00	2.47
Liver (g)	1.32	0.04	1.34	0.04	1.06	0.04	1.05	0.04
Omental Fat (mg)	543.80	57.60	518.70	43.80	262.20	23.00	279.30	20.80
Testis (mg)	95.00	2.31	92.60	4.28				
T. Anterior (mg)	51.10	0.79	52.30	1.57	43.90	0.98	42.90	0.83
EDL (mg)	9.35	0.35	10.20	0.59	8.47	0.36	8.61	0.63
Soleus (mg)	8.70	0.28	9.66*	0.37	7.80	0.27	8.39	0.45
Plantaris (mg)	17.50	0.57	17.90	0.44	13.90	0.40	13.67	0.64
Gastrocnemius (mg)	128.30	2.13	138.10*	2.48	112.70	3.48	113.30	2.28

* significantly different from EX within sex and generation ($p < 0.05$)

Figures

Figure 4.1 Experimental Design.

Figure 4.2. Blood glucose concentration during an intraperitoneal glucose tolerance test in: (A) F1 males at 8 weeks, (B) F1 males at 28 weeks, (C) F1 females at 8 weeks, (D) F1 females at 28 weeks, (E) F2 males at 8 weeks, (F) F2 males at 28 weeks, (G) F2 females at 8 weeks, and (H) F2 females at 28 weeks. Mice were fasted 6 hours and given a 2 g/kg body mass glucose load at time 0. Glucose levels were determined before and 15, 30, 60, 90, and 120 min after injection. Area under the curve for concentration vs. time was calculated using the linear trapezoidal rule. Values are means ± SE. *significant difference between EX and SED at timepoint ($p < 0.05$).

Figure 4.3. Relative gastrocnemius muscle mRNA levels. *Hk2*, *Cd36*, *Cox1*, *CS*, *Cytc*, *Ppara*, *Ppard*, *Pparg*, *Pgc1a*, *Adipoq*, *Cidec*, and *Scd1* determined by RT-PCR in: (A) F0 females, (B) F1 males, (C) F2 males, and (D) F2 females at 8 weeks of age. Average expression level in EX was set to 1.0 *significantly different from EX within sex and generation ($p < 0.05$)

Figure 4.4. Relative gastrocnemius muscle mRNA levels. *Hk2*, *Cd36*, *Cox1*, *CS*, *Cytc*, *Ppara*, *Ppard*, *Pparg*, *Pgc1a*, *Adipoq*, *Cidec*, and *Scd1* determined by RT-PCR in: (A) F0 females, (B) F1 males, (C) F2 males, and (D) F2 females at 28 weeks of age. Average expression level in EX was set to 1.0 *significantly different from EX within sex and generation ($p < 0.05$)

Figure 4.1

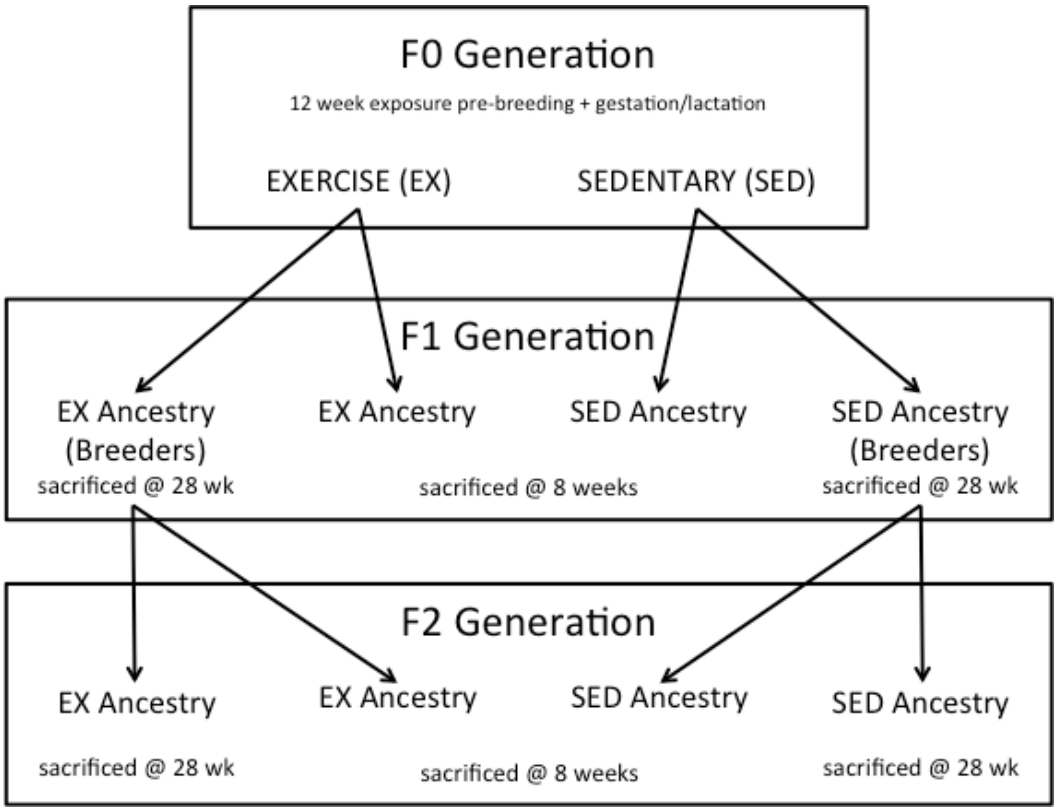

F0 Generation
12 week exposure pre-breeding + gestation/lactation
EXERCISE (EX)
SEDENTARY (SED)
F1 Generation
EX Ancestry (Breeders)
sacrificed @ 28 wk
EX Ancestry
SED Ancestry
sacrificed @ 8 weeks
SED Ancestry (Breeders)
sacrificed @ 28 wk
F2 Generation
EX Ancestry
sacrificed @ 28 wk
EX Ancestry
SED Ancestry
sacrificed @ 8 weeks
SED Ancestry
sacrificed @ 28 wk

Figure 4.2

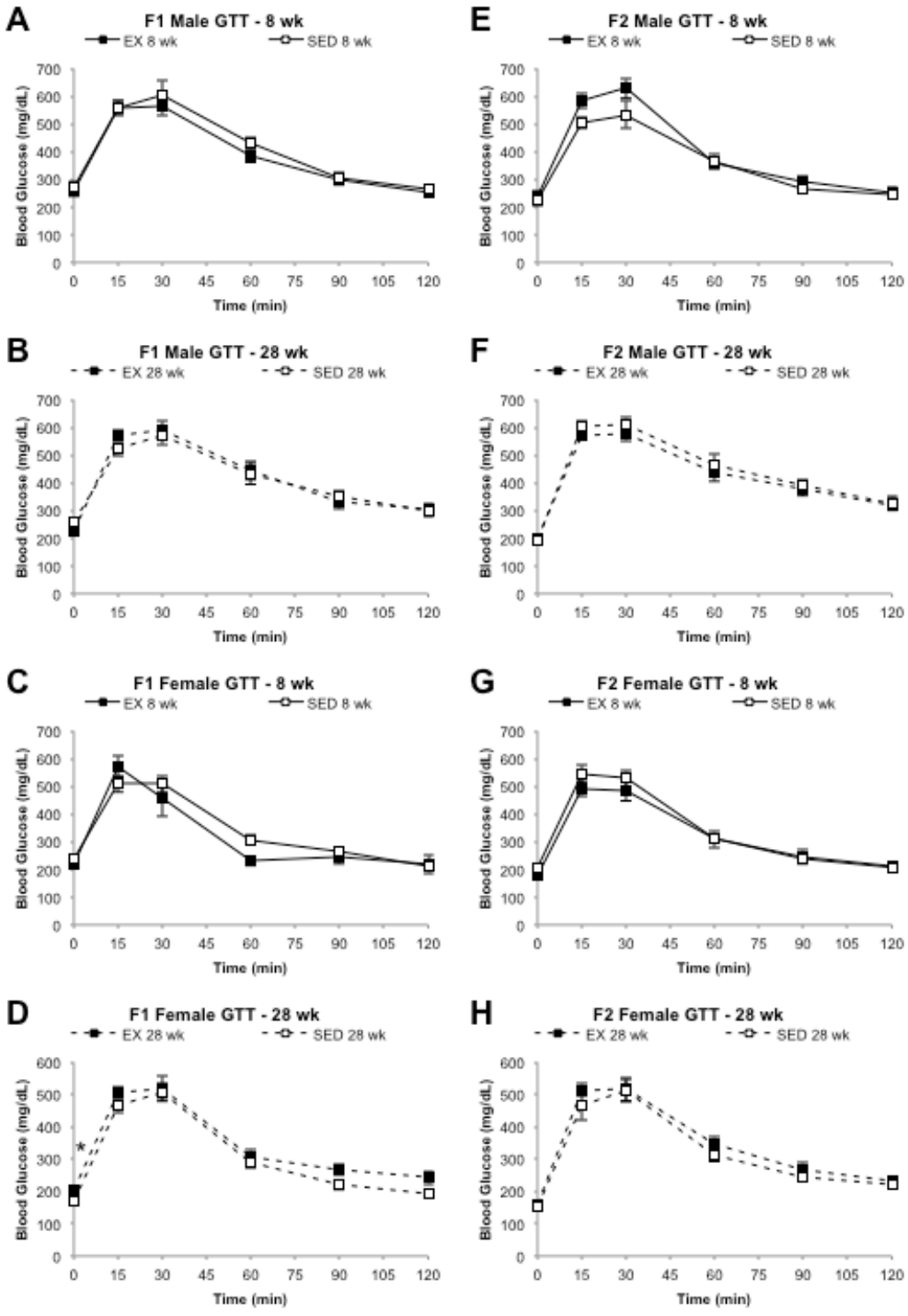

A
F1 Male GTT - 8 wk
EX 8 wk
SED 8 wk
B
F1 Male GTT - 28 wk
EX 28 wk
SED 28 wk
C
F1 Female GTT - 8 wk
EX 8 wk
SED 8 wk
D
F1 Female GTT - 28 wk
EX 28 wk
SED 28 wk
E
F2 Male GTT - 8 wk
EX 8 wk
SED 8 wk
F
F2 Male GTT - 28 wk
EX 28 wk
SED 28 wk
G
F2 Female GTT - 8 wk
EX 8 wk
SED 8 wk
H
F2 Female GTT - 28 wk
EX 28 wk
SED 28 wk
Blood Glucose (mg/dL)
Time (min)
0 15 30 45 60 75 90 105 120
0 100 200 300 400 500 600 700

Figure 4.3

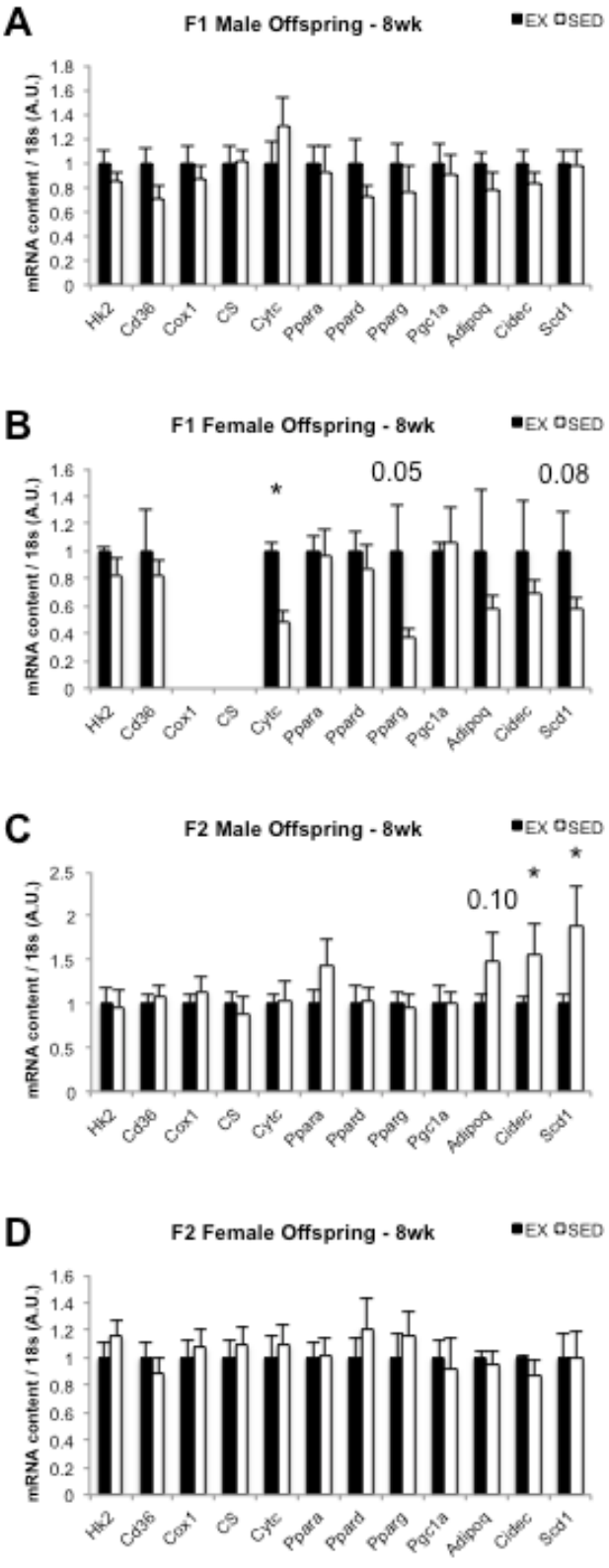
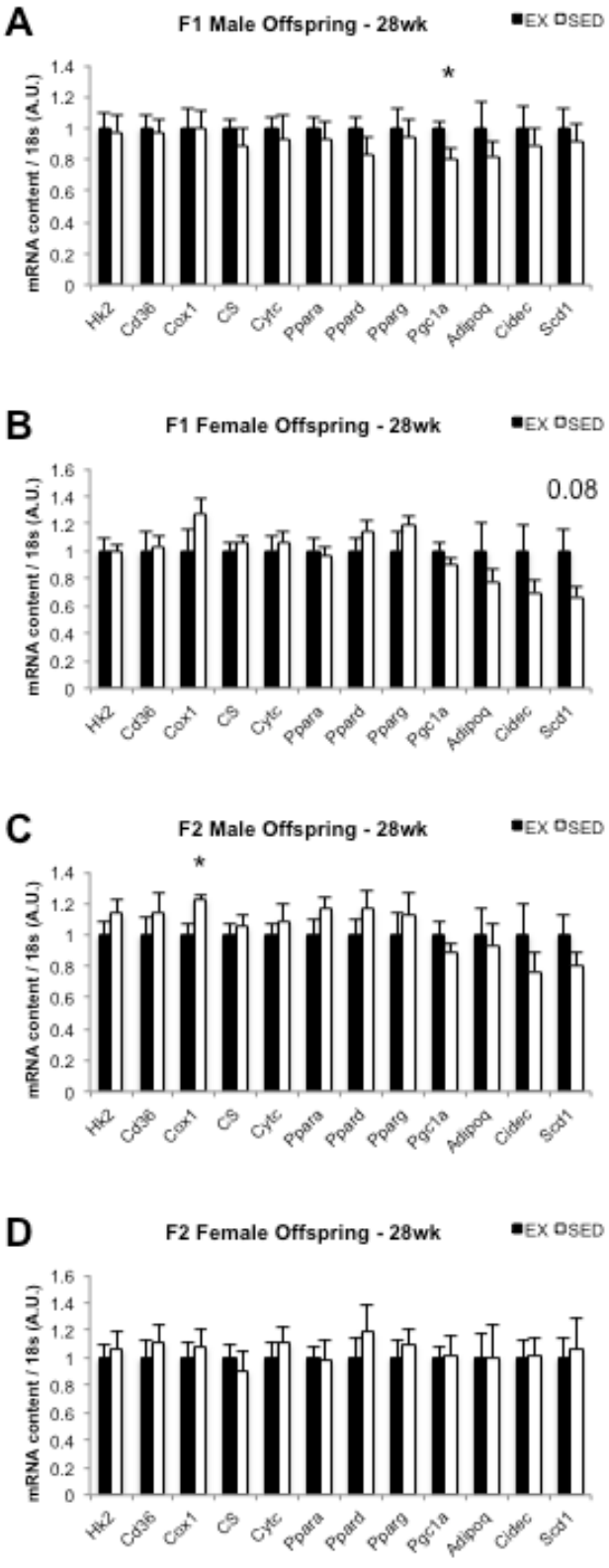

A
F1 Male Offspring - 8wk
EX SED
mRNA content / 18s (A.U.)
Hk2
Cd36
Cox1
CS
Cytc
Ppara
Ppard
Pparg
Pgc1a
Adipoq
Cidec
Scd1
B
F1 Female Offspring - 8wk
EX SED
mRNA content / 18s (A.U.)
*
0.05
0.08
C
F2 Male Offspring - 8wk
EX SED
mRNA content / 18s (A.U.)
0.10
*
*
D
F2 Female Offspring - 8wk
EX SED
mRNA content / 18s (A.U.)

Figure 4.4


A
F1 Male Offspring - 28wk
EX SED
*
mRNA content / 18s (A.U.)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Hk2
Cd36
Cox1
CS
Cytc
Ppara
Ppard
Pparg
Pgc1a
Adipoq
Cidec
Scd1
B
F1 Female Offspring - 28wk
EX SED
0.08
mRNA content / 18s (A.U.)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Hk2
Cd36
Cox1
CS
Cytc
Ppara
Ppard
Pparg
Pgc1a
Adipoq
Cidec
Scd1
C
F2 Male Offspring - 28wk
EX SED
*
mRNA content / 18s (A.U.)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Hk2
Cd36
Cox1
CS
Cytc
Ppara
Ppard
Pparg
Pgc1a
Adipoq
Cidec
Scd1
D
F2 Female Offspring - 28wk
EX SED
mRNA content / 18s (A.U.)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Hk2
Cd36
Cox1
CS
Cytc
Ppara
Ppard
Pparg
Pgc1a
Adipoq
Cidec
Scd1

Chapter 5: Effects of Exercise Ancestry on Metabolic Phenotypes in Skeletal Muscle, Liver, and Adipose Tissue over Two Generations of Mature Mouse Offspring.

Title: Effects of Exercise Ancestry on Metabolic Phenotypes in Skeletal Muscle, Liver, and Adipose Tissue over Two Generations of Mature Mouse Offspring.

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Abstract

Recent evidence suggests that parental exercise can influence metabolism in the mature offspring of rodents, though the underlying physiological mechanism remains unclear. The purpose of this study was to examine carbohydrate and lipid storage combined with related gene expression patterns in metabolically active tissues of two generations of mature mouse offspring exposed to either exercise or sedentary ancestry. F0 C57BL/6 mice were exposed to voluntary exercise or sedentary lifestyle and bred with like-exposed mates to produce an F1 generation. F1 mice of both ancestries were bred with like-exposed F1 offspring to produce an F2 generation. All offspring remained sedentary until sacrifice at 28 weeks, thus exercise ancestry was the only distinguishing feature between offspring groups. Exercise ancestry was not associated with significant differences in triglyceride or glycogen storage in skeletal muscle or liver in offspring, but there were tendencies toward lower serum TAG and glycerol in F1 male offspring of exercise compared to sedentary parents. Exercise ancestry did not significantly affect gene expression in skeletal muscle, liver, or adipose tissue. Overall, these results indicate no adverse effects of exercise ancestry on glycogen or triglyceride storage or associated gene expression through two generations of mature mouse offspring.

Introduction

The Developmental Origin of Health and Disease hypothesis states that early life events can influence adult health and disease risk (131). The effects of maternal or early life nutrition on offspring metabolic phenotypes have been well studied in both humans and animals. Parental exercise has more recently been investigated as a potential environmental influence (33, 34, 90, 205).

We previously developed a novel model to investigate the effects of voluntary parental exercise on multiple generations of mouse offspring (90). Using this model, we have demonstrated broad, small effects of parental exercise on whole-body and skeletal muscle phenotypes in two generations of mouse offspring at 8 (90) Chapter 4) and 28 (Chapter 4) weeks of age. While our previous studies have focused primarily on skeletal muscle, it is possible that other tissues may be more sensitive to developmental programming. Due to the hallmark obesity and insulin resistance observed in many models of altered maternal nutrition, offspring adipose and hepatic tissue are frequently studied.

Other researchers (33) have documented effects of perinatal maternal voluntary exercise on body composition, insulin sensitivity, and glucose dynamics in one generation of mouse offspring. They observed a more dramatic difference in glucose uptake in adipose tissue than skeletal muscle when comparing offspring of exercised and sedentary dams. This observation suggests a greater influence of adipose tissue insulin sensitivity over skeletal muscle in the enhancement of whole-body glucose disposal in the offspring of exercised dams. Limited information exists regarding the effects of parental exercise on offspring liver; however, both adipose

and hepatic tissue work in concert with skeletal muscle to maintain metabolic homeostasis by regulating the uptake, storage, and release of carbohydrate and lipid. Thus, the purpose of this study was to investigate whether exercise ancestry is associated with underlying differences in the control of carbohydrate and lipid storage and/or release. We hypothesized that mature mouse offspring with exercise ancestry would have larger skeletal muscle and liver glycogen stores along with a more glycogenic and less glycogenolytic gene expression pattern. We further hypothesized mature mouse offspring with an exercise ancestry would have lower TAG content in skeletal muscle, liver, and serum along with a less lipogenic and more lipolytic gene expression pattern in skeletal muscle, liver, and adipose tissues.

Methods

Experimental Design

The experiments for Specific Aim 3 utilized tissues from the same mice examined in Specific Aim 2, thus specific experimental design and animal handling details can be found in Chapter 4. Briefly, C57BL/6 mice (F0) were exposed to EX (computer-monitored voluntary wheel running, N=20) or a SED condition (no wheel access, N=20) for 12 weeks prior to breeding. EX males were bred with EX females and SED males were bred with SED females to obtain F1 pups. EX mice had continued access to the running wheel during breeding, pregnancy, and lactation. F1 pups were bred with like-ancestry F1 offspring to obtain F2 generation pups. F1 and F2 offspring were sacrificed at 28 weeks without EX exposure (EX ancestry was the

only distinguishing feature). A visual summary of the experimental design is provided in Figure 5.1.

Animal Numbers

For the F1 generation, we obtained a total of 10 EX male offspring, 10 SED male offspring, 10 EX female offspring, and 9 SED female offspring. For the F2 generation, we obtained a total of 11 EX male offspring, 9 SED male offspring, 10 EX female offspring, and 8 SED female offspring. Six to ten offspring per group were used for each analysis.

Tissue Harvesting and Preservation

Animals were euthanized under isoflurane anesthesia after a 6-hour fast. Approximately 1 ml of blood was obtained from a cardiac puncture and allowed to coagulate. The coagulated blood was centrifuged at 1750 x g for 15 minutes to obtain serum. Serum was removed to a fresh tube and stored at -80°C until analysis. Liver and omental fat pads were dissected, weighed, and flash frozen in liquid nitrogen and then stored at -80°C until analysis.

Serum

Serum TAG and glycerol were measured using the Serum Triglyceride Determination Kit (TR0100; Sigma-Aldrich, St. Louis, MO). Serum insulin was measured using an ultrasensitive mouse-specific ELISA kit (ALPCO).

Tissue Triglyceride

Liver and gastrocnemius muscle TAG were assessed using a method based on Rector et al. (168). Frozen samples of liver and powdered gastrocnemius muscle were weighed and homogenized in 1 ml lipid extraction solution (1:2 vol/vol methanol-chloroform) and rotated overnight at 4°C. One ml of 4 mM $MgCl_2$ was added, vortexed, and centrifuged at 1000 x g for 1 h at 4°C. The organic phase was removed, evaporated overnight, and resuspended in butanol-Triton X-114 (3:2 vol/vol). TAG content was measured using a commercially available kit (Sigma TR0100) and expressed relative to the wet tissue weight of the sample.

Glycogen Content

Glycogen content in liver and gastrocnemius was assessed by measuring the glucose released from glycogen breakdown by amyloglucosidase (132). Samples of frozen liver or powdered gastrocnemius muscle were weighed and mechanically homogenized at 4ºC in 0.03N HCl, then boiled. Four µl of homogenate was incubated for 30 minutes at room temperature with 20 µl 0.1 M acetate buffer (pH 4.7) and amyloglucosidase (10 µg/ml in 20mM Tris/0.02% BSA, pH 7.5) to hydrolyze glycogen in the sample. Following the incubation, 200 µl glucose reagent cocktail (50 mM Tris, 25 mM HCl, 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.3 mM ATP, 0.05 mM NADP, 1 U/ml hexokinase, and 0.1 U/ml glucose-6-phosphate dehydrogenase, all from Sigma) was added to each sample and allowed to stand 10 minute at room temperature. Glucose content was measured spectrophotometrically at 340 nm and

absorbance was compared to a glucose standard curve and expressed relative to the wet tissue weight of the sample.

Total Protein

Sections of frozen liver, adipose tissue, and powdered gastrocnemius muscle were mechanically homogenized in Mueller buffer (50 mM Hepes (pH 7.4), 0.1% Triton-X100, 4 mM EGTA, 10 mM EDTA, 15 mM $Na_4P_2O_7 \cdot H_2O$, 100 mM β-glycerophosphate, 25 mM NaF, 50µg/ml leupeptin, 50µg/ml pepstatin, 40µg/ml aprotinin, 5 mM Na_3VO_4, and 0.1% NP-40 and centrifuged for 10 min at 4°C and 13,000 x g. The supernatant was collected and stored at -80°C until analysis. Total protein content was determined using a BCA assay (Thermo). To examine differences in total protein banding patterns, equal amounts of protein (5 µg) from EX and SED samples within a generation and sex were loaded onto 4-20% gradient polyacrylamide gels (BioRad) and electrophoresed to separate proteins by size. After electrophoresis, the gels were be stained with GelCode Blue (Thermo) and imaged (GelDox XR, BioRad) and visually inspected for differences in banding patterns between EX and SED groups.

Gene Expression

Sections of frozen liver and adipose tissue were mechanically homogenized in TRIzol reagent (15596-026, Life Technologies, Grand Island, NY). RNA was isolated by chloroform extraction, DNase-treated, and quantified spectrophotometrically. Reverse transcription was performed with 1µg of total RNA with the High-Capacity

cDNA RT kit (4368813, Life Technologies, Grand Island, NY). Real-time quantitative PCR was used to assess the mRNA expression levels of diglyceride acyltransferase 2 (*Dgat2*), adipose triglyceride lipase (*Atgl*), hormone-sensitive lipase (*Hsl*), glucokinase (*Gck*), glycogen synthase 2 (*Gys2*), glycogen phosphorylase, liver (*Pygl*), and phosphoenolpyruvate carboxykinase (*Pepck*) in adipose, liver, and/or gastrocnemius muscle as appropriate. Primer and probe sequences were designed for each gene's mRNA sequence using the PrimeTime qPCR Assay designer (IDT). qPCR data was be normalized to expression of β-actin using the -ΔCt method (176) and expressed as fold induction ($2^{-\Delta Ct}$) of mRNA expression.

Statistical Analysis

A three-way factorial design was used to analyze the data. Specifically, a 2 (condition, EX vs. SED) x 2 (generation, F1 vs. F2), x 2 (sex, male vs. female) ANOVA was be used to determine main effects and interactions for these factors. Pre-planned contrasts were used to compare EX and SED offspring within sex and generation groups regardless of the significance of the overall ANOVA. Statistical significance was accepted at $p < 0.05$. Statistical analyses were performed using SPSS Statistics Version 21.0 (IBM).

Results

Body and tissue masses for these animals have been reported previously (see Chapter 4, Table 4.3). Briefly, there was no effect of exercise ancestry on body mass, but liver and soleus mass were lower in F1 and F2 EX males compared to SED males of the same generation.

Carbohydrate Metabolism

Fasting blood glucose and intraperitoneal glucose tolerance for these animals have been reported previously (see Chapter 4, Figure 4.2). Briefly, fasting blood glucose was higher in F1 EX females relative to F1 SED females, but no effect of exercise ancestry was observed for glucose tolerance in either sex or generation.

Fasting insulin levels and muscle and liver glycogen content were assessed to investigate the potential effect of exercise ancestry on carbohydrate metabolism. No significant differences in serum insulin were observed (Table 5.1). Similarly, no differences in muscle or liver glycogen content were observed (Figures 5.2 & 5.3).

We examined the mRNA expression of genes encoding enzymes critical to liver glycogen storage (*Gck* and *Gys2*) and breakdown (*Pygl*) (Figure 5.4). Exercise ancestry did not significantly affect the mRNA expression of any of these genes. We observed a main effect of generation for *Gys2* ($p < 0.05$, Figure 5.4B), where *Gys2* expression was higher in F1 compared to F2 offspring. *Pepck* mRNA expression was higher in females compared to male offspring; expression was also higher in F1 compared to F2 offspring ($p < 0.05$, Figure 5.4D). *Gck* and *Pygl* levels were not affected by generation or sex. It is critical to note that main effects of generation and offspring sex were observed for housekeeping gene expression in liver ($p < 0.05$, data not shown); this potentially confounds the observed differences in *Gys2* and *Pepck* expression.

Lipid Metabolism

Circulating levels of TAG and glycerol were measured in all offspring following a 6-hr fast (Figure 5.5). Preplanned contrasts between offspring of EX and SED ancestry indicated tendencies towards higher serum TAG ($p = 0.061$) and glycerol ($p = 0.079$) in F1 SED male offspring compared to F1 EX male offspring.

No significant differences in muscle triglyceride content were observed (Figure 5.6). The mRNA expression of selected genes critical for TAG synthesis (*Dgat2*) and breakdown (*Atgl* & *Hsl*) were examined in liver. *Hsl* expression was higher in female compared to male offspring ($p < 0.05$ Figure 5.7C), but no differences in mRNA expression were detected for *Dgat2* or *Atgl*. No significant differences in liver triglyceride content were observed (Figure 5.8). The mRNA expression of selected genes critical for TAG synthesis (*Dgat2*) and breakdown (*Atgl* & *Hsl*) were examined in liver. *Dgat2* expression was significantly higher in F1 compared to F2 offspring; expression was also higher in female compared to male offspring ($p < 0.05$, Figure 5.9A). No differences in liver *Atgl* or *Hsl* expression were detected. It is critical to note that main effects of generation and offspring sex were observed for housekeeping gene expression in liver ($p < 0.05$, data not shown); this potentially confounds the observed main effects in *Dgat2* expression.

The mRNA expression of selected genes critical for TAG synthesis (*Dgat2*) and breakdown (*Atgl* & *Hsl*) were also examined in adipose tissue. Significant main effects of sex and generation were observed for *Dgat2* expression in adipose tissue, where expression was higher in female compared to male offspring as well as in F2 compared to F1 offspring (both $p < 0.05$, Figure 5.10A). Preplanned contrasts also

indicated a tendency ($p = 0.09$) for higher mRNA expression of *Dgat2* in F1 SED male offspring compared to F1 EX male offspring. *Atgl* expression was also higher in female compared to male offspring ($p < 0.05$, Figure 5.10B). No differences in *Hsl* expression were observed.

Total Protein

Total protein gel images from muscle, liver, and adipose tissue are included in Supplementary Figures 5.1, 5.2, and 5.3, respectively. Visual inspection of these gels yielded one band of interest at ~75kDa that was more prominent in the skeletal muscle of F1 EX males relative to other offspring groups (Figure 5.1). Identification of this band was outside the scope of this study but a follow-up study of this protein could be of interest.

Discussion

This study was the first to investigate the effects of parental exercise on metabolite storage and associated gene expression in offspring skeletal muscle, liver, and adipose tissue. Contrary to our hypotheses, offspring glycogen and TAG storage were not affected by parental exercise, nor did parental exercise influence the expression of genes related to glycogen and TAG storage and breakdown pathways in skeletal muscle, liver, or adipose tissue.

No differences in muscle or liver glycogen were detected in the present study. This is in contrast to our hypothesis and to the greater liver glycogen stores others have previously observed in the offspring of exercise-trained rats, though this effect was observed at 28 days of age (169). We are not aware of any published data

reporting muscle glycogen content in the offspring of exercised animals. The present study is the first to examine the skeletal muscle or liver glycogen content or the expression of genes related to liver glycogen storage and breakdown and gluconeogenesis in the offspring of exercised mice. The glucokinase enzyme (encoded by *Gck*) catalyzes the critical first step towards glycogen storage by phosphorylating glucose. Glycogen synthase 2 (encoded by *Gys2*) is the enzyme responsible for most glycogen formation in the liver, which glycogen phosphorylase (encoded by *Pygl*), catalyzes the rate-limiting step of glycogen breakdown). None of these genes were differentially regulated by exercise ancestry in the present study; while many additional factors beyond transcriptional control of these rate-limiting enzymes influence glycogen storage, the lack of differential gene expression for *Gck*, *Gys2* and *Pygl* is consistent with the lack of difference in liver glycogen content between EX and SED ancestry offspring. The expression of *Pck*, which encodes phosphoenolpyruvate carboxykinase was examined based on this enyzme's role in regulating gluconeogenesis in the liver. No differences in *Pck* expression were detected between EX and SED ancestry offspring. While blood glucose was higher in F1 EX compared to F1 SED female offspring, this effect does not appear to be related to altered *Pck* expression. Differences in the translational or post-translational control of Pck or other gluconeogenic enzymes could be responsible for the higher glucose observed in F1 EX offspring; alternatively, the difference in baseline glucose could be related to tissue glucose uptake or metabolism rather than gluconeogenesis.

We observed tendencies towards lower serum TAG and glycerol in F1 EX compared to F1 SED male offspring, though these magnitude of difference is likely

not physiologically relevant. Higher serum TAG levels have been observed in the offspring of dams exposed to protein-restriction (136), high-fat diet (18) and stress (27). No differences in skeletal muscle or liver TAG were observed in the present study, refuting our hypothesis that TAG content would be lower in both skeletal muscle and liver in offspring with exercise ancestry. We did not measure TAG content in adipose tissue as TAG is the primary constituent of adipose tissue and there were no observed differences in omental adipose tissue mass (Chapter 4). The present study is the first to examine TAG content in skeletal muscle or liver or the expression of genes related to TAG storage and breakdown in the offspring of exercised mice. Diacylglycerol transferase (encoded by *Dgat2*) is critical for lipogenesis as it catalyzes the final step of TAG formation (adding a third fatty acid to diacylglycerol). Conversely, adipose triglyceride lipase and hormone-sensitive lipase (encoded by *Atgl* and *Hsl*, respectively) are responsible for catalyzing the opposing reactions leading to the fatty acid liberation from TAG. Exercise ancestry did not affect the expression of any of these TAG-related genes in offspring skeletal muscle or liver. While many additional factors beyond mRNA expression of these selected genes influence the control of TAG storage and breakdown, the lack of differential gene expression for *Dgat2*, *Atgl*, and *Hsl* is consistent with the absence of an exercise ancestry effect on skeletal muscle or liver TAG content. In adipose tissue, the only exercise ancestry-related difference we observed was a tendency for higher *Dgat2* expression in F1 SED compared to F1 EX male offspring; this tendency reflects only a 4% greater mRNA expression level for *Dgat2* in F1 SED compared to F1 EX males, which is likely not sufficient to be physiologically relevant.

Parental exercise did not affect the metabolic phenotypes examined in the present study, but Carter et al. (33) found voluntary maternal exercise significantly improved whole body glucose tolerance and insulin-stimulated glucose uptake in skeletal muscle and liver in mature mouse offspring, suggesting maternal exercise can program offspring metabolism. While the Carter et al. studies used an outbred mouse strain, we used the inbred C57Bl/6 strain of mice in the present study to control for the potentially confounding effects of parental genetic variation. Very recent evidence, however, suggests inbred animals do not respond the same as animals from outbred strains to environmental stressors during development. Maternal vinclozoin exposure resulted in higher offspring disease in an outbred, but not in an inbred mouse model (89). The causative mechanism for this differential response remained to be identified, but we speculate that the stress of continued inbreeding could reduce offspring susceptibility to temporary environmental stressors during developmentally sensitive periods.

A primary limitation of this study is the investigation of metabolic storage and gene expression in only the fasted state. Future studies should investigate the phenotypes examined in the present study in offspring exposed to an acute or chronic metabolic (i.e. diet or exercise) stress. Alternatively, tissues responses to an acute *ex vivo* stress could be investigated.

In conclusion, parental exercise does not influence the basal storage nor the transcriptional control of glycogen or TAG in skeletal muscle, liver, or adipose tissue in mouse offspring at 28 weeks of age. Our results together with the physiological findings of others using similar models continue to support a potential for parental

exercise to influence metabolically active tissues in mouse offspring, but importantly, our results indicate no adverse effects of parental exercise through multiple generations of mouse offspring.

Tables

Table 5.1. Fasting Serum Insulin (ng/ml)

Generation	Exercise (EX)		Sedentary (SED)	
	Male	Female	Male	Female
F1 Offspring	0.50 ± 0.08	0.72 ± 0.15	0.72 ± 0.11	0.56 ± 0.14
F2 Offspring	0.58 ± 0.08	0.62 ± 0.08	0.56 ± 0.11	0.59 ± 0.10

Values are means ± SE.

Figures

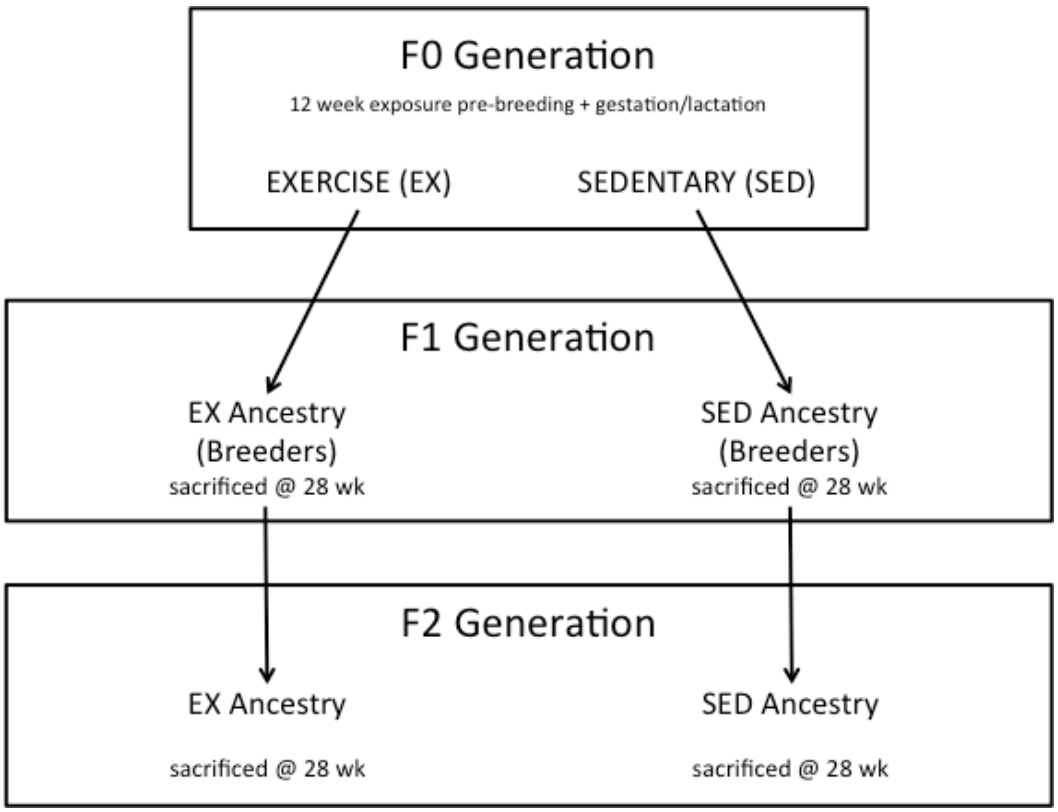


Figure 5.1. Experimental Design

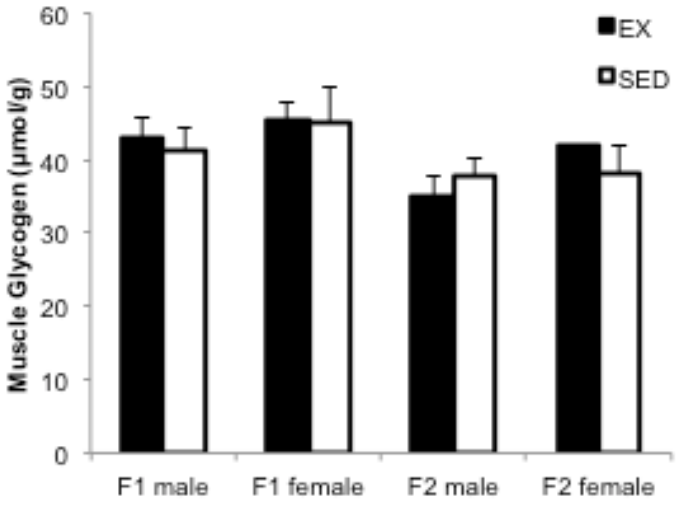


Figure 5.2. Gastrocnemius glycogen content following a 6-hour fast. Values are means ± SE expressed in µmol per gram wet weight.

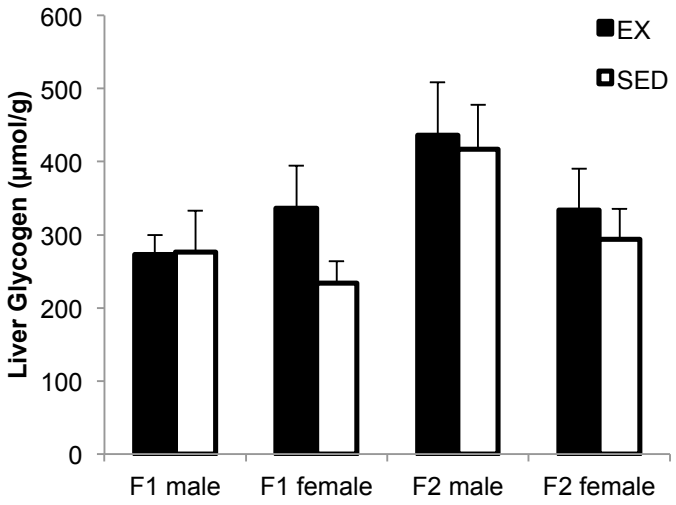


Figure 5.3. Liver glycogen content following a 6-hour fast. Values are means ± SE expressed in µmol per gram wet weight.

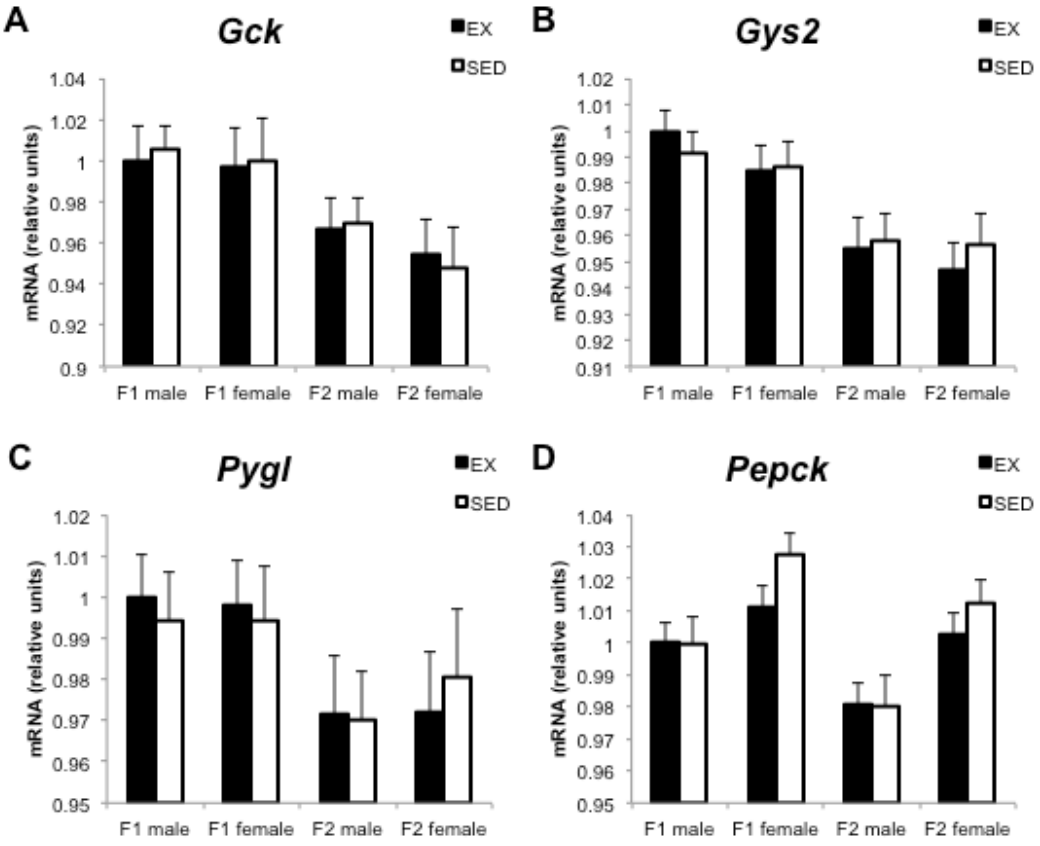


Figure 5.4. Liver mRNA levels of *Gck* (A), *Gys2* (B), *Pygl* (C), and *Pepck* (D) determined by RT-qPCR and normalized to *ActB* expression. Average expression level in F1 EX male was set to 1.0 and all other groups are expressed relative to this value. Values are means ± SE. *a significantly different from EX within sex and generation ($p < 0.05$)

Other significant findings ($p < 0.05$) (not indicated above):
B (G*ys2*): Main effect of generation – higher *Gys2* expression in F1 compared to F2 offspring.
D (*Pepck*): Main effect of offspring sex – higher expression of *Pepck* in female compared to male offspring. Main effect of generation – higher expression of *Pepck* in F1 compared to F2 offspring.

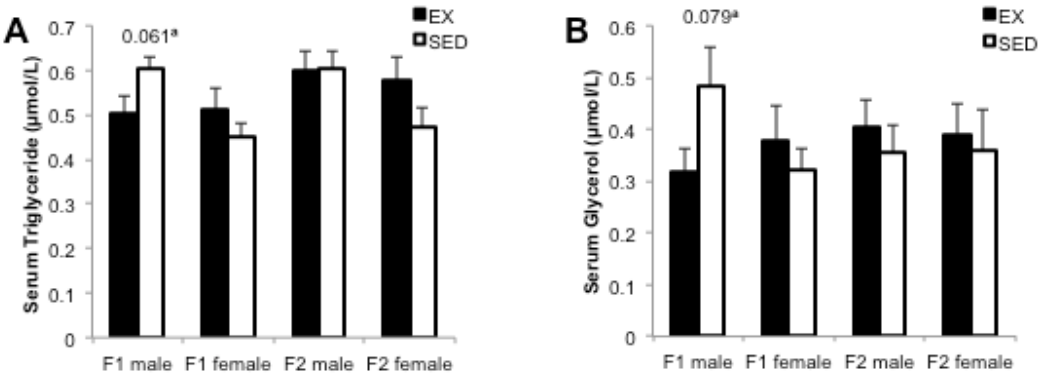


Figure 5.5. Serum triglyceride (A) and glycerol (B) content following a 6-hour fast. Values are means ± SE. [a]compared to EX within sex and generation.

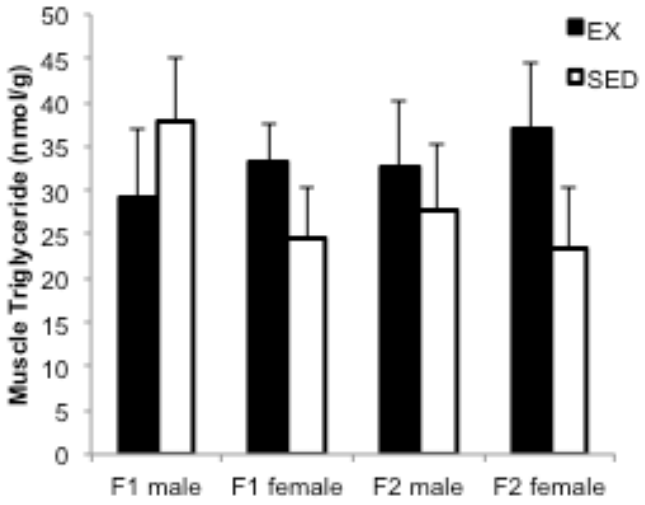


Figure 5.6. Gastrocnemius triglyceride content following a 6-hour fast. Values are means ± SE expressed in nmol per gram wet weight.

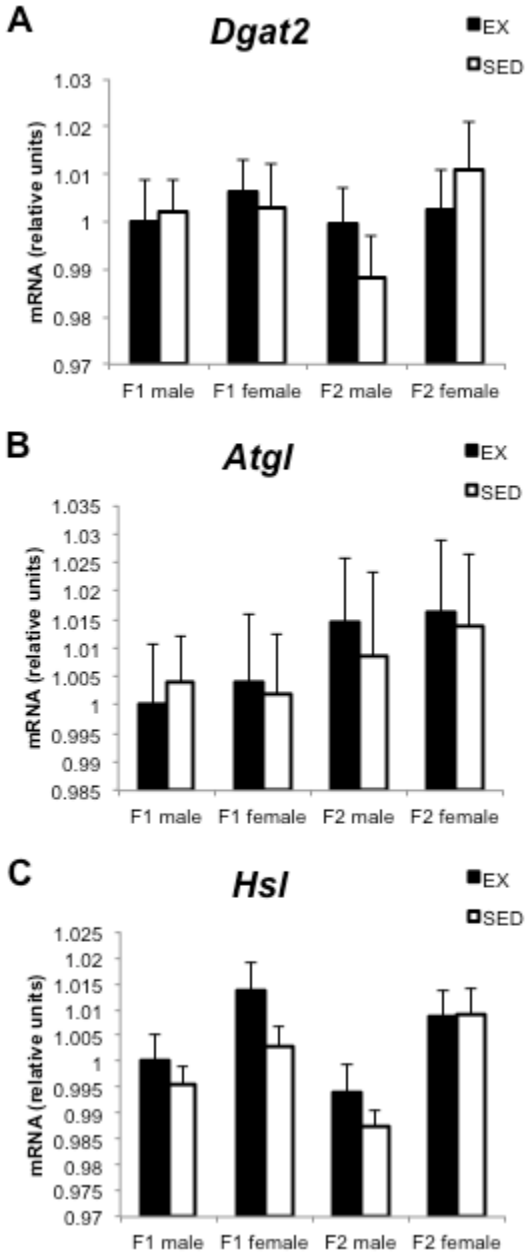


Figure 5.7. Relative gastrocnemius mRNA levels of *Dgat2* (A), *Atgl* (B), and *Hsl* (C) determined by RT-qPCR and normalized to *ActB* expression. Average expression level in F1 EX male was set to 1.0 and all other groups are expressed relative to this value. Values are means ± SE. *a significantly different from EX within sex and generation ($p < 0.05$)

Other significant ($p < 0.05$) findings (not indicated above):
C (*Hsl*): Main effect of offspring sex – higher expression of *Hsl* in female compared to male offspring.

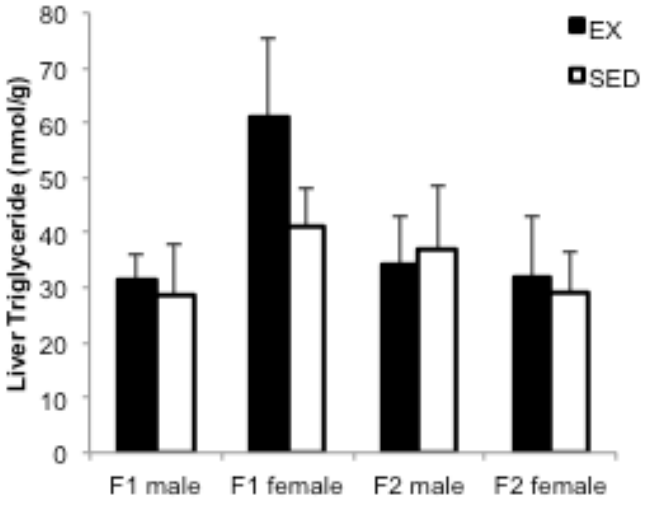


Figure 5.8. Liver triglyceride content following a 6-hour fast. Values are means ± SE expressed in nmol per gram wet weight.

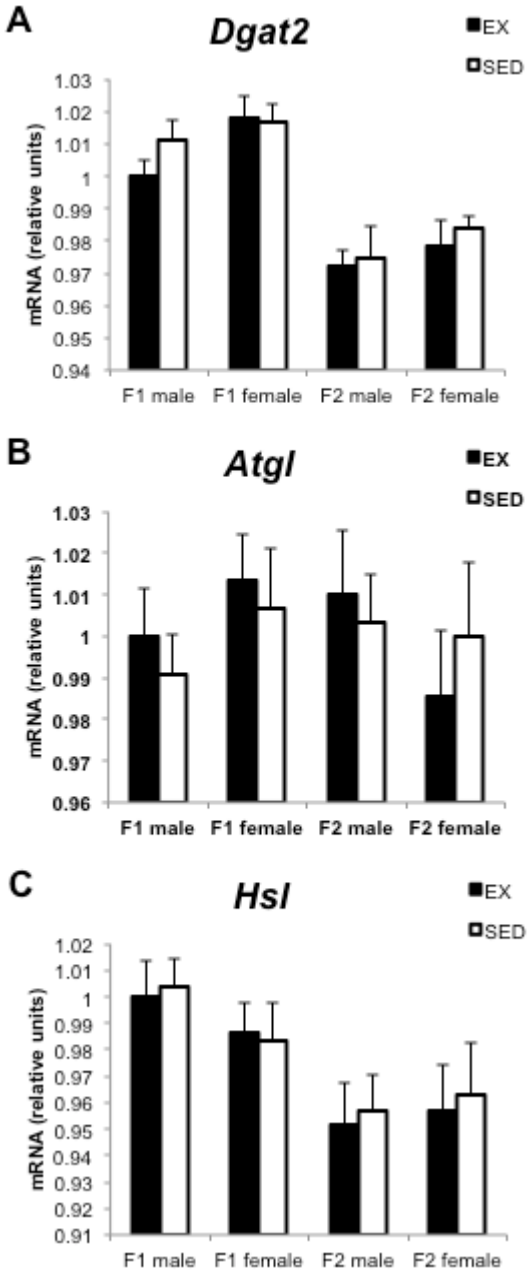


Figure 5.9. Relative liver mRNA levels of *Dgat2* (A), *Atgl* (B), and *Hsl* (C) determined by RT-qPCR and normalized to *ActB* expression. Average expression level in F1 EX male was set to 1.0 and all other groups are expressed relative to this value. Values are means ± SE. *a significantly different from EX within sex and generation ($p < 0.05$)

Other significant ($p < 0.05$) findings (not indicated above):
A (*Dgat2*): Main effect of generation – higher expression of Dgat2 in F1 compared to F2 offspring.

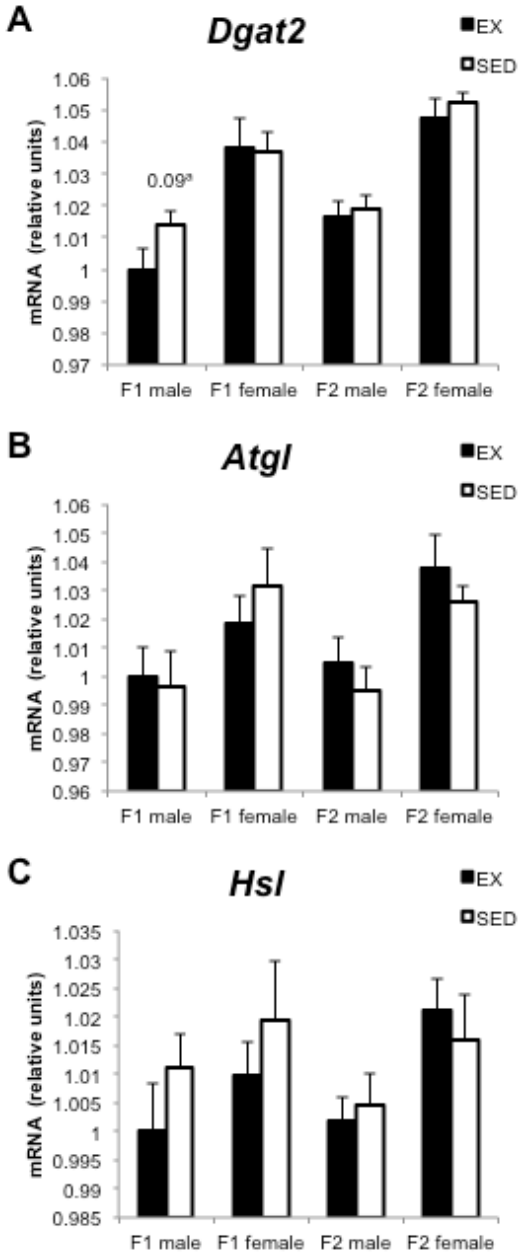
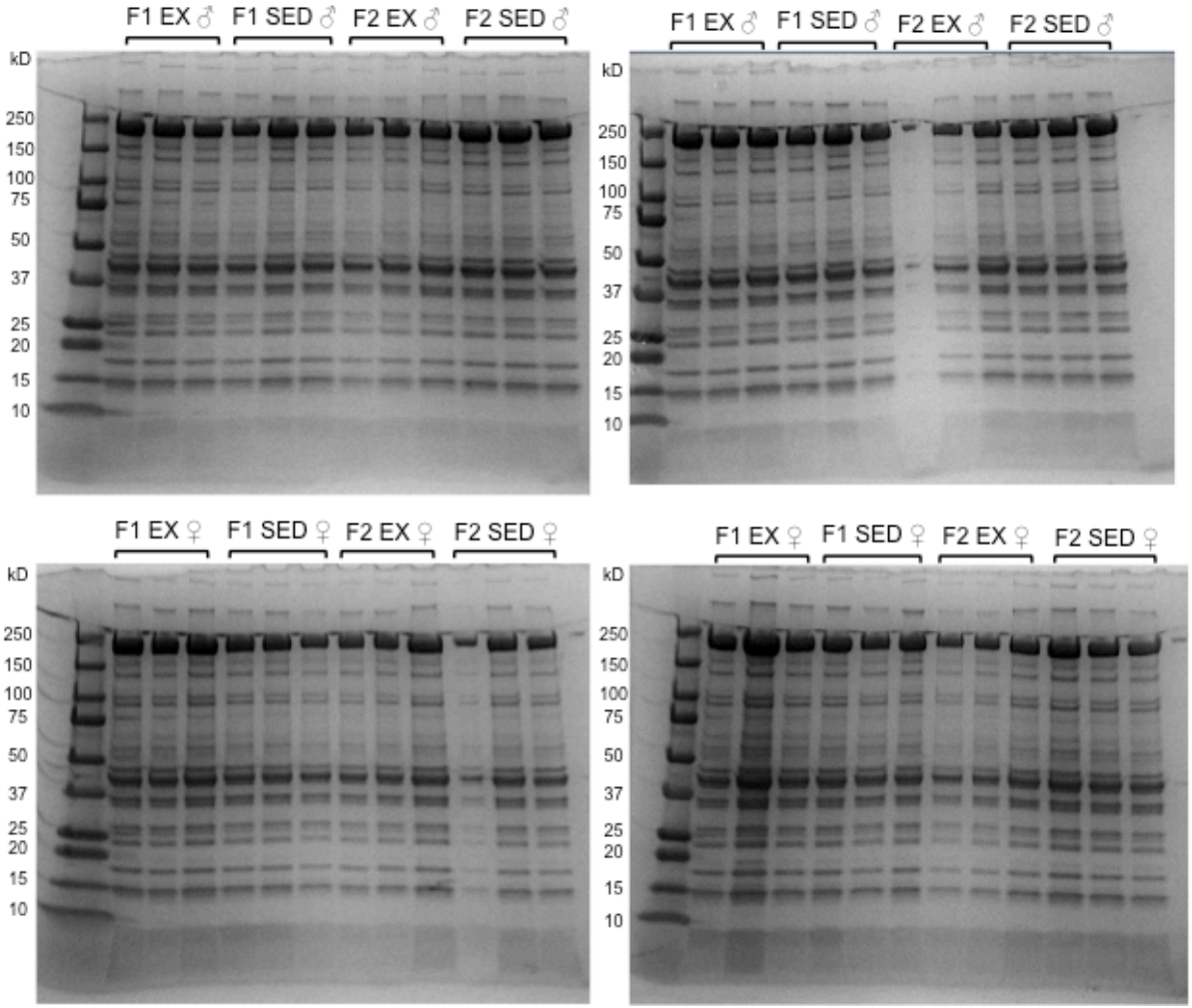


Figure 5.10. Relative adipose tissue mRNA levels of *Dgat2* (A), *Atgl* (B), and *Hsl* (C) determined by RT-qPCR and normalized to *ActB* expression. Average expression level in F1 EX male was set to 1.0 and all other groups are expressed relative to this value. Values are means ± SE. *a significantly different from EX within sex and generation ($p < 0.05$)

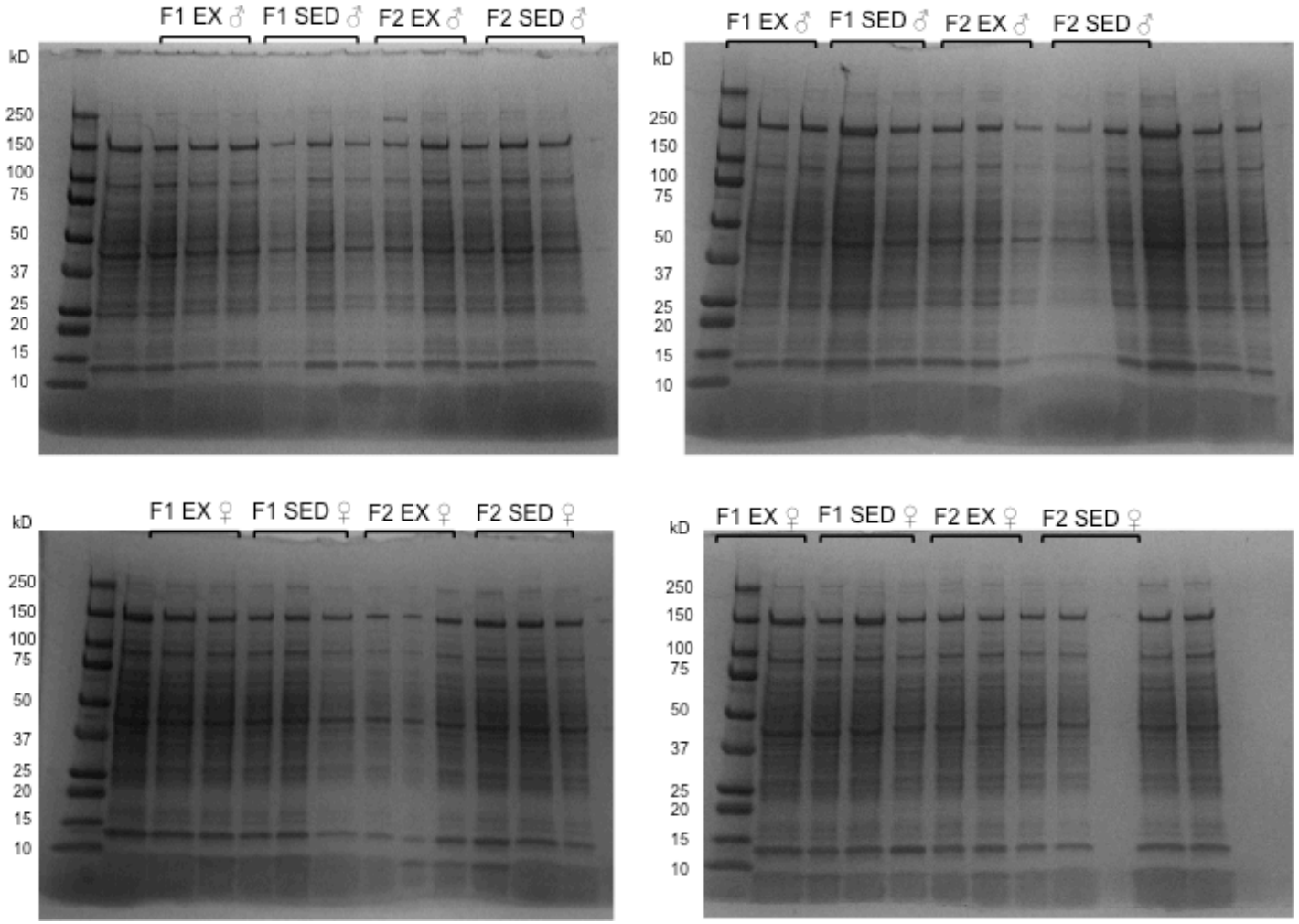
Other significant ($p < 0.05$) findings (not indicated above):
A (*Dgat2*): Significant main effects of Sex and Generation. Significantly higher expression in female compared to male offspring. Significantly higher expression in F2 compared to F1 offspring.

Supplementary Material

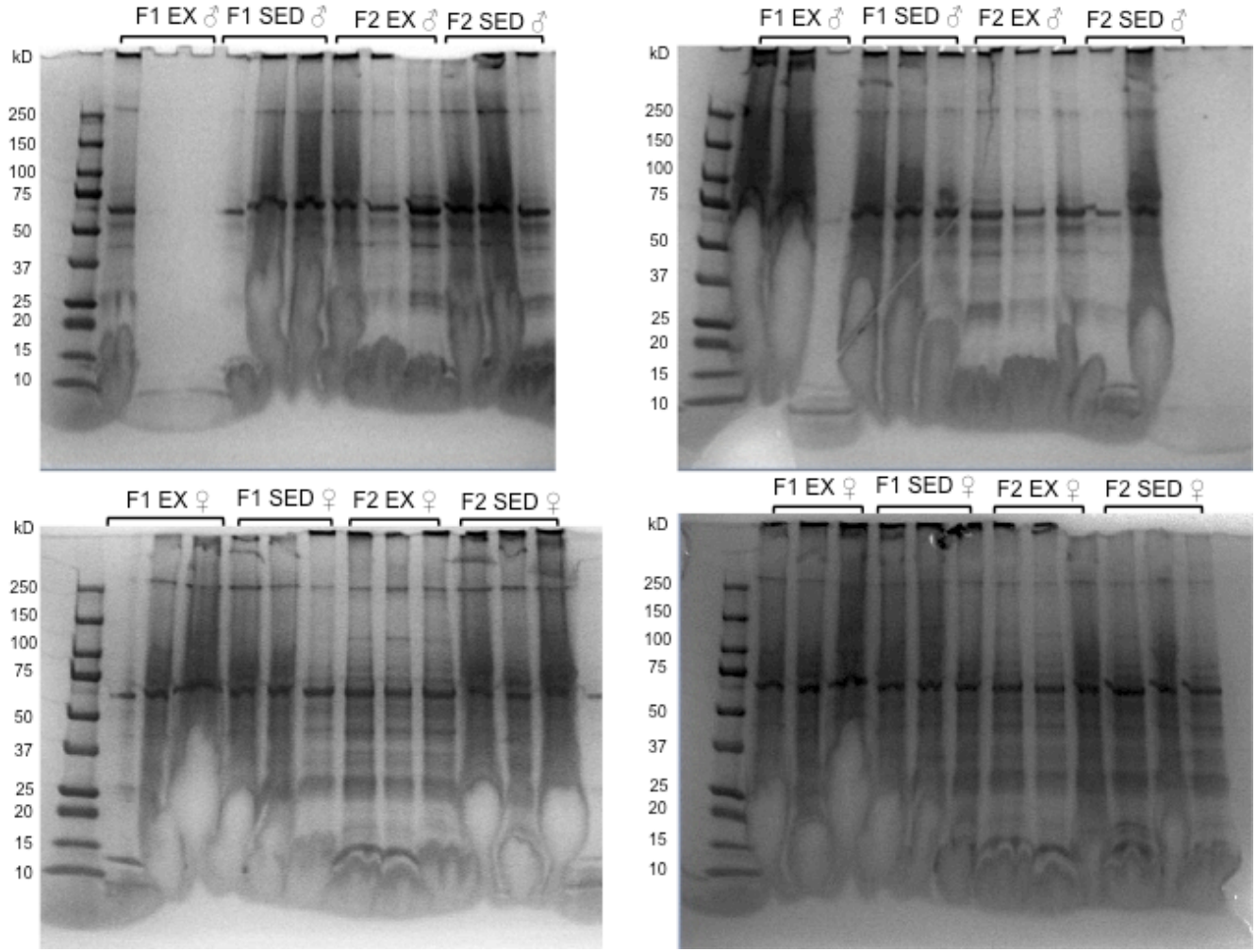
The following total protein gel images serve as supplementary material for Chapter 5 of this dissertation but will not be included in the manuscript submitted for publication.



Supplementary Figure 5.1. Gastrocnemius total protein content gel. 5 ug total protein was loaded onto 4-20% gradient gel.



Supplementary Figure 5.2. Liver total protein content gel. 5 ug total protein was loaded onto 4-20% gradient gel.



Supplementary Figure 5.3. Adipose tissue total protein content gel. 5 ug total protein was loaded onto 4-20% gradient gel.

Chapter 6: Summary, Limitations, and Future Directions

Summary

The overall aim of this dissertation research was to investigate the effect of parental exercise on metabolic health in multiple generations of mouse offspring. In Specific Aim 1 (Chapter 3), we used a novel breeding paradigm to examine the effect of parental exercise on several phenotypes from the whole body to the transcriptional level in 8-week old offspring. These included body mass and morphology, glucose tolerance, and skeletal muscle gene expression. Exposure of F0 (parent) mice to voluntary exercise was associated with lower body and fat mass, but higher fasting serum insulin in F1 female offspring. In F2 offspring, females with exercise ancestry had impaired glucose tolerance compared to F2 SED females. We also observed alterations in gene expression, including the relatively consistent pattern of upregulation of three lipogenic genes in both generations of male offspring. Interestingly, these genes were generally downregulated in female offspring, indicated a potential sex-specific effect of exercise ancestry on lipogenesis.

From this study, we concluded that EX ancestry can affect whole-body and transcription-level offspring phenotypes across two generations of young mouse offspring in a generation- and sex-dependent manner. Next, we aimed to extend these findings by investigating the same health-related phenotypes in mature offspring (Specific Aim 2, Chapter 4). We used a similar breeding paradigm, but in this study we sacrificed one subset of offspring at 8 weeks of age and a second subset of offspring at 28 weeks of age. We hypothesized that the effect of exercise ancestry would be more profound in mature offspring; however, our hypotheses were not

supported. In fact, we observed fewer differences between EX and SED offspring at 28 compared to 8 weeks of age. Further, many of our findings were not consistent with our previous observations in 8-week old offspring (90). Stanford et al. (189) observed differences in liver glycogen content and gene expression at 4 weeks and 52 weeks of age between the offspring of trained and untrained dams; interestingly, these differences were not observed at intermediate ages. While the Stanford et al. data are not published, these observations combined with our findings that more differences between EX and SED offspring existed at 8 compared to 28 weeks further support an age-specific response. We speculate overt differences may be present at birth through sexual maturity, but that these differences are managed by the offspring during adult life until a metabolic challenge is presented. Additional experiments could be designed to test this hypothesis where phenotypes of interest are compared in EX- and SED-exposed offspring at more frequent samplings of offspring age.

The final investigation of this dissertation research (Chapter 5) aimed to expand our scope of interest into metabolically active tissues beyond skeletal muscle. We examined phenotypes related to carbohydrate and lipid storage and breakdown in skeletal muscle, liver, and adipose tissue. We hypothesized that mature mouse offspring with exercise ancestry would have greater skeletal muscle and liver glycogen stores combined with a more glycogenic and less glycogenolytic gene expression pattern. We further hypothesized that mature mouse offspring with an exercise ancestry would have lower TAG content in skeletal muscle and liver combined with a less lipogenic and more lipolytic gene expression pattern in these and adipose tissues. Contrary to our hypotheses, we did not observe any differences

in glycogen or TAG content in liver or skeletal muscle, though we did observe a tendency towards lower serum TAG and glycerol in F1 EX compared to F1 SED male offspring. No differences in mRNA expression of either glycogen- or TAG-related pathway enzymes were detected.

We did not observe a consistent beneficial effect of parental exercise on glucose tolerance at either 8 or 28 weeks of age in the studies comprising this dissertation, but using a very similar model, Carter et al. (33) found voluntary maternal exercise significantly improved glucose tolerance and insulin-stimulated glucose uptake in mature mouse offspring, a result that was further supported by enhanced glucose disposal during a hyperinsulinemic-euglycemic clamp in a similar rat model (34). While the Carter et al. studies used outbred rodent strains, we used the inbred C57Bl/6 strain of mice for all of the studies comprising this dissertation. The advantage of inbred strains is that all mice of the strain are considered to be (autosomally) genetically identical. This trait was important to us as it allowed us to eliminate genetic variability while trying to elucidate the effect of an environmental stimulus (i.e., parental exercise). Very recent evidence, however, suggests inbred animals do not respond the same as animals from outbred strains to environmental stressors during development. In a study of the effects of maternal vinclozoin exposure on offspring disease risk, exposure to the drug induced adult-onset disease in an outbred but not in an inbred mouse model (89). The causative mechanism for this differential response has not been identified; however, we speculate that the stress of continued inbreeding could render the inbred rodent less susceptible to temporary environmental stressors, even during developmentally sensitive periods.

Unpublished findings from Stanford et al. indicate maternal exercise is associated with lower offspring percent body fat and fasting insulin and better glucose tolerance in male offspring at 52 weeks of age, suggesting that inbred mice may, in fact, be affected by maternal exercise, but on a longer timeframe (189).

Overall, while no overt effects of parental exercise on offspring health were consistently detected, some small differences in skeletal muscle gene expression were observed. It is possible that parental exercise is "priming" offspring metabolic physiology through alterations in gene expression. Bruce et al. (26) examined maternal high fat feeding during gestation in mice; they found while body fat and liver histology were normal in offspring weaned onto a control diet, these mice presented with similar lipogenic gene expression patterns in liver to mice weaned onto a high-fat diet that had already developed nonalcoholic steatohepatitis. By 30 weeks of age, the mice weaned onto the control diet had developed nonalcoholic fatty liver disease, an effect possibly primed by the altered lipogenic gene expression prior to measurable dysfunction (26). The skeletal muscle gene expression differences observed in our studies may indicate a similar priming of metabolic physiology through basal control of metabolic gene expression that has yet to be exposed by age or metabolic challenge. We also speculated that the detection of overt differences could be related to offspring age, and thus present from birth through sexual maturity and then suppressed during "young adult" life. If our speculation is accurate, the lack of more substantial phenotypic differences between EX and SED offspring across these three studies could be due to our testing of offspring at intermediate ages rather than closer to birth and/or at older ages. Overall, our results together with the

physiological findings of others using similar models continue to support a potential for parental exercise to influence metabolically active tissues in mouse offspring. Importantly, our results indicate no adverse effects of parental exercise through multiple generations of mouse offspring.

Limitations

In our model, both parents were exposed to exercise prior to breeding and dams continued to exercise throughout gestation and lactation. Given that these were some of the first studies of their kind when designed and implemented, we took an approach to maximize the parental exercise exposure conditions rather than narrow the exposure in a more mechanistic fashion. As a result, we cannot identify specific effects of maternal versus paternal exercise, or identify whether a critical period for exercise exposure occurs.

All of the studies in the dissertation examined the effect of voluntary exercise. Wheel running exercise was chosen because it is less stressful for the animal (129) and maternal stress alone has a negative effect on offspring metabolism (27). The use of voluntary exercise, however, imparts a number of limitations on the standardization of exercise intensity, duration, and overall volume between breeding pairs. This variability in exposure to the intervention could contribute to variability in offspring phenotypes.

Inbred mice were chosen as the model organism for all of the studies comprising this dissertation research. Here, the use of an animal model was critical for ethical and logistical limitations: mice provide a number of advantages in that

they are time- and cost-effective and share basic metabolic processes with humans. The use of inbred mice allowed us to control for genetic and confounding pre- and post-natal environmental factors that would be impossible to control for in a human study. Mice, however, pose a number of limitations in the ability to translate findings to human health. Most notably, mice and other rodents bear young in large litters in an undeveloped state, while human offspring are born in a fully developed state, and generally as singleton births. Additionally, brown adipose tissue, which is detectable only neonatally in humans, contributes substantially to metabolic regulation in rodents throughout the life cycle (30).

Future Directions

When examining our results with those of others, we argue continued research in this area is warranted. In particular, the potential limitation of strain type-specific responses should be investigated by repeating the same experimental model used here in an outbred strain of mouse. Further, offspring should be exposed to one or more acute or chronic metabolic challenges to determine if the small gene expression differences observed here do, in fact, represent a priming of metabolic physiology. Potential metabolic challenges could include an acute exercise bout, chronic exercise training, an acute glucose or lipid challenge, or a chronic high-caloric/high-fat diet. Alternatively (or in addition) tissues could be exposed to an acute metabolic stress such as muscle contraction, insulin stimulation, or lipid incubation *ex vivo*. These metabolic challenges would allow for examination of stimulated gene expression

and/or protein signaling patterns, which may shed light on the impact of phenotypes previously observed at baseline.

Lastly, the role of parental exercise as an intervention to prevent deleterious developmental programming from other stressors could be examined. Vega et al. recently studied exercise as an intervention in obese rat dams before and during pregnancy. Despite no change in maternal caloric intake or body mass, maternal exercise partially prevented the higher fat mass and serum insulin and completely prevented the higher glucose and fat cell size observed in the offspring of obese dams that did not exercise (205). An investigation of the underlying mechanisms responsible for this rescue effect would help further elucidate the role of parental exercise in developmental programming.

Appendices

UNIVERSITY OF MARYLAND
GRADUATE STUDIES AND RESEARCH
Institutional Animal Care & Use Committee

James M Dietz

Jmdietz@.umd.edu
phone: (301) 405-6949

October 17, 2007

Dr. Espen Spangenburg
Kinesiology Department
Health & Human Perf. Building
CAMPUS

Dear Dr. Spangenburg:

This letter is to inform you that the members of the Institutional Animal Care & Use Committee (IACUC), at their **May 17, 2007** meeting, reviewed your protocol:

Exercise Ancestry & Gene Expression

R-07-38

At that time the Committee requested additional information before your protocol could receive approval. You have now provided this office with that information. The approval date is **October 17, 2007.**

Please note that an approved protocol is valid for three (3) years unless there is a change in the protocol. Thus, this protocol is valid until **October 17, 2010.** Moreover, federal laws indicate that protocols must be reviewed yearly. Thus, in order to keep your approved protocol active you **MUST** submit a protocol renewal/update by the first of the month of the anniversary of your approval **(October & October 2009)**. All subsequent work extending beyond the approval date of the protocol must be submitted to the IACUC as a new protocol.

Sincerely,

James M. Dietz
Chair, IACUC
Professor, Biology

UNIVERSITY OF
MARYLAND
GRADUATE STUDIES AND RESEARCH
Institutional Animal Care & Use Committee

James M Dietz

Jmdietz@.umd.edu
phone: (301) 405-6949

October 16, 2007

Dr. Espen Spangenburg
Kinesiology Department
Health & Human Perf. Building
CAMPUS

Dear Dr. Spangenburg:

This letter is to inform you that the members of the Institutional Animal Care & Use Committee (IACUC), at their **July 19, 2007** meeting, reviewed your protocol:

Exercise Ancestry, Brain Function & Gene Expression

R-07-60

At that time the Committee requested additional information before your protocol could receive approval. You have now provided this office with that information. The approval date is **October 3, 2007.**

Please note that an approved protocol is valid for three (3) years unless there is a change in the protocol. Thus, this protocol is valid until **October 3, 2010.** Moreover, federal laws indicate that protocols must be reviewed yearly. Thus, in order to keep your approved protocol active you **MUST** submit a protocol renewal/update by the first of the month of the anniversary of your approval **(October & October 2009).** All subsequent work extending beyond the approval date of the protocol must be submitted to the IACUC as a new protocol.

Sincerely,

James M. Dietz

James M. Dietz
Chair, IACUC
Professor, Biology

UNIVERSITY OF
MARYLAND
DIVISION OF RESEARCH
INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

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W. Ray Stricklin
IACUC Chair
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Phone: (301)405-7044

September 27, 2011

Dr. Stephen Roth
Kinesiology
University of Maryland
sroth1@umd.edu

Dr. Roth,

This letter is to inform you that on **September 22, 2011** the members of the Institutional Animal Care & Use Committee (IACUC) reviewed and approved the annual review for the protocol:

Role of Maternal Exercise Environment on Transgenerational Offspring Health

R-10-93

Please note that an approved protocol is valid for three (3) years unless there is a change in the protocol. Thus, this protocol is valid until **November 18, 2013**. Federal laws indicate that protocols must be reviewed yearly. You must submit your next annual review in November, 2012. All work extending beyond the approval date of the protocol must be submitted to the IACUC as a new protocol.

Sincerely,

W. Ray Stricklin
IACUC Chair

CC: Doug Powell, Amanda Underwood

Appendix B – Statistical Outputs

Statistical Outputs for Chapter 5 are provided. A 3-way factorial ANOVA (Generation|Sex|Condition) was performed for each outcome using the factor coding below. Pre-planned contrasts (EX vs. SED) were performed within each sex and generation.

Generation
F1 offspring = 1.0
F2 offspring = 2.0

Sex
Male = 0.0
Female = 1.0

Condition
Exercise Ancestry = 0.0
Sedentary Ancestry = 1.0

ANOVA Outputs

Univariate Analysis of Variance – Serum Glycerol

Between-Subjects Factors

		N
Generation	1.0	39
	2.0	37
Sex	.0	39
	1.0	37
Condition	.0	40
	1.0	36

Descriptive Statistics

Dependent Variable: serumglycerol

Generation	Sex	Condition	Mean	Std. Deviation	N
		.0	.318930336608312	.141669463707052	10
	.0	1.0	.482036927150370	.238077069052338	10
		Total	.400483631879341	.208222698240905	20
		.0	.379663827860347	.207652821885312	10
1.0	1.0	1.0	.321141936479400	.127497625342006	9
		Total	.351942931943056	.172295804790899	19
		.0	.349297082234330	.175791767853203	20
	Total	1.0	.405823510516752	.205857952994907	19
		Total	.376835598577049	.190641589130643	39
		.0	.403270637734111	.173243582715196	10
	.0	1.0	.355756412504061	.155450865831142	9
		Total	.380763899467245	.162298375151254	19
		.0	.390728922683521	.179749825814701	10
2.0	1.0	1.0	.359256306480272	.224013112338260	8
		Total	.376741093259855	.195006101909018	18
		.0	.396999780208816	.171938869579581	20
	Total	1.0	.357403421434043	.184500131132619	17
		Total	.378806858609596	.176442370651246	37
		.0	.361100487171212	.159986613759330	20
	.0	1.0	.422219841265276	.208030889090001	19
		Total	.390876582755499	.185081763178849	39
		.0	.385196375271934	.189108633563021	20
Total	1.0	1.0	.339078110597457	.174547792708363	17
		Total	.364006902313391	.181543901634739	37
		.0	.373148431221573	.173323779108113	40
	Total	1.0	.382958468449917	.194824106524777	36
		Total	.377795290961315	.182643650692135	76

Tests of Between-Subjects Effects

Dependent Variable: serumglycerol

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.188[a]	7	.027	.787	.600
Intercept	10.699	1	10.699	314.366	.000
Generation	6.186E-005	1	6.186E-005	.002	.966
Sex	.014	1	.014	.414	.522
Condition	.001	1	.001	.023	.881
Generation * Sex	.010	1	.010	.288	.593
Generation * Condition	.040	1	.040	1.169	.283
Sex * Condition	.050	1	.050	1.466	.230
Generation * Sex * Condition	.067	1	.067	1.959	.166
Error	2.314	68	.034		
Total	13.349	76			
Corrected Total	2.502	75			

a. R Squared = .075 (Adjusted R Squared = -.020)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: serumglycerol

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
.376	.021	.334	.419

2. Generation

Dependent Variable: serumglycerol

Generation	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.0	.375	.030	.316	.434
2.0	.377	.030	.316	.438

3. Sex

Dependent Variable: serumglycerol

Sex	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	.390	.030	.331	.449
1.0	.363	.030	.302	.423

4. Condition

Dependent Variable: serumglycerol

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	.373	.029	.315	.431
1.0	.380	.031	.318	.441

5. Generation * Sex

Dependent Variable: serumglycerol

Generation	Sex	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	.400	.041	.318	.483
	1.0	.350	.042	.266	.435
2.0	.0	.380	.042	.295	.464
	1.0	.375	.044	.288	.462

6. Generation * Condition

Dependent Variable: serumglycerol

Generation	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	.349	.041	.267	.432
	1.0	.402	.042	.317	.486
2.0	.0	.397	.041	.315	.479
	1.0	.358	.045	.268	.447

7. Sex * Condition

Dependent Variable: serumglycerol

Sex	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
.0	.0	.361	.041	.279	.443
	1.0	.419	.042	.334	.503
1.0	.0	.385	.041	.303	.468
	1.0	.340	.045	.251	.430

8. Generation * Sex * Condition

Dependent Variable: serumglycerol

Generation	Sex	Condition	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	.0	.0	.319	.058	.203	.435
		1.0	.482	.058	.366	.598
	1.0	.0	.380	.058	.263	.496
		1.0	.321	.061	.198	.444
2.0	.0	.0	.403	.058	.287	.520
		1.0	.356	.061	.233	.478
	1.0	.0	.391	.058	.274	.507
		1.0	.359	.065	.229	.489

Univariate Analysis of Variance – Serum TAG

Between-Subjects Factors

		N
Generation	1.0	39
	2.0	37
Sex	.0	39
	1.0	37
Condition	.0	40
	1.0	36

Descriptive Statistics

Dependent Variable: serumTAG

Generation	Sex	Condition	Mean	Std. Deviation	N
		.0	.502215777721161	.132109264957748	10
	.0	1.0	.603219976200266	.089472225370143	10
		Total	.552717876960714	.121424109622637	20
		.0	.512826418347634	.147304027591453	10
1.0	1.0	1.0	.450754223688553	.097483134993441	9
		Total	.483423799824911	.126833406126018	19
		.0	.507521098034398	.136290119803230	20
	Total	1.0	.530999356589455	.119764332484381	19
		Total	.518959223997118	.127370036959878	39
		.0	.597404579441088	.151163786185192	10
	.0	1.0	.604304142054437	.119652434023811	9
		Total	.600672793310569	.133419460618141	19
		.0	.577041485261310	.161747262589509	10
2.0	1.0	1.0	.472054440513694	.125574878418583	8
		Total	.530380576484592	.152398636014198	18
		.0	.587223032351199	.152727359921678	20
	Total	1.0	.542068988388205	.136700451455650	17
		Total	.566476579719553	.145384091140146	37
		.0	.549810178581125	.146545245956774	20
	.0	1.0	.603733528446979	.101813205734507	19
		Total	.576080528515772	.128037114075064	39
		.0	.544933951804472	.154129773685826	20
Total	1.0	1.0	.460777855135678	.108492106050076	17
		Total	.506267637118810	.139917879796772	37
		.0	.547372065192798	.148465329165649	40
	Total	1.0	.536226682716643	.126296087239864	36
		Total	.542092673493567	.137611259036412	76

Tests of Between-Subjects Effects

Dependent Variable: serumTAG

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.254[a]	7	.036	2.114	.054
Intercept	22.026	1	22.026	1284.020	.000
Generation	.039	1	.039	2.274	.136
Sex	.102	1	.102	5.966	.017
Condition	.004	1	.004	.241	.625
Generation * Sex	.000	1	.000	.008	.929
Generation * Condition	.022	1	.022	1.292	.260
Sex * Condition	.089	1	.089	5.202	.026
Generation * Sex * Condition	.003	1	.003	.180	.672
Error	1.166	68	.017		
Total	23.754	76			
Corrected Total	1.420	75			

a. R Squared = .179 (Adjusted R Squared = .094)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: serumTAG

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
.540	.015	.510	.570

2. Generation

Dependent Variable: serumTAG

Generation	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.0	.517	.021	.475	.559
2.0	.563	.022	.520	.606

3. Sex

Dependent Variable: serumTAG

Sex	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	.577	.021	.535	.619
1.0	.503	.022	.460	.546

4. Condition

Dependent Variable: serumTAG

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	.547	.021	.506	.589
1.0	.533	.022	.489	.576

5. Generation * Sex

Dependent Variable: serumTAG

Generation	Sex	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	.553	.029	.494	.611
	1.0	.482	.030	.422	.542
2.0	.0	.601	.030	.541	.661
	1.0	.525	.031	.463	.587

6. Generation * Condition

Dependent Variable: serumTAG

Generation	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	.508	.029	.449	.566
	1.0	.527	.030	.467	.587
2.0	.0	.587	.029	.529	.646
	1.0	.538	.032	.475	.602

7. Sex * Condition

Dependent Variable: serumTAG

Sex	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
.0	.0	.550	.029	.491	.608
	1.0	.604	.030	.544	.664
1.0	.0	.545	.029	.486	.603
	1.0	.461	.032	.398	.525

8. Generation * Sex * Condition

Dependent Variable: serumTAG

Generation	Sex	Condition	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	.0	.0	.502	.041	.420	.585
		1.0	.603	.041	.521	.686
	1.0	.0	.513	.041	.430	.595
		1.0	.451	.044	.364	.538
2.0	.0	.0	.597	.041	.515	.680
		1.0	.604	.044	.517	.691
	1.0	.0	.577	.041	.494	.660
		1.0	.472	.046	.380	.564

Univariate Analysis of Variance – Muscle Glycogen

Between-Subjects Factors

		N
Generation	1.0	31
	2.0	31
Sex	.0	31
	1.0	31
Condition	.0	31
	1.0	31

Descriptive Statistics

Dependent Variable: mglycogen

Generation	Sex	Condition	Mean	Std. Deviation	N
		.0	42.908363094977630	7.805700033505358	7
	.0	1.0	41.149723317670370	9.354141475860452	8
		Total	41.970421880413750	8.407564461467505	15
		.0	45.404043658493954	6.702385997036197	8
1.0	1.0	1.0	45.103807728768494	13.433100090671456	8
		Total	45.253925693631220	10.256548019513666	16
		.0	44.239392728853005	7.087616655418660	15
	Total	1.0	43.126765523219430	11.367134261238201	16
		Total	43.665133525945350	9.400426415571427	31
		.0	35.115992284582970	7.226508401075842	8
	.0	1.0	37.673137914317586	7.095063956860495	8
		Total	36.394565099450276	7.043164386580833	16
		.0	41.959248365264500	10.017538580879764	8
2.0	1.0	1.0	38.301876556065324	9.392493409103231	7
		Total	40.252474854304886	9.568212222046057	15
		.0	38.537620324923730	9.148169709017509	16
	Total	1.0	37.966549280466540	7.942511994750059	15
		Total	38.261295625992830	8.447936448111864	31
		.0	38.752431996100476	8.271390352415482	15
	.0	1.0	39.411430615993970	8.218782762200657	16
		Total	39.092560316045520	8.112563486867360	31
		.0	43.681646011879224	8.423688263295737	16
Total	1.0	1.0	41.929573181507020	11.847779298532170	15
		Total	42.833868835892666	10.088478539654485	31
		.0	41.296542455857260	8.583525713957485	31
	Total	1.0	40.629886696080940	10.045722190049338	31
		Total	40.963214575969076	9.272462406198501	62

Tests of Between-Subjects Effects

Dependent Variable: mglycogen

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	739.320[a]	7	105.617	1.266	.285
Intercept	103631.254	1	103631.254	1242.092	.000
Generation	446.962	1	446.962	5.357	.024
Sex	187.132	1	187.132	2.243	.140
Condition	9.636	1	9.636	.115	.735
Generation * Sex	1.009	1	1.009	.012	.913
Generation * Condition	.887	1	.887	.011	.918
Sex * Condition	21.841	1	21.841	.262	.611
Generation * Sex * Condition	56.844	1	56.844	.681	.413
Error	4505.372	54	83.433		
Total	109279.759	62			
Corrected Total	5244.692	61			

a. R Squared = .141 (Adjusted R Squared = .030)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: mglycogen

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
40.952	1.162	38.622	43.282

2. Generation

Dependent Variable: mglycogen

Generation	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.0	43.641	1.643	40.347	46.936
2.0	38.263	1.643	34.968	41.557

3. Sex

Dependent Variable: mglycogen

Sex	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	39.212	1.643	35.917	42.506
1.0	42.692	1.643	39.398	45.987

4. Condition

Dependent Variable: mglycogen

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	41.347	1.643	38.052	44.642
1.0	40.557	1.643	37.263	43.852

5. Generation * Sex

Dependent Variable: mglycogen

Generation	Sex	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	42.029	2.364	37.290	46.768
	1.0	45.254	2.284	40.676	49.832
2.0	.0	36.395	2.284	31.816	40.973
	1.0	40.131	2.364	35.392	44.869

6. Generation * Condition

Dependent Variable: mglycogen

Generation	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	44.156	2.364	39.417	48.895
	1.0	43.127	2.284	38.549	47.705
2.0	.0	38.538	2.284	33.959	43.116
	1.0	37.988	2.364	33.249	42.726

7. Sex * Condition

Dependent Variable: mglycogen

Sex	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
.0	.0	39.012	2.364	34.273	43.751
	1.0	39.411	2.284	34.833	43.990
1.0	.0	43.682	2.284	39.103	48.260
	1.0	41.703	2.364	36.964	46.442

8. Generation * Sex * Condition

Dependent Variable: mglycogen

Generation	Sex	Condition	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	.0	.0	42.908	3.452	35.987	49.830
		1.0	41.150	3.229	34.675	47.624
	1.0	.0	45.404	3.229	38.929	51.879
		1.0	45.104	3.229	38.629	51.578
2.0	.0	.0	35.116	3.229	28.641	41.591
		1.0	37.673	3.229	31.199	44.148
	1.0	.0	41.959	3.229	35.485	48.434
		1.0	38.302	3.452	31.380	45.223

Univariate Analysis of Variance – Liver Glycogen

Between-Subjects Factors

		N
Generation	1.0	31
	2.0	32
Sex	.0	31
	1.0	32
Condition	.0	30
	1.0	33

Descriptive Statistics

Dependent Variable: lglycogen

Generation	Sex	Condition	Mean	Std. Deviation	N
		.0	272.870713396449700	71.538636603772390	7
	.0	1.0	276.014159671276900	161.653448249562360	8
		Total	274.547218076357500	123.539007864003600	15
		.0	335.942136047587550	165.031253809945980	8
1.0	1.0	1.0	234.041169163533800	84.869005895978900	8
		Total	284.991652605560660	137.259284683793900	16
		.0	306.508805477056500	129.891459787694700	15
	Total	1.0	255.027664417405400	126.593582295963150	16
		Total	279.937893962397900	128.726145673067400	31
		.0	435.720915520973700	192.089981500090180	7
	.0	1.0	417.247436298536740	180.907335110834100	9
		Total	425.329583458352940	179.732175848793560	16
		.0	333.491571598409300	160.723227432436120	8
2.0	1.0	1.0	293.470489586875150	117.932684598151070	8
		Total	313.481030592642200	137.740641678790600	16
		.0	381.198598762272600	177.528975805959600	15
	Total	1.0	358.999461375401840	162.800557862224800	17
		Total	369.405307025497560	167.449836620060070	32
		.0	354.295814458711600	162.887097350135750	14
	.0	1.0	350.784717885708600	181.869338390751440	17
		Total	352.370374402548630	170.707999218136280	31
		.0	334.716853822998360	157.373211413113580	16
Total	1.0	1.0	263.755829375204540	103.892072303267870	16
		Total	299.236341599101370	136.036296306276770	32
		.0	343.853702119664660	157.488460357586580	30
	Total	1.0	308.588893153342900	153.455519573787770	33
		Total	325.381659327781800	155.146825289613960	63

Tests of Between-Subjects Effects

Dependent Variable: lglycogen

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	276285.763[a]	7	39469.395	1.785	.109
Intercept	6609499.176	1	6609499.176	298.928	.000
Generation	127581.422	1	127581.422	5.770	.020
Sex	41090.416	1	41090.416	1.858	.178
Condition	24200.042	1	24200.042	1.094	.300
Generation * Sex	59756.534	1	59756.534	2.703	.106
Generation * Condition	1586.480	1	1586.480	.072	.790
Sex * Condition	15683.245	1	15683.245	.709	.403
Generation * Sex * Condition	6822.807	1	6822.807	.309	.581
Error	1216087.555	55	22110.683		
Total	8162386.445	63			
Corrected Total	1492373.319	62			

a. R Squared = .185 (Adjusted R Squared = .081)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: lglycogen

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
324.850	18.789	287.196	362.503

2. Generation

Dependent Variable: lglycogen

Generation	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.0	279.717	26.751	226.106	333.328
2.0	369.983	26.390	317.095	422.870

3. Sex

Dependent Variable: lglycogen

Sex	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	350.463	26.854	296.647	404.279
1.0	299.236	26.286	246.558	351.915

4. Condition

Dependent Variable: lglycogen

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	344.506	27.209	289.979	399.034
1.0	305.193	25.918	253.252	357.135

5. Generation * Sex

Dependent Variable: lglycogen

Generation	Sex	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	274.442	38.479	197.329	351.556
	1.0	284.992	37.174	210.493	359.490
2.0	.0	426.484	37.468	351.397	501.572
	1.0	313.481	37.174	238.982	387.980

6. Generation * Condition

Dependent Variable: lglycogen

Generation	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	304.406	38.479	227.293	381.520
	1.0	255.028	37.174	180.529	329.526
2.0	.0	384.606	38.479	307.493	461.720
	1.0	355.359	36.127	282.959	427.759

7. Sex * Condition

Dependent Variable: lglycogen

Sex	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
.0	.0	354.296	39.741	274.653	433.938
	1.0	346.631	36.127	274.231	419.030
1.0	.0	334.717	37.174	260.218	409.216
	1.0	263.756	37.174	189.257	338.254

8. Generation * Sex * Condition

Dependent Variable: lglycogen

Generation	Sex	Condition	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	.0	.0	272.871	56.202	160.239	385.502
		1.0	276.014	52.572	170.657	381.371
	1.0	.0	335.942	52.572	230.585	441.299
		1.0	234.041	52.572	128.684	339.398
2.0	.0	.0	435.721	56.202	323.090	548.352
		1.0	417.247	49.566	317.916	516.579
	1.0	.0	333.492	52.572	228.135	438.849
		1.0	293.470	52.572	188.113	398.828

Univariate Analysis of Variance – Muscle TAG

Between-Subjects Factors

		N
Generation	1.0	32
	2.0	28
Sex	.0	31
	1.0	29
Condition	.0	30
	1.0	30

Descriptive Statistics

Dependent Variable: mTAG

Generation	Sex	Condition	Mean	Std. Deviation	N
		.0	29.077184013365443	22.571682157342714	8
	.0	1.0	37.894692237738270	20.001901160269520	8
		Total	33.485938125551860	21.099592418193918	16
		.0	33.358234066839074	12.171384645731933	8
1.0	1.0	1.0	24.480363786496960	16.874942831556726	8
		Total	28.919298926668016	14.934547021803729	16
		.0	31.217709040102264	17.657243339937730	16
	Total	1.0	31.187528012117617	19.172297811070404	16
		Total	31.202618526109937	18.130651264024490	32
		.0	32.560512436899600	20.051840145144457	7
	.0	1.0	27.765341904798590	21.266906352177430	8
		Total	30.003088153112397	20.114440261965370	15
		.0	36.856009967060730	20.424952891126896	7
2.0	1.0	1.0	23.413279959723518	17.176975443268702	6
		Total	30.651673040597405	19.498156352505050	13
		.0	34.708261201980170	19.572566180317390	14
	Total	1.0	25.900172499766416	19.026601276490900	14
		Total	30.304216850873296	19.464437396450844	28
		.0	30.702737277681383	20.743539948344280	15
	.0	1.0	32.830017071268430	20.618626600547437	16
		Total	31.800688138887590	20.360151823150970	31
		.0	34.990529486942520	16.003896091820163	15
Total	1.0	1.0	24.023042146451200	16.343646848657563	14
		Total	29.695880426015670	16.828200088315850	29
		.0	32.846633382311960	18.333835942954700	30
	Total	1.0	28.720095439687057	18.963253368760242	30
		Total	30.783364410999510	18.609149277678817	60

Tests of Between-Subjects Effects

Dependent Variable: mTAG

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1477.742[a]	7	211.106	.579	.770
Intercept	55898.443	1	55898.443	153.357	.000
Generation	16.493	1	16.493	.045	.832
Sex	78.388	1	78.388	.215	.645
Condition	310.778	1	310.778	.853	.360
Generation * Sex	76.469	1	76.469	.210	.649
Generation * Condition	306.691	1	306.691	.841	.363
Sex * Condition	644.109	1	644.109	1.767	.190
Generation * Sex * Condition	75.983	1	75.983	.208	.650
Error	18953.984	52	364.500		
Total	77288.657	60			
Corrected Total	20431.726	59			

a. R Squared = .072 (Adjusted R Squared = -.053)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: mTAG

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
30.676	2.477	25.705	35.646

2. Generation

Dependent Variable: mTAG

Generation	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.0	31.203	3.375	24.430	37.975
2.0	30.149	3.627	22.871	37.426

3. Sex

Dependent Variable: mTAG

Sex	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	31.824	3.435	24.932	38.717
1.0	29.527	3.570	22.363	36.691

4. Condition

Dependent Variable: mTAG

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	32.963	3.493	25.953	39.973
1.0	28.388	3.513	21.339	35.437

5. Generation * Sex

Dependent Variable: mTAG

Generation	Sex	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	33.486	4.773	23.908	43.064
	1.0	28.919	4.773	19.342	38.497
2.0	.0	30.163	4.940	20.249	40.077
	1.0	30.135	5.311	19.478	40.792

6. Generation * Condition

Dependent Variable: mTAG

Generation	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	31.218	4.773	21.640	40.795
	1.0	31.188	4.773	21.610	40.765
2.0	.0	34.708	5.103	24.469	44.947
	1.0	25.589	5.155	15.244	35.934

7. Sex * Condition

Dependent Variable: mTAG

Sex	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
.0	.0	30.819	4.940	20.905	40.733
	1.0	32.830	4.773	23.252	42.408
1.0	.0	35.107	4.940	25.193	45.021
	1.0	23.947	5.155	13.602	34.292

8. Generation * Sex * Condition

Dependent Variable: mTAG

Generation	Sex	Condition	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	.0	.0	29.077	6.750	15.532	42.622
		1.0	37.895	6.750	24.350	51.440
	1.0	.0	33.358	6.750	19.813	46.903
		1.0	24.480	6.750	10.936	38.025
2.0	.0	.0	32.561	7.216	18.080	47.041
		1.0	27.765	6.750	14.220	41.310
	1.0	.0	36.856	7.216	22.376	51.336
		1.0	23.413	7.794	7.773	39.054

Univariate Analysis of Variance – Liver TAG

Between-Subjects Factors

		N
Generation	1.0	26
	2.0	26
Sex	.0	24
	1.0	28
Condition	.0	28
	1.0	24

Descriptive Statistics

Dependent Variable: ITAG

Generation	Sex	Condition	Mean	Std. Deviation	N
		.0	31.341600401068310	12.137002031950830	7
	.0	1.0	28.816129934279310	20.833933185883524	5
		Total	30.289321039906223	15.487985082469852	12
		.0	60.877570806833280	35.734383479022945	6
1.0	1.0	1.0	41.324183684469100	18.822059566610633	8
		Total	49.704206736910890	27.977302580900588	14
		.0	44.973586742190610	28.992828623461786	13
	Total	1.0	36.513393780549950	19.785283929121500	13
		Total	40.743490261370270	24.697963870009854	26
		.0	34.033692933051780	23.288877549320926	7
	.0	1.0	37.160362432525960	25.690773489283927	5
		Total	35.336471891166020	23.204246847946155	12
		.0	31.896907455680630	31.066162652206636	8
2.0	1.0	1.0	29.237043685111360	17.482270703869737	6
		Total	30.756965839722370	25.280200360679405	14
		.0	32.894074011787160	26.762209478908830	15
	Total	1.0	32.838552206663450	20.831293081934234	11
		Total	32.870584017311740	23.972041435355330	26
		.0	32.687646667060050	17.895933321219026	14
	.0	1.0	32.988246183402640	22.485402666935823	10
		Total	32.812896465536120	19.464912335142213	24
		.0	44.317191749031764	35.104306846566466	14
Total	1.0	1.0	36.143980827601500	18.623701041680192	14
		Total	40.230586288316630	27.886422710599433	28
		.0	38.502419208045914	27.975003712769514	28
	Total	1.0	34.829091392518635	19.909994556183385	24
		Total	36.807037139341006	24.423541854654150	52

Tests of Between-Subjects Effects

Dependent Variable: ITAG

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4759.118[a]	7	679.874	1.166	.342
Intercept	68429.834	1	68429.834	117.326	.000
Generation	710.682	1	710.682	1.218	.276
Sex	806.094	1	806.094	1.382	.246
Condition	368.056	1	368.056	.631	.431
Generation * Sex	2139.274	1	2139.274	3.668	.062
Generation * Condition	400.542	1	400.542	.687	.412
Sex * Condition	410.149	1	410.149	.703	.406
Generation * Sex * Condition	99.578	1	99.578	.171	.681
Error	25662.861	44	583.247		
Total	100869.394	52			
Corrected Total	30421.979	51			

a. R Squared = .156 (Adjusted R Squared = .022)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: ITAG

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
36.836	3.401	29.982	43.690

2. Generation

Dependent Variable: ITAG

Generation	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.0	40.590	4.809	30.897	50.283
2.0	33.082	4.809	23.389	42.775

3. Sex

Dependent Variable: ITAG

Sex	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	32.838	5.000	22.762	42.914
1.0	40.834	4.611	31.540	50.127

4. Condition

Dependent Variable: ITAG

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	39.537	4.588	30.291	48.783
1.0	34.134	5.021	24.015	44.254

5. Generation * Sex

Dependent Variable: ITAG

Generation	Sex	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	30.079	7.071	15.829	44.329
	1.0	51.101	6.521	37.958	64.244
2.0	.0	35.597	7.071	21.347	49.847
	1.0	30.567	6.521	17.424	43.710

6. Generation * Condition

Dependent Variable: ITAG

Generation	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	46.110	6.718	32.570	59.649
	1.0	35.070	6.884	21.196	48.944
2.0	.0	32.965	6.250	20.370	45.560
	1.0	33.199	7.312	18.462	47.935

7. Sex * Condition

Dependent Variable: ITAG

Sex	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
.0	.0	32.688	6.454	19.679	45.696
	1.0	32.988	7.637	17.597	48.380
1.0	.0	46.387	6.521	33.244	59.530
	1.0	35.281	6.521	22.138	48.424

8. Generation * Sex * Condition

Dependent Variable: ITAG

Generation	Sex	Condition	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	.0	.0	31.342	9.128	12.945	49.738
		1.0	28.816	10.800	7.049	50.583
	1.0	.0	60.878	9.859	41.007	80.748
		1.0	41.324	8.538	24.116	58.532
2.0	.0	.0	34.034	9.128	15.637	52.430
		1.0	37.160	10.800	15.394	58.927
	1.0	.0	31.897	8.538	14.689	49.105
		1.0	29.237	9.859	9.367	49.107

Univariate Analysis of Variance - Insulin

Between-Subjects Factors

		N
Generation	1.0	38
	2.0	35
Sex	.0	37
	1.0	36
Condition	.0	40
	1.0	33

Descriptive Statistics

Dependent Variable: insulin

Generation	Sex	Condition	Mean	Std. Deviation	N
		.0	.501950000000100	.266863099526597	10
	.0	1.0	.719800000000100	.348641745444713	10
		Total	.610875000000100	.322179742045143	20
		.0	.720800000000100	.488024031739886	10
1.0	1.0	1.0	.562125000000100	.402250432833038	8
		Total	.650277777777878	.446426735383813	18
		.0	.611375000000100	.398941160250713	20
	Total	1.0	.649722222222322	.370777609825333	18
		Total	.629539473684311	.381141624057228	38
		.0	.583300000000100	.260522359885005	10
	.0	1.0	.559642857142957	.285227645090490	7
		Total	.573558823529512	.262354774253456	17
		.0	.625950000000100	.278946176608500	10
2.0	1.0	1.0	.585062500000100	.292257523122138	8
		Total	.607777777777878	.277131324901905	18
		.0	.604625000000100	.263602665258968	20
	Total	1.0	.573200000000100	.278829492188939	15
		Total	.591157142857243	.266631927709348	35
		.0	.542625000000100	.260048521535277	20
	.0	1.0	.653852941176571	.324779481182423	17
		Total	.593729729729830	.292796492526968	37
		.0	.673375000000100	.389924988569089	20
Total	1.0	1.0	.573593750000100	.339866994511679	16
		Total	.629027777777878	.366837156251139	36
		.0	.608000000000100	.333767288037892	40
	Total	1.0	.614939393939494	.329461913522925	33
		Total	.611136986301470	.329539643757051	73

Tests of Between-Subjects Effects

Dependent Variable: insulin

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.411[a]	7	.059	.515	.820
Intercept	26.439	1	26.439	231.977	.000
Generation	.025	1	.025	.223	.638
Sex	.019	1	.019	.164	.687
Condition	3.229E-005	1	3.229E-005	.000	.987
Generation * Sex	5.324E-005	1	5.324E-005	.000	.983
Generation * Condition	.017	1	.017	.150	.699
Sex * Condition	.174	1	.174	1.524	.222
Generation * Sex * Condition	.145	1	.145	1.269	.264
Error	7.408	65	.114		
Total	35.084	73			
Corrected Total	7.819	72			

a. R Squared = .053 (Adjusted R Squared = -.050)

Estimated Marginal Means

1. Grand Mean

Dependent Variable: insulin

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
.607	.040	.528	.687

2. Generation

Dependent Variable: insulin

Generation	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.0	.626	.055	.516	.736
2.0	.588	.058	.473	.704

3. Sex

Dependent Variable: insulin

Sex	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	.591	.056	.479	.703
1.0	.623	.057	.510	.737

4. Condition

Dependent Variable: insulin

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.0	.608	.053	.501	.715
1.0	.607	.059	.488	.725

5. Generation * Sex

Dependent Variable: insulin

Generation	Sex	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	.611	.075	.460	.762
	1.0	.641	.080	.482	.801
2.0	.0	.571	.083	.405	.738
	1.0	.606	.080	.446	.765

6. Generation * Condition

Dependent Variable: insulin

Generation	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.0	.0	.611	.075	.461	.762
	1.0	.641	.080	.481	.801
2.0	.0	.605	.075	.454	.755
	1.0	.572	.087	.398	.747

7. Sex * Condition

Dependent Variable: insulin

Sex	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
.0	.0	.543	.075	.392	.693
	1.0	.640	.083	.474	.806
1.0	.0	.673	.075	.523	.824
	1.0	.574	.084	.405	.742

8. Generation * Sex * Condition

Dependent Variable: insulin

Generation	Sex	Condition	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	.0	.0	.502	.107	.289	.715
		1.0	.720	.107	.507	.933
	1.0	.0	.721	.107	.508	.934
		1.0	.562	.119	.324	.801
2.0	.0	.0	.583	.107	.370	.797
		1.0	.560	.128	.305	.814
	1.0	.0	.626	.107	.413	.839
		1.0	.585	.119	.347	.823

Pre-planned contrasts

Generation = 1.0, Sex = .0

Group Statistics[a]

	Condition	N	Mean	Std. Deviation	Std. Error Mean
Bactin_F	.0	7	1.000000000000100	.064304394662600	.024304776640889
	1.0	7	1.067543310475806	.091094988445845	.034430669301778
ATGL_B_F	.0	7	1.000000000000100	.026557184924215	.010037672404552
	1.0	7	.996636127815681	.031675478059184	.011972205372017
DGAT_B_F	.0	7	1.000000000000100	.017488282168248	.006609949353621
	1.0	7	1.014144917402574	.010669075292003	.004032531420300
HSL_B_F	.0	7	1.000000000000100	.022045381025052	.008332370821484
	1.0	7	1.011176650725896	.015445193853625	.005837734555473
Bactin_L	.0	7	1.000000000000100	.073596846945222	.027816993470854
	1.0	7	1.002716171092484	.051747591581476	.019558751181652
ATGL_L	.0	7	1.000000000000100	.029909040591162	.011304554765312
	1.0	7	.990991389350246	.024925364812586	.009420902376014
DGAT_L	.0	7	1.000000000000100	.013741792628059	.005193909408928
	1.0	7	1.011355158003877	.015412642503295	.005825431301500
GCK_L	.0	7	1.000000000000100	.044512556710106	.016824165039291
	1.0	7	1.005983760465029	.030166597211674	.011401902017654
GYS_L	.0	7	1.000000000000100	.020956824471719	.007920935117462
	1.0	7	.991765806134653	.020582440374365	.007779431229403
HSL_L	.0	7	1.000000000000100	.037029684018791	.013995905005923
	1.0	7	1.003864628436157	.028794724910419	.010883383026274
PEPCK_L	.0	7	1.000000000000100	.017026974865587	.006435591582075
	1.0	7	.999696152824187	.021990754483271	.008311723929407
PYGL_L	.0	7	1.000000000000100	.026928741039486	.010178107415853
	1.0	7	.994430870323691	.031160261193012	.011777471700709
Bactin_M	.0	8	1.000000000000100	.033811449458879	.011954152597124
	1.0	8	1.004311636428912	.037069474041256	.013106038234860
ATGL_M	.0	8	1.000000000000100	.030684512323720	.010848613370817
	1.0	8	1.003971107606967	.023415687325203	.008278695646962
DGAT_M	.0	8	1.000000000000100	.024743329825582	.008748088154467
	1.0	8	1.002029147044994	.018736465629224	.006624340951011
HK_M	.0	8	1.000000000000100	.008293585476718	.002932225265533
	1.0	8	1.001842809182919	.015644286619299	.005531090577731
HSL_M	.0	8	1.000000000000100	.014154049508470	.005004212194409
	1.0	8	.995407415187559	.010064312551086	.003558271826492

a. Generation = 1.0, Sex = .0

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Bactin_F	Equal variances assumed	.817	.384	-1.603	12	.135	-.06754331047580	.042144906645152
	Equal variances not assumed			-1.603	10.790	.138	-.06754331047580	.042144906645152
ATGL_B_F	Equal variances assumed	.604	.452	.215	12	.833	.003363872184519	.015623334111821
	Equal variances not assumed			.215	11.646	.833	.003363872184519	.015623334111821
DGAT_B_F	Equal variances assumed	1.264	.283	-1.827	12	.093	-.01414491740257	.007742915478846
	Equal variances not assumed			-1.827	9.923	.098	-.01414491740257	.007742915478846
HSL_B_F	Equal variances assumed	1.904	.193	-1.099	12	.294	-.01117665072589	.010173865943980
	Equal variances not assumed			-1.099	10.747	.296	-.01117665072589	.010173865943980
Bactin_L	Equal variances assumed	.966	.345	-.080	12	.938	-.00271617109248	.034004850735456
	Equal variances not assumed			-.080	10.767	.938	-.00271617109248	.034004850735456
ATGL_L	Equal variances assumed	.111	.744	.612	12	.552	.009008610649954	.014715514262816
	Equal variances not assumed			.612	11.622	.552	.009008610649954	.014715514262816
DGAT_L	Equal variances assumed	.555	.471	-1.455	12	.171	-.01135515800387	.007804636109135
	Equal variances not assumed			-1.455	11.845	.172	-.01135515800387	.007804636109135
GCK_L	Equal variances assumed	4.475	.056	-.294	12	.773	-.00598376046502	.020323776688595
	Equal variances not assumed			-.294	10.551	.774	-.00598376046502	.020323776688595
GYS_L	Equal variances assumed	.015	.904	.742	12	.473	.008234193865547	.011102286403581
	Equal variances not assumed			.742	11.996	.473	.008234193865547	.011102286403581
HSL_L	Equal variances assumed	.419	.529	-.218	12	.831	-.00386462843615	.017729449597491
	Equal variances not assumed			-.218	11.313	.831	-.00386462843615	.017729449597491
PEPCK_L	Equal variances assumed	.361	.559	.029	12	.977	.000303847176013	.010511973824601

	Equal variances not assumed			.029	11.292	.977	.000303847176013	.010511973824601
PYGL_L	Equal variances assumed	.163	.693	.358	12	.727	.005569129676509	.015566075620669
	Equal variances not assumed			.358	11.753	.727	.005569129676509	.015566075620669
Bactin_M	Equal variances assumed	.282	.604	-.243	14	.811	-.00431163642891	.017738940287613
	Equal variances not assumed			-.243	13.883	.812	-.00431163642891	.017738940287613
ATGL_M	Equal variances assumed	1.573	.230	-.291	14	.775	-.00397110760696	.013646582490990
	Equal variances not assumed			-.291	13.088	.776	-.00397110760696	.013646582490990
DGAT_M	Equal variances assumed	4.973	.043	-.185	14	.856	-.00202914704499	.010973191850719
	Equal variances not assumed			-.185	13.041	.856	-.00202914704499	.010973191850719
HK_M	Equal variances assumed	5.837	.030	-.294	14	.773	-.00184280918292	.006260264210595
	Equal variances not assumed			-.294	10.647	.774	-.00184280918292	.006260264210595
HSL_M	Equal variances assumed	1.596	.227	.748	14	.467	.004592584812641	.006140312539065
	Equal variances not assumed			.748	12.637	.468	.004592584812641	.006140312539065

a. Generation = 1.0, Sex = .0

Generation = 1.0, Sex = 1.0

Group Statistics[a]

	Condition	N	Mean	Std. Deviation	Std. Error Mean
Bactin_F	.0	8	.993353028065093	.040244618542039	.014228621338735
	1.0	7	1.008055252386596	.043070980164195	.016279300319813
ATGL_B_F	.0	8	1.018301532728459	.026794334261449	.009473227726889
	1.0	7	1.031191071889615	.034617172409869	.013084061327028
DGAT_B_F	.0	8	1.037827632696701	.027523188205545	.009730916510072
	1.0	7	1.036762671673279	.016627296822186	.006284527481028
HSL_B_F	.0	8	1.009771069315450	.016287367502822	.005758454004526
	1.0	7	1.019286073055554	.027354111613206	.010338882380583
Bactin_L	.0	8	.898215256797683	.035035619393439	.012386962028151
	1.0	8	.900541819049234	.058873414942672	.020814895468851
ATGL_L	.0	8	1.013422323810248	.031695530908170	.011206062419302
	1.0	8	1.006854469343553	.041078360202201	.014523393529564
DGAT_L	.0	8	1.018223300423874	.017830919940986	.006304182202598
	1.0	8	1.016858936951206	.014649475050985	.005179371574752
GCK_L	.0	8	.997213036561659	.053747182349335	.019002498554507
	1.0	8	.999901868499849	.059013865231677	.020864552144739
GYS_L	.0	8	.984833921632115	.026742907854379	.009455045746304
	1.0	8	.986378209690991	.027126619483947	.009590708293948
HSL_L	.0	8	.986391840644081	.032399022539453	.011454784270796
	1.0	8	.983341598315252	.040425437121493	.014292550360584
PEPCK_L	.0	8	1.011361391000865	.019230890845221	.006799146662522
	1.0	8	1.027505287634216	.019526449838364	.006903642546668
PYGL_L	.0	8	.998147963714333	.030776504030762	.010881137350748
	1.0	8	.994062220059818	.037689459909899	.013325236340839
Bactin_M	.0	8	1.017361388888912	.029141924402926	.010303226181132
	1.0	8	1.000434564338897	.033857787907480	.011970535712742
ATGL_M	.0	8	1.003953274646893	.034119265857380	.012062982128495
	1.0	8	1.002019179959337	.029603918133326	.010466565630948
DGAT_M	.0	8	1.006313703148757	.019122151715779	.006760701574617
	1.0	8	1.002953659540020	.025593730023973	.009048750027969
HK_M	.0	8	.991945863203459	.014946769719915	.005284481112958
	1.0	8	.989376103941930	.012547146775283	.004436086284737
HSL_M	.0	8	1.013843645669117	.014372153445719	.005081323580926
	1.0	8	1.002839275152262	.011325522344625	.004004176825247

a. Generation = 1.0, Sex = 1.0

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Bactin_F	Equal variances assumed	.001	.975	-.683	13	.506	-.014702224321603	.021516059638269
	Equal variances not assumed			-.680	12.444	.509	-.014702224321603	.021621037997793
ATGL_B_F	Equal variances assumed	.706	.416	-.812	13	.431	-.012889539161256	.015864930089235
	Equal variances not assumed			-.798	11.282	.441	-.012889539161256	.016153473446094
DGAT_B_F	Equal variances assumed	.291	.599	.089	13	.931	.001064961023522	.011976525133489
	Equal variances not assumed			.092	11.685	.928	.001064961023522	.011583869033483
HSL_B_F	Equal variances assumed	.281	.605	-.832	13	.420	-.009515003740204	.011435226908104
	Equal variances not assumed			-.804	9.515	.441	-.009515003740204	.011834368652401
Bactin_L	Equal variances assumed	4.565	.051	-.096	14	.925	-.002326562251650	.024221822839420
	Equal variances not assumed			-.096	11.405	.925	-.002326562251650	.024221822839420
ATGL_L	Equal variances assumed	.285	.602	.358	14	.726	.006567854466795	.018344067012482
	Equal variances not assumed			.358	13.154	.726	.006567854466795	.018344067012482
DGAT_L	Equal variances assumed	.428	.524	.167	14	.870	.001364363472767	.008158958460014
	Equal variances not assumed			.167	13.492	.870	.001364363472767	.008158958460014
GCK_L	Equal variances assumed	.334	.572	-.095	14	.925	-.002688831938290	.028220993737149
	Equal variances not assumed			-.095	13.879	.925	-.002688831938290	.028220993737149
GYS_L	Equal variances assumed	.023	.881	-.115	14	.910	-.001544288058976	.013467723476638
	Equal variances not assumed			-.115	13.997	.910	-.001544288058976	.013467723476638
HSL_L	Equal variances assumed	1.024	.329	.167	14	.870	.003050242328930	.018316360951314
	Equal variances not assumed			.167	13.366	.870	.003050242328930	.018316360951314
PEPCK_L	Equal variances assumed	.013	.911	-1.666	14	.118	-.016143896633451	.009689616904183

	Equal variances not assumed			-1.666	13.997	.118	-.016143896633451	.009689616904183
PYGL_L	Equal variances assumed	1.993	.180	.237	14	.816	.004085743654615	.017203519220893
	Equal variances not assumed			.237	13.462	.816	.004085743654615	.017203519220893
Bactin_M	Equal variances assumed	.065	.803	1.072	14	.302	.016926824550114	.015793992370148
	Equal variances not assumed			1.072	13.696	.302	.016926824550114	.015793992370148
ATGL_M	Equal variances assumed	.884	.363	.121	14	.905	.001934094687656	.015970739930824
	Equal variances not assumed			.121	13.727	.905	.001934094687656	.015970739930824
DGAT_M	Equal variances assumed	3.471	.084	.297	14	.770	.003360043608836	.011295439913912
	Equal variances not assumed			.297	12.958	.771	.003360043608836	.011295439913912
HK_M	Equal variances assumed	.520	.483	.372	14	.715	.002569759261628	.006899608840962
	Equal variances not assumed			.372	13.592	.715	.002569759261628	.006899608840962
HSL_M	Equal variances assumed	.527	.480	1.701	14	.111	.011004370516955	.006469411208247
	Equal variances not assumed			1.701	13.274	.112	.011004370516955	.006469411208247

a. Generation = 1.0, Sex = 1.0

Generation = 2.0, Sex = .0

Group Statistics[a]

	Condition	N	Mean	Std. Deviation	Std. Error Mean
Bactin_F	.0	6	1.036359849485396	.062848961062169	.025657980911120
	1.0	8	1.029891198020192	.078023448868659	.027585454893360
ATGL_B_F	.0	6	1.004513890937867	.023159551466762	.009454847294275
	1.0	8	.994947730997337	.022728803067811	.008035845388816
DGAT_B_F	.0	6	1.016533014020804	.011626990929492	.004746699170263
	1.0	8	1.018676551550157	.013715732832303	.004849243847397
HSL_B_F	.0	6	1.001779379050077	.010038579692783	.004098232998323
	1.0	8	1.004594477801851	.015397049956505	.005443679217321
Bactin_L	.0	8	.870623191286473	.070089213824291	.024780279191660
	1.0	8	.869448083956214	.038268300727422	.013529887474488
ATGL_L	.0	8	1.010115858669212	.043297436760819	.015307955570850
	1.0	8	1.003163565868960	.032887593452653	.011627520173703
DGAT_L	.0	8	.972402493638986	.012818301620105	.004531953999500
	1.0	8	.974815664207705	.026727874903579	.009449730795578
GCK_L	.0	8	.966870299758867	.041675630761936	.014734560561061
	1.0	8	.969563649709480	.034968626528267	.012363276473523
GYS_L	.0	8	.955186337466962	.033163125093200	.011724935319435
	1.0	8	.958201310473710	.028193966878124	.009968072584100
HSL_L	.0	8	.951462513795181	.046122562984348	.016306788526033
	1.0	8	.957186509110302	.037433897202469	.013234881279118
PEPCK_L	.0	8	.980926643906309	.018712570013606	.006615892575089
	1.0	8	.980449370950800	.027529804679639	.009733255786922
PYGL_L	.0	8	.971382996771378	.040070634104512	.014167108550937
	1.0	8	.970327781963943	.032303877692607	.011421145487596
Bactin_M	.0	8	1.001871218990701	.035645573216510	.012602613270403
	1.0	8	.974777707379136	.029019860909740	.010260070219248
ATGL_M	.0	8	1.014373590873626	.032453352977956	.011473992981541
	1.0	8	1.008696075975440	.040888730307212	.014456349237234
DGAT_M	.0	8	.999460517195259	.021115901866744	.007465598700487
	1.0	8	.988387604212467	.024908189744401	.008806374937738
HK_M	.0	8	.994510932826376	.016386586255399	.005793533130910
	1.0	8	.993249656528409	.012372882052486	.004374474401132
HSL_M	.0	8	.994151662718090	.014657703580344	.005182280799207
	1.0	8	.987638164268174	.008006691252768	.002830792839914

a. Generation = 2.0, Sex = .0

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Bactin_F	Equal variances assumed	.980	.342	.166	12	.871	.006468651465303	.038933034870899
	Equal variances not assumed			.172	11.891	.867	.006468651465303	.037673456253785
ATGL_B_F	Equal variances assumed	.174	.684	.773	12	.454	.009566159940630	.012372417631957
	Equal variances not assumed			.771	10.805	.457	.009566159940630	.012408422481121
DGAT_B_F	Equal variances assumed	.651	.436	-.308	12	.763	-.002143537529453	.006959577524000
	Equal variances not assumed			-.316	11.745	.758	-.002143537529453	.006785743798872
HSL_B_F	Equal variances assumed	2.836	.118	-.388	12	.705	-.002815098751874	.007251304025369
	Equal variances not assumed			-.413	11.853	.687	-.002815098751874	.006813894417225
Bactin_L	Equal variances assumed	5.232	.038	.042	14	.967	.001175107330359	.028233315283312
	Equal variances not assumed			.042	10.833	.968	.001175107330359	.028233315283312
ATGL_L	Equal variances assumed	1.267	.279	.362	14	.723	.006952292800352	.019223234096984
	Equal variances not assumed			.362	13.060	.723	.006952292800352	.019223234096984
DGAT_L	Equal variances assumed	4.981	.042	-.230	14	.821	-.002413170568819	.010480268086350
	Equal variances not assumed			-.230	10.058	.823	-.002413170568819	.010480268086350
GCK_L	Equal variances assumed	.894	.360	-.140	14	.891	-.002693349950713	.019234289175500
	Equal variances not assumed			-.140	13.590	.891	-.002693349950713	.019234289175500
GYS_L	Equal variances assumed	.427	.524	-.196	14	.847	-.003014973006847	.015389495745006
	Equal variances not assumed			-.196	13.647	.848	-.003014973006847	.015389495745006
HSL_L	Equal variances assumed	1.732	.209	-.273	14	.789	-.005723995315221	.021001748367776
	Equal variances not assumed			-.273	13.431	.789	-.005723995315221	.021001748367776
PEPCK_L	Equal variances assumed	1.336	.267	.041	14	.968	.000477272955609	.011768870072265

	Equal variances not assumed			.041	12.330	.968	.000477272955609	.011768870072265
PYGL_L	Equal variances assumed	1.502	.241	.058	14	.955	.001055214807536	.018197514361621
	Equal variances not assumed			.058	13.397	.955	.001055214807536	.018197514361621
Bactin_M	Equal variances assumed	.360	.558	1.667	14	.118	.027093511611665	.016250996958523
	Equal variances not assumed			1.667	13.447	.119	.027093511611665	.016250996958523
ATGL_M	Equal variances assumed	2.975	.107	.308	14	.763	.005677514898285	.018456395861810
	Equal variances not assumed			.308	13.314	.763	.005677514898285	.018456395861810
DGAT_M	Equal variances assumed	1.259	.281	.959	14	.354	.011072912982892	.011545016392357
	Equal variances not assumed			.959	13.635	.354	.011072912982892	.011545016392357
HK_M	Equal variances assumed	1.667	.218	.174	14	.865	.001261276298067	.007259549051045
	Equal variances not assumed			.174	13.024	.865	.001261276298067	.007259549051045
HSL_M	Equal variances assumed	.389	.543	1.103	14	.289	.006513498450016	.005905033647992
	Equal variances not assumed			1.103	10.836	.294	.006513498450016	.005905033647992

a. Generation = 2.0, Sex = .0

Generation = 2.0, Sex = 1.0

Group Statistics[a]

	Condition	N	Mean	Std. Deviation	Std. Error Mean
Bactin_F	.0	6	1.078192405137686	.085470752794388	.034893288713027
	1.0	7	1.068933773437087	.098147319312739	.037096199821370
ATGL_B_F	.0	6	1.038051121158411	.027758008532666	.011332159530201
	1.0	7	1.025734452935959	.016096666769139	.006083968172665
DGAT_B_F	.0	6	1.047249700784456	.015168882773683	.006192670460662
	1.0	7	1.052581731889449	.007327763230741	.002769634167906
HSL_B_F	.0	6	1.021251400079305	.013358547763253	.005453604287487
	1.0	7	1.015998535056647	.020823141041205	.007870407530098
Bactin_L	.0	8	.844263833677950	.042075545878379	.014875951906428
	1.0	7	.848620766417655	.045368798187672	.017147793898128
ATGL_L	.0	8	.985411631608985	.045217951992477	.015986960242689
	1.0	7	.999933171303813	.046711643374241	.017655341671402
DGAT_L	.0	8	.978392846271543	.022486860896215	.007950305913721
	1.0	7	.983882518207711	.009381774361515	.003545977402504
GCK_L	.0	8	.954058609983800	.048143471310848	.017021287516945
	1.0	7	.947719975755863	.053627458089369	.020269273935635
GYS_L	.0	8	.946586014246090	.029508301930956	.010432760198404
	1.0	7	.956284175102703	.033098305085559	.012509983439224
HSL_L	.0	8	.956949435588711	.048594948085329	.017180908661337
	1.0	7	.962960972173394	.051039999138144	.019291306376702
PEPCK_L	.0	8	1.002580029382289	.019754246116744	.006984180693254
	1.0	7	1.012693447055715	.019320176230839	.007302340227597
PYGL_L	.0	8	.972027008528020	.041540200029995	.014686678566592
	1.0	7	.980346389391121	.044978658437832	.017000334933179
Bactin_M	.0	8	1.011041111905017	.046466472419400	.016428378872853
	1.0	8	1.028636647070024	.051286365168399	.018132468296557
ATGL_M	.0	8	1.016414931092114	.035188504491547	.012441015072958
	1.0	8	1.013841175632985	.035758265379806	.012642455966829
DGAT_M	.0	8	1.002354040975030	.023904195203136	.008451409263537
	1.0	8	1.010792279359233	.028507451387654	.010078906095343
HK_M	.0	8	.989347918067758	.010628012143611	.003757569728705
	1.0	8	.987531000319498	.013524304866751	.004781563841121
HSL_M	.0	8	1.008520547925025	.014320139738795	.005062933958485
	1.0	8	1.009069524894072	.013648536290185	.004825486282095

a. Generation = 2.0, Sex = 1.0

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Bactin_F	Equal variances assumed	.052	.823	.180	11	.861	.009258631700699	.051518229728597
	Equal variances not assumed			.182	10.990	.859	.009258631700699	.050928083003324
ATGL_B_F	Equal variances assumed	2.220	.164	.999	11	.339	.012316668222552	.012334880554786
	Equal variances not assumed			.958	7.760	.367	.012316668222552	.012862056925042
DGAT_B_F	Equal variances assumed	.894	.365	-.828	11	.425	-.005332031105093	.006437254965492
	Equal variances not assumed			-.786	6.968	.458	-.005332031105093	.006783807253892
HSL_B_F	Equal variances assumed	.338	.573	.530	11	.607	.005252865022758	.009915263465732
	Equal variances not assumed			.549	10.296	.595	.005252865022758	.009575234431259
Bactin_L	Equal variances assumed	.640	.438	-.193	13	.850	-.004356932739804	.022578812527547
	Equal variances not assumed			-.192	12.406	.851	-.004356932739804	.022701118489915
ATGL_L	Equal variances assumed	.284	.603	-.611	13	.552	-.014521539694928	.023762435164401
	Equal variances not assumed			-.610	12.608	.553	-.014521539694928	.023817934153349
DGAT_L	Equal variances assumed	4.396	.056	-.600	13	.559	-.005489671936269	.009154937565987
	Equal variances not assumed			-.631	9.618	.543	-.005489671936269	.008705246685778
GCK_L	Equal variances assumed	.349	.565	.241	13	.813	.006338634228038	.026264706389014
	Equal variances not assumed			.239	12.232	.815	.006338634228038	.026468239356069
GYS_L	Equal variances assumed	.091	.767	-.600	13	.559	-.009698160856713	.016156110403717
	Equal variances not assumed			-.595	12.193	.562	-.009698160856713	.016289326904625
HSL_L	Equal variances assumed	.621	.445	-.234	13	.819	-.006011536584783	.025742052516273
	Equal variances not assumed			-.233	12.534	.820	-.006011536584783	.025832888420516
PEPCK_L	Equal variances assumed	.088	.772	-.999	13	.336	-.010113417673527	.010120727563252

	Equal variances not assumed			-1.001	12.810	.335	-.010113417673527	.010104600573736
PYGL_L	Equal variances assumed	.285	.602	-.372	13	.716	-.008319380863201	.022338060006247
	Equal variances not assumed			-.370	12.385	.717	-.008319380863201	.022465749824051
Bactin_M	Equal variances assumed	.031	.864	-.719	14	.484	-.017595535165108	.024467898130277
	Equal variances not assumed			-.719	13.866	.484	-.017595535165108	.024467898130277
ATGL_M	Equal variances assumed	.064	.803	.145	14	.887	.002573755459229	.017737264414709
	Equal variances not assumed			.145	13.996	.887	.002573755459229	.017737264414709
DGAT_M	Equal variances assumed	.569	.463	-.642	14	.532	-.008438238384303	.013153351915670
	Equal variances not assumed			-.642	13.587	.532	-.008438238384303	.013153351915670
HK_M	Equal variances assumed	.099	.757	.299	14	.770	.001816917748360	.006081338917731
	Equal variances not assumed			.299	13.259	.770	.001816917748360	.006081338917731
HSL_M	Equal variances assumed	.027	.871	-.078	14	.939	-.000548976969146	.006994184593367
	Equal variances not assumed			-.078	13.968	.939	-.000548976969146	.006994184593367

a. Generation = 2.0, Sex = 1.0

ANOVA

Tests of Between-Subjects Effects

Dependent Variable: Bactin_F

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.054[a]	7	.008	1.468	.201
Intercept	59.404	1	59.404	11236.692	.000
Generation	.018	1	.018	3.417	.071
Sex	.000	1	.000	.036	.851
Condition	.004	1	.004	.725	.399
Generation * Sex	.019	1	.019	3.540	.066
Generation * Condition	.008	1	.008	1.572	.216
Sex * Condition	.003	1	.003	.507	.480
Generation * Sex * Condition	.002	1	.002	.410	.525
Error	.254	48	.005		
Total	60.142	56			
Corrected Total	.308	55			

a. R Squared = .176 (Adjusted R Squared = .056)

Tests of Between-Subjects Effects

Dependent Variable: ATGL_B_F

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.014[a]	7	.002	2.716	.019
Intercept	56.949	1	56.949	79991.992	.000
Generation	.000	1	.000	.356	.553
Sex	.012	1	.012	16.703	.000
Condition	.000	1	.000	.186	.668
Generation * Sex	.000	1	.000	.160	.691
Generation * Condition	.001	1	.001	1.200	.279
Sex * Condition	.000	1	.000	.222	.640
Generation * Sex * Condition	.000	1	.000	.439	.511
Error	.034	48	.001		
Total	57.530	56			
Corrected Total	.048	55			

a. R Squared = .284 (Adjusted R Squared = .179)

Tests of Between-Subjects Effects

Dependent Variable: DGAT_B_F

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.016[a]	7	.002	8.517	.000
Intercept	58.567	1	58.567	217165.844	.000
Generation	.002	1	.002	6.885	.012
Sex	.014	1	.014	50.227	.000
Condition	.000	1	.000	1.357	.250
Generation * Sex	1.511E-005	1	1.511E-005	.056	.814
Generation * Condition	2.720E-005	1	2.720E-005	.101	.752
Sex * Condition	.000	1	.000	.464	.499
Generation * Sex * Condition	.000	1	.000	1.087	.302
Error	.013	48	.000		
Total	59.191	56			
Corrected Total	.029	55			

a. R Squared = .554 (Adjusted R Squared = .489)

Tests of Between-Subjects Effects

Dependent Variable: HSL_B_F

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.003[a]	7	.000	1.241	.300
Intercept	56.591	1	56.591	166127.874	.000
Generation	9.952E-006	1	9.952E-006	.029	.865
Sex	.002	1	.002	6.043	.018
Condition	.000	1	.000	.847	.362
Generation * Sex	.000	1	.000	.429	.515
Generation * Condition	.000	1	.000	1.360	.249
Sex * Condition	8.198E-005	1	8.198E-005	.241	.626
Generation * Sex * Condition	3.554E-005	1	3.554E-005	.104	.748
Error	.016	48	.000		
Total	57.182	56			
Corrected Total	.019	55			

a. R Squared = .153 (Adjusted R Squared = .030)

Tests of Between-Subjects Effects

Dependent Variable: Bactin_L

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.201[a]	7	.029	10.108	.000
Intercept	49.676	1	49.676	17458.754	.000
Generation	.129	1	.129	45.302	.000
Sex	.060	1	.060	21.041	.000
Condition	6.420E-005	1	6.420E-005	.023	.881
Generation * Sex	.023	1	.023	8.199	.006
Generation * Condition	3.287E-006	1	3.287E-006	.001	.973
Sex * Condition	2.510E-005	1	2.510E-005	.009	.926
Generation * Sex * Condition	3.328E-005	1	3.328E-005	.012	.914
Error	.151	53	.003		
Total	49.986	61			
Corrected Total	.352	60			

a. R Squared = .572 (Adjusted R Squared = .515)

Tests of Between-Subjects Effects

Dependent Variable: ATGL_L

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.005[a]	7	.001	.484	.842
Intercept	60.896	1	60.896	42446.032	.000
Generation	.000	1	.000	.106	.746
Sex	1.732E-006	1	1.732E-006	.001	.972
Condition	6.086E-005	1	6.086E-005	.042	.838
Generation * Sex	.003	1	.003	2.166	.147
Generation * Condition	.001	1	.001	.354	.554
Sex * Condition	.001	1	.001	.378	.541
Generation * Sex * Condition	.000	1	.000	.240	.626
Error	.076	53	.001		
Total	61.257	61			
Corrected Total	.081	60			

a. R Squared = .060 (Adjusted R Squared = -.064)

Tests of Between-Subjects Effects

Dependent Variable: DGAT_L

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.021[a]	7	.003	9.437	.000
Intercept	60.078	1	60.078	192924.048	.000
Generation	.018	1	.018	57.160	.000
Sex	.001	1	.001	4.585	.037
Condition	.000	1	.000	.976	.328
Generation * Sex	7.134E-005	1	7.134E-005	.229	.634
Generation * Condition	4.138E-006	1	4.138E-006	.013	.909
Sex * Condition	8.826E-005	1	8.826E-005	.283	.597
Generation * Sex * Condition	.000	1	.000	.761	.387
Error	.017	53	.000		
Total	60.343	61			
Corrected Total	.037	60			

a. R Squared = .555 (Adjusted R Squared = .496)

Tests of Between-Subjects Effects

Dependent Variable: GCK_L

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.028[a]	7	.004	1.825	.102
Intercept	58.360	1	58.360	26630.908	.000
Generation	.026	1	.026	11.775	.001
Sex	.002	1	.002	.820	.369
Condition	2.399E-005	1	2.399E-005	.011	.917
Generation * Sex	.001	1	.001	.288	.594
Generation * Condition	.000	1	.000	.066	.799
Sex * Condition	.000	1	.000	.066	.799
Generation * Sex * Condition	3.124E-005	1	3.124E-005	.014	.905
Error	.116	53	.002		
Total	58.722	61			
Corrected Total	.144	60			

a. R Squared = .194 (Adjusted R Squared = .088)

Tests of Between-Subjects Effects

Dependent Variable: GYS_L

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.022[a]	7	.003	4.014	.001
Intercept	57.439	1	57.439	73773.699	.000
Generation	.020	1	.020	26.243	.000
Sex	.001	1	.001	1.177	.283
Condition	3.443E-005	1	3.443E-005	.044	.834
Generation * Sex	9.560E-005	1	9.560E-005	.123	.727
Generation * Condition	.000	1	.000	.459	.501
Sex * Condition	.000	1	.000	.330	.568
Generation * Sex * Condition	9.094E-006	1	9.094E-006	.012	.914
Error	.041	53	.001		
Total	57.683	61			
Corrected Total	.063	60			

a. R Squared = .346 (Adjusted R Squared = .260)

Tests of Between-Subjects Effects

Dependent Variable: HSL_L

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.022[a]	7	.003	1.907	.087
Intercept	57.778	1	57.778	34465.338	.000
Generation	.020	1	.020	11.910	.001
Sex	.000	1	.000	.296	.589
Condition	.000	1	.000	.089	.766
Generation * Sex	.002	1	.002	1.167	.285
Generation * Condition	.000	1	.000	.068	.796
Sex * Condition	4.169E-005	1	4.169E-005	.025	.875
Generation * Sex * Condition	4.924E-005	1	4.924E-005	.029	.865
Error	.089	53	.002		
Total	58.051	61			
Corrected Total	.111	60			

a. R Squared = .201 (Adjusted R Squared = .096)

Tests of Between-Subjects Effects

Dependent Variable: PEPCK_L

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.014[a]	7	.002	4.698	.000
Intercept	60.977	1	60.977	142832.239	.000
Generation	.004	1	.004	8.522	.005
Sex	.008	1	.008	19.257	.000
Condition	.001	1	.001	1.443	.235
Generation * Sex	.000	1	.000	.482	.490
Generation * Condition	3.653E-005	1	3.653E-005	.086	.771
Sex * Condition	.001	1	.001	1.625	.208
Generation * Sex * Condition	3.256E-005	1	3.256E-005	.076	.783
Error	.023	53	.000		
Total	61.255	61			
Corrected Total	.037	60			

a. R Squared = .383 (Adjusted R Squared = .301)

Tests of Between-Subjects Effects

Dependent Variable: PYGL_L

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.009[a]	7	.001	.974	.460
Intercept	58.948	1	58.948	44944.994	.000
Generation	.008	1	.008	6.200	.016
Sex	6.764E-005	1	6.764E-005	.052	.821
Condition	5.425E-006	1	5.425E-006	.004	.949
Generation * Sex	.000	1	.000	.120	.730
Generation * Condition	.000	1	.000	.207	.651
Sex * Condition	.000	1	.000	.085	.771
Generation * Sex * Condition	5.910E-005	1	5.910E-005	.045	.833
Error	.070	53	.001		
Total	59.235	61			
Corrected Total	.078	60			

a. R Squared = .114 (Adjusted R Squared = -.003)

Tests of Between-Subjects Effects

Dependent Variable: Bactin_M

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.014[a]	7	.002	1.375	.234
Intercept	64.616	1	64.616	45284.699	.000
Generation	3.342E-005	1	3.342E-005	.023	.879
Sex	.006	1	.006	4.103	.048
Condition	.000	1	.000	.343	.561
Generation * Sex	.002	1	.002	1.720	.195
Generation * Condition	9.717E-006	1	9.717E-006	.007	.935
Sex * Condition	.001	1	.001	.385	.537
Generation * Sex * Condition	.004	1	.004	3.046	.086
Error	.080	56	.001		
Total	64.710	64			
Corrected Total	.094	63			

a. R Squared = .147 (Adjusted R Squared = .040)

Tests of Between-Subjects Effects

Dependent Variable: ATGL_M

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.002[a]	7	.000	.290	.955
Intercept	65.016	1	65.016	59292.086	.000
Generation	.002	1	.002	1.716	.196
Sex	8.442E-005	1	8.442E-005	.077	.782
Condition	3.862E-005	1	3.862E-005	.035	.852
Generation * Sex	2.689E-005	1	2.689E-005	.025	.876
Generation * Condition	.000	1	.000	.097	.757
Sex * Condition	7.848E-006	1	7.848E-006	.007	.933
Generation * Sex * Condition	8.116E-005	1	8.116E-005	.074	.787
Error	.061	56	.001		
Total	65.080	64			
Corrected Total	.064	63			

a. R Squared = .035 (Adjusted R Squared = -.086)

Tests of Between-Subjects Effects

Dependent Variable: DGAT_M

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.002[a]	7	.000	.600	.753
Intercept	64.197	1	64.197	115816.837	.000
Generation	.000	1	.000	.191	.663
Sex	.001	1	.001	1.910	.172
Condition	1.573E-005	1	1.573E-005	.028	.867
Generation * Sex	.000	1	.000	.588	.446
Generation * Condition	1.700E-006	1	1.700E-006	.003	.956
Sex * Condition	.000	1	.000	.360	.551
Generation * Sex * Condition	.001	1	.001	1.119	.295
Error	.031	56	.001		
Total	64.230	64			
Corrected Total	.033	63			

a. R Squared = .070 (Adjusted R Squared = -.047)

Tests of Between-Subjects Effects

Dependent Variable: HSL_M

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.004[a]	7	.001	3.822	.002
Intercept	64.184	1	64.184	392933.666	.000
Generation	.000	1	.000	.989	.324
Sex	.003	1	.003	19.943	.000
Condition	.000	1	.000	2.846	.097
Generation * Sex	.000	1	.000	1.292	.261
Generation * Condition	9.278E-005	1	9.278E-005	.568	.454
Sex * Condition	4.234E-007	1	4.234E-007	.003	.960
Generation * Sex * Condition	.000	1	.000	1.111	.296
Error	.009	56	.000		
Total	64.197	64			
Corrected Total	.014	63			

a. R Squared = .323 (Adjusted R Squared = .239)

References

1. **Aiken CE**, **Ozanne SE**. Sex differences in developmental programming models. *Reproduction* 145: R1–13, 2013.

2. **Aksu I**, **Baykara B**, **Ozbal S**, **Cetin F**, **Sisman AR**, **Dayi A**, **Gencoglu C**, **Tas A**, **Büyük E**, **Gonenc-Arda S**, **Uysal N**. Maternal treadmill exercise during pregnancy decreases anxiety and increases prefrontal cortex VEGF and BDNF levels of rat pups in early and late periods of life. *Neurosci. Lett.* 516: 221–225, 2012.

3. **Aldoretta PW**, **Carver TD**, **Hay WW**. Ovine uteroplacental glucose and oxygen metabolism in relation to chronic changes in maternal and fetal glucose concentrations. *Placenta* 15: 753–764, 1994.

4. **Allen DL**, **Harrison BC**, **Maass A**, **Bell ML**, **Byrnes WC**, **Leinwand LA**. Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. *J. Appl. Physiol.* 90: 1900–1908, 2001.

5. **Alwan A**, **Alwan A**. *Global status report on noncommunicable diseases 2010.* World Health Organization, 2011.

6. **Anderson D**, **Schmid TE**, **Baumgartner A**. Male-mediated developmental toxicity. *Asian J. Androl.* 16: 81–88, 2014.

7. **Anderson LM**, **Riffle L**, **Wilson R**, **Travlos GS**, **Lubomirski MS**, **Alvord WG**. Preconceptional fasting of fathers alters serum glucose in offspring of mice. *Nutrition* 22: 327–331, 2006.

8. **Ashino NG**, **Saito KN**, **Souza FD**, **Nakutz FS**, **Roman EA**, **Velloso LA**, **Torsoni AS**, **Torsoni MA**. Maternal high-fat feeding through pregnancy and lactation predisposes mouse offspring to molecular insulin resistance and fatty liver. *The Journal of Nutritional Biochemistry* 23: 341–348, 2012.

9. **Augusto V**, **Padovani CR**, **Campos GER**. Skeletal muscle fiber types in C57BL6J mice. *Braz. J. Morphol. Sci* 21: 89–94, 2004.

10. **Baldwin KM**. Effects of chronic exercise on biochemical and functional properties of the heart. *Med Sci Sports Exerc* 17: 522–528, 1985.

11. **Barker DJ**, **Osmond C**, **Golding J**, **Kuh D**, **Wadsworth ME**. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ* 298: 564–567, 1989.

12. **Barnes SK**, **Ozanne SE**. Pathways linking the early environment to long-term health and lifespan. *Progress in Biophysics and Molecular Biology* 106: 323–336, 2011.

13. **Barry JA**, **Kay AR**, **Navaratnarajah R**, **Iqbal S**, **Bamfo JEAK**, **David AL**, **Hines M**, **Hardiman PJ**. Umbilical vein testosterone in female infants born to mothers with polycystic ovary syndrome is elevated to male levels. *J Obstet Gynaecol* 30: 444–446, 2010.

14. **Bayol SA**, **Macharia R**, **Farrington SJ**, **Simbi BH**, **Stickland NC**. Evidence that a maternal "junk food" diet during pregnancy and lactation can reduce muscle force in offspring. *Eur J Nutr* 48: 62–65, 2009.

15. **Bayol SA**, **Simbi BH**, **Bertrand JA**, **Stickland NC**. Offspring from mothers fed a "junk food" diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females. *J. Physiol. (Lond.)* 586: 3219–3230, 2008.

16. **Bell RJ**, **Palma SM**, **Lumley JM**. The effect of vigorous exercise during pregnancy on birth-weight. *Aust N Z J Obstet Gynaecol* 35: 46–51, 1995.

17. **Bellinger L**, **Sculley DV**, **Langley-Evans SC**. Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats. *Int J Obes (Lond)* 30: 729–738, 2006.

18. **Benkalfat NB**, **Merzouk H**, **Bouanane S**, **Merzouk SA**, **Bellenger J**, **Gresti J**, **Tessier C**, **Narce M**. Altered adipose tissue metabolism in offspring of dietary obese rat dams. *Clin. Sci.* 121: 19–28, 2011.

19. **Benyshek DC**, **Johnston CS**, **Martin JF**. Glucose metabolism is altered in the adequately-nourished grand-offspring (F3 generation) of rats malnourished during gestation and perinatal life. *Diabetologia* 49: 1117–1119, 2006.

20. **Berger SL**. The complex language of chromatin regulation during transcription. *Nature* 447: 407–412, 2007.

21. **Bernal AJ**, **Jirtle RL**. Epigenomic disruption: the effects of early developmental exposures. *Birth Defects Research Part A: Clinical and Molecular Teratology* 88: 938–944, 2010.

22. **Bertram C**, **Trowern AR**, **Copin N**, **Jackson AA**, **Whorwood CB**. The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 2 11beta-hydroxysteroid dehydrogenase: potential molecular mechanisms underlying the programming of hypertension in utero. *Endocrinology* 142: 2841–2853, 2001.

23. **Bonen A**, **Campagna P**, **Gilchrist L**, **Young DC**, **Beresford P**. Substrate and endocrine responses during exercise at selected stages of pregnancy. *J. Appl. Physiol.* 73: 134–142, 1992.

24. **Bouskila M**, **Hunter RW**, **Ibrahim AFM**, **Delattre L**, **Peggie M**, **van Diepen JA**, **Voshol PJ**, **Jensen J**, **Sakamoto K**. Allosteric regulation of glycogen synthase controls glycogen synthesis in muscle. *Cell Metabolism* 12: 456–466, 2010.

25. **Brasaemle DL**, **Subramanian V**, **Garcia A**, **Marcinkiewicz A**, **Rothenberg A**. Perilipin A and the control of triacylglycerol metabolism. *Mol. Cell. Biochem.* 326: 15–21, 2009.

26. **Bruce KD**, **Cagampang FR**, **Argenton M**, **Zhang J**, **Ethirajan PL**, **Burdge GC**, **Bateman AC**, **Clough GF**, **Poston L**, **Hanson MA**, **McConnell JM**, **Byrne CD**. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. *Hepatology* 50: 1796–1808, 2009.

27. **Brunton PJ**, **Sullivan KM**, **Kerrigan D**, **Russell JA**, **Seckl JR**, **Drake AJ**. Sex-specific effects of prenatal stress on glucose homoeostasis and peripheral metabolism in rats. *Journal of Endocrinology* 217: 161–173, 2013.

28. **Burdge GC**, **Lillycrop KA**. Environment-physiology, diet quality and energy balance: The influence of early life nutrition on future energy balance. *Physiology & Behavior* (January 3, 2014). doi: 10.1016/j.physbeh.2013.12.007.

29. **Burns SP**, **Desai M**, **Cohen RD**, **Hales CN**, **Iles RA**, **Germain JP**, **Going TC**, **Bailey RA**. Gluconeogenesis, glucose handling, and structural changes in livers of the adult offspring of rats partially deprived of protein during pregnancy and lactation. *Journal of Clinical Investigation* 100: 1768–1774, 1997.

30. **Cannon B**, **Nedergaard J**. Brown adipose tissue: function and physiological significance. *Physiological Reviews* 84: 277–359, 2004.

31. **Carone BR**, **Fauquier L**, **Habib N**, **Shea JM**, **Hart CE**, **Li R**, **Bock C**, **Li C**, **Gu H**, **Zamore PD**, **Meissner A**, **Weng Z**, **Hofmann HA**, **Friedman N**, **Rando OJ**. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* 143: 1084–1096, 2010.

32. **Carter LG**, **Lewis KN**, **Wilkerson DC**, **Tobia CM**, **Ngo Tenlep SY**, **Shridas P**, **Garcia-Cazarin ML**, **Wolff G**, **Andrade FH**, **Charnigo RJ**, **Esser KA**, **Egan JM**, **de Cabo R**, **Pearson KJ**. Perinatal exercise improves glucose homeostasis in adult offspring. *AJP: Endocrinology and Metabolism* 303: E1061–E1068, 2012.

33. **Carter LG**, **Lewis KN**, **Wilkerson DC**, **Tobia CM**, **Ngo Tenlep SY**, **Shridas P**, **Garcia-Cazarin ML**, **Wolff G**, **Andrade FH**, **Charnigo RJ**, **Esser KA**, **Egan JM**, **de Cabo R**, **Pearson KJ**. Perinatal exercise improves glucose homeostasis in adult offspring. *AJP: Endocrinology and Metabolism* 303: E1061–8, 2012.

34. **Carter LG**, **Qi NR**, **de Cabo R**, **Pearson KJ**. Maternal exercise improves insulin sensitivity in mature rat offspring. *Med Sci Sports Exerc* 45: 832–840, 2013.

35. **Caruso A**, **Paradisi G**, **Ferrazzani S**, **Lucchese A**, **Moretti S**, **Fulghesu AM**. Effect of maternal carbohydrate metabolism on fetal growth. *Obstet Gynecol* 92: 8–12, 1998.

36. **Carver TD**, **Anderson SM**, **Aldoretta PW**, **Hay WW**. Effect of low-level basal plus marked "pulsatile" hyperglycemia on insulin secretion in fetal sheep. *Am. J. Physiol.* 271: E865–71, 1996.

37. **Chen J-H**, **Ozanne SE**, **Hales CN**. Methods of cellular senescence induction using oxidative stress. *Methods Mol. Biol.* 371: 179–189, 2007.

38. **Clapp JF**, **Capeless EL**. Neonatal morphometrics after endurance exercise during pregnancy. *Am. J. Obstet. Gynecol.* 163: 1805–1811, 1990.

39. **Clapp JF**, **Capeless EL**. The changing glycemic response to exercise during pregnancy. *Am. J. Obstet. Gynecol.* 165: 1678–1683, 1991.

40. **Clapp JF**, **Kim H**, **Burciu B**, **Lopez B**. Beginning regular exercise in early pregnancy: effect on fetoplacental growth. *Am. J. Obstet. Gynecol.* 183: 1484–1488, 2000.

41. **Clapp JF**, **Kim H**, **Burciu B**, **Schmidt S**, **Petry K**, **Lopez B**. Continuing regular exercise during pregnancy: effect of exercise volume on fetoplacental growth. *Am. J. Obstet. Gynecol.* 186: 142–147, 2002.

42. **Clapp JF**, **Lopez B**, **Harcar-Sevcik R**. Neonatal behavioral profile of the offspring of women who continued to exercise regularly throughout pregnancy. *Am. J. Obstet. Gynecol.* 180: 91–94, 1999.

43. **Clapp JF**, **Simonian S**, **Lopez B**, **Appleby-Wineberg S**, **Harcar-Sevcik R**. The one-year morphometric and neurodevelopmental outcome of the offspring of women who continued to exercise regularly throughout pregnancy. *Am. J. Obstet. Gynecol.* 178: 594–599, 1998.

44. **Clapp JF**. A clinical approach to exercise during pregnancy. *Clin Sports Med* 13: 443–458, 1994.

45. **Clapp JF**. Morphometric and neurodevelopmental outcome at age five years of the offspring of women who continued to exercise regularly throughout pregnancy. *J. Pediatr.* 129: 856–863, 1996.

46. **Clapp JF**. Exercise during pregnancy. A clinical update. *Clin Sports Med* 19: 273–286, 2000.

47. **Clapp JF**. The effects of maternal exercise on fetal oxygenation and feto-placental growth. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 110 Suppl 1: S80–5, 2003.

48. **Collings CA**, **Curet LB**, **Mullin JP**. Maternal and fetal responses to a maternal aerobic exercise program. *Am. J. Obstet. Gynecol.* 145: 702–707, 1983.

49. **Da Costa D**, **Rippen N**, **Dritsa M**, **Ring A**. Self-reported leisure-time physical activity during pregnancy and relationship to psychological well-being. *J Psychosom Obstet Gynaecol* 24: 111–119, 2003.

50. **Dahri S**, **Snoeck A**, **Reusens-Billen B**, **Remacle C**, **Hoet JJ**. Islet function in offspring of mothers on low-protein diet during gestation. *Diabetes* 40 Suppl 2: 115–120, 1991.

51. **Daniel ZCTR**, **Brameld JM**, **Craigon J**, **Scollan ND**, **Buttery PJ**. Effect of maternal dietary restriction during pregnancy on lamb carcass characteristics and muscle fiber composition. *Journal of Animal Science* 85: 1565–1576, 2007.

52. **Danielsen I**, **Granström C**, **Rytter D**, **Hammer Bech B**, **Brink Henriksen T**, **Vaag AA**, **Olsen SF**. Does physical activity during pregnancy adversely influence markers of the metabolic syndrome in adult offspring? A prospective study over two decades. *J Epidemiol Community Health* 67: 648–654, 2013.

53. **Das UG**, **Schroeder RE**, **Hay WW**, **Devaskar SU**. Time-dependent and tissue-specific effects of circulating glucose on fetal ovine glucose transporters. *Am. J. Physiol.* 276: R809–17, 1999.

54. **Dayi A**, **Agilkaya S**, **Ozbal S**, **Cetin F**, **Aksu I**, **Gencoglu C**, **Cingoz S**, **Pekcetin C**, **Tugyan K**, **Kayatekin BM**, **Uysal N**. Maternal aerobic exercise during pregnancy can increase spatial learning by affecting leptin expression on offspring's early and late period in life depending on gender. *Scientific World Journal* 2012: 429803, 2012.

55. **De Bono JP**, **Adlam D**, **Paterson DJ**, **Channon KM**. Novel quantitative phenotypes of exercise training in mouse models. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290: R926–34, 2006.

56. **de Gusmão Correia ML**, **Volpato AM**, **Águila MB**, **Mandarim-de-Lacerda CA**. Developmental origins of health and disease: experimental and human evidence of fetal programming for metabolic syndrome. *J Hum Hypertens* 26: 405–419, 2012.

57. **de Rooij SR**, **Painter RC**, **Roseboom TJ**, **Phillips DIW**, **Osmond C**, **Barker DJP**, **Tanck MW**, **Michels RPJ**, **Bossuyt PMM**, **Bleker OP**. Glucose tolerance at age 58 and the decline of glucose tolerance in comparison with age 50 in people prenatally exposed to the Dutch famine. *Diabetologia* 49: 637–643, 2006.

58. **Delaigle AM**, **Jonas J-C**, **Bauche IB**, **Cornu O**, **Brichard SM**. Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. *Endocrinology* 145: 5589–5597, 2004.

59. **Delaigle AM**, **Senou M**, **Guiot Y**, **Many MC**, **Brichard SM**. Induction of adiponectin in skeletal muscle of type 2 diabetic mice: In vivo and in vitro studies. *Diabetologia* 49: 1311–1323, 2006.

60. **Denadai BS**, **Piçarro IDC**, **Madjian S**, **Bergamaschi CT**, **Santos VC**, **da Silva AC**, **Russo AK**. High intensity exercise during pregnancy of rats. Effects on mother and offspring. *Comp. Biochem. Physiol. A Physiol.* 109: 727–740, 1994.

61. **Dewey KG**, **Lovelady CA**, **Nommsen-Rivers LA**, **McCrory MA**, **Lönnerdal B**. A randomized study of the effects of aerobic exercise by lactating women on breast-milk volume and composition. *N. Engl. J. Med.* 330: 449–453, 1994.

62. **Dobrzyn P**, **Pyrkowska A**, **Jazurek M**, **Szymanski K**, **Langfort J**, **Dobrzyn A**. Endurance training-induced accumulation of muscle triglycerides is coupled to upregulation of stearoyl-CoA desaturase 1. *Journal of Applied Physiology* 109: 1653–1661, 2010.

63. **Dörner G**, **Plagemann A**, **Rückert J**, **Götz F**, **Rohde W**, **Stahl F**, **Kürschner U**, **Gottschalk J**, **Mohnike A**, **Steindel E**. Teratogenetic maternofoetal transmission and prevention of diabetes susceptibility. *Exp. Clin. Endocrinol.* 91: 247–258, 1988.

64. **Drake AJ**, **Liu L**. Intergenerational transmission of programmed effects: public health consequences. *Trends Endocrinol. Metab.* 21: 206–213, 2010.

65. **Drake AJ**, **Walker BR**, **Seckl JR**. Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288: R34–8, 2005.

66. **Du M**, **Tong J**, **Zhao J**, **Underwood KR**, **Zhu M**, **Ford SP**, **Nathanielsz PW**. Fetal programming of skeletal muscle development in ruminant animals. *Journal of Animal Science* 88: E51–60, 2010.

67. **Du M**, **Zhao JX**, **Yan X**, **Huang Y**, **Nicodemus LV**, **Yue W**, **McCormick RJ**, **Zhu MJ**. Fetal muscle development, mesenchymal multipotent cell differentiation, and associated signaling pathways. *Journal of Animal Science* 89: 583–590, 2011.

68. **Dunn GA**, **Bale TL**. Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. *Endocrinology* 150: 4999–5009, 2009.

69. **Dunn GA**, **Bale TL**. Maternal high-fat diet effects on third-generation female body size via the paternal lineage. *Endocrinology* 152: 2228–2236, 2011.

70. **Epstein CJ**, **Smith S**, **Travis B**, **Tucker G**. Both X chromosomes function before visible X-chromosome inactivation in female mouse embryos. *Nature* 274: 500–503, 1978.

71. **Erkek S**, **Hisano M**, **Liang C-Y**, **Gill M**, **Murr R**, **Dieker J**, **Schübeler D**, **van der Vlag J**, **Stadler MB**, **Peters AHFM**. Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. *Nat. Struct. Mol. Biol.* 20: 868–875, 2013.

72. **Fagundes ATS**, **Moura EG**, **Passos MCF**, **Santos-Silva AP**, **de Oliveira E**, **Trevenzoli IH**, **Casimiro-Lopes G**, **Nogueira-Neto JF**, **Lisboa PC**. Temporal evaluation of body composition, glucose homeostasis and lipid profile of male rats programmed by maternal protein restriction during lactation. *Horm. Metab. Res.* 41: 866–873, 2009.

73. **Fahey AJ**, **Brameld JM**, **Parr T**, **Buttery PJ**. The effect of maternal undernutrition before muscle differentiation on the muscle fiber development of the newborn lamb. *Journal of Animal Science* 83: 2564–2571, 2005.

74. **Fleten C**, **Stigum H**, **Magnus P**, **Nystad W**. Exercise during pregnancy, maternal prepregnancy body mass index, and birth weight. *Obstet Gynecol* 115: 331–337, 2010.

75. **Flück M**. Functional, structural and molecular plasticity of mammalian skeletal muscle in response to exercise stimuli. *J. Exp. Biol.* 209: 2239–2248, 2006.

76. **Fowden AL**, **Li J**, **Forhead AJ**. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proc. Nutr. Soc.* 57: 113–122, 1998.

77. **Franko KL**, **Forhead AJ**, **Fowden AL**. Differential effects of prenatal stress and glucocorticoid administration on postnatal growth and glucose metabolism in rats. *Journal of Endocrinology* 204: 319–329, 2010.

78. **Gallou-Kabani C**, **Gabory A**, **Tost J**, **Karimi M**, **Mayeur S**, **Lesage J**, **Boudadi E**, **Gross M-S**, **Taurelle J**, **Vigé A**, **Breton C**, **Reusens B**, **Remacle C**, **Vieau D**, **Ekström TJ**, **Jais J-P**, **Junien C**. Sex- and diet-specific changes of imprinted gene expression and DNA methylation in mouse placenta under a high-fat diet. *PLoS ONE* 5: e14398, 2010.

79. **Gauguier D**, **Bihoreau MT**, **Picon L**, **Ktorza A**. Insulin secretion in adult rats after intrauterine exposure to mild hyperglycemia during late gestation. *Diabetes* 40 Suppl 2: 109–114, 1991.

80. **Gauguier D**, **Bihoreau MT**, **Picon L**, **Ktorza A**. Insulin secretion in adult rats after intrauterine exposure to mild hyperglycemia during late gestation. *Diabetes* 40 Suppl 2: 109–114, 1991.

81. **George LA**, **Zhang L**, **Tuersunjiang N**, **Ma Y**, **Long NM**, **Uthlaut AB**, **Smith DT**, **Nathanielsz PW**, **Ford SP**. Early maternal undernutrition programs increased feed intake, altered glucose metabolism and insulin secretion, and liver function in aged female offspring. *AJP: Regulatory, Integrative and Comparative Physiology* 302: R795–804, 2012.

82. **Girard J**, **Ferré P**, **Foufelle F**. Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. *Annu. Rev. Nutr.* 17: 325–352, 1997.

83. **Gluckman P**, **Hanson M**. *Developmental Origins of Health and Disease*. Cambridge University Press, 2006.

84. **Gluckman PD**, **Lillycrop KA**, **Vickers MH**, **Pleasants AB**, **Phillips ES**, **Beedle AS**, **Burdge GC**, **Hanson MA**. Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. *Proc. Natl. Acad. Sci. U.S.A.* 104: 12796–12800, 2007.

85. **Godfrey KM**, **Sheppard A**, **Gluckman PD**, **Lillycrop KA**, **Burdge GC**, **McLean C**, **Rodford J**, **Slater-Jefferies JL**, **Garratt E**, **Crozier SR**, **Emerald BS**, **Gale CR**, **Inskip HM**, **Cooper C**, **Hanson MA**. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes* 60: 1528–1534, 2011.

86. **Gosby AK**, **Maloney CA**, **Phuyal JL**, **Denyer GS**, **Bryson JM**, **Caterson ID**. Maternal protein restriction increases hepatic glycogen storage in young rats. *Pediatric Research* 54: 413–418, 2003.

87. **Greenberg AS**, **Coleman RA**, **Kraemer FB**, **McManaman JL**, **Obin MS**, **Puri V**, **Yan Q-W**, **Miyoshi H**, **Mashek DG**. The role of lipid droplets in metabolic disease in rodents and humans. *J. Clin. Invest.* 121: 2102–2110, 2011.

88. **Grün F**, **Blumberg B**. Endocrine disrupters as obesogens. *Molecular and Cellular Endocrinology* 304: 19–29, 2009.

89. **Guerrero-Bosagna C**, **Covert TR**, **Haque MM**, **Settles M**, **Nilsson EE**, **Anway MD**, **Skinner MK**. Epigenetic transgenerational inheritance of vinclozolin induced mouse adult onset disease and associated sperm epigenome biomarkers. *Reprod. Toxicol.* 34: 694–707, 2012.

90. **Guth LM**, **Ludlow AT**, **Witkowski S**, **Marshall MR**, **Lima LCJ**, **Venezia AC**, **Xiao T**, **Ting Lee M-L**, **Spangenburg EE**, **Roth SM**. Sex-specific effects of exercise ancestry on metabolic, morphological and gene expression phenotypes in multiple generations of mouse offspring. *Experimental Physiology* 98: 1469–1484, 2013.

91. **Habbout A**, **Li N**, **Rochette L**, **Vergely C**. Postnatal overfeeding in rodents by litter size reduction induces major short- and long-term pathophysiological consequences. *Journal of Nutrition* 143: 553–562, 2013.

92. **Hackett JA**, **Surani MA**. Beyond DNA: programming and inheritance of parental methylomes. *Cell* 153: 737–739, 2013.

93. **Hales CN**, **Barker DJ**, **Clark PM**, **Cox LJ**, **Fall C**, **Osmond C**, **Winter PD**. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 303: 1019–1022, 1991.

94. **Hales CN**, **Barker DJ**. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 35: 595–601, 1992.

95. **Hardie DG**. Organismal carbohydrate and lipid homeostasis. *Cold Spring Harb Perspect Biol* 4, 2012.

96. **Harrison M**, **Langley-Evans SC**. Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. *BJN* 101: 1020–1030, 2009.

97. **Heijmans BT**, **Tobi EW**, **Stein AD**, **Putter H**, **Blauw GJ**, **Susser ES**, **Slagboom PE**, **Lumey LH**. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proceedings of the National Academy of Sciences* 105: 17046–17049, 2008.

98. **Holloszy JO**. Exercise-induced increase in muscle insulin sensitivity. *J. Appl. Physiol.* 99: 338–343, 2005.

99. **Holson JF**, **Scott WJ**, **Gaylor DW**, **Wilson JG**. Reduced interlitter variability in rats resulting from a restricted mating period, and reassessment of the "litter effect". *Teratology* 14: 135–141, 1976.

100. **Hopkins SA**, **Baldi JC**, **Cutfield WS**, **McCowan L**, **Hofman PL**. Exercise training in pregnancy reduces offspring size without changes in maternal insulin sensitivity. *J. Clin. Endocrinol. Metab.* 95: 2080–2088, 2010.

101. **Hoppeler H**, **Flück M**. Plasticity of skeletal muscle mitochondria: structure and function. *Med Sci Sports Exerc* 35: 95–104, 2003.

102. **Houghton PE**, **Mottola MF**, **Plust JH**, **Schachter CL**. Effect of maternal exercise on fetal and placental glycogen storage in the mature rat. *Can J Appl Physiol* 25: 443–452, 2000.

103. **Hulver MW**, **Berggren JR**, **Carper MJ**, **Miyazaki M**, **Ntambi JM**, **Hoffman EP**, **Thyfault JP**, **Stevens R**, **Dohm GL**, **Houmard JA**, **Muoio DM**. Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. *Cell Metabolism* 2: 251–261, 2005.

104. **Hyatt MA**, **Gardner DS**, **Sebert S**, **Wilson V**, **Davidson N**, **Nigmatullina Y**, **Chan LLY**, **Budge H**, **Symonds ME**. Suboptimal maternal nutrition, during early fetal liver development, promotes lipid accumulation in the liver of obese offspring. *Reproduction* 141: 119–126, 2011.

105. **Ivaska KK**, **Hentunen TA**, **Vääräniemi J**, **Ylipahkala H**, **Pettersson K**, **Väänänen HK**. Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption in vitro. *J. Biol. Chem.* 279: 18361–18369, 2004.

106. **James DE**, **Burleigh KM**, **Kraegen EW**, **Chisholm DJ**. Effect of acute exercise and prolonged training on insulin response to intravenous glucose in vivo in rat. *J. Appl. Physiol.* 55: 1660–1664, 1983.

107. **Jimenez-Chillaron JC**, **Isganaitis E**, **Charalambous M**, **Gesta S**, **Pentinat-Pelegrin T**, **Faucette RR**, **Otis JP**, **Chow A**, **Diaz R**, **Ferguson-Smith A**, **Patti ME**. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. *Diabetes* 58: 460–468, 2009.

108. **Juhl M**, **Olsen J**, **Andersen PK**, **Nøhr EA**, **Andersen A-MN**. Physical exercise during pregnancy and fetal growth measures: a study within the Danish National Birth Cohort. *Am. J. Obstet. Gynecol.* 202: 63.e1–8, 2010.

109. **Kaati G**, **Bygren LO**, **Edvinsson S**. Cardiovascular and diabetes mortality determined by nutrition during parents“ and grandparents” slow growth period. *Eur J Hum Genet* 10: 682–688, 2002.

110. **Kaati G**, **Bygren LO**, **Pembrey M**, **Sjöström M**. Transgenerational response to nutrition, early life circumstances and longevity. *Eur J Hum Genet* 15: 784–790, 2007.

111. **Kalisiak B**, **Spitznagle T**. What effect does an exercise program for healthy pregnant women have on the mother, fetus, and child? *PM R* 1: 261–266, 2009.

112. **Kardel KR**. Effects of intense training during and after pregnancy in top-level athletes. *Scandinavian Journal of Medicine & Science in Sports* 15: 79–86, 2005.

113. **Kim E**, **Lee J-H**, **Ntambi JM**, **Hyun C-K**. Inhibition of stearoyl-CoA desaturase1 activates AMPK and exhibits beneficial lipid metabolic effects in vitro. *Eur. J. Pharmacol.* 672: 38–44, 2011.

114. **Kuzawa CW**, **Sweet E**. Epigenetics and the embodiment of race: developmental origins of US racial disparities in cardiovascular health. *Am. J. Hum. Biol.* 21: 2–15, 2009.

115. **Langley-Evans SC**, **Gardner DS**, **Jackson AA**. Maternal protein restriction influences the programming of the rat hypothalamic-pituitary-adrenal axis. *J. Nutr.* 126: 1578–1585, 1996.

116. **Langley-Evans SC**, **Phillips GJ**, **Benediktsson R**, **Gardner DS**, **Edwards CR**, **Jackson AA**, **Seckl JR**. Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. *Placenta* 17: 169–172, 1996.

117. **Langley-Evans SC**. Developmental programming of health and disease. *Proc Nutr Soc* 65: 97–105, 2006.

118. **Langley-Evans SC**. Nutritional programming of disease: unravelling the mechanism. *J. Anat.* 215: 36–51, 2009.

119. **Lawrence JC**, **Roach PJ**. New insights into the role and mechanism of glycogen synthase activation by insulin. *Diabetes* 46: 541–547, 1997.

120. **Lee H-H**, **Kim H**, **Lee J-W**, **Kim Y-S**, **Yang H-Y**, **Chang H-K**, **Lee T-H**, **Shin M-C**, **Lee M-H**, **Shin M-S**, **Park S**, **Baek S**, **Kim C-J**. Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups. *Brain Dev.* 28: 147–154, 2006.

121. **Lee M-LT**. *Analysis of Microarray Gene Expression Data*. Springer, 2004.

122. **Leick L**, **Wojtaszewski JFP**, **Johansen ST**, **Kiilerich K**, **Comes G**, **Hellsten Y**, **Hidalgo J**, **Pilegaard H**. PGC-1 is not mandatory for exercise- and training-induced adaptive gene responses in mouse skeletal muscle. *AJP: Endocrinology and Metabolism* 294: E463–E474, 2007.

123. **Levin MC**, **Monetti M**, **Watt MJ**, **Sajan MP**, **Stevens RD**, **Bain JR**, **Newgard CB**, **Farese RV**, **Farese RV**. Increased lipid accumulation and insulin resistance in transgenic mice expressing DGAT2 in glycolytic (type II) muscle. *Am. J. Physiol. Endocrinol. Metab.* 293: E1772–81, 2007.

124. **Li J**, **Huang J**, **Li J-S**, **Chen H**, **Huang K**, **Zheng L**. Accumulation of endoplasmic reticulum stress and lipogenesis in the liver through generational effects of high fat diets. *J. Hepatol.* 56: 900–907, 2012.

125. **Liang L**, **Zhao M**, **Xu Z**, **Yokoyama KK**, **Li T**. Molecular cloning and characterization of CIDE-3, a novel member of the cell-death-inducing DNA-fragmentation-factor (DFF45)-like effector family. *Biochem. J.* 370: 195–203, 2003.

126. **Lillycrop KA**, **Phillips ES**, **Jackson AA**, **Hanson MA**, **Burdge GC**. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J. Nutr.* 135: 1382–1386, 2005.

127. **Lillycrop KA**, **Slater-Jefferies JL**, **Hanson MA**, **Godfrey KM**, **Jackson AA**, **Burdge GC**. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br. J. Nutr.* 97: 1064–1073, 2007.

128. **Liu Y**, **Chewchuk S**, **Lavigne C**, **Brûlé S**, **Pilon G**, **Houde V**, **Xu A**, **Marette A**, **Sweeney G**. Functional significance of skeletal muscle adiponectin production, changes in animal models of obesity and diabetes, and regulation by rosiglitazone treatment. *AJP: Endocrinology and Metabolism* 297: E657–64, 2009.

129. **Liu Y-F**, **Chen H-I**, **Wu C-L**, **Kuo Y-M**, **Yu L**, **Huang A-M**, **Wu F-S**, **Chuang J-I**, **Jen CJ**. Differential effects of treadmill running and wheel running on spatial or aversive learning and memory: roles of amygdalar brain-derived neurotrophic factor and synaptotagmin I. *J. Physiol. (Lond.)* 587: 3221–3231, 2009.

130. **Lovelady CA**, **Lönnerdal B**, **Dewey KG**. Lactation performance of exercising women. *Am. J. Clin. Nutr.* 52: 103–109, 1990.

131. **Low FM**, **Gluckman PD**, **Hanson MA**. Developmental plasticity and epigenetic mechanisms underpinning metabolic and cardiovascular diseases. *Epigenomics* 3: 279–294, 2011.

132. **Lowry OH**, **Passonneau JV**. *A flexible system of enzymatic analysis*. Academic Pr, 1972.

133. **Lumey LH**, **Stein AD**, **Kahn HS**, **Romijn JA**. Lipid profiles in middle-aged men and women after famine exposure during gestation: the Dutch Hunger Winter Families Study. *American Journal of Clinical Nutrition* 89: 1737–1743, 2009.

134. **Ma Q**. Beneficial effects of moderate voluntary physical exercise and its biological mechanisms on brain health. *Neurosci Bull* 24: 265–270, 2008.

135. **Magee TR**, **Han G**, **Cherian B**, **Khorram O**, **Ross MG**, **Desai M**. Down-regulation of transcription factor peroxisome proliferator-activated receptor in programmed hepatic lipid dysregulation and inflammation in intrauterine growth-restricted offspring. *Am. J. Obstet. Gynecol.* 199: 271.e1–5, 2008.

136. **Maloney CA**, **Gosby AK**, **Phuyal JL**, **Denyer GS**, **Bryson JM**, **Caterson ID**. Site-specific changes in the expression of fat-partitioning genes in weanling rats exposed to a low-protein diet in utero. *Obes. Res.* 11: 461–468, 2003.

137. **Mao J**, **Zhang X**, **Sieli PT**, **Falduto MT**, **Torres KE**, **Rosenfeld CS**. Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *Proceedings of the National Academy of Sciences* 107: 5557–5562, 2010.

138. **Martin JF**, **Johnston CS**, **Han CT**, **Benyshek DC**. Nutritional origins of insulin resistance: a rat model for diabetes-prone human populations. *J. Nutr.* 130: 741–744, 2000.

139. **Matthews SG**, **Phillips DI**. Transgenerational inheritance of stress pathology. *Exp. Neurol.* 233: 95–101, 2012.

140. **McClelland GB**. Fat to the fire: the regulation of lipid oxidation with exercise and environmental stress. *Comp. Biochem. Physiol. B, Biochem. Mol. Biol.* 139: 443–460, 2004.

141. **McManus EJ**, **Sakamoto K**, **Armit LJ**, **Ronaldson L**, **Shpiro N**, **Marquez R**, **Alessi DR**. Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. *EMBO J.* 24: 1571–1583, 2005.

142. **McMillen IC**, **Adam CL**, **Mühlhäusler BS**. Early origins of obesity: programming the appetite regulatory system. *J. Physiol. (Lond.)* 565: 9–17, 2005.

143. **Millard LAC**, **Lawlor DA**, **Fraser A**, **Howe LD**. Physical activity during pregnancy and offspring cardiovascular risk factors: findings from a prospective cohort study. *BMJ Open* 3: e003574, 2013.

144. **Mokdad AH**, **Marks JS**, **Stroup DF**, **Gerberding JL**. Actual causes of death in the United States, 2000. *JAMA* 291: 1238–1245, 2004.

145. **Monetti M**, **Levin MC**, **Watt MJ**, **Sajan MP**, **Marmor S**, **Hubbard BK**, **Stevens RD**, **Bain JR**, **Newgard CB**, **Farese RV**, **Hevener AL**, **Farese RV**. Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metabolism* 6: 69–78, 2007.

146. **Monteiro ACT**, **Paes ST**, **Santos dos JA**, **de Lira KDS**, **de Moraes SRA**. Effects of physical exercise during pregnancy and protein malnutrition during pregnancy and lactation on the development and growth of the offspring's femur. *J Pediatr (Rio J)* 86: 233–238, 2010.

147. **Mueller BR**, **Bale TL**. Sex-specific programming of offspring emotionality after stress early in pregnancy. *Journal of Neuroscience* 28: 9055–9065, 2008.

148. **Ng S-F**, **Lin RCY**, **Laybutt DR**, **Barres R**, **Owens JA**, **Morris MJ**. Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* 467: 963–966, 2010.

149. **Nguyen LT**, **Muhlhausler BS**, **Botting KJ**, **Morrison JL**. Maternal undernutrition alters fat cell size distribution, but not lipogenic gene expression, in the visceral fat of the late gestation guinea pig fetus. *Placenta* 31: 902–909, 2010.

150. **Oben JA**, **Mouralidarane A**, **Samuelsson A-M**, **Matthews PJ**, **Morgan ML**, **McKee C**, **Soeda J**, **Fernandez-Twinn DS**, **Martin-Gronert MS**, **Ozanne SE**, **Sigala B**, **Novelli M**, **Poston L**, **Taylor PD**. Maternal obesity during pregnancy and lactation programs the development of offspring non-alcoholic fatty liver disease in mice. *J. Hepatol.* 52: 913–920, 2010.

151. **Ozanne SE**, **Smith GD**, **Tikerpae J**, **Hales CN**. Altered regulation of hepatic glucose output in the male offspring of protein-malnourished rat dams. *Am. J. Physiol.* 270: E559–64, 1996.

152. **Painter RC**, **Osmond C**, **Gluckman P**, **Hanson M**, **Phillips DIW**, **Roseboom TJ**. Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. *BJOG: An International Journal of Obstetrics & Gynaecology* 115: 1243–1249, 2008.

153. **Patin V**, **Lordi B**, **Vincent A**, **Thoumas JL**, **Vaudry H**, **Caston J**. Effects of prenatal stress on maternal behavior in the rat. *Brain Res. Dev. Brain Res.* 139: 1–8, 2002.

154. **Peixoto-Silva N**, **Frantz EDC**, **Mandarim-de-Lacerda CA**, **Pinheiro-Mulder A**. Maternal protein restriction in mice causes adverse metabolic and hypothalamic effects in the F1 and F2 generations. *BJN* 106: 1364–1373, 2011.

155. **Pembrey ME**, **Bygren LO**, **Kaati G**, **Edvinsson S**, **Northstone K**, **Sjöström M**, **Golding J**, **ALSPAC Study Team**. Sex-specific, male-line transgenerational responses in humans. *Eur J Hum Genet* 14: 159–166, 2006.

156. **Pentinat T**, **Ramon-Krauel M**, **Cebria J**, **Diaz R**, **Jimenez-Chillaron JC**. Transgenerational inheritance of glucose intolerance in a mouse model of neonatal overnutrition. *Endocrinology* 151: 5617–5623, 2010.

157. **Peterside IE**, **Selak MA**, **Simmons RA**. Impaired oxidative phosphorylation in hepatic mitochondria in growth-retarded rats. *Am. J. Physiol. Endocrinol. Metab.* 285: E1258–66, 2003.

158. **Petrik J**, **Reusens B**, **Arany E**, **Remacle C**, **Coelho C**, **Hoet JJ**, **Hill DJ**. A low protein diet alters the balance of islet cell replication and apoptosis in the fetal and neonatal rat and is associated with a reduced pancreatic expression of insulin-like growth factor-II. *Endocrinology* 140: 4861–4873, 1999.

159. **Phillips DI**, **Barker DJ**, **Fall CH**, **Seckl JR**, **Whorwood CB**, **Wood PJ**, **Walker BR**. Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? *Journal of Clinical Endocrinology & Metabolism* 83: 757–760, 1998.

160. **Pilegaard H**, **Ordway GA**, **Saltin B**, **Neufer PD**. Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *Am. J. Physiol. Endocrinol. Metab.* 279: E806–14, 2000.

161. **Pilkis SJ**, **Granner DK**. Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Physiol.* 54: 885–909, 1992.

162. **Pinto ML**, **Shetty PS**. Influence of exercise-induced maternal stress on fetal outcome in Wistar rats: inter-generational effects. *Br. J. Nutr.* 73: 645–653, 1995.

163. **Postic C**, **Dentin R**, **Girard J**. Role of the liver in the control of carbohydrate and lipid homeostasis. *Diabetes Metab.* 30: 398–408, 2004.

164. **Poudevigne MS**, **O'Connor PJ**. Physical activity and mood during pregnancy. *Med Sci Sports Exerc* 37: 1374–1380, 2005.

165. **Puntschart A**, **Claassen H**, **Jostarndt K**, **Hoppeler H**, **Billeter R**. mRNAs of enzymes involved in energy metabolism and mtDNA are increased in endurance-trained athletes. *Am. J. Physiol.* 269: C619–25, 1995.

166. **Puri V**, **Konda S**, **Ranjit S**, **Aouadi M**, **Chawla A**, **Chouinard M**, **Chakladar A**, **Czech MP**. Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage. *J. Biol. Chem.* 282: 34213–34218, 2007.

167. **Ravelli AC**, **van Der Meulen JH**, **Osmond C**, **Barker DJ**, **Bleker OP**. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am. J. Clin. Nutr.* 70: 811–816, 1999.

168. **Rector RS**, **Thyfault JP**, **Morris RT**, **Laye MJ**, **Borengasser SJ**, **Booth FW**, **Ibdah JA**. Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294: G619–26, 2008.

169. **Rodgers CD**, **Mottola MF**, **Corbett K**, **Taylor AW**. Skeletal muscle metabolism in the offspring of trained rats. *J Sports Med Phys Fitness* 31: 389–395, 1991.

170. **Rosa BV**, **Blair HT**, **Vickers MH**, **Dittmer KE**, **Morel PCH**, **Knight CG**, **Firth EC**. Moderate Exercise during Pregnancy in Wistar Rats Alters Bone and Body Composition of the Adult Offspring in a Sex-Dependent Manner. *PLoS ONE* 8: e82378, 2013.

171. **Rosa BV**, **Blair HT**, **Vickers MH**, **Morel PC**, **Cockrem JF**, **Firth EC**. Voluntary exercise in pregnant rats improves post-lactation maternal bone parameters but does not affect offspring outcomes in early life. *J Musculoskelet Neuronal Interact* 12: 199–208, 2012.

172. **Rosa BV**, **Firth EC**, **Blair HT**, **Vickers MH**, **Morel PCH**. Voluntary exercise in pregnant rats positively influences fetal growth without initiating a maternal physiological stress response. *AJP: Regulatory, Integrative and Comparative Physiology* 300: R1134–41, 2011.

173. **Rowell LB**, **Blackmon JR**. Human cardiovascular adjustments to acute hypoxaemia. *Clin Physiol* 7: 349–376, 1987.

174. **Ryan EA**, **O'Sullivan MJ**, **Skyler JS**. Insulin action during pregnancy. Studies with the euglycemic clamp technique. *Diabetes* 34: 380–389, 1985.

175. **Saal Vom FS**, **Moyer CL**. Prenatal effects on reproductive capacity during aging in female mice. *Biology of Reproduction* 32: 1116–1126, 1985.

176. **Schmittgen TD**, **Livak KJ**. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 3: 1101–1108, 2008.

177. **Schmutz S**, **Däpp C**, **Wittwer M**, **Vogt M**, **Hoppeler H**, **Flück M**. Endurance training modulates the muscular transcriptome response to acute exercise. *Pflugers Arch - Eur J Physiol* 451: 678–687, 2006.

178. **Schulz KM**, **Pearson JN**, **Neeley EW**, **Berger R**, **Leonard S**, **Adams CE**, **Stevens KE**. Maternal stress during pregnancy causes sex-specific alterations in offspring memory performance, social interactions, indices of anxiety, and body mass. *Physiology & Behavior* 104: 340–347, 2011.

179. **Schweiger M**, **Schreiber R**, **Haemmerle G**, **Lass A**, **Fledelius C**, **Jacobsen P**, **Tornqvist H**, **Zechner R**, **Zimmermann R**. Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. *J. Biol. Chem.* 281: 40236–40241, 2006.

180. **Seckl JR**, **Holmes MC**. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal "programming" of adult pathophysiology. *Nat Clin Pract Endocrinol Metab* 3: 479–488, 2007.

181. **Selak MA**, **Storey BT**, **Peterside I**, **Simmons RA**. Impaired oxidative phosphorylation in skeletal muscle of intrauterine growth-retarded rats. *Am. J. Physiol. Endocrinol. Metab.* 285: E130–7, 2003.

182. **Shankar K**, **Kang P**, **Harrell A**, **Zhong Y**, **Marecki JC**, **Ronis MJJ**, **Badger TM**. Maternal overweight programs insulin and adiponectin signaling in the offspring. *Endocrinology* 151: 2577–2589, 2010.

183. **Shi Y**, **Cheng D**. Beyond triglyceride synthesis: the dynamic functional roles of MGAT and DGAT enzymes in energy metabolism. *AJP: Endocrinology and Metabolism* 297: E10–8, 2009.

184. **Simmons RA**, **Suponitsky-Kroyter I**, **Selak MA**. Progressive accumulation of mitochondrial DNA mutations and decline in mitochondrial function lead to beta-cell failure. *J. Biol. Chem.* 280: 28785–28791, 2005.

185. **Skinner MK**, **Guerrero-Bosagna C**. Environmental signals and transgenerational epigenetics. *Epigenomics* 1: 111–117, 2009.

186. **Skinner MK**, **Manikkam M**, **Guerrero-Bosagna C**. Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends Endocrinol. Metab.* 21: 214–222, 2010.

187. **Skinner MK**. Role of epigenetics in developmental biology and transgenerational inheritance. *Birth Defects Res. C Embryo Today* 93: 51–55, 2011.

188. **Snoeck A**, **Remacle C**, **Reusens B**, **Hoet JJ**. Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biol. Neonate* 57: 107–118, 1990.

189. **Stanford KI**, **Lee M-Y**, **Getchell KM**, **Hirshman MF**, **Goodyear LJ**. Maternal Exercise During Pregnancy Improves Glucose Metabolism of Offspring. *FASEB Journal* 27: 1134.4, 2013.

190. **Stark MJ**, **Wright IMR**, **Clifton VL**. Sex-specific alterations in placental 11beta-hydroxysteroid dehydrogenase 2 activity and early postnatal clinical course following antenatal betamethasone. *AJP: Regulatory, Integrative and Comparative Physiology* 297: R510–4, 2009.

191. **Stein AD**, **Kahn HS**, **Rundle A**, **Zybert PA**, **van der Pal-de Bruin K**, **Lumey LH**. Anthropometric measures in middle age after exposure to famine during gestation: evidence from the Dutch famine. *Am. J. Clin. Nutr.* 85: 869–876, 2007.

192. **Stein Z**. *Famine and human development*. Oxford University Press, USA, 1975.

193. **Swallow JG**, **Rhodes JS**, **Garland T**. Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. *Integr. Comp. Biol.* 45: 426–437, 2005.

194. **Tchirikov M**, **Tchirikov M**, **Buchert R**, **Wilke F**, **Brenner W**. Glucose uptake in the placenta, fetal brain, heart and liver related to blood flow redistribution during acute hypoxia. *J. Obstet. Gynaecol. Res.* 37: 979–985, 2011.

195. **Thompson D**, **Karpe F**, **Lafontan M**, **Frayn K**. Physical activity and exercise in the regulation of human adipose tissue physiology. *Physiological Reviews* 92: 157–191, 2012.

196. **Tiidus P**, **Tupling AR**, **Houston M**. *Biochemistry Primer for Exercise Science, 4E*. Human Kinetics, 2012.

197. **Tobi EW**, **Lumey LH**, **Talens RP**, **Kremer D**, **Putter H**, **Stein AD**, **Slagboom PE**, **Heijmans BT**. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Human Molecular Genetics* 18: 4046–4053, 2009.

198. **Tomas E**, **Tsao T-S**, **Saha AK**, **Murrey HE**, **Zhang Cc CC**, **Itani SI**, **Lodish HF**, **Ruderman NB**. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc. Natl. Acad. Sci. U.S.A.* 99: 16309–16313, 2002.

199. **Treadway J**, **Dover EV**, **Morse W**, **Newcomer L**, **Craig BW**. Influence of exercise training on maternal and fetal morphological characteristics in the rat. *J. Appl. Physiol.* 60: 1700–1703, 1986.

200. **Treadway JL**, **Lederman SA**. The effects of exercise on milk yield, milk composition, and offspring growth in rats. *Am. J. Clin. Nutr.* 44: 481–488, 1986.

201. **Tseng BS**, **Marsh DR**, **Hamilton MT**, **Booth FW**. Strength and aerobic training attenuate muscle wasting and improve resistance to the development of disability with aging. *J Gerontol A Biol Sci Med Sci* 50 Spec No: 113–119, 1995.

202. **Vaiserman A**, **Lumey LH**. *Early Life Nutrition and Adult Health and Development*. Nova Science Pub Incorporated, 2013.

203. **van Schaftingen E**, **Gerin I**. The glucose-6-phosphatase system. *Biochem. J.* 362: 513–532, 2002.

204. **van Straten EME**, **Bloks VW**, **van Dijk TH**, **Baller JFW**, **Huijkman NCA**, **Kuipers I**, **Verkade HJ**, **Plösch T**. Sex-dependent programming of glucose and fatty acid metabolism in mouse offspring by maternal protein restriction. *Gend Med* 9: 166–179.e13, 2012.

205. **Vega CC**, **Reyes-Castro LA**, **Bautista CJ**, **Larrea F**, **Nathanielsz PW**, **Zambrano E**. Exercise in obese female rats has beneficial effects on maternal and male and female offspring metabolism. *Int J Obes Relat Metab Disord* (August 16, 2013). doi: 10.1038/ijo.2013.150.

206. **Vickers MH**. Developmental programming of the metabolic syndrome - critical windows for intervention. *WJG* 2: 137–148, 2011.

207. **Vulliamy TJ**. Premature aging. *Cell. Mol. Life Sci.* 66: 3091–3094, 2009.

208. **Wang YH**, **Bower NI**, **Reverter A**, **Tan SH**, **De Jager N**, **Wang R**, **McWilliam SM**, **Cafe LM**, **Greenwood PL**, **Lehnert SA**. Gene expression patterns during intramuscular fat development in cattle. *Journal of Animal Science* 87: 119–130, 2009.

209. **Wang ZQ**, **Yu Y**, **Zhang XH**, **Floyd EZ**, **Cefalu WT**. Human adenovirus 36 decreases fatty acid oxidation and increases de novo lipogenesis in primary cultured human skeletal muscle cells by promoting Cidec/FSP27 expression. *Int J Obes Relat Metab Disord* 34: 1355–1364, 2010.

210. **Watt MJ**, **Hoy AJ**. Lipid metabolism in skeletal muscle: generation of adaptive and maladaptive intracellular signals for cellular function. *AJP: Endocrinology and Metabolism* 302: E1315–28, 2012.

211. **Yan X**, **Huang Y**, **Zhao J-X**, **Long NM**, **Uthlaut AB**, **Zhu M-J**, **Ford SP**, **Nathanielsz PW**, **Du M**. Maternal obesity-impaired insulin signaling in sheep and induced lipid accumulation and fibrosis in skeletal muscle of offspring. *Biology of Reproduction* 85: 172–178, 2011.

212. **Yao W**, **Jee WS**, **Chen JL**, **Li CY**, **Frost HM**. A novel method to “exercise” rats: making rats rise to erect bipedal stance for feeding - raised cage model. *J Musculoskelet Neuronal Interact* 1: 241–247, 2001.

213. **Young JC**, **Treadway JL**. The effect of prior exercise on oral glucose tolerance in late gestational women. *Eur J Appl Physiol Occup Physiol* 64: 430–433, 1992.

214. **Zambrano E**, **Martínez-Samayoa PM**, **Bautista CJ**, **Deás M**, **Guillén L**, **Rodríguez-González GL**, **Guzmán C**, **Larrea F**, **Nathanielsz PW**. Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. *J. Physiol. (Lond.)* 566: 225–236, 2005.

215. **Zheng S**, **Rollet M**, **Pan Y-X**. Protein restriction during gestation alters histone modifications at the glucose transporter 4 (GLUT4) promoter region and induces GLUT4 expression in skeletal muscle of female rat offspring. *The Journal of Nutritional Biochemistry* 23: 1064–1071, 2012.

216. **Zhu MJ**, **Ford SP**, **Means WJ**, **Hess BW**, **Nathanielsz PW**, **Du M**. Maternal nutrient restriction affects properties of skeletal muscle in offspring. *J. Physiol. (Lond.)* 575: 241–250, 2006.

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EDUCATION

Anticipated January 2014	**Ph.D. Kinesiology -- University of Maryland, College Park, MD** Advisor: Stephen M. Roth, Ph.D. Co-Advisor: James M. Hagberg, Ph.D.
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RESEARCH EXPERIENCE

2009-2013	**Pre-Doctoral Research Fellow**, National Institutes of Health T32 Institutional Training Grant, Functional Genomics Laboratory, University of Maryland, College Park. (advisor: Stephen M. Roth, Ph.D.)
2007-2009	**Graduate Research Assistant**, Human Performance Laboratory, Ball State University (advisor: Anthony D. Mahon, Ph.D.)
2006-2007	**Undergraduate Research Assistant**, Human Performance Laboratory, Ball State University (advisor: Anthony D. Mahon, Ph.D.)

PUBLICATIONS

Guth LM, SM Roth. Genetic influence on athletic performance. Invited Review. *Curr Opin Pediatr* 25(6):653-8, 2013.

Guth LM, AT Ludlow, S Witkowski, MR Marshall, LC Lima, AC Venezia, T Xiao, M-LT Lee, EE Spangenburg, and SM Roth. Transgenerational Effects of Exercise Ancestry in Mice. *Exp Physiol* 98(10):1469-84, 2013.

Ludlow AT, LC Lima, J Wang, ED Hanson, **LM Guth**, EE Spangenburg, and SM Roth. Exercise alters mRNA expression of telomere-repeat binding factor 1 in skeletal muscle via p38 MAPK. *J Appl Physiol* 113(11):1737-46, 2012.

Ludlow AT, S Witkowski, MR Marshall, J Wang, LC Lima, **LM Guth**, EE Spangenburg, SM Roth. Chronic Exercise Modifies Age-Related Telomere Dynamics in a Tissue-Specific Fashion. *J Gerontol A Biol Sci Med Sci* 67(9):911-26, 2012.

Mahon AD, **LM Guth**, MP Rogowski, and KA Craft. Fuel use responses in young boys and girls during submaximal exercise. *Children and Exercise XXVII*, CA Williams and N. Armstrong (eds.). London: Routledge, 2011, pp. 65-69.

Lee JD, LE Sterrett, **LM Guth**, AR Konopka, and AD Mahon. The effect of pre-exercise carbohydrate supplementation on anaerobic exercise performance in adolescent males. *Pediatr Exerc Sci* 23(3):344-354, 2011.

Mahon AD, **LM Guth**, and KA Craft. Physiological and perceptual responses in children during variable-intensity exercise. *Children and Exercise XXV*, G. Baquet and S. Berthoin (eds.). London: Routledge, 2010, pp. 171-177.

ABSTRACTS/PRESENTATIONS

National and International Meetings

Guth LM, AC Venezia, MP Marini, EP Beltran, EE Spangenburg, and SM Roth. Effects of exercise ancestry on multiple generations of mature mouse offspring. Poster presentation, *Experimental Biology 2013*, Boston, MA.

Venezia AC, **LM Guth**, MP Marini, EP Beltran, EE Spangenburg, and SM Roth. Impact of parental voluntary wheel running on offspring hippocampal gene expression in C57BL/6 mice. Poster presentation, *Neuroscience 2012*, New Orleans, LA, October 2012.

Bradshaw T, **LM Guth**, JM Hagberg. Genetic variants in slc30a8 gene had no effect on type 2 diabetes related phenotypes in response to exercise training. Poster presentation, *Annual Biomedical Research Conference for Minority Students*, San Jose, CA, November 2012.

Guth LM, AC Venezia, MP Marini, EP Beltran, EE Spangenburg, and SM Roth. Effects of Physical Activity Ancestry on Aspects of Body Composition and Glucose Tolerance in Mice. Poster presentation, *2012 American College of Sports Medicine Annual Meeting*, San Francisco, CA, May 2012.

Venezia AC, **LM Guth**, MP Marini, EP Beltran, EE Spangenburg, and SM Roth. Effects of parental physical activity on hippocampal gene expression in C57BL/6 Mice. Poster presentation, *2012 American College of Sports Medicine Annual Meeting*, San Francisco, CA, May 2012.

Marini MP, **LM Guth**, AC Venezia, EP Beltran, EE Spangenburg, and SM Roth. Effects of chronic exercise on DNA methyltransferase expression in mouse testes. Poster presentation, *2012 American College of Sports Medicine Annual Meeting*, San Francisco, CA, May 2012.

Mahon AD, **LM Guth**, MP Rogowski, and KA Craft. Fuel use responses in young boys and girls during submaximal exercise. Oral presentation, *27th Pediatric Work Physiology Meeting*, Mawgan Porth, UK, September 2011.

Guth LM, AT Ludlow, S Witkowski, MR Marshall, L Lima, AC Venezia, T Xiao, M-LT Lee, EE Spangenburg, and SM Roth. Exercise ancestry decreases lipogenesis-related gene expression in skeletal muscle of male offspring. Oral and poster presentation, *Experimental Biology 2011*, Washington, D.C. *FASEB J* 25:862.3, March 2011.

Ludlow AT, S Witkowski, MR Marshall, J Wang, **Guth LM**, EE Spangenburg, and SM Roth. Exercise modifies age-related telomere dynamics in multiple tissues of CAST/Ei mice. Poster presentation, *Experimental Biology 2011*, Washington, D.C. *FASEB J* 25:lb564, March 2011.

Guth LM, AT Ludlow, S Witkowski, MR Marshall, L Lima, K Perret, N Caffes, AC Venezia, EE Spangenburg, and SM Roth. Transgenerational effects of physical activity ancestry on mouse body composition, glucose metabolism, and gene expression. Poster presentation, *ACSM's Conference on Integrative Physiology of Exercise*, Miami Beach, Florida, September 2010.

Marshall MR, AT Ludlow, S Witkowski, J Wang, **LM Guth**, S Frank, EE Spangenburg, and SM Roth. Chronic wheel running alters telomere biology in CAST/Ei mouse liver tissue. Poster presentation, *2010 American College of Sports Medicine Annual Meeting*, Baltimore, MD. Med Sci Sports Exerc 42(5): Supplement, June 2010.

Ludlow AT, S Witkowski, MR Marshall, J Wang, **LM Guth**, EE Spangenburg, and SM Roth. Year Long Wheel Running Alters Telomere Dynamics and Markers of DNA Damage in Mice. Poster presentation, *2010 American College of Sports Medicine Annual Meeting*, Baltimore, MD. Med Sci Sports Exerc 42(5): Supplement, June 2010.

Guth LM, KA Craft, AD Mahon. Effects of carbohydrate supplementation on variable-intensity exercise responses in boys and men. Oral presentation, *2010 American College of Sports Medicine Annual Meeting*, Baltimore, MD. Med Sci Sports Exerc 42(5): Supplement, June 2010.

Mahon AD, **LM Guth**, and KA Craft. Physiological and perceptual responses in children during variable-intensity exercise. Oral presentation, *25th Pediatric Work Physiology Meeting*, Le Touquet, France, 2009.

Hanna LE, **LM Guth**, KA Craft and AD Mahon. The metabolic response to rest and acute exercise in adolescent boys with non-insulin-dependent diabetes mellitus (NIDDM) relatives. Oral presentation, *15th North American Society of Pediatric Exercise Medicine Scientific Meeting*, Colorado Springs, CO, 2008.

Mahon AD, LE Hanna, **LM Guth**, and KA Craft. The influence of a positive family history for non-insulin dependent diabetes mellitus (NIDDM) on ventilatory threshold in adolescent boys. Oral presentation, *15th North American Society of Pediatric Exercise Medicine Scientific Meeting*, Colorado Springs, CO, 2008.

Guth LM, LE Hanna, KA Craft, and AD Mahon. Effects of a family history of non-insulin dependent diabetes mellitus on body composition in adolescents. Poster presentation, *15th North American Society of Pediatric Exercise Medicine Scientific Meeting*, Colorado Springs, CO, 2008.

Guth LM, LE Hanna, JD Lee, and AD Mahon. The effects of carbohydrate supplementation on fatigue during intermittent cycling. Oral presentation, *2008 American College of Sports Medicine Annual Meeting*, Indianapolis, IN. Med Sci Sports Exerc 40(5): Supplement, May 2008.

Mahon AD, JD Lee, LE Hanna, **LM Guth**, and AR Konopka. Pre-exercise carbohydrate supplementation and perceived exertion in adolescent males performing high-intensity intermittent exercise. Oral presentation*, 24th Pediatric Work Physiology Meeting*, Tallinn (Laulasmaa), Estonia, 2007.

Lee JD, LE Hanna, **LM Guth**, AR Konopka, and AD Mahon. The effects of pre-exercise carbohydrate supplementation on anaerobic exercise performance in adolescent males. Oral presentation, *24th Pediatric Work Physiology Meeting*, Tallinn (Laulasmaa), Estonia, 2007.

Regional and Local Meetings

Bradshaw T, **LM Guth**, JM Hagberg. Genetic variants in SLC30A8 gene had no effect on Type 2 Diabetes related phenotypes in response to exercise training. Poster presentation, *University of Maryland School of Public Health Summer Training and Research program poster session*. August 2012.

McKinney M, **LM Guth**, JM Hagberg. Genetic variation in PPAR gamma will affect obesity related phenotype in response to exercise training. Poster presentation, *University of Maryland School of Public Health Summer Training and Research program poster session*. August 2012.

Beltran EP, **LM Guth**, AC Venezia, MP Marini, EE Spangenburg, SM Roth. The Potential of Exercise Ancestry to have Transgenerational effects on body composition, glucose tolerance, and gene expression in mouse liver. *Howard Hughes Medical Institute Undergraduate Research Symposium*. University of Maryland, February 2012.

***Guth LM**, AT Ludlow, S Witkowski, MR Marshall, L Lima, AC Venezia, T Xiao, M-LT Lee, EE Spangenburg, and SM Roth. Exercise ancestry decreases lipogenesis-related gene expression in skeletal muscle of male offspring. Poster presentation, *University of Maryland School of Public Health Research Interaction Day*, September 2011.

Guth LM, AT Ludlow, S Witkowski, MR Marshall, L Lima, AC Venezia, T Xiao, M-LT Lee, EE Spangenburg, and SM Roth. Exercise ancestry decreases lipogenesis-related gene expression in skeletal muscle of male offspring. Poster presentation, *University of Maryland Graduate Research Interaction Day*, April 2011.

Guth LM, AT Ludlow, S Witkowski, MR Marshall, L Lima, K Perret, N Caffes, AC Venezia, EE Spangenburg, and SM Roth. Transgenerational effects of physical activity ancestry on mouse body composition, glucose metabolism, and gene expression. Poster presentation, *University of Maryland Bioscience Day*, November 2010.

Guth LM, AT Ludlow, S Witkowski, MR Marshall, L Lima, K Perret, N Caffes, AC Venezia, EE Spangenburg, and SM Roth. Transgenerational effects of physical activity ancestry on mouse body composition, glucose metabolism, and gene expression. Poster presentation, *University of Maryland School of Public Health Research Interaction Day*, October 2010.

***Guth LM**, LE Hanna, JD Lee, and AD Mahon. The effects of carbohydrate supplementation on fatigue during intermittent cycling. Poster presentation, *2007 Midwest Regional Chapter American College of Sports Medicine Annual Meeting*, Columbus, OH, 2007.

*Honor/Award received

GRANTS

Effects of Exercise Ancestry on Mouse Offspring Glycemia. Graduate Research Initiative Fund (GRIF), Department of Kinesiology, University of Maryland. $2500. 2012.

The effects of chronic exercise on metabolic gene expression and DNA methylation patterns in CAST/Ei mouse skeletal muscle. Graduate Research Initiative Fund (GRIF), Department of Kinesiology, University of Maryland. $2500. 2010.

TEACHING

2013 EXSC 340, **Exercise Prescription and Testing for General Populations, Trinity Washington University, Adjunct Faculty**

The course provides students with theoretical principles and practical experiences in exercise prescription and assessment in low-risk health populations. Health-related fitness, rather than performance based fitness is emphasized. The course content is based upon guidelines published by the American College of Sports Medicine (ACSM) and provides a foundation for future ACSM certification as a Health/Fitness instructor.

2012 **KNES 497, Independent Studies Seminar (Physical activity and the obesity and diabetes epidemic), University of Maryland, Co-Instructor** with James M. Hagberg, Ph.D.

Seminar discussions of contemporary issues vital to the discipline, critiques of research in the student's area/areas of special interest, and completion of a major project where the students will be asked to demonstrate the ability to carry out the investigative process in terms of problem solving and critical writing under faculty direction.

2008-2009 **PEP 493L, Advanced Concepts in Exercise Physiology Laboratory, Ball State University,** Instructor

The effect of exercise on the anatomical structures and the physiological functions in humans during acute and chronic activity.

SERVICE

2008-2009	Undergraduate Curriculum Committee, School of Physical Education, Sport & Exercise Science, Ball State University
2010-2013	Functional Genomics Laboratory Manager
2010-2011	University of Maryland Kinesiology Graduate Committee
2010-2012	University of Maryland School of Public Health Orientation Student Panel (Panel Member)
2011-2012	University of Maryland Kinesiology Graduate Student Council
2012	Kinesiology Orientation Student Panel (Panel Member)
2012-2013	School of Public Health Dean's Student Advisory Committee

Mentoring:

2009-2010	Kat Perret (Undergraduate Kinesiology Honors Student)
2010-2011	Nick Caffes (Undergraduate Kinesiology Honors Student)
2010-2012	Michael Marini (Exercise Physiology Master of Arts Student)
2010-2013	Estefan Beltran (Undergraduate Research Assistant, Maryland-HHMI Research Fellow)
2011	Elizabeth Antman (Undergraduate Research Assistant)
2011-2012	Erika Olney (Undergraduate Research Assistant)
2011-2012	Angel Baez (University of Maryland STAR)
2011-2012	Tancia Bradshaw (University of Maryland STAR)
2012-2013	Tripti Soni (University of Maryland STAR)
2012-2013	Martell McKinney (University of Maryland STAR)
2012-2013	Kelsey Corrigan (Exercise Physiology Master of Arts Student)
2012-2013	Craig Foote (Undergraduate Research Assistant)
2013	Nigell Essix (University of Maryland STAR)
2013	Yolanda Pine (University of Maryland STAR)
2013	Chris Leon (Undergraduate Research Assistant)

Assisted with Manuscript Peer Review for *Journal of Applied Physiology*, *Sports Medicine*, *Medicine and Science in Sports and Exercise*.

MEMBERSHIP IN PROFESSIONAL ORGANIZATIONS

2007-2008	Midwest Chapter of the American College of Sports Medicine
2008-present	American College of Sports Medicine
2012-present	Delta Omega (Honorary Society in Public Health)

HONORS AND AWARDS

2007	Outstanding Poster Presentation, Undergraduate (Midwest ACSM, Columbus, OH)
2008	Internal Travel Award, Ball State University (North American Society of Pediatric Exercise Medicine Annual Meeting, Colorado Springs, CO)
2011	2nd Place in Communication (University of Maryland School of Public Health Research Interaction Day, College Park, MD)
2012	Inducted into Delta Omega (Honorary Society in Public Health), University of Maryland Gamma Zeta Chapter