Summer 8-15-2017

The Effect of Dietary Fat on Obesity, Gene Expression, and DNA Methylation in Two Generations of Mice

Madeline Rose Keleher
Washington University in St. Louis

WASHINGTON UNIVERSITY IN ST. LOUIS

Division of Biology and Biomedical Sciences

Evolution, Ecology, and Population Biology

Dissertation Examination Committee:
Jim Cheverud, Chair
Jenny Duncan
Allan Larson
Kelle Moley
Ken Olsen
Ting Wang

The Effect of Dietary Fat on Obesity, Gene Expression, and DNA Methylation in Two Generations of Mice

by

Madeline Rose Keleher

A dissertation presented to
The Graduate School
of Washington University in
partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

August 2017

St. Louis, Missouri

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Jim Cheverud for being my advisor and supporting me these six long years. Jim, your comments on my earlier drafts of this dissertation made the final product far better. I am also indebted to my committee members. I had never done wet lab work before my lab rotation with Jenny Duncan, my co-advisor who personally showed me how to use a micropipettor and extract DNA, and patiently taught me how to troubleshoot when I made mistakes. Allan Larson has given me wise advice and is one of the best teachers I've ever had. Kelle Moley gave me good feedback and helped when I was having difficulties getting the high-fat-fed mice to reproduce. Ting Wang is charismatic and has endless energy for science; he introduced me to epigenetics and has been a great role model for how to give an engaging science talk. Ken Olsen always managed to respond to my emails within 10 minutes, submitted many letters of recommendation for me, helped me feel connected to Wash U's program even during the four years I spent in Chicago, and is a superb teacher and scientist.

A special thank you to my middle school science teacher, Jim Gravagna, who nurtured my interest in science and convinced me to join the school Science Olympiad team. He is a gifted teacher and an amazing coach. Thank you to Charles Roseman, who started my career in research by letting me into his lab when I was a sophomore at the University of Illinois. What I thought was just a semester-long honors project turned into a 2.5-year research position with a hilarious, brilliant scientist. It hadn't occurred to me to do research or to apply to graduate school until Charles encouraged me to.

I also want to thank Stan Braude, who treated me as a co-teacher instead of just a TA in his human biology class, let me co-author a book with him to teach the general public to be more informed biomedical consumers, inspired me with his creative teaching techniques, and has

given me so much support and encouragement over the years. I am extremely thankful for the members of the Cheverud lab in both its St. Louis and Chicago incarnations, including Eric Schmidt, Bing Wang, and Susan Pletscher (Susan was the first person who got to know me in St. Louis and she taught me how to take care of mice). Mihaela Pavlicev has been a mentor and a friend, and I am grateful for her guidance and her eternally cheerful attitude. I also want to thank my wonderful team of undergrads, who worked tirelessly to help collect data for my thesis: Lauren Hicks, Rabab Zaidi, Amer Ahmed, Cassondra Pavlatos, Kayna Patel, Shyam Shah, Elsa Oakley, Carlee Bettler, and Samir El Idrissi. Lauren and Rabab, on the days when I really didn't feel up to spending hours glucose testing or necropsying mice, your impressive work ethic and sunny dispositions made the time fly by.

I would also like to thank the employees of Loyola's Animal Care Facility, particularly James Robinson and Beth McGeehan, for ensuring that my mice were well taken care of. Thank you to Chris Calderaro and Virginia Lorenzo for helping me with ordering all the supplies necessary for my research, especially when it was an urgent last-minute request or involved exceedingly complicated paperwork. I am grateful to Catherine Putonti for allowing me to run my methylation analysis on her giant computer, and to Jason Shapiro for showing me how to use said computer. Stefan Kanzok spent several hours teaching me everything I needed to know about how RT-qPCR works and he generously let me use the thermal cycler machine in his lab. Alec Lindstrom patiently showed me how to use the machine and let me observe him work. Sushma Reddy has always looked out for me at Loyola, and I am so happy to have become friends with two wonderful scientists in her lab, Jane Younger and Lynika Strozier. Lynika, it has been great finishing up our theses at the same time, and Jane I've thoroughly enjoyed our Chicago adventures.

I owe a big thank you to Xiaoyun Xing, Daofeng Li, and Bo Zhang for all of their help with the methylation data. I also want to thank the American Heart Association for awarding me a pre-doctoral fellowship and Thermo Fisher Scientific for giving me a scholarship—the funding was much needed and deeply appreciated! A huge thank you to Shirley McTigue at Wash U for helping me submit my grant applications and enthusiastically cheering me on along the way (especially when my applications kept getting rejected in the beginning).

Thank you to my friends in Wash U's EEPB program for all of the support and advice—about science and life in general—during this process, especially Kimberley Sukhum, Boahemaa Adu-Oppong, and Elizabeth Atkinson. I am happy to have shared the Wash U graduate school experience with Claudia Henriquez, Arthur Porto, Sara Wright, Kevin Stiles, Michael Stevens, and Jessica Joganic—your friendship made it so much better. And I never would have gotten through this without Devin Dobias, my best friend and partner in everything. Devin, thank you for pushing me to be a better scientist and person, for helping me learn R, for answering all of my questions about statistics, for our many stimulating science discussions over beers, for cheering me up whenever graduate school got tough, for being an excellent travel companion, for turning me into a birder, and for being a model of integrity and perseverance.

Finally, I would like to thank my family. Mom, you listened to the nitty-gritty details about my research, kept my spirits up, and have been my personal cheerleader each step of the way. Dad, you instilled your love of science and the natural world in me at a very early age—thank you for our weekend trips to the zoo when I was a baby, for encouraging me to question everything, for having us watch all those David Attenborough series as a family, for being able to talk about any science topic with me, for reading my entire dissertation, and for all of your help and love during this PhD journey. Shannon and Katrina, thank you for being such kind,

empathetic, hilarious sisters—you are excellent listeners and always know just what to say to make me laugh. To my aunts, uncles, grandma, and cousins, thank you for your encouragement and understanding throughout this process—getting asked how my mice were doing instead of how I was doing at family gatherings showed that you all understood how much my research meant to me. Marian, thank you for teaching me how to write a grant application—that certainly came in handy! Michelle, thanks for your weekly phone messages and two decades of friendship. Janine, thanks for your endless stream of encouragement and for going to such great lengths to understand my work—I think you know more about DNA methylation and obese mice than any other graduate of Harvard Business School.

It is said that it takes a village to raise a child, but I also think it takes a village to make a scientist. Thank you to everyone who has been a part of my village.

Madeline Rose Keleher

Washington University

August 2017

ABSTRACT OF THE DISSERTATION

The Effect of Dietary Fat on Obesity, Gene Expression, and DNA Methylation in Two Generations of Mice

by

Madeline Rose Keleher

Doctor of Philosophy in Evolution, Ecology, and Population Biology

Washington University in St. Louis, 2017

Professor James Cheverud, Chairperson

As obesity rates continue rising nationally and globally, it is crucial to understand how a high-fat diet disrupts the regulation of the genome and leads to adverse health effects. Uncovering the underlying gene expression and DNA methylation changes induced by an individual's high-fat diet and a maternal high-fat diet can pinpoint new targets for epigenetic therapies and reveal the physiological and behavioral changes in obesity. The goal of this dissertation is to gain deeper insight into the DNA methylation and gene expression changes that occur in response to a high-fat diet.

I studied the response to dietary fat within two generations of the inbred SM/J mouse strain by feeding them either a low-fat diet or a high-fat diet that was nearly isocaloric but had approximately three times as many calories from fat. I measured their weights weekly for 17 weeks, tested their glucose and insulin tolerance, assessed serum biomarkers, and weighed their organs at necropsy. Diet strongly affected all of these traits. I isolated RNA from the liver tissue and used RNA-sequencing to uncover 4,356 genes that were differentially expressed due to diet. Nearly 200 of these genes had a significant sex-by-diet interaction, highlighting the importance

of sex differences in the response to a high-fat diet. The dysregulated genes were enriched for pathways involved in cytokine-cytokine receptor interaction, chemokine signaling, and oxidative phosphorylation. I extracted DNA from the liver tissue and identified differentially methylated regions across the genome using MeDIP and MRE sequencing. More than 7,000 genes in the liver had differentially methylated regions at the FDR-adjusted $q < 0.05$ level. The differentially methylated regions occurred in differentially expressed genes significantly more often than expected by chance alone. These findings underscore the major effect that dietary fat has on gene expression and DNA methylation patterns in the liver, and illustrate how different this effect is depending on sex.

I then mated low-fat-fed males with females on either diet, and cross-fostered all offspring to low-fat-fed SM/J nurses in order to study the effect of prenatal maternal diet on adult offspring metabolic traits, behavior, gene expression, and DNA methylation. I weaned the offspring onto a high fat or low-fat diet at 3 weeks of age. I then measured the same traits in the offspring as I measured in the parental generation, as well as additional morphological and behavioral traits. I measured the long-bone lengths and weights, anxiety with the Open Field Test, behavior with instantaneous scan sampling, and nest quality with the Deacon Scale. Compared to low-fat-fed offspring, high-fat-fed offspring had longer, heavier bones, had increased anxiety levels, built lower-quality nests, and had reduced activity levels in adulthood, including exploratory and self-maintenance behaviors. Maternal diet did not have much of an impact on offspring bones or behavior, only affecting whether or not the offspring built their nests inside a hut.

Although maternal diet did not widely affect offspring behavior, it had major effects on the obesity and diabetes-related traits in the adult daughters. Compared to high-fat-fed daughters

of low fat mothers, high-fat-fed daughters of high-fat mothers weighed more, had heavier livers and reproductive fat pads, and had higher leptin levels in their serum. These physiological changes were accompanied by 46 differentially expressed genes and 1,700 differentially methylated genomic regions in liver cells, as well as 45 differentially expressed genes and 4,103 differentially methylated genomic regions in heart cells. Although the obesity traits were only altered by maternal diet only in the daughters, all offspring experienced changes in the expression of dozens of genes in the liver, particularly genes involved in RNA processing, immune response, and cellular respiration in mitochondria. Maternal obesity also altered DNA methylation in thousands of regions in the genome. Over 7,300 genes contained at least one differentially methylated region due to maternal diet in high-fat-fed offspring, and over 9,300 genes did in low-fat-fed offspring. Regardless of maternal diet, an offspring high-fat diet reduced overall variation in methylation, increased body size—as measured by the weights of the organs and body, and the weights and lengths of the long bones—decreased insulin sensitivity, and altered the expression of 3,908 genes. While the mice were more affected by their own individual diets, their maternal diet impacted their DNA methylation and gene expression into adulthood. The findings of this dissertation improve our understanding of the epigenetic architecture of obesity and identify new targets for therapies in the future.

CHAPTER 1

Introduction of the Dissertation

The human obesity rate has been climbing over the last several decades. While it was 14% in the United States in the 1980s (Bray and Popkin 2014), it had passed 37% by 2014 (Flegal *et al.* 2016), and it is predicted to reach 42% by 2030 (Finkelstein *et al.* 2012). The prevalence of obesity may be higher than estimated, since many studies are based on self-reported weights, which people tend to underestimate (Cawley *et al.* 2015). The increasing prevalence of obesity is a major public health concern, because obesity raises the risk of developing hypertension by 5 times (Haslam and James 2005), it elevates the risk for many cancers (Lauby-Secretan *et al.* 2016), it predisposes people to diabetes (Haslam and James 2005), it raises the risk of stroke and heart attack (Cawley and Meyerhoefer 2011), and it decreases life expectancy by a decade on average (Buchwald 2005).

Obesity is a complex condition with a myriad of causes. A major factor leading to obesity is the environment, particularly an individual's diet and physical activity level (Hill and Peters 1998). Diet has been changing considerably in the United States, both in terms of macronutrient composition and total calorie intake. From the 1970s to 2000, the average energy intake increased by 168 kcal/day for men and 335 kcal/day for women (CDC 2004). Sugar partially accounts for the higher energy intake; while the average American consumed 4 lbs of sugar annually in 1776, it had increased to 20 lbs per year by 1850 and 120 lbs per year by 1994 (Bray and Popkin 2014).

In addition to environmental factors such as diet and physical activity, another factor leading to obesity is genetic. Obesity is highly heritable (BMI has a heritability of 64-84%), and genes such as *MC4R*, *POMC*, and *LEP* are known to be involved in monogenic obesity syndromes (O'Rahilly and Farooqi 2006). A study of more than 2,000 first-degree relatives

revealed that having a parent or sibling with a BMI of at least 40 increases a person's risk of being obese by 5 times (Lee *et al.* 1997). A study of more than 3,000 children in Germany revealed that offspring BMI was significantly correlated with maternal BMI (r = 0.254) and paternal BMI (r = 0.159) (Danielzik *et al.* 2002). The authors found that the odds ratio for being overweight in children was higher for those with at least one overweight parent, and was highest for children with two obese parents (an odds ratio of 7.6 in sons and 6.3 in daughters). A longitudinal study that followed girls from the ages of 5 to 13 showed that daughters with two overweight parents had higher levels of disinhibited eating and had an 8 times higher risk of being overweight at age 13 than daughters of lean parents (Francis *et al.* 2007). The health risks that come with having two overweight parents are important to consider, because researchers have identified assortative mating in terms of weight, with a strong association between maternal and paternal obesity prior to conception (Soubry *et al.* 2015).

Childhood weight has a strong genetic component, as illustrated by a study of 177 pairs of twins where genes accounted for 90% of body weight variance at 5 months of age and 87% of variance at 5 years of age (Dubois *et al.* 2007). Mutations in 5 genes account for at least 5% of severe childhood obesity cases: *MC4R*, *POMC*, *PCSK1*, *LEP*, and *LEPR* (Bouchard 2009). However, obesity disorders are more commonly caused by many genes of small effect rather than a single gene of large effect (Bouchard 2009). More than 75 genomic loci linked to obesity have been identified through genome-wide association studies (Gorkin and Ren 2014). Interestingly, many of these loci are in non-protein coding regions of genes, as is the case with *FTO* (Gorkin and Ren 2014). Although GWAS studies have consistently identified an association between obesity and SNPs in *FTO*, the expression of *FTO* itself does not impact obesity. Rather, an intron of *FTO* regulates the expression of the homeobox gene *IRX3* (Smemo

et al. 2014).

Modern increases in obesity are not due to genetic changes but to the changes in environment as discussed above. Nevertheless, there is genetic variation in response to obesogenic environments. For instance, the *FTO* gene participates in gene-by-environment interactions. The effects of physical activity on obesity change depending on the *FTO* genotype in humans. Compared to TT homozygotes, AA homozygotes have 1.95 kg/m^2 higher BMIs, but only if they are physically inactive (Andreasen *et al.* 2008). A gene-by-environment interaction has also been identified in the response to caloric excess. When Bouchard *et al.* (1990) assigned 12 pairs of identical male twins to a diet with an excess of 1,000-kcal/day for 100 days, each of the men gained weight, but there was 3 times more variance between pairs of twins than within pairs. Numerous gene-by-diet interactions have also been discovered in mice, and most of the genes affecting obesity in a cross of LG/J and SM/J mice (the latter strain is the focus of this dissertation) are involved in gene-by-diet interactions (Ehrich *et al.* 2000, Cheverud *et al.* 2011).

Many less-studied causes of obesity have been identified as well. Sleep deprivation has been shown to cause impaired glucose and insulin sensitivity (Knutson *et al*. 2007), and lower rates of obesity have been linked to urbanization and living at a higher elevation in the United States (Voss *et al.* 2013). Recently, the gut microbiome has also been implicated in obesity (Parekh *et al.* 2015, Barlow *et al.* 2015), and researchers have shown that transferring gut microbiota from obese people to mice causes the mice to gain significantly more adipose tissue than if they receive microbiota from a lean person (Ridaura *et al.* 2013). Additionally, several studies have suggested a relationship between obesity and infection with certain viruses. Obesity has resulted in mice after they were infected with canine distemper virus, and in chickens after being infected with Rous-associated virus-7 (RAV-7) (McAllister *et al.* 2009). Marmosets

infected with adenovirus-36 (Ad-36) gained 4 times as much weight as uninfected marmosets. In a study of 502 people in the United States, 30% of obese individuals were found to have been naturally infected by Ad-36, compared to only 11% of non-obese individuals (Atkinson *et al.* 2005).

Parental effects also contribute to obesity risk through environmental and genetic mechanisms. Paternal obesity has been shown to impact offspring in numerous ways. Children have an increased risk of developing early-onset obesity if they inherit a paternal allele of the *INS* gene that carries a class I variable nucleotide tandem repeat polymorphism (Le Stunff *et al.* 2001). Epigenetic changes associated with paternal obesity can also impact offspring. High-fat-fed male mice had 25% less DNA methylation in their sperm and altered gene expression in their testes, which affected their progeny by increasing adiposity in their daughters by 67% and in their grandsons by 24% (Fullston *et al*. 2013). In addition to methylation, the tRNA in sperm is also susceptible to diet. When the tRNA from high-fat-fed male mice was inserted into eggs fertilized by the sperm of lean males, the offspring went on to develop insulin resistance (Chen *et al.* 2016).

Maternal high-fat diet also has substantial effects on offspring, although our understanding of this has only begun to develop only in the last few decades. The prevailing view in the mid-1900s was that the fetus was a "perfect parasite," shielded by the placenta from any dangerous substances in the mother's body, including alcohol and cigarette smoke (Almond and Currie 2011). In the 1960s and 1970s, nearly half of pregnant women smoked (Abel 1980, Fertig 2010, CDC 2001), and in the 1980s, a third of women consumed alcohol during pregnancy (Serdula *et al.* 1991). Since the emergence of the fetal origins of adult disease hypothesis, the prevalence of smoking and drinking has dropped drastically: by the 2000s only 8.4% of pregnant

women smoked (Curtin and Matthews 2016), and alcohol consumption had dropped to 8-14% (Zhao *et al.* 2012). Now that we have a better understanding of the relationship between the mother, fetus, and environmental inputs, the next step is to reduce maternal obesity during pregnancy.

The fetal origins of adult disease hypothesis originated from research that Dr. David Barker published in the 1980s. Barker and colleagues compiled the birth weights of 5,654 men born in Hertfordshire, England between 1911-1930 and catalogued the cause of death in the over 1,000 men who had died by 1987 (Barker *et al.* 1989). They found that men with the lowest weights at birth and one year of age had the highest death rates of ischemic heart disease (those weighing 18 lbs or less at one year had a 3 times higher rate of death from heart disease than those weighing at least 27 lbs), leading the authors to conclude that risk of heart disease is affected by processes influencing prenatal and early postnatal growth. Subsequent research extended the fetal origins hypothesis to include type 2 diabetes and hypertension (Hales *et al.* 1991, Barker *et al.* 2002).

To specify the underlying mechanism, Barker proposed the thrifty phenotype hypothesis. This hypothesis states that exposure to poor nutrition in the prenatal and early postnatal environment permanently changes glucose and insulin metabolism, resulting in metabolic syndrome later in life (Hales and Barker 2001). While Barker's thrifty phenotype hypothesis originally focused on the connection between low birth weight and adult cardiovascular disease, high birth weight has now been added to it. Many studies noted a U-shaped curve, with both low and high birth weight infants having an increased risk of metabolic syndrome as adults (Pettitt and Jovanovic 2001, Harder *et al.* 2007). Compared to infants with intermediate birth weights, large for gestational age infants have higher BMIs as children,

adolescents, and adults (Skelton *et al.* 2014)

Maternal obesity during pregnancy is harmful to both a mother and her offspring. Negative effects to the mother include increasing the risk of: preeclampsia by 10-15% (Barton and Sibai 2008), gestational diabetes (while 2.3% of lean women develop gestational diabetes, the incidence is 4.8% in overweight women, 5.5% in obese women, and 11.5% in extremely obese women) (Kim *et al.* 2010), miscarriage (10.7% of lean women have at least one miscarriage, compared to 11.8% of overweight and 13.6% of obese women) (Boots and Stephenson 2011), gestational hypertension (affecting 5% of lean women, 10% of obese women, and 12% of morbidly obese women) (Weiss *et al.* 2004), C-section (21% of lean women have C-sections, compared to 34% of obese and 47% of morbidly obese women) (Weiss *et al.* 2004), and longer time to dilate from 4 to 10 cm (6.2 hours for lean women compared to 7.5 for overweight and 7.9 hours for obese women) (Vahratian *et al.* 2004).

The risks to the offspring include stillbirth (the risk is 2-5 times higher with an obese mother, and 25% of stillbirths after 36 weeks of gestation are associated with obesity) (Yao *et al.* 2014, Chu *et al. 2*007), neural tube defects (the odds ratios is 1.22 with an overweight mother, 1.70 with an obese mother, and 3.11 with a severely obese mother) (Rasmussen *et al.* 2008), and being born large for gestational age (Weiss *et al.* 2004, Gaudet *et al.* 2014, Wang *et al.* 2015), which comes with an elevated risk of hypoglycemia and hypomagnesemia *in utero*, and cardiovascular disease and obesity later in life (Stirrat and Reynolds 2014). The effects of maternal obesity last through childhood, increasing the risk of childhood obesity (9% of 4-year-olds with lean mothers were found to be obese, compared to 24% of those whose mothers were obese during pregnancy) (Whitaker 2004), increasing the levels of insulin, cholesterol, and blood pressure (Gaillard *et al.* 2014), and raising the risk of anxiety, attention deficit hyperactivity

disorder, and autism (Sullivan *et al.* 2014). As adults, people whose mothers were obese during pregnancy have a higher risk of cancer, cardiovascular disease, hypertension, and type 2 diabetes (Hochner *et al.* 2012, Eriksson *et al.* 2014, Stirrat and Reynolds 2014, Galliard 2015).

Maternal obesity can affect offspring development through a variety of mechanisms, including by altering the glucose consumption of oocytes (Jungheim *et al.* 2010, Leary *et al.* 2015), causing the placenta to be inflamed and transport more glucose to the fetus (Bar *et al.* 2012, Sferruzzi-Perri *et al.* 2013), and causing mitochondrial dysfunction (Igosheva *et al.* 2010, Luzzo *et al.* 2012, Grindler and Moley 2013). Saben *et al.* (2016) showed that feeding female mice a diet high in fat and sugar induced mitochondrial dysfunction in 3 generations of offspring, which appeared to be transmitted through mitochondrial abnormalities observed in the F_1 and F_2 germ cells. Postnatally, one way that maternal obesity affects offspring is through lactation. Mouse dams fed a high-fat diet have higher levels of leptin and fat in their milk (Sun *et al.* 2012), and maternal obesity delays the onset of lactogenesis II in women. This may be because obese women have a higher risk of edema, prolonged labor, dysfunctional labor, insulin imbalance, and C-sections, which are all associated with a delayed onset of lactogenesis (Babendure *et al.* 2015).

The present study is an investigation of a subset of non-genetic maternal effects. In Chapters 2 and 4, I studied the maternal effects a high-fat diet on offspring behavior and obesity. When maternal effects are present, the offspring's phenotype is determined not only by its own environment and genetics, but by its mother's environment and genetics as well. In some cases, the maternal environment that an individual experiences during development can have effects lasting throughout life. This is an important evolutionary strategy that helps some organisms cope with environmental heterogeneity. For instance, when seed beetle mothers are forced to lay

eggs on thick-coated seeds, they lay larger eggs to give the offspring enough energy provisions to bore through the seed (Fox *et al.* 1999). Females of many species can also manipulate the number of eggs they lay in a clutch, and female parasitic wasps, alligators, and collared flycatchers can manipulate offspring sex ratio to increase fitness based on environmental cues (Mousseau and Fox 1998). Maternal effects are not always adaptive, however, as in the case of prenatal metabolic programming of obesity in humans. Children of obese mothers have a higher future risk of insulin resistance, obesity, and cardiometabolic disease (Tam *et al.* 2014). One mechanism through which maternal effects can persist in offspring is via epigenetics.

When Conrad Waddington first coined the term 'epigenetics,' he used it to describe the developmental processes connecting genotype to phenotype (Waddington 1942). The meaning of the term has developed over the decades, and now usually specifically refers to factors other than DNA sequence that control gene expression, such as histone modifications, RNA interference, and DNA methylation. A textbook example of maternal diet affecting the methylation of offspring DNA involves the A^{vy} allele of the agouti gene in mice. Normally agouti is expressed only in the skin and only during early development, but mice carrying the genetically dominant A^{vy} allele continuously express agouti throughout the body and throughout development due to a cryptic promoter inserted by a transposable element, causing obesity and diabetes in adulthood (Miltenberger *et al.* 1997, Morgan *et al.* 1999). Expression of the agouti locus is directly correlated with the level of methylation at the cryptic promoter, which can be modulated by maternal diet. When an A^{vy}/a dam is fed a diet rich in methyl-donors during pregnancy, her offspring are born with increased agouti methylation, reduced agouti expression, and they do not become obese as adults (Wolff *et al.* 1998, Waterland and Jirtle 2003, Waterland *et al.* 2004). It appears that genes with cryptic promoters are not the only ones affected epigenetically by

maternal diet. In humans, DNA sampled from cord blood reveals differences in methylation at several imprinted genes between newborns with obese parents versus those whose parents are not obese (Soubry *et al.* 2015), and children born after their mothers had gastric bypass surgery had methylation differences in over 5,000 genes compared to their siblings born before the weight-loss surgery, as well as lower BMIs (Guénard *et al.* 2013).

Many unanswered questions remain in the field of obesity epigenetics. Which genes become dysregulated by a high-fat diet? How are changes in DNA methylation associated with dysregulated gene expression? Which type of genomic regions do these diet-responsive methylated regions reside in? How are these factors affected by a prenatal maternal high-fat diet? How are the effects different in different tissues? How does a different genetic background influence the development of obesity? My dissertation attempts to answer some of these questions in the context of the inbred SM/J mouse strain, which is known to have an extensive obesogenic response to a high-fat diet (Cheverud *et al.* 1999, Ehrich *et al.* 2003, Partridge *et al.* 2014).

Dietary fat has substantial, wide-ranging effects on the body. Some of these effects—such as insulin resistance and weight gain—are better understood than others, such as anxiety and altered activity patterns. Chapter 2 of this dissertation investigates the effect that a maternal high-fat diet and an individual's high-fat diet have on behavior. To determine whether dietary fat increased anxiety, I performed Open Field Testing on 98 high-fat-fed and low-fat-fed mice. I used an ethogram to measure behavior with instantaneous scan sampling, and rated the quality of the nests the mice built according to the Deacon Scale (Deacon 2006). Chapter 3 addresses the direct effect that an individual's diet has on obesity traits, DNA methylation, and gene expression in the liver. Although circadian variation in DNA methylation can be an issue in the

brain (Azzi *et al.* 2014), there is little to none in the liver (Vollmers *et al.* 2012). I identified methylated regions, genes, and gene pathways that were dysregulated due to a high-fat diet. Chapter 4 takes this a step further by investigating the response of these traits to a maternal high-fat diet. I discovered thousands of differentially methylated regions due to maternal diet and classified each region's location in the genome, any regulatory elements it contained, the gene nearest to it, and whether the nearest gene had been previously associated with diabetes, obesity, or cardiovascular disease. I identified dozens of genes that were differentially expressed due to maternal diet and found which of these genes contained differentially methylated regions.

This dissertation adds to the field of obesity epigenetics in several ways. For financial reasons, most studies measure methylation and expression in only one tissue type, even though epigenetic changes due to diet differ across tissues. In Chapter 4, I measured methylation and expression in both the liver and the heart in the high-fat-fed daughters. And while most obesity studies take a candidate-gene approach to investigate methylation, I took a genome-wide approach and identified thousands of methylation changes across the genome, not only in genes and enhancers but in intergenic regions as well. Most mouse obesity research is conducted in C57BL/6 mice, but I used the less-studied SM/J strain, which allowed me to compare my findings with those of other studies to get a better picture of how genetic background affects gene expression, methylation, and obesity.

LITERATURE CITED

Abel, E. L. 1980. Smoking during pregnancy: a review of effects on growth and development of offspring. *Human Biology* 52(4): 593-625.

Almond, D., and J. Currie. 2011. Killing me softly: the fetal origins hypothesis. *J Econ Prospect* 25(3): 153-172.

Andreasen, C. H., K. L. Stender-Petersen, M. S. Mogensen, S. S. Torekov, L. Wegner, G. Andersen, A. L. Nielsen, A. Albrechtsen, K. Borch-Johnsen, S. S. Rasmussen, J. O. Clausen, A. Sandbæk, T. Lauritzen, L. Hansen, T. Jørgensen, O. Pedersen, and T. Hansen. 2008. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* 57: 95-101.

Atkinson, R. L., N. V. Dhurandhar, D. B. Allison, R. L. Bowen, B. A. Israel, J. B. Albu, and A. S. Augustus. 2005. Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. *Int J Obes* 29(3): 281-286.

Azzi, A., R. Dallmann, A. Casserly, H. Rehrauer, A. Patrignani, B. Maier, A. Kramer, and S. A. Brown. 2014. Circadian behavior is light-reprogrammed by plastic DNA methylation. *Nat Neurosci* 17(3): 377-382.

Babendure J. B., E. Reifsnider, E. Mendias, M. W. Moramarco, and Y. R. Davila. 2015. Reduced breastfeeding rates among obese mothers: a review of contributing factors, clinical considerations and future directions. *Int Breastfeed J* 10: 21.

Bar, J., L. Schreiber, E. Saruhanov, A. Ben-Haroush, A. Golan, and M. Kovo. 2012. Placental histopathological findings in obese and nonobese women with complicated and uncomplicated pregnancies. *Arch Gynecol Obstet* 286(6): 1343-1347.

Barker, D. J., P. D. Winter, C. Osmond, B. Margetts, and S. J. Simmonds. 1989. Weight in infancy and death from ischaemic heart disease. *Lancet* 2(8663): 577-580.

Barker, D. J., J. G. Eriksson, T. Forsén, and C. Osmond. 2002. Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol* 31(6): 1235-1239.

Barton, J. R., and B. M. Sibai. 2008. Prediction and prevention of recurrent preeclampsia. *Obstetrics and Gynecology* 112: 359-372.

Boots, C., and M. D. Stephenson. 2011. Does obesity increase the risk of miscarriage in spontaneous conception: a systematic review. *Semin Reprod Med* 29(6): 507-513.

Bouchard, C. 2009. Childhood obesity: are genetic differences involved? *Am J Clin Nutr* 89(5): 1494S-1501S.

Bray, G. A., and B. M. Popkin. 2014. Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes? Health be damned! Pour on the sugar. *Diabetes Care* 37(4): 950-956.

Buchwald, H. 2005. Bariatric surgery for morbid obesity: health implications for patients, health professionals, and third-party payers. *J Am Coll Surg* 200(4): 593-604.

Cawley, J., and C. Meyerhoefer. 2012. The medical care costs of obesity: an instrumental variables approach. *J Health Econ* 31: 219-230.

Cawley, J., J. C. Maclean, M. Hammer, and N. Wintfeld. 2015. Reporting error in weight and its implications for bias in economic models. *Econ Hum Biol* 19: 27-44.

CDC. 2001.Women and smoking: a report of the surgeon general. *MMWR Recommendations and Reports* 51(RR12): 1-30.

CDC. 2004. Trends in intake of energy and macronutrients–United States, 1971–2000. *MMWR Morb Mortal Wkly Rep* 53: 80-82.

Chen, Q., M. Yan, Z. Cao, X. Li, Y. Zhang, J. Shi, G. H. Feng, H. Peng, X. Zhang, Y. Zhang, J. Qian, E. Duan, Q. Zhai, and Q. Zhou. 2016. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science* 351(6271): 397-400.

Cheverud, J. M., L. S. Pletscher, T. T. Vaughn, and B. Marshall. 1999. Differential response to dietary fat in large (LG/J) and small (SM/J) inbred mouse strains. *Physiol Genomics* 15(1): 33-39.

Cheverud, J. M., H. A. Lawson, G. L. Fawcett, B. Wang, L. S. Pletscher, A. Fox, T. J. Maxwell, T. H. Ehrich, J. P. Kenney-Hunt, J. B. Wolf, and C. F. Semenkovich. 2011. Diet-dependent genetic and genomic imprinting effects on obesity in mice. *Obesity* 19: 160-170.

Chu, S. Y., S. Y. Kim, J. Lau, C. H. Schmid, P. M. Dietz, W. M. Callaghan, and K. M. Curtis. 2007. Maternal obesity and risk of stillbirth: a metaanalysis. *Am J Obstet Gynecol* 197(3): 223-228.

Curtin, S. C., and T. J. Matthews. 2016. Smoking prevalence and cessation before and during pregnancy: data from the birth certificate, 2014. *Natl Vital Stat Rep* 65(1): 1-14.

Danielzik, S., K. Langnäse, M. Mast, C. Spethmann, and M. J. Müller. 2002. Impact of parental BMI on the manifestation of overweight 5-7 year old children. *Eur J Nutr* 41(3): 132-138.

Deacon, R. M. 2006. Assessing nest building in mice. *Nat Protoc* 1(3): 1117-1119.
Ehrich, T. E., J. P. Kenney, T. T. Vaughn, L. S. Pletscher, and J. M. Cheverud. 2003. Diet, obesity, and hyperglycemia in LG/J and SM/J mice. *Obesity Research* 11(11): 1400-1410.

Dubois L., M. Girard, A. Girard, R. Tremblay, M. Boivin, and D. Pérusse. 2007. Genetic and environmental influences on body size in early childhood: a twin birth-cohort study. *Twin Res Hum Genet* 10: 479-485.

Ehrich, T.H., T. Hrbek, J. P. Kenney-Hunt, L. S. Pletscher, B. Wang, C. F. Semenkovich, and J. M. Cheverud. 2005. Fine-mapping gene-by-diet interactions on chromosome 13 in a LG/J x SM/J murine model of obesity. *Diabetes* 54(6): 1863-1872.

Eriksson, J. G., S. Sandboge, M. K. Salonen, E. Kajantie, and C. Osmond. 2014. Long-term consequences of maternal overweight in pregnancy on offspring later health: findings from the

Helsinki Birth Cohort Study. *Ann Med* 46(6): 434-438.

Fertig, A. R. 2010. Selection and the effect of prenatal smoking. *Health Econ* 19(2): 209-226.

Finkelstein, E. A., O. A. Khavjou, H. Thompson, J. G. Trogdon, L. Pan, B. Sherry, and W. Dietz. 2012. Obesity and severe obesity forecasts through 2030. *Am J Prev Med* 42(6): 563-570.

Flegal, K. M., D. Kruszon-Moran, M. D. Carroll, C. D. Fryar, and C. L. Ogden. 2016. Trends in obesity among adults in the United States, 2005 to 2014. *JAMA* 315(21): 2284-2291.

Francis, L. A., A. K. Ventura, M. Marini, and L. L. Birch. 2007. Parent overweight predicts daughters' increase in BMI and disinhibited overeating from 5 to 13 years. *Obesity* 15(6): 1544-1553.

Fox, C. W., M. E. Czesak, T. A. Mousseau, and D. A. Roff. 1999. The evolutionary genetics of an adaptive maternal effect: egg size plasticity in a seed beetle. *Evolution* 53: 552-560.

Fullston, T., E. M. C. O. Teague, N. O. Palmer, M. J. DeBlasio, M. Mitchell, M. Corbett, C. G. Print, J. A. Owens, and M. Lane. 2013. Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. *FASEB J* 27(10): 4226-4243.

Gaillard, R. 2015. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. *Eur J Epidemiol* 30: 1141-1152.

Gaillard, R., E. A. Steegers, L. Duijts, J. F. Felix, A. Hofman, O. H. Franco, and V. W. V. Jaddoe. 2014. Childhood cardiometabolic outcomes of maternal obesity during pregnancy: the Generation R Study. *Hypertension* 63(4): 683-691.

Gaudet, L., Z. M. Ferraro, S. W. Wen, and M. Walker. 2014. Maternal obesity and occurrence of fetal macrosomia: a systematic review and meta-analysis. *Biomed Res Int* 2014: 640291.

Gorkin, D. U., and B. Ren. 2014. Genetics: closing the distance on obesity culprits. *Nature* 507(7492): 309-310.

Grindler, N. M., and K. H. Moley. 2013. Maternal obesity, infertility and mitochondrial dysfunction: potential mechanisms emerging from mouse model systems. *Mol Hum Reprod* 19(8): 486-494.

Guénard, F., Y. Deshaies, K. Cianflone, J. G. Kral, P. Marceau, and M.-C. Vohl. 2013. Differential methylation in glucoregulatory genes of offspring born before vs. after maternal gastrointestinal bypass surgery. *PNAS* 110(28): 11439-11444.

Hales, C. N., D. J. Barker, P. M. Clark, L. J. Cox, C. Fall, C. Osmond, and P. D. Winter. 1991. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 303(6809): 1019-1022.

Hales, C. N., and D. J. Barker. 2001. The thrifty phenotype hypothesis. *Br Med Bull* 60: 5-20.

Harder, T., E. Rodekamp, K. Schellong, J. W. Dudenhausen, and A. Plagemann. 2007. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. *Am J Epidemiol* 165(8): 849-857.

Haslam, D. W., and W. P. James. 2005. Obesity. *Lancet* 366: 1197-1209.

Hill, J. O., and J. C. Peters. 1998. Environmental contributions to the obesity epidemic. *Science* 280(5368): 1371-1374.

Hochner, H., Y. Friedlander, R. Calderon-Margalit, V. Meiner, Y. Sagy, M. Avgil-Tsadok, A. Burger, B. Savitsky, D. S. Siscovick, and O. Manor. 2012. Associations of maternal prepregnancy body mass index and gestational weight gain with adult offspring cardiometabolic risk factors: the Jerusalem Perinatal Family Follow-up Study. *Circulation* 125(11): 1381-1389.

Igosheva, N., A. Y. Abramov, L. Poston, J. J. Eckert, T. P. Fleming, M. R. Duchen, and J. McConnell. 2010. Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One* 5(4): e10074.

Jungheim, E. S., E. L. Schoeller, K. L. Marquard, E. D. Louden, J. E. Schaffer, and K. H. Moley. 2010. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. *Endocrinology* 151(8): 4039-4046.

Kim, S. Y., L. England, H. G. Wilson, C. Bish, G. A. Satten, and P. Dietz. 2010. Percentage of gestational diabetes mellitus attributable to overweight and obesity. *Am J Public Health* 100(6): 1047-1052.

Knutson, K. L., K. Spiegel, P. Penev, and E. Van Cauter. 2007. The metabolic consequences of sleep deprivation. *Sleep Med Rev* 11(3): 163-178.

Lauby-Secretan, B., C. Scoccianti, D. Loomis, Y. Grosse, F. Bianchini, and K. Straif. 2016. Body fatness and cancer—Viewpoint of the IARC working group. *The New England Journal of Medicine* 375: 794-798.

Le Stunff, C., D. Fallin, and P. Bougnères. 2001. Paternal transmission of the very common class I INS VNTR alleles predisposes to childhood obesity. *Nature Genetics* 29: 96-99.

Leary, C., H. J. Leese, and R. G. Sturmey. 2015. Human embryos from overweight and obese women display phenotypic and metabolic abnormalities. *Hum. Reprod* 30(1): 122-132.

Lee, J. H., D. R. Reed, and R. A. Price. 1997. Familial risk ratios for extreme obesity: implications for mapping human obesity genes. *Int J Obes Relat Metab Disord* 21: 935-940.

Luzzo, K. M., Q. Wang, S. H. Purcell, M. Chi, P. T. Jimenez, N. Grindler, T. Schedl, and K. H. Moley. 2012. High fat diet induced developmental defects in the mouse: oocyte meiotic

aneuploidy and fetal growth retardation/brain defects. *PLoS One* 7(11): e49217.

McAllister, E. J., N. V. Dhurandhar, S. W. Keith, L. J. Aronne, J. Barger, M. Baskin, R. M. Benca, J. Biggio, M. M. Boggiano, J. C. Eisenmann, M. Elobeid, K. R. Fontaine, P. Gluckman, E. C. Hanlon, P. Katzmarzyk,A. Pietrobelli, D. T. Redden, D. M. Ruden, C. Wang, R. A. Waterland, S. M. Wright, and D. B. Allison. 2009. Ten putative contributors to the obesity epidemic. *Crit Rev Food Sci Nutr* 49: 868-913.

Miltenberger, R. J., R. L. Mynatt, J. E. Wilkinson, and R. P. Woychik. 1997. The role of the agouti gene in the yellow obese syndrome. *J Nutr* 127: 1902S-1907S.

Morgan, H. D., H.G. Sutherland, D. I. Martin, and E. Whitelaw. 1999. Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 23: 314-318.

Mousseau, T. A. and C. W. Fox. 1998. The adaptive significance of maternal effects. *Trends Ecol Evol* 13: 403-407.

O'Rahilly, S., and I. S. Farooqi. 2006. Genetics of obesity. *Philos Trans R Soc Lond B Biol Sci* 361(1471): 1095-1105.

Parekh, P. J., L. A. Balart, and D. A. Johnson. The influence of the gut microbiome on obesity, metabolic syndrome and gastrointestinal disease. 2015. *Clin Transl Gastroenterol* 6: e91.

Partridge, C, G. L. Fawcett, B. Wang, C. F. Semenkovich, and J. M. Cheverud. 2014. The effect of dietary fat intake on hepatic gene expression in LG/J and SM/J mice. *BMC Genomics* 15: 99.

Pettitt, D. J., and L. Jovanovic. 2001. Birth weight as a predictor of type 2 diabetes mellitus: the U-shaped curve. *Curr Diab Rep* 1(1): 78-81.

Rasmussen, S. A., S. Y. Chu, S. Y. Kim, C. H. Schmid, and J. Lau. 2008. Maternal obesity and risk of neural tube defects: a metaanalysis. *Am J Obstet Gynecol* 198(6): 611-619.

Ridaura, V. K., J. J. Faith, F. E. Rey, J. Cheng, A. E. Duncan, A. L. Kau, N. W. Griffin, V. Lombard, B. Henrissat, J. R. Bain, M. J. Muehlbauer, O. Ilkayeva, C. F. Semenkovich, K. Funai, D. K. Hayashi, B. J. Lyle, M. C. Martini, L. K. Ursell, J. C. Clemente, W. Van Treuren, W. A. Walters, R. Knight, C. B. Newgard, A. C. Heath, and J. I. Gordon. 2013. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 341(6150): 1241214.

Sabin, J. L., A. L. Boudoures, Z. Asghar, A. Thompson, A. Drury, W. Zhang, M. Chi, A. Cusumano, S. Scheaffer, and K. H. Moley. 2016. Maternal metabolic syndrome programs mitochondrial dysfunction via germline changes across three generations. *Cell Reports* 16(1): 1-8.

Serdula, M., D. F. Williamson, J. S. Kendrick, R. F. Anda, and T. Byers. 1991. Trends in alcohol consumption by pregnant women. 1985 through 1988. *JAMA* 265(7): 876-879.

Sferruzzi-Perri, A. N., O. R. Vaughan, M. Haro, W. G. Cooper, B. Musial, M. Charalambous, D. Pestana, S. Ayyar, A. C. Ferguson-Smith, G. J. Burton, M. Constancia, and A. L. Fowden. 2013. An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB J* 27(10): 3928-3937.

Skelton, M. R., N. Siitonen, P. Würtz, J. S. Viikari, M. Juonala, I. Seppälä, T. Laitinen, T. Lehtimäki, L. Taittonen, M. Kähönen, D. S. Celermajer, and O. T. Raitakari. 2014. High birth weight is associated with obesity and increased carotid wall thickness in young adults: the cardiovascular risk in young Finns study. *Arterioscler Thromb Vasc Biol* 34(5): 1064-1068.

Smemo, S., J. J. Tena, K.-H. Kim, E. R. Gamazon, N. J. Sakabe, C. Gómez-Marín, I. Aneas, F. L. Credidio, D. R. Sobreira, N. F. Wasserman, J. H. Lee, V. Puviindran, D. Tam, M. Shen, J. E. Son, N. A. Vakili, H.-K. Sung, S. Naranjo, R. D. Acemel, M. Manzanares, A. Nagy, N. J. Cox, C.-C. Hui, J. L. Gomez-Skarmeta and M. A. Nóbrega. 2014. Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature* 507: 371-375.

Soubry, A., S. K. Murphy, F. Wang, Z. Huang, A. C. Vidal, B. F. Fuemmeler, J. Kurtzberg, A. Murtha, R. L. Jirtle, J. M. Schildkraut, and C. Hoyo. 2015. Newborns of obese parents have altered DNA methylation patterns at imprinted genes. *Int J Obes (Lond)* 39(4): 650-657.

Stirrat, L. I., and R. M. Reynolds. 2014. Effects of maternal obesity on early and long-term outcomes for offspring. *Research and Reports in Neonatology* 4: 43-53.

Sullivan, E. L., E. K. Nousen, and K. A. Chamlou. 2014. Maternal high fat diet consumption during the perinatal period programs offspring behavior. *Physiol Behav* 123: 236-242.

Tam, C. H. T., Y. Wang, J. Luan, H. M. Lee, A. O. Y, Luk, G. E. Tutino, P. C. Y. Tong, A. P. S. Kong, W. Y. So, J. C. N. Chan, and R. C. W. Ma. 2014. Maternal history of diabetes is associated with increased cardiometabolic risk in Chinese. *Nutrition & Diabetes* 4: e112.

Vahratian, A., J. Zhang, J. F. Troendle, D. A. Savitz, and A. M. Siega-Riz. 2004. Maternal prepregnancy overweight and obesity and the pattern of labor progression in term nulliparous women. *Obstet Gynecol* 104(5 Pt 1): 943-951.

Vollmers, C., R. J. Schmitz, J. Nathanson, G. Yeo, J. R. Ecker, and S. Panda. 2012. Circadian oscillations of protein-coding and regulatory RNAs in a highly dynamic mammalian liver epigenome. *Cell Metab* 16(6): 833-845.

Voss, J. D., P. Masuoka, B. J. Webber, A. I. Scher, and R. L. Atkinson. 2013. Association of elevation, urbanization and ambient temperature with obesity prevalence in the United States. *Int J Obes (Lond)* 37(10): 1407-1412.

Waddington, C.H. 1942. The Epigenotype. *Endeavour* 1: 18-20.

Wang, L.-F. H.-J. Wang, D. Ao, Z. Liu, Y. Wang, and H.-X. Yang. 2015. Influence of pre-pregnancy obesity on the development of macrosomia and large for gestational age in women

with or without gestational diabetes mellitus in Chinese population. *Journal of Perinatology* 35: 985-990.

Waterland, R. A., and R. L. Jirtle. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23(15): 5293-5300.

Waterland R. A., M. Travisano, K. G. Tahiliani, M. T. Rached, and S. Mirza. 2008. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes (Lond)* 32(9): 1373-1379.

Weiss, J. L., F. D. Malone, D. Emig, R. H. Ball, D. A. Nyberg, C. H. Comstock, G. Saade, K. Eddleman, S. M. Carter, S. D. Craigo, S. R. Carr, and M. E. D'Alton; FASTER Research Consortium. 2004. Obesity, obstetric complications and cesarean delivery rate—a population-based screening study. *Am J Obstet Gynecol* 190(4): 1091-1097.

Whitaker, R. C. 2004. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics* 114(1): e29-e36.

Wolff, G. L., R. L. Kodell, S. R. Moore, and C. A. Cooney. 1998. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J* 12: 949-957.

Yao, R., C. V. Ananth, B. Y. Park, L. Pereira, and L. A. Plante. Perinatal Research Consortium. 2014. Obesity and the risk of stillbirth: a population-based cohort study. *Am J Obstet Gynecol* 210(5): 457.e1-457.e9.

Zhao, G., E. S. Ford, J. Tsai, C. Li, I. B. Ahluwalia, W. S. Pearson, L. S. Balluz, and J. B. Croft. 2012. Trends in health-related behavioral risk factors among pregnant women in the United States: 2001-2009. *Journal of Women's Health* 21(3): 255-263.

CHAPTER 2

The Effect of Dietary Fat on Behavior in Mice

ABSTRACT

Research has shown that obesity alters certain aspects of behavior, and that maternal obesity can increase offspring risk of metabolic disease and anxiety. However, the extent to which behavior is impacted by both an individual's diet and that of its mother is not well understood. In this study, I fed a high fat (HF) or low fat (LF) diet to genetically identical female SM/J mice and mated them with LF-fed males. I cross-fostered all offspring to LF-fed SM/J nurses and weaned them onto an HF or LF diet at 3 weeks of age. I then measured offspring anxiety with the Open Field Test, behavior with instantaneous scan sampling, and nest quality with the Deacon Scale. The offspring's own diet had major behavioral effects. HF-fed mice had increased anxiety levels (they produced more fecal boli and urinations in the Open Field Test), built lower quality nests, and had lower activity levels in adulthood than LF-fed mice. The only trait that prenatal maternal diet affected was whether the offspring built their nests inside or outside a hut. Offspring diet, but not prenatal maternal diet, affected a wide range of behaviors in these mice.

INTRODUCTION

Obesity is a systemic inflammatory condition that can induce extensive behavioral changes, in part due to its effect on the brain. Obesity is tightly linked to Alzheimer's disease and other types of cognitive dysfunction in humans (Beydoun *et al.* 2008), and is associated with lower cognitive performance in men based on tests of learning and memory (Elias *et al.* 2003). Obesity's effects in mice include increased anxiety (Sharma and Fulton 2013, André *et al.* 2014), diminished spatial memory (André *et al.* 2014), reduced object-location memory (Heyward *et al.* 2012), impaired learning of contextual fear conditioning and passive avoidance (Hwang *et al.*

2010), and increased depressive-like behavior (Sharma and Fulton 2013). André *et al.* (2014) found that obese C57BL/6 mice had impaired spatial working memory and increased anxiety behavior in the Elevated Plus Maze. High-fat-fed C57BL/6J mice also showed increased depressive-like behavior in the Forced Swim Test in addition to increased anxiety in the Elevated Plus Maze and Open Field Test (Sharma and Fulton 2013). Even when lean male C57BL/6J mice were given gut microbiota from obese mice, they had reduced exploratory behavior and increased anxiety (Bruce-Keller *et al.* 2015). Certain behaviors can lead to obesity, but these studies illustrate that obesity can also alter behavior.

Compounding its consequences for public health, the effects of obesity are not limited to one generation. Children of obese mothers have a higher future risk of insulin resistance, obesity, and cardiometabolic disease (Tam *et al.* 2014). But while much attention has been paid to how maternal obesity affects offspring risk of metabolic disease, its impact on offspring behavior is only recently beginning to be understood.

From rodents to humans, maternal obesity during pregnancy fundamentally alters the neurological programming that regulates behavior. In humans, this results in an increased risk of offspring anxiety, attention deficit hyperactivity disorder, and autism (Sullivan *et al.* 2014). In rodents, offspring of obese dams exhibit significant deficits in reversal learning accompanied by striatal disturbance (Wu *et al.* 2013), as well as long-term impairments in spatial learning thought to be caused by reduced hippocampal production of brain-derived neurotrophic factor (BDNF), which is critical for spatial learning and memory (Tozuka *et al.* 2010).

Several mechanisms have been suggested for how maternal obesity impacts offspring behavior. One indirect route is through maternal behavior: high-fat-fed rat dams nurse their young more, which can lead to over-eating behavior later in life (Bertino 1982). In humans,

parental eating habits can influence children's eating habits (Savage *et al.* 2007). Evidence for a hormonal aspect of transmission is provided by studies such as Rodriguez *et al.* (2012), where pregnant high-fat-fed rat dams had elevated levels of corticosterone during pregnancy and whose offspring had learning deficits. Maternal steroids can disrupt fetal development when they pass through the placenta, which may account for the impaired learning exhibited by the rat offspring in that study. Maternal obesity also affects offspring behavior through inflammation. Obesity is associated with chronically elevated levels of inflammatory cytokines, which are known to affect the serotonergic and dopaminergic systems (Mehta *et al.* 2014). Exposure to maternal cytokines in the womb has been shown to have a neuroinflammatory effect on offspring. Bilbo and Tsang (2010) found that rats born to obese mothers had inflammation of the hippocampus and increased anxiety as adults. Similarly, Kang *et al.* (2014) found that female mice born to mothers on a high-fat diet exhibited higher levels of anxiety, brain tissue inflammation, and inflammatory cytokines. The researchers concluded that the results supported a link between inflammatory cytokines and behavioral changes due to maternal diet.

The effects of a maternal high-fat diet on behavior are complex and ambiguous, and its impact on offspring anxiety is far from resolved in the field (Table 2.1). In some cases, maternal high-fat diet is credited with increasing anxiety in offspring (Kang *et al.* 2014, Peleg-Raibstein *et al.* 2012, Bilbo and Tsang 2010), while in others it has been shown to have an anxiolytic effect (Rodriguez *et al.* 2012, Sasaki *et al.* 2014, Bellisario *et al.* 2014, Wright *et al.* 2011). This discrepancy can be attributed to several causes, including the fact that behavior is a highly variable and notoriously difficult trait to measure, the small sample sizes that are often used in these types of experiments, the variation in the fat content of high-fat diets (ranging from 16% to 60% of calories from fat), the multitude of tools being used to measure anxiety, and the lack of

standardization in how these tools are used.

Common tools for measuring anxiety in rodents are the Elevated Plus Maze, the Open Field Test, the Light/Dark Box, and the Water Maze. When researchers use multiple tests in a study, oftentimes one test shows no difference in anxiety traits while another does. For instance, while not finding an effect of maternal diet in the Open Field Test, Peleg-Raibstein *et al.* (2012) did find increased anxiety behavior as measured by the Elevated Plus Maze and a test of food neophobia. This contrasted with Rodriguez *et al.* (2012), who found no effects of maternal high-fat diet in the Elevated Plus Maze but did find an effect in the Open Field Test. These discrepancies suggest that conclusions drawn about the effect of a maternal high-fat diet on anxiety can be highly inconsistent.

The results from a timed test like the Open Field Test are difficult to compare across studies, because the duration of the test varies. In studies of maternal obesity the test has been run for 5 minutes (Wright *et al.* 2011, Ramírez-López *et al.* 2016), 10 minutes (Fernandez *et al.* 2012, Rodriguez *et al.* 2012), 15 minutes (Kang *et al.* 2014, Bellisario *et al.* 2014, Sasaki *et al.* 2014), and one hour (Peleg-Raibstein *et al.* 2012). Anxiety traits measured in the Open Field Test include the number of fecal boli and urination events, the degree of thigmotaxis (wall-hugging), and the number of rears (standing on hind legs). Higher values for each of these traits indicate higher anxiety levels; however, results can be mixed and interpreted in different ways. For instance, Fernandes *et al.* (2012) found that exposure to a maternal high-fat diet resulted in adult male C57BL/6J mice (n = 9) traveling further and rearing more in the outer zone of the Open Field arena compared to control males (n = 8), but they concluded that there were no differences in anxiety in the mice. Kang *et al.* (2014) found that in C57BL/6J mice, the sons traveled further (n = 28), the daughters exhibited more thigmotaxis (n = 29), and both sexes

reared more if they had high-fat-fed mothers, concluding that maternal high-fat diet induces offspring anxiety. However, researchers found the opposite in Wistar rats with high-fat-fed mothers (n = 6), who displayed less thigmotaxis in the Open Field Test than rats with mothers on a control diet (Rodriguez *et al.* 2012), as well as in Long Evans rats with high-fat-fed mothers (n = 20), who had more entries into the center arena, indicating lower levels of anxiety (Sasaki *et al.* 2014).

Even less is understood about the effect of maternal high-fat diet on offspring activity levels, as few studies have investigated this. Maternal high-fat diet was found to decrease locomotor activity in daughters (with no effect in sons) in Sprague-Dawley rats (Khan *et al*. 2003), but it increased locomotor activity in one of three mouse strains in an Open Field Test and in all three strains during a swim test (Raygada *et al.* 1998). More research must be done to understand the direction and magnitude of the effect of maternal obesity on offspring activity levels, if indeed there is a consistent effect. In humans, it has become clear that children's diet, behavior, and weight are influenced by those of their parents through non-genetic mechanisms (Oken 2009, Skouteris *et al.* 2011). However, it is challenging to determine how much of this is due to the prenatal environment versus the postnatal environment, which makes it difficult to know the most effective time to target health interventions. It is far more feasible to investigate this phenomenon with a cross-fostering design in mice, as I have done in the present experiment. Here, I studied the effect of maternal high-fat diet during pregnancy, while controlling for postnatal environment by cross-fostering all pups to a low-fat-fed SM/J nurse.

In this study I also investigated nest building, since it plays an important role in the fitness of mice and has been used to assess their well-being (Jirkof 2014). Carter *et al*. (2000) showed that nest size is correlated with locomotor activity, where mice selected for high levels of

voluntary wheel running built smaller nests. Nests are important for thermoregulation, and both males and females build them (Deacon 2006). Mouse pups particularly rely on nests, since they are born hairless and nests reduce heat loss (Gaskill *et al.* 2013). Nest quality affects mouse fitness, as illustrated by Lynch (1980) who found that mice selected for poor nest building became less fertile over 15 generations, whereas mice selected for good nest building became more fertile and increased their litter size and body weight. A more recent study revealed that mice in cages lacking enrichment materials for hiding and nest building produced pups that weighed less and had lower survival rates to weaning age (Whitaker *et al.* 2009). Several quantitative trait loci have been identified that contribute to genetic variation in nest building (Sauce *et al.* 2012). In addition to genetics, nest building is affected by hormones (Lisk *et al.* 1969, Bond *et al.* 2002) and lesions on the hippocampus, which are also both known to be disrupted by obesity (Stranahan *et al.* 2008, Kanoski *et al.* 2011). In fact, maternal obesity has been shown to alter hippocampal gene expression in offspring and to impair their spatial learning (Tozuka *et al.* 2010). Because of this potential connection between obesity and nest building, I tested whether a mouse's diet and mother's diet affected nest quality. By measuring the offspring's nest building, activity levels, and anxiety, I have generated a fuller picture of the effect of dietary fat on behavior.

METHODS

Animal Rearing

I used the inbred SM/J mouse strain from The Jackson Laboratory (Bar Harbor, Maine) which has been shown to be hyper-responsive to a high fat (HF) diet (Cheverud *et al.* 1999, Ehrich *et al.* 2003, Partridge *et al.* 2014). Using a strain with maximal response to an HF diet increases our power to detect effects. At three weeks of age, 30 male mice and 15 female mice

were weaned onto a low fat (LF) diet, and 15 female mice were weaned onto an HF diet to create an F_0 generation. In the LF diet, 15% of the calories came from fat (Research Diets D12284), whereas 42% of the calories came from fat in the HF diet (Harlan Teklad diet TD.88137) (Table 2.2). An LF diet was used instead of standard mouse chow because it was specifically tailored to have nearly the same number of calories per gram as the HF diet. Erich *et al.* (2003) has shown that SM/J mice consume the same amount of food whether they are on the HF diet or the LF diet. The F_0 mice were raised for 7 weeks on the diet and then mated. To limit paternal effects, sires were removed from the cages after abdominal palpation revealed the dam to be pregnant. To avoid confounding the prenatal and postnatal maternal obesity effects, all pups were cross-fostered within 24 hours of birth to an LF-fed SM/J nurse. Pups born to LF mothers were also cross-fostered to an LF nurse to control for the effect of cross-fostering. Half of the pups from each litter were weaned onto an HF diet and the other half onto an LF diet. This produced four F_1 diet treatment groups: HF-HF, LF-HF, HF-LF, and LF-LF, where the first diet listed is the mother's diet and the second diet listed is the offspring's diet (Figure 2.1). Ten to 15 offspring of each sex were assigned to each diet treatment group, for a total of 98 F_1 mice. After weaning, each mouse was housed with one other mouse of the same sex and diet in a cage that contained a wooden gnawing block (Bio Serve), a red privacy hut (Alt Design), a 2" x 2" cotton nestlet for nesting material (Ancare), and food and water *ad libitum*. Procedures followed an approved Institutional Animal Care and Use Committee protocol (Project #1188).

Open Field Test

The Open Field Test was conducted using a 17.5" (L) x 13" (W) x 15" (H) opaque plastic box with a grid on the floor that subdivided it into 48 rectangles measuring 2.16" x 2.18" each (Figure 2.2). The mice were brought into the testing room at 9:00 am and allowed to acclimate,

with testing beginning at 11:30 am. Open Field Tests are short in duration to assess exploratory behavior and response to a novel environment rather than baseline activity levels (Gould *et al.* 2009). To reduce exposure to other types of novelty, no cage changes were performed within 24 hours prior to testing, and the entire arena was sanitized with 70% ethanol after each mouse was tested. A 10-week old mouse was then placed into a corner of the arena, and the mouse's movements were observed and video recorded for 5 minutes by 1-2 female researchers. I note that the researchers were female because Sorge *et al.* (2014) showed that male experimenters induce a stress response in mice and rats, which includes increasing fecal boli production. I collected the following measurements in the Open Field Test: the number of times the mouse reared, the number of times the mouse crossed any of the 8 squares in the center of the arena, the total number of squares the mouse crossed, the number of times the mouse urinated, and the number of fecal boli produced. The number of squares crossed was determined by reviewing the video of the session, because the mice often moved too quickly to count the squares with the unaided eye. The center:total distance ratio was calculated by dividing the number of center squares crossed by the total number of squares crossed. A low center:total distance ratio and high levels of rearing, urination, and fecal boli production were interpreted as indicators of anxiety.

Ethogram Data

A behavioral ethogram was created by listing all mouse behaviors witnessed during 5 hours of observation, for a total of 19 behaviors (Table 2.3). The offspring were observed 3-4 times per week, and the observation times were categorized as a morning session (between 8:00 and 10:30 am) or an afternoon session (between 2:30 and 5:00 pm). Each session consisted of 20 observations by instantaneous scan sampling. Specifically, a researcher marked on the ethogram checklist which behavior the mouse was performing at the instant it was observed, then moved

on to the next mouse, and continued until all of the mice had been observed once. The observer then returned to the first mouse and checked off the behavior it was then performing, completing this cycle 20 times. The mice were observed for an average of 26 sessions between the ages of 3-14 weeks. Since some mice were observed more than others, I calculated an average of each behavior per age group for analysis (3-5 weeks, 6-8 weeks, 9-11 weeks, and 12-14 weeks).

Nest Quality

At 13 weeks of age, each mouse was housed alone in a fresh cage and given a 2" x 2" cotton nestlet. Twenty-four hours later, a photograph was taken of the nest that the mouse had built and it was rated for quality using the Deacon Scale, which ranges from 1 to 5 (Deacon 2006). A Deacon score of 1 indicates a poor quality nest, where over 90% of the nestlet remains unused; a score of 2 means that 50-90% of the nestlet is still intact; 3 indicates the nestlet is mostly shredded but there is no identifiable nest site; 4 means that more than 90% of the nestlet is torn and the nest walls are higher than the mouse's body; and a score of 5 is a near perfect nest (Deacon 2006). Since there was a privacy hut in the cage, I also noted whether the nest was built inside of the hut or outside of it.

Statistical Analysis

The behavior data were not normally distributed, as determined by the Shapiro-Wilk test of normality. I thus used a non-parametric approach, the Kruskal-Wallis test, for the analysis. I also randomized the relevant phenotypes over the factors to obtain a null distribution of ANOVA parameters under the hypothesis of no treatment effects on the phenotypes. Using just the offspring of HF mothers, I randomized the trait values 9,999 times, then tested the difference between the LF- and HF-fed offspring using a 2-sample t-test for each behavior, and compared the t-test statistic from the observed values to those of the randomized values. I did another

randomization to determine if offspring sex had a significant effect on behavior. I repeated this procedure for the offspring of LF mothers. The t-test statistics for each of these tests were normally distributed, even though the raw data were not. The p-values from the randomization procedure were nearly identical to those resulting from an ANOVA. Since the ANOVA was so robust to the non-normally distributed data, I report those p-values. For each set of traits (Open Field Test, ethogram, and nest quality) I ran a General Linear Model in SYSTAT (Version 12, Systat Software, San Jose, CA) to test the full model, which included the effects of maternal diet, offspring diet, offspring sex, nurse ID, parity, and their two- and three-way interactions. For the ethogram data I also included age period in the model. I then ran a reduced model for the ethogram data that included just nurse, offspring diet, and age period, since those were the only three variables with a statistically significant effect.

RESULTS

Open Field Test

The direct effects of an offspring's diet had a significant effect on the Open Field Test traits. Both urination frequency ($p = 0.018$) and fecal boli production ($p = 0.040$) were significantly different between the four diet-sex groups based on the Kruskal-Wallis test. A general linear model revealed that offspring diet was significant on a multivariate level ($p = 0.028$), as well as for the individual traits of urination frequency ($p = 0.007$) and fecal boli production ($p = 0.042$). The average urination frequency was 3.8 times higher in HF-fed females and 1.9 times higher in HF-fed males compared to mice on an LF diet (Figure 2.4A). The average fecal boli production was more than 4 times higher in mice on an HF diet than those on an LF diet for both sexes (Figure 2.4B). The two diets had equal fiber content, so this was not a factor in the difference in fecal boli production.

Prenatal maternal diet did not have much of an effect on anxiety. There were 8 groups of offspring based on their sex, their mother's diet, and their own diet, and the only Open Field Test trait that the eight groups differed significantly for was urination frequency ($p = 0.039$) based on the Kruskal-Wallis test. A maternal HF diet did appear to increase urination frequency in HF sons (1.2 average urinations) compared to LF sons (0.42 average urinations), but a t-test showed only borderline significance ($p = 0.058$) so this result was interpreted with caution (Figure 2.3). A general linear model run on the Open Field Test traits indicated again that maternal diet was only borderline significant on the multivariate level ($p = 0.054$).

Ethogram Data

Testing the full model revealed that offspring diet ($p = 4.60 \times 10^{-8}$), age period ($p = 2.08 \times 10^{-10}$), and nurse ID ($p = 0.01$) had statistically significant effects on the offspring ethogram traits. Since sex did not have a significant effect, the males and females were analyzed together. Maternal diet also did not have significant effect. The fact that nurse ID was significant means that even though all pups were cross-fostered to genetically identical LF-fed SM/J nurses, the nurses differed in some other way that had a lasting impact on their fostered offspring, no matter what diet the offspring or the biological mother had. This random environmental maternal effect could be an interesting avenue to explore in a future study, but in the present study I controlled for it by including nurse ID in the model.

The most commonly observed behaviors in the mice were sleeping (62.5% of the time), autogrooming (7.4%), climbing on the ceiling bars (7.4%), walking (5.9%), and eating (3.7%). Many of the behaviors were performed too infrequently to detect much of a difference due to diet. For example, behaviors observed less than 1% of the time were: motionless but alert, being groomed, rearing, drinking, gnawing, digging, carrying, nesting, allogrooming, running, fighting,

and mounting. To incorporate these rare behaviors into the analysis in a more meaningful way, in addition to analyzing the individual behaviors, I grouped them into four larger behavior categories: self maintenance (drinking, eating, and autogrooming), inactive (sleeping, resting, motionless but alert, and sitting), exploring (walking, climbing on the ceiling bars, gnawing, digging, carrying, nest arrangement, running, and rearing), and social interaction (allogrooming, being groomed, fighting, and mounting) (Table 2.3). I calculated the average percent of time a mouse spent performing behaviors in these four categories during each of four age periods for which each animal was observed (3-5 weeks, 6-8 weeks, 9-11 weeks, and 12-14 weeks). The Social Interaction category could be analyzed only for the first three age period groups, because the mice were housed in pairs until 12 weeks of age, after which they were housed individually to measure nest-building ability.

HF-fed offspring spent less time performing self-maintenance behaviors than LF-fed mice at all four age periods (Figure 2.5A). Although they differed in self-maintenance early on, significant differences in other behaviors did not manifest until later in life. The mice spent an equal amount of time exploring the cage until 12-14 weeks of age, when LF mice increased their time exploring and HF mice decreased it ($p = 0.0001$). This change meant that HF mice explored only half as often as LF mice in adulthood (Figure 2.5B). Instead of performing self-maintenance and exploration behaviors as often, the HF mice spent more time being inactive as adults. Mice on an LF diet became more active with age (they were inactive 75.8% of the time at 3-6 weeks old, and 66.6% of the time at 12-14 weeks old), whereas mice on an HF diet never increased their activity levels (they were inactive 80% of the time at both 3-6 weeks and 12-14 weeks of age) (Figure 2.5C). The difference in activity levels between the two diet groups became detectable at 9-11 weeks of age ($p = 0.026$), but weight differences were detectable at 4 weeks,

indicating that the reduced activity levels followed the weight gain from an HF diet. Neither group of mice performed social interaction behaviors frequently, but at 9-11 weeks of age the HF mice performed them significantly more often than the LF mice (1% of the time versus 0.2% of the time, $p = 0.003$) (Figure 2.5D). This was not due to differences in fighting or mounting, but rather because the HF mice spent more time grooming each other ($p = 0.005$) and being groomed ($p = 0.025$) as adults.

The differences in the four behavior summary categories appear to be primarily driven by a significant difference in the following individual behaviors at 12-14 weeks of age: sleeping, climbing on the ceiling, and walking, with HF-fed mice sleeping more and climbing and walking less than LF-fed mice (Figure 2.6).

Nest Quality

Mice on an HF diet built poorer quality nests than mice on an LF diet ($p = 0.040$) (Figure 2.7B). The difference was driven by the males, where HF-fed males scored an average of 2.8 out of 5 on the Deacon scale, compared to 3.8 for LF-fed males. Maternal diet did not affect the offspring's Deacon score, although it did affect where they built their nests. Regardless of their own diets, offspring of LF mothers were 2.5 to 3.5 times more likely to build their nests inside the hut than offspring of HF diet mothers ($p = 0.020$) (Figure 2.7A). In other words, having an HF-fed mother reduced the offspring's probability of building a nest inside the hut (11% of offspring of HF-fed mothers built their nests inside the hut, compared to 38% of offspring of LF-fed mothers). Although offspring diet did not significantly affect where the nests were built ($p = 0.075$), it is interesting to note that a paired t-test of just the sons shows that HF-fed sons built their nests inside the hut less often ($p = 0.021$) (Figure 2.7C).

DISCUSSION

A high fat (HF) diet in offspring induced anxiety, reduced levels of activity and exploration, and reduced nest quality, indicating that diet influences a wide range of behaviors in mice. This is generally similar to findings in previous studies (Sharma and Fulton 2013, André *et al.* 2014, Hwang *et al.* 2010, Bruce-Keller *et al.* 2015).

In the Open Field Test, the higher levels of urination and fecal boli production in both sexes of HF-fed mice support the hypothesis that obesity increases anxiety. However, there was no difference in rearing or the center:total squares ratio between the diet treatment groups, indicating a limited manifestation of this anxiety. The results of the Open Field Test can be difficult to compare across studies, since rodents may show a significant difference in only one or two of the multiple anxiety measures, and there is not a standardized way of interpreting the collective findings. In the present study, the mice showed increased anxiety for two of the five measures. Although I found no effect of an HF diet on the number of squares crossed in the center of the arena, Bruce-Keller *et al.* (2015) found that HF-fed male mice (n = 10) spent less time in the inner zone of the Open Field Test than LF-chow-fed mice (n = 10), with the total distance traveled unchanged. Similarly, Sharma and Fulton (2013) found that HF-fed mice (n = 8) entered the inner zone less often and spent less time in it than LF-fed mice (n = 8). Both of those studies were conducted with C57BL/6J mice, whereas ours was with SM/J mice, so it is possible that the manifestation of anxiety is dependent on genetic background. Nevertheless, although different aspects of the Open Field Test came out as significant in these studies, all of them detected increased anxiety due to an HF diet.

In addition to being more anxious, HF mice performed fewer self-maintenance behaviors at all ages, and by 11 weeks of age they explored the cage half as often and were far less active than low-fat (LF) mice. In fact, while LF mice became more active as they aged, HF mice

became less active. HF mice slept significantly more and spent less time walking and climbing as adults. The lower activity levels in HF mice developed several weeks after they began to weigh more than the LF mice. This suggests that weight gain can lead to inactivity, and not just the other way around. The reduced activity levels in the HF-fed mice may compound the effect of the diet to lead to further weight gain.

The mice on an HF diet also built poorer quality nests. This could potentially be influenced by several factors, such as thermoregulatory changes due to obesity, hormonal changes, the observed reduction in activity levels, or alterations in brain regions known both to affect nesting behavior and be impaired by obesity, such as the hippocampus.

An HF diet has a definitive effect on behavior in SM/J mice, while maternal prenatal HF diet has little effect on offspring behavior. However, there was a significant effect of nurse ID on offspring behavior, despite the nurses all being genetically identical and LF-fed. This environmental maternal effect persisted through adulthood, indicating that the rearing and lactation environment has a lasting effect on murine behavior, more so than the prenatal maternal diet.

Findings on the effect that maternal obesity has on offspring anxiety are varied in rodents, ranging from an anxiolytic effect to an anxiety-inducing effect. The present study revealed no effect of maternal diet on offspring anxiety in the Open Field Test, other than a marginal increase in urination in HF sons. If there is an effect of maternal diet, the effect size must be small, as our sample size gave us 80% power to detect differences of 0.4 residual standard deviation units. By not detecting an effect of maternal obesity on anxiety, my findings suggest that the effect of maternal diet identified in other rodent studies may principally be due to postnatal maternal diet, since I only varied prenatal diet. This is supported by an experiment

by Kang *et al.* (2014), who found that the increased anxiety in mice with HF mothers was reduced in those whose mothers were transferred to a control diet during lactation. Postnatal maternal diet in rodents may thus have a stronger effect on offspring behavior than prenatal diet. If this scales up to humans, it would support there being a strong emphasis on postnatal maternal dietary interventions for obesity and not just prenatal ones.

Maternal diet did not affect the offspring's behavior patterns as measured by instantaneous scan sampling, consistent with the outcome of the Open Field Test. It did, however, have an unexpected effect on offspring nesting behavior. Mice with mothers on an HF diet were less likely to build their nests inside huts (not a single HF-fed male with a HF mother built a nest inside the hut). The connection between maternal HF diet and building nests outside huts is unclear. Perhaps thermoregulation plays a role, although maternal diet only affected the weight of HF daughters and not of LF daughters or sons (data shown in Chapter 3). Alternatively, anxiety could play a role if having a nest separate from the hut provides a second hiding place. This has yet to be established, however.

While this study did not investigate the effects of an HF diet on parenting behavior, the lasting effect of nurse ID as well as the observed changes in behavior due to dietary fat give reason to predict that parental HF diet could have major effects on the pups. For instance, pup survival could be reduced if the father and mother's poorer nest building failed to keep the pups warm and hidden from predators. Higher levels of inactivity in the mothers could lead to a reduction in pup grooming and arch-backed nursing, which would negatively affect offspring weight. Higher levels of maternal anxiety could increase offspring stress response into adulthood, as seen in rats (Liu *et al.* 1997). It will be important in the future to study the effect of a postnatal maternal HF diet and to determine its underlying mechanisms. In the meantime, it is

clear that an individual mouse's own diet affects a vast array of behaviors—including anxiety, nest building, and activity patterns—while prenatal maternal diet does not.

LITERATURE CITED

André, C., A. L. Dinel, G. Ferreira, S. Layé, and N. Castanon. 2014. Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: focus on brain indoleamine 2,3-dioxygenase activation. *Brain Behav Immun* 41: 10-21.

Barua, S., S. Kuizon, K. K. Chadman, M. J. Flory, W. Brown, and M. A. Junaid. 2014. Single-base resolution of mouse offspring brain methylome reveals epigenome modifications caused by gestational folic acid. *Epigenetics & Chromatin* 7(1): 3.

Bellisario, V., A. Berry, S. Capoccia, C. Raggi, P. Panetta, I. Branchi, G. Piccaro, M. Giorgio, P. G. Pelicci, and F. Cirulli. 2014. Gender-dependent resiliency to stressful and metabolic challenges following prenatal exposure to high-fat diet in the p66Shc−/− mouse. *Front Behav Neurosci* 8: 285.

Bertino, M. 1982. Effects of high fat, protein supplemented diets on maternal behavior in rats. *Physiology & Behavior* 29(6): 999–1005.

Beydoun, M. A., H. A. Beydoun, and Y. Wang. 2008. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. *Obes Rev* 9(3): 204-218.

Bilbo, S. D., and V. Tsang. 2014. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. *FASEB J* 24(6): 2104-2115.

Bond, T.L., P. E. Neumann, W. B. Mathieson, and R. E. Brown. 2002. Nest building in nulligravid, primigravid and primiparous C57BL/6J and DBA/2J mice (*Mus musculus*). *Physiol Behav* 75: 551-555.

Bruce-Keller, A. J., J. M. Salbaum, M. Luo, E. Blanchard 4th, C. M. Taylor, D. A. Welsh, and H. R. Berthoud. 2015. Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biol Psychiatry* 77(7): 607-615.

Carter, P. A, J. G. Swallow, S. J. Davis, and T. Garland Jr. 2000. Nesting behavior of house mice (*Mus domesticus*) selected for increased wheel-running activity. *Behavior genetics* 30(2): 85-94.

Chen, H., D. Simar, and M. J. Morris. 2009. Hypothalamic neuroendocrine circuitry is programmed by maternal obesity: interaction with postnatal nutritional environment. *PLoS One* 4(7): e6259.

Deacon, R. M. 2006. Assessing nest building in mice. *Nat Protoc* 1(3): 1117-1119.

Cheverud, J. M., L. S. Pletscher, T. T. Vaughn, and B. Marshall. 1999. Differential response to dietary fat in large (LG/J) and small (SM/J) inbred mouse strains. *Physiol Genomics* 15(1): 33-39.

Ehrich, T. E., J. P. Kenney, T. T. Vaughn, L. S. Pletscher, and J. M. Cheverud. 2003. Diet, obesity, and hyperglycemia in LG/J and SM/J Mice. *Obesity Research* 11(11): 1400-1410.

Elias, M. F., P. K. Elias, L. M. Sullivan, P. A. Wolf, and R. B. D'Agostino. 2003. Lower cognitive function in the presence of obesity and hypertension: the Framingham heart study. *Int J Obes Relat Metab Disord* 27(2): 260-268.

Fernandes, C., H. Grayton, L. Poston, A. M. Samuelsson, P. D. Taylor, D. A. Collier, and A. Rodriguez. 2012. Prenatal exposure to maternal obesity leads to hyperactivity in offspring. *Mol Psychiatry* 17(12): 1159-1160.

Gaskill, B. N., C. J. Gordon, E. A. Pajor, J. R. Lucas, J. K. Davis, and J. P. Garner. 2013. Impact of nesting material on mouse body temperature and physiology. *Physiol Behav* 110-111: 87-95.

Heyward, F. D., R. G. Walton, M. S. Carle, M. A. Coleman, W. T. Garvey, and J. D. Sweatt. 2012. Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression. *Neurobiol Learn Mem* 98(1): 25-32.

Hwang, L. L., C. H. Wang, T. L. Li, S. D. Chang, L. C. Lin, C. P. Chen, C. T. Chen, K. C. Liang, I. K. Ho, W. S. Yang, and L. C. Chiou. 2010. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. *Obesity* 18(3): 463-469.

Jirkof, P. 2014. Burrowing and nest building behavior as indicators of well-being in mice. *J Neurosci Methods* 234: 139-146.

Kang, S. S., A. Kurti, D. A. Fair, and J. D. Fryer. 2014. Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring. *J Neuroinflammation* 11: 156.

Kanoski, S. E., and T. L. Davidson. 2011. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiol Behav* 103(1): 59-68.

Khan, I. Y., P. D. Taylor, V. Dekou, P. T. Seed, L. Lakasing, D. Graham, A. F. Dominiczak, M. A. Hanson, and L. Poston. 2003. Gender-linked hypertension in offspring of lard-fed pregnant rats. *Hypertension* 41(1): 168-175.

Kohsaka, A., A. D. Laposky, K. M. Ramsey, C. Estrada, C. Joshu, Y. Kobayashi, F. W. Turek, and J. Bass. 2007. High-fat diet disrupts behavioral and molecular circadian rhythms in mice.

Cell Metab 6(5): 414-421.

Hwang, L. L., C. H. Wang, T. L. Li, S. D. Chang, L. C. Lin, C. P. Chen, C. T. Chen, K. C. Liang, I. K. Ho, W. S. Yang, and L. C. Chiou. 2010. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. *Obesity* 18(3): 463-469.

Lisk, R.D., R. A. Pretlow, and S. M. Friedman. 1969. Hormonal stimulation necessary for elicitation of maternal nest-building in the mouse (*Mus musculus*). *Anim Behav* 17: 730-737.

Liu, D., J. Diorio, B. Tannenbaum, C. Caldji, D. Francis, A. Freedman, S. Sharma, D. Pearson, P. M. Plotsky, and M. J. Meaney. 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 277(5332): 1659-1662.

Lynch, C. B. 1980. Response to divergent selection for nesting behavior in *Mus musculus*. *Genetics* 96(3): 757-765.

Meaney, M. J., and M. Szyf. 2005. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin Neurosci* 7(2): 103-123.

Mehta, S. H., J. M. Kerver, R. J. Sokol, D. P. Keating, and N. Paneth. 2014. The association between maternal obesity and neurodevelopmental outcomes of offspring. *J Pediatr* 165(5): 891-896.

Oken, E. 2009. Maternal and child obesity: the causal link. *Obstet Gynecol Clin N Am* 36: 361-377.

Page, K. C., R. E. Malik, J. A. Ripple, and E. K. Anday. 2009. Maternal and postweaning diet interaction alters hypothalamic gene expression and modulates response to a high-fat diet in male offspring. *Am J Physiol Regul Integr Comp Physiol* 297(4): R1049-R1057.

Partridge, C, G. L. Fawcett, B. Wang, C. F. Semenkovich, and J. M. Cheverud. 2014. The effect of dietary fat intake on hepatic gene expression in LG/J AND SM/J mice. *BMC Genomics* 15: 99.

Peleg-Raibstein, D., E. Luca, C. Wolfrum. 2012. Maternal high-fat diet in mice programs emotional behavior in adulthood. *Behav Brain Res* 233(2): 398-404.

Pollock, K. E., D. Stevens, K. A. Pennington, R. Thaisrivongs, J. Kaiser, M. R. Ellersieck, D. K. Miller, and L. C. Schulz. 2015. Hyperleptinemia during pregnancy decreases adult weight of offspring and is associated with increased offspring locomotor activity in mice. Endocrinology. 156(10): 3777-3790.

Ramírez-López, M. T., M. Vázquez, L. Bindila, E. Lomazzo, C. Hofmann, R. N. Blanco, F. Alén, M. Antón, J. Decara, D. Ouro, L. Orio, J. Suarez, B. Lutz, F. Rodríguez de Fonseca, and R.

Gómez de Heras. 2016. Exposure to a highly caloric palatable diet during pregestational and gestational periods affects hypothalamic and hippocampal endocannabinoid levels at birth and induces adiposity and anxiety-like behaviors in male rat offspring. *Front Behav Neurosci* 9: 339.

Raygada, M., E. Cho, and L. Hilakivi-Clarke. 1998. High maternal intake of polyunsaturated fatty acids during pregnancy in mice alters offsprings' aggressive behavior, immobility in the swim test, locomotor activity and brain protein kinase C activity. *J Nutr* 128(12): 2505-2511.

Rodriguez, A., J. Miettunen, T. B. Henriksen, J. Olsen, C. Obel, A. Taanila, H. Ebeling, K. M. Linnet, I. Moilanen, and M-R. Järvelin. 2008. Maternal adiposity prior to pregnancy is associated with ADHD symptoms in offspring: evidence from three prospective pregnancy cohorts. *International Journal of Obesity* 32: 550-557.

Rodriguez, J. S., G. L. Rodríguez-González, L. A. Reyes-Castro, C. Ibáñez, A. Ramírez, R. Chavira, F. Larrea, P. W. Nathanielsz, and E. Zambrano. 2012. Maternal obesity in the rat programs male offspring exploratory, learning and motivation behavior: prevention by dietary intervention pre-gestation or in gestation. *Int J Dev Neurosci* 30(2): 75-81.

Sasaki, A., W. de Vega, S. Sivanathan, S. St-Cyr, P. O. McGowan. 2014. Maternal high-fat diet alters anxiety behavior and glucocorticoid signaling in adolescent offspring. *Neuroscience* 272: 92-101.

Sauce B., R. A. de Brito, and A. C. Peripato. 2012. Genetic architecture of nest building in mice LG/J × SM/J. *Front Genet* 3: 90.

Savage, J. S., J. O. Fisher, and L. L. Birch. 2007. Parental influence on eating behavior: conception to adolescence. *J Law Med Ethics* 35(1): 22-34.

Sharma, S., and S. Fulton. 2013. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *International Journal of Obesity* 37: 382-389.

Skouteris, H., M. McCabe, B. Swinburn, V. Newgreen, P. Sacher, and P. Chadwick. 2011. Parental influence and obesity prevention in pre-schoolers: a systematic review of interventions. *Obes Rev* 12(5): 315-328.

Smith, S. M., and W. W. Vale. 2006. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci* 8(4): 383-395.

Sorge, R. E., L. J. Martin, K. A. Isbester, S. G. Sotocinal, S. Rosen, A. H. Tuttle, J. S. Wieskopf, E. L. Acland, A. Dokova, B. Kadoura, P. Leger, J. C. Mapplebeck, M. McPhail, A. Delaney, G. Wigerblad, A. P. Schumann, T. Quinn, J. Frasnelli, C. I. Svensson, W. F. Sternberg, and J. S. Mogil. 2014. Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nat Methods* 11(6): 629-362.

Spencer, S. J., and A. Tilbrook. 2009. Neonatal overfeeding alters adult anxiety and stress

responsiveness. *Psychoneuroendocrinology* 34(8): 1133-1143.

Stachowiak, E. K., S. Oommen, V. T. Vasu, M. Srinivasan, M. Stachowiak, K. Gohil, and M. S. Patel. 2013. Maternal obesity affects gene expression and cellular development in fetal brains. *Nutr Neurosci* 16(3): 96-103.

Stranahan, A. M., T. V. Arumugam, R. G. Cutler, K. Lee, J. M. Egan, and M. P. Mattson. 2008. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nature Neuroscience* 11: 309-317.

Sullivan, E. L., B. Grayson, D. Takahashi, N. Robertson, A. Maier, C. L. Bethea, M. S. Smith, K. Coleman, and K. L. Grove. 2010. Chronic consumption of a high-fat diet during pregnancy causes perturbations in the serotonergic system and increased anxiety-like behavior in nonhuman primate offspring. *J Neurosci* 30(10): 3826-3830.

Sullivan, E. L., E. K. Nousen, and K. A. Chamlou. 2014. Maternal high fat diet consumption during the perinatal period programs offspring behavior. *Physiol Behav* 123: 236-242.

Tam, C. H. T., Y. Wang, J. Luan, H. M. Lee, A. O. Y, Luk, G. E. Tutino, P. C. Y. Tong, A. P. S. Kong, W. Y. So, J. C. N. Chan, and R. C. W. Ma.2014. Maternal history of diabetes is associated with increased cardiometabolic risk in Chinese. *Nutrition & Diabetes* 4: e112.

Tozuka, Y., M. Kumon, E. Wada, M. Onodera, H. Mochizuki, and K. Wada. 2010. Maternal obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring. *Neurochem Int* 57(3): 235-247.

Vickers, M. H., B. H. Breier, D. McCarthy, and P. D. Gluckman. 2003. Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. *Am J Physiol Regul Integr Comp Physiol* 285(1): R271-R273.

Vucetic, Z., J. Kimmel, K. Totoki, E. Hollenbeck, and T. M. Reyes. 2010. Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. *Endocrinology* 151(10): 4756-4764.

Whitaker, J., S. S. Moy, V. Godfrey, J. Nielsen, D. Bellinger, and J. Bradfield. 2009. Effects of cage size and enrichment on reproductive performance and behavior in C57BL/6Tac mice. *Lab Animal* 38(1): 24-34.

Wright, T., S. C. Langley-Evans, and J. P. Voigt. 2011. The impact of maternal cafeteria diet on anxiety-related behaviour and exploration in the offspring. *Physiol Behav* 103(2): 164-172.

Wu, T., S. Deng, W. G. Li, Y. Yu, F. Li, and M. Mao. 2013. Maternal obesity caused by overnutrition exposure leads to reversal learning deficits and striatal disturbance in rats. *PLoS One* 8(11): e78876.

TABLES AND FIGURES

Study	Species (Strain)	Maternal Diet Conclusion	Length of Time on Maternal Diet	Diet attributes	Offspring sample size
Kang *et al.* (2014)	Mice (C57BL/6J)	Induces anxiety	Gestation and lactation	HF (60% cal from fat) vs. C (10% cal from fat)	22 C ♂, 21 HF ♂, 7 HF-C (lactation) ♂, 30 C ♀, 16 HF ♀, 13 HF-C (lactation) ♀
Fernandes *et al.* (2012)	Mice (C57BL/6J)	No effect on anxiety (but induced hyperactivity)	6 weeks prior to pregnancy, through lactation	Western diet (16% fat, 33% sugar) vs. C	9 HF ♂, 8 C ♂ (all raised on C diet)
Rodriguez *et al.* (2012)	Rats (Wistar)	Anxiolytic	Gestation and lactation	HF (25% cal from fat) vs. C (5% cal from fat)	6 HF ♂, 6 C ♂ (all on C diet)
Bellisario *et al.* (2014)	Mice (P66Shc WT and KO on C57BL/6J background)	Anxiolytic in daughters, anxiety inducing in sons	10 weeks prior to mating until 3 days prior to giving birth	HF (58% cal from fat) vs. C (10.5 % cal from fat) vs. Standard Diet (17% cal from fat)	15 HF-wt (10 ♀, 11 ♂), 16 C-wt (15 ♀, 10 ♂), n=12 for the OFT
Peleg-Raibstein *et al.* (2012)	Mice (C57BL/6N)	Induces anxiety	3 weeks prior to mating, through lactation	HF (60% cal from fat) vs. chow	12 HF ♂, 9 HF ♀, 12 C ♂, 10 C ♀
Bilbo and Tsang (2010)	Rats (Sprague-Dawley)	Induces anxiety	4 weeks prior to mating, through lactation	High-saturated-fat (60% cal from fat) vs. high-trans-fat (60% cal from fat) vs. C (10% cal from fat)	8 per group
Sasaki *et al.* (2014)	Rats (Long Evans)	Anxiolytic	4 weeks prior to mating, through lactation	HF (60% cal from fat) vs. C (13.5% fat)	13 HF ♀, 7 HF ♂, 13 C ♀, 13 C ♂
Wright *et al.* (2011)	Rats (Wistar)	Anxiolytic	8 weeks prior to mating, then some switched to chow	Cafeteria diet, different each day, % fat not reported	5-12 per group
Ramírez-López *et al.* 2016	Rats (Wistar)	Induces anxiety	8 weeks prior to mating, then throughout pregnancy and lactation	Chow vs. free-choice of chow and cafeteria diet (chocolate) (24.45% cals from fat)	15 from chow moms, 17 from free-choice moms, all weaned on chow

Study	Length of OFT	OFT total distance traveled	OFT Center:total ratio	OFT Time in Center	OFT Rearing	OFT boarder entries	Elevated Plus Maze
Kang *et al.* (2014)	15 min	Increased in ♂, NS in ♀	NS in ♂, lower in ♀	NA	Increased in both sexes	NA	NA
Fernandes *et al.* (2012)	10 min	Traveled more	NA	No diff	Increased (in outer part)	NA	NA
Rodriguez *et al.* (2012)	10 min	No diff in ♂, ♀ not tested	NA	No diff in ♂, ♀ not tested	NA	Increased in HF ♂, ♀ not tested	No diff
Bellisario *et al.* (2014)	Three 5-min intervals	HF ♂ and daughters traveled less	NA	No diff	NA	NA	HF ♂ more anxious (groomed more, immobile more), ♀ less anxious (immobile less)
Peleg-Raibstein *et al.* (2012)	1 hr	No diff	NA	NA	NA	NA	More anxious (spent less time in open arms)
Bilbo and Tsang (2010)	NA	NA	NA	NA	NA	NA	SFD and TFD ♂ spent less time in the open arms
Sasaki *et al.* (2014)	15 min	NA	HF offspring had a higher ratio	NA	NA	NA	HF offspring entered the open arms more
Wright *et al.* (2011)	5 min	Pregnancy diet: no diff Lactation diet: reduced tot distance traveled in ♂ (no diff in ♀)	NA	♂ entered center sooner (no diff in ♀)	Pregnancy diet: ♂ increased Lactation diet: ♂ decreased (♀ decreased for both)	NA	Reduced locomotor activity in ♂ and ♀, reduced grooming in ♂
Ramírez-López *et al.* 2016	5 min	No diff in total distance traveled or mean speed	NA	Offspring from free-choice dams spent less time in center of arena	NA	No diff	Offspring of free-choice dams spent less time in open arms, entered less often into the open arms, spent more time in closed arms and entered the closed arms more

Table 2.1 Compilation of the literature studying the effect of maternal high-fat diet on anxiety in rodent offspring. HF = High-fat diet, C = Control diet, WT = Wild Type, KO = Knock-Out, Cal = Calories, OFT = Open Field Test, NS = Not Significant, diff = difference.

Component	High-fat diet	Low-fat diet
Energy from fat, %	42	15
Casein, g/kg	195	197
Sugars, g/kg	341	307
Corn starch, g/kg	150	313
Cellulose, g/kg	50	30
Corn oil, g/kg	0	58
Hydrogenated coconut oil, g/kg	0	7
Anhydrous milk fat, g/kg	210	0
Cholesterol, g/kg	1.5	0
Kilojoules per gram	18.95	16.99

Table 2.2. Composition of diets.

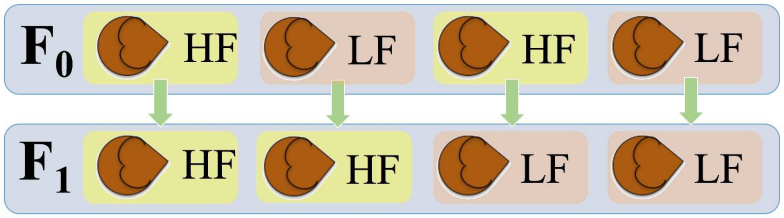


Figure 2.1. Breeding design.

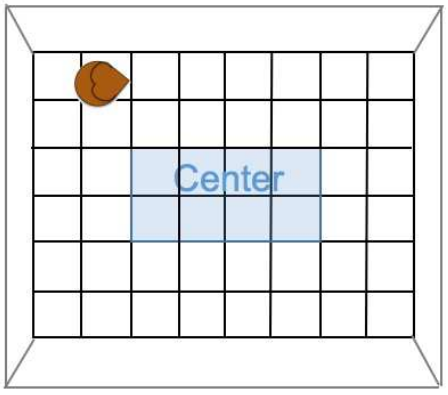


Figure 2.2. Diagram of the Open Field Test arena.

Behavior Category	Factor	Age 3-5 weeks	Age 6-8 weeks	Age 9-11 weeks	Age 12-14 weeks
Self maintenance (drinking, eating, autogrooming)	High fat avg	7.3%	6.2%	6.7%	10.0%
	Low fat avg	12.1%	10.8%	14.6%	13.4%
	Offspring Diet	**0.00864**	**0.0134**	**8.00E-04**	**8.60E-04**
	Nurse	**0.00178**	**0.00066**	0.36662	**0.00126**
Inactive (resting, motionless but alert, sitting, sleeping)	High fat avg	80.6%	81.7%	75.4%	79.8%
	Low fat avg	75.8%	77.3%	67.5%	66.6%
	Offspring Diet	0.10326	0.64236	**2.58E-02**	**0.00001**
	Nurse	**0.00002**	**0.02823**	**0.00897**	**1.17E-06**
Explore (gnawing, digging, carrying, nest arrangement, climbing, running, rearing, walking)	High fat avg	11.5%	11.8%	16.9%	10.1%
	Low fat avg	11.7%	11.7%	17.7%	19.8%
	Offspring Diet	0.56773	0.51643	0.17565	**0.00012**
	Nurse	**0.00002**	0.1679	**0.00005**	**2.96E-09**
Social interaction (allogrooming, being groomed, fighting, mounting)	High fat avg	0.7%	0.3%	1.0%	NA
	Low fat avg	0.4%	0.3%	0.2%	NA
	Offspring Diet	0.12934	0.50193	**0.00333**	NA
	Nurse	0.17361	0.57082	0.10353	NA
	Sample size	n = 36 HF 41 LF	n = 35 HF 40 LF	n = 36 HF 35 LF	n = 35 HF 38 LF

Table 2.3. This table indicates how the 19 ethogram behaviors were grouped into 4 larger behavioral categories. It shows the average percent of time that mice on a high-fat diet and mice on a low-fat diet were observed performing behaviors in each of these summary categories at each of the four age periods. Since sex did not have a significant effect on the behaviors, males and females were analyzed together. The p-values are from an ANOVA showing the effect of nurse ID and offspring diet on the summary ethogram categories. Nurse ID affected how often the mice performed self-maintenance, inactive, and exploration behaviors throughout their lives. Offspring diet affected self-maintenance behaviors throughout life, and inactive, exploration, and social interaction behaviors later in adulthood.

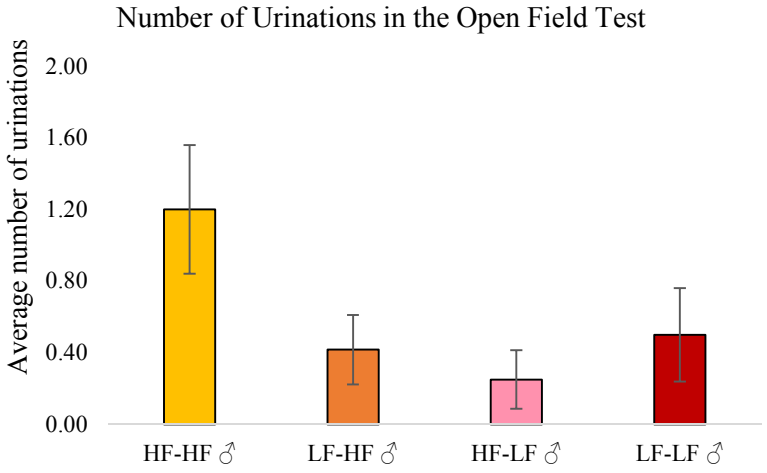
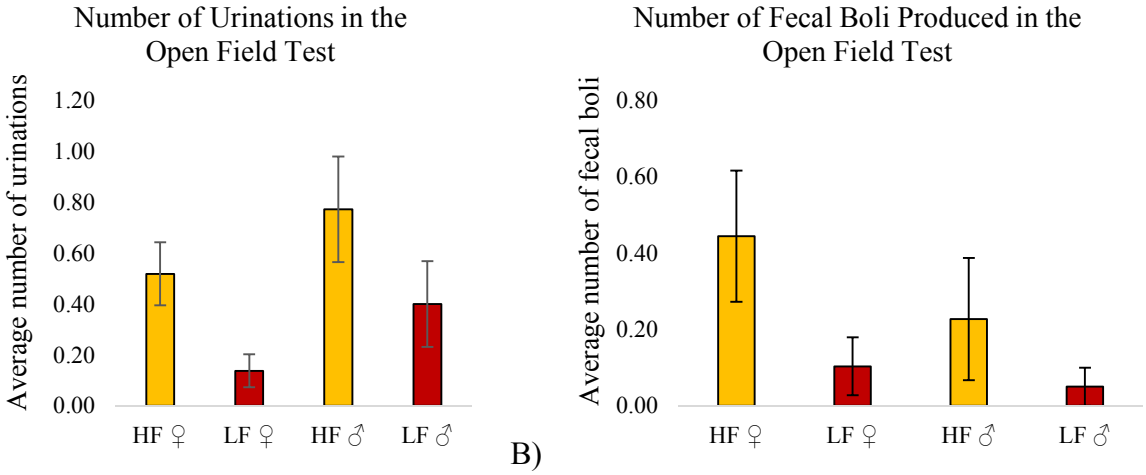


Figure 2.3. In the Open Field Test, high fat male mice born to high fat mothers (HF-HF ♂) had a borderline significant elevation in anxiety through increased urination compared to high fat males born to low fat mothers (LF-HF ♂) ($p = 0.058$). The first diet listed is the maternal diet, and the second diet listed is the offspring diet. Error bars represent ± a single standard error, HF = High-fat diet, LF = Low-fat diet.



A) B)

Figure 2.4. (A) In the Open Field Test, high fat mice urinated more than low fat mice ($p = 0.007$). (B) High fat mice also produced more fecal boli than low fat mice ($p = 0.042$). Sample size: n = 27 HF diet females, n = 29 LF diet females, n = 22 HF diet males, n = 20 LF diet males. Error bars represent ± a single standard error, HF = High-fat diet, LF = Low-fat diet.

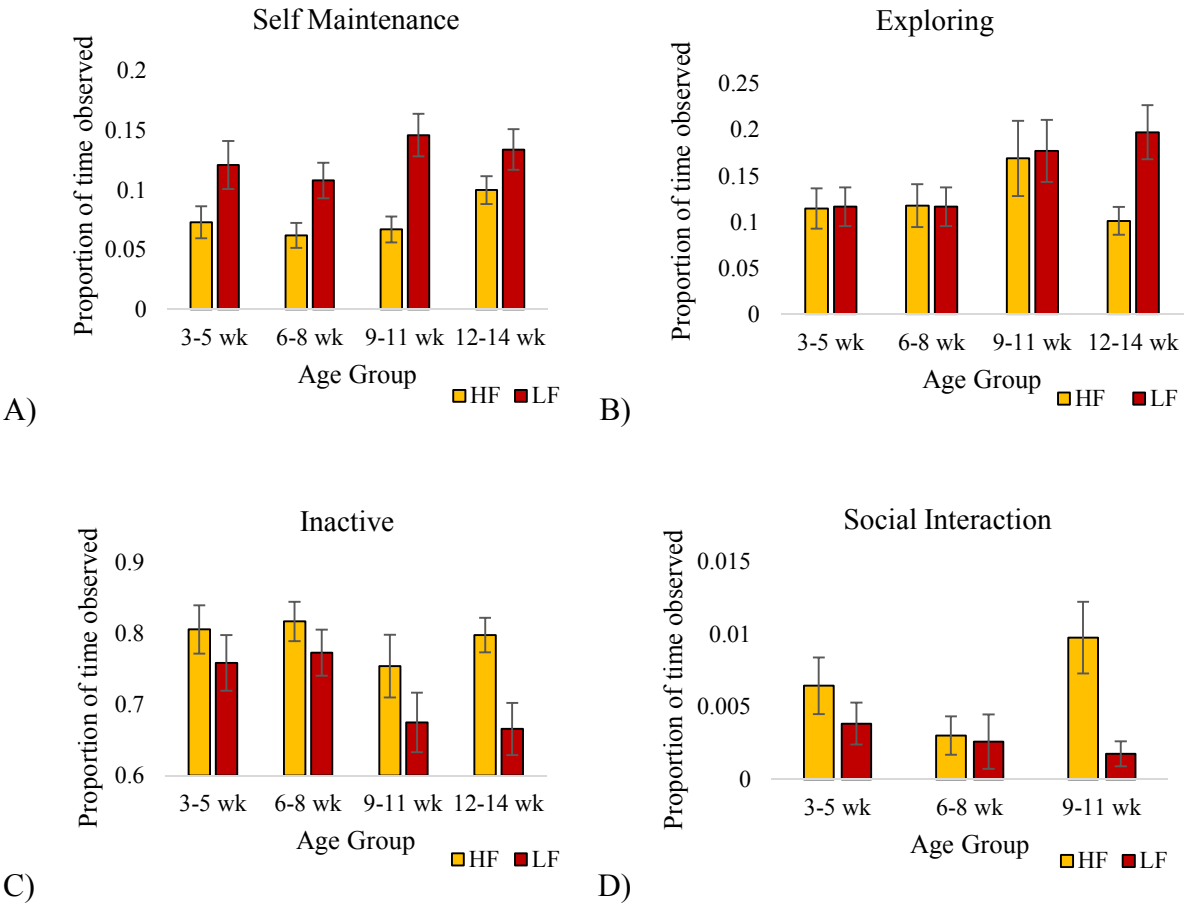
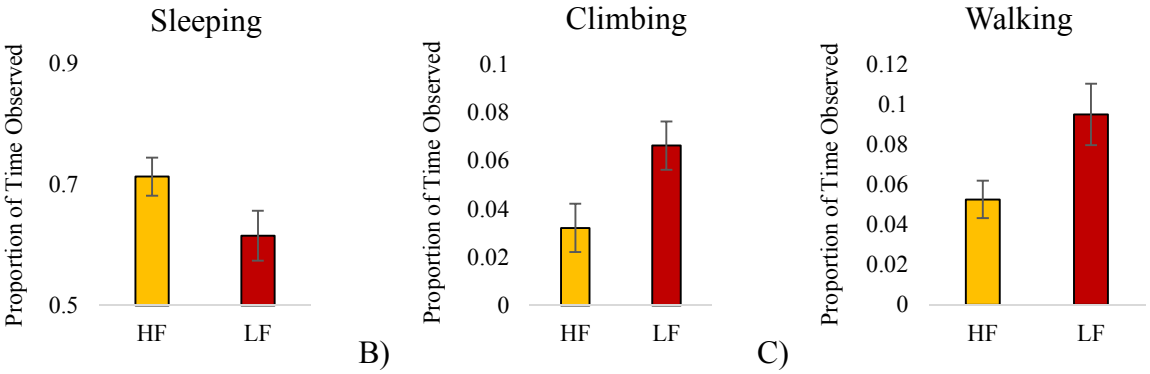
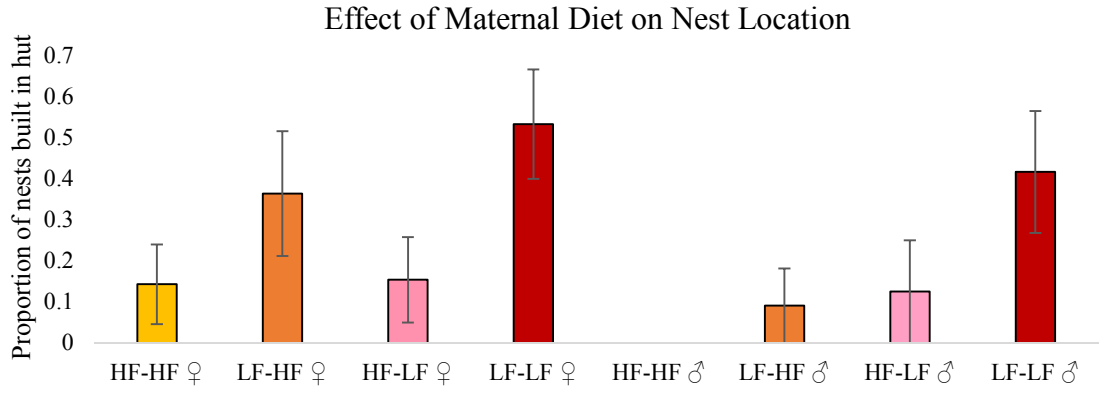


Figure 2.5. (A) High fat mice performed self-maintenance behaviors significantly less often than low fat mice at every age group. (B) By 12-14 weeks of age, high fat mice spent less time exploring than low fat mice ($p = 0.0001$). (C) Low fat mice became less inactive in adulthood, whereas high fat mice never decreased their level of inactivity ($p = 0.00001$). (D) At 9-11 weeks of age, high fat mice engaged in more social interaction behaviors than low fat mice ($p = 0.0033$). Error bars represent ± a single standard error, HF = High-fat diet, LF = Low-fat diet.

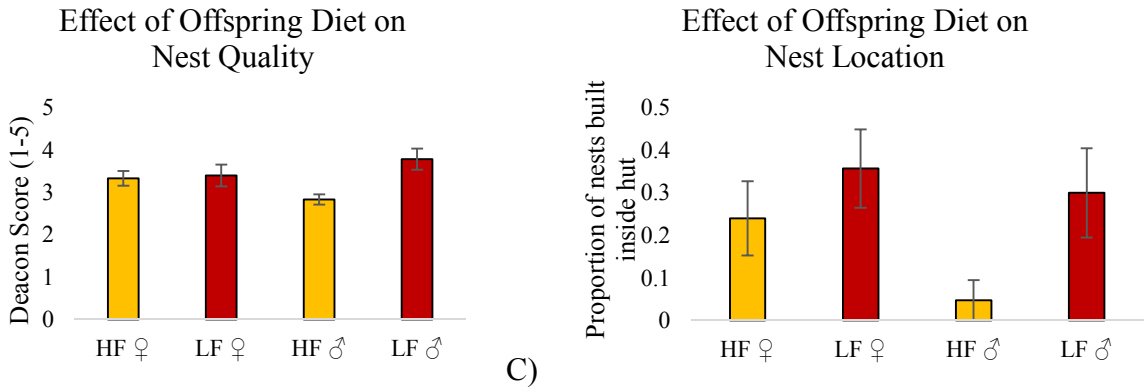


A) B) C)

Figure 2.6. These graphs contrast the behavior of the high fat and low fat mice at 12-14 weeks of age. (A) High fat mice spent more time sleeping than low fat mice ($p = 0.0017$). (B) High fat mice spent less time climbing on the ceiling bars than LF mice ($p = 0.014$). (C) High fat mice also spent less time walking ($p = 0.0031$). Error bars represent the standard error, HF = High-fat diet, LF = Low-fat diet, and sample size is n = 35 high-fat diet mice and n = 38 low-fat diet mice.



A)



B) C)

Figure 2.7. (A) Offspring of high fat mothers built their nests inside of a hut less often ($p = 0.020$). (B) Offspring diet has a significant effect on nest quality ($p = 0.040$), with this difference being driven by the sons. High fat sons built lower quality nests than low fat sons. (C) High fat offspring showed a nonsignificant trend ($p = 0.075$) of building nests in the hut less often, although a paired t-test of just the sons showed a statistically significant difference ($p = 0.021$). Error bars represent the standard error, HF = High-fat diet, LF = Low-fat diet, sample size: n = 14 HF-HF♀, 11 LF-HF♀, 13 HF-LF♀, 15 LF-LF♀, 10 HF-HF♂, 11 LF-HF♂, 8 HF-LF♂, and 12 LF-LF♂.

CHAPTER 3

A High-Fat Diet Alters Genome-wide DNA Methylation and Gene Expression in SM/J Mice

ABSTRACT

Discovering the gene expression and methylation changes induced by a high-fat diet can help to identify new targets for epigenetic therapies and inform about the physiological changes in obesity. In this study, I investigated the effects of dietary fat on obesity, gene expression, and DNA methylation in an inbred mouse strain (SM/J). I weaned the male and female mice onto a high fat or low-fat diet and measured their weights weekly, tested their glucose and insulin tolerance as adults, assessed serum biomarkers, and weighed their organs at necropsy. All of these traits were strongly affected by diet. Liver gene expression data from RNA sequencing revealed 4,356 genes that were differentially expressed due to diet, with 184 genes exhibiting a strong sex-by-diet interaction, indicating that expression levels due to diet differed in the two sexes. Dietary fat caused the dysregulation of several pathways, including those involved in cytokine-cytokine receptor interaction, chemokine signaling, and oxidative phosphorylation. MeDIP and MRE sequencing of DNA from the liver identified 7,000 genes with differentially methylated regions at the FDR-adjusted $q < 0.05$ level. At the $q < 0.01$ level there were 2,356 differentially methylated regions in the females and 1,539 in the males due to diet. In the females, 174 of the differentially methylated regions occurred in genes that were also differentially expressed, as did 240 differentially methylated regions in the males. These genes highlight potential targets for epigenetic treatments of obesity in the future. This study emphasizes the substantial effect that dietary fat has on gene expression and methylation patterns in the liver, and shows that this effect is different in males and females.

INTRODUCTION

In the last three decades, the number of obese adults in the United States has more than doubled. This rise in obesity is expected to continue: while 35% of adults are obese today, 42%

are predicted to be obese by 2030 (Ogden *et al.* 2014, Yang and Colditz 2015, Finkelstein *et al.* 2012). This is a major threat to public health, since obesity is associated with cancer, stroke, asthma, type 2 diabetes, hypertension, heart attack, and other serious health conditions (Cawley and Meyerhoefer 2011). As a result, the average life expectancy for the morbidly obese is 9 years lower for women and 12 years lower for men (Buchwald 2005). The best studied causes of obesity are genetics, the environment, and their interaction (Martinez 2000, Cheverud *et al.* 2004, Ehrich *et al.* 2005, Bell *et al.* 2005, Pérusse *et al.* 2005, O'Rahilly and Farooqi 2006). The environment changes the expression of genes via the epigenome, and thus environmental factors causing obesity may do so by inducing epigenetic modifications that change gene expression. Epigenetic variation between individuals may hold the key to more accurate predictions of obesity risk, and better understanding it could lead to new tools for fighting obesity.

Health problems can result from dysregulated gene expression, which can be caused by genetics and epigenetics. While much research has focused on the genetic variants underlying disrupted gene expression in obesity (Pérusse *et al.* 2005, Emilsson *et al.* 2008), far less is known about how diet changes gene expression through epigenetics to cause obesity. A high-fat diet has been shown to remodel chromatin in C57BL/6J and DBA/2J mouse livers (Leung *et al.* 2014). Differences in H3K9 and H3K4 histone methylation have been linked to cardiac hypertrophy, and alterations in DNA methylation have been linked to heart failure, atherosclerosis, and diabetes (Khalil 2014). Technological advances have made epigenetic studies more feasible, and new journals and scientific meetings have been created to address the explosion of epigenetics research (Bird 2007). Epigenetics in this sense is the study of changes in gene expression that persist across cell divisions in an individual's lifetime and are not due to changes in the underlying DNA sequence (Russo *et al.* 1996). The epigenome is a dynamic body that allows the

static genome to adjust to the ever-changing environment by regulating gene expression. Diet is an example of an environmental factor, and a high-fat diet has been linked to changes in the epigenetic profile of many metabolic genes (Strakovsky *et al.* 2011, Fullston *et al.* 2013, Yoon *et al.* 2017, Zhang *et al.* 2017, Zhou *et al.* 2017, Zwamborn *et al.* 2017). It is becoming clear that epigenetic differences between individuals impact their obesity risk (Campion *et al.* 2009). We need to characterize these epigenetic differences to understand this important aspect of obesity.

Gene expression is regulated by many types of epigenetic mechanisms, including DNA methylation, non-coding RNA, and histone modifications (where various chemical groups such as acetyl, methyl, phosphate, and ubiquitin groups are added to histone tails). The best-characterized epigenetic mechanism is DNA methylation; however, even this is still not well-understood. DNA methylation is the addition of a methyl (CH_3) group to DNA, usually to a cytosine (Parle-McDermott and Ozaki 2011). In animals, DNA methylation occurs at CpG doublets, and in plants it occurs at CpNpG triplets. Vertebrate genomes have distinct clusters of CpG doublets at the promoters of many genes (Bird 2002), including 60% of human genes (Illingworth and Bird 2009). When the majority of the cytosines in promoter regions are methylated, gene expression tends to be much lower than when these regions are hypomethylated (Razin and Cedar 1991). This is not always the case, however, and methylation at other regulatory regions can actually increase expression (Barua *et al.* 2014). To fill in the gaps of our understanding of epigenetics, it is important to explore the methylation profile of not just promoter regions in candidate genes but of the entire genome, as I do in this study.

Some variation in methylation is heritable. For example, numerous methylation QTLs have been discovered (Voisin *et al.* 2015, Volkov *et al.* 2016), epistasis can involve genomic imprinting (Lawson *et al.* 2013), and in an analysis of 648 monozygotic and dizygotic twins,

Grundberg *et al.* (2013) found that methylation variation in adipose tissue had a heritability of 0.34, and 6% of methylation QTLs regulated both methylation and gene expression. Methylation levels are also known to change with age, exercise, diet, and BMI. Comparing blood samples from obese and lean preschool children revealed that the promoters of 392 genes were differentially methylated (Ding *et al.* 2015). However, changes in DNA methylation do not always imply changes in gene expression, or vice versa. Rats fed a diet high in fat and sugar had higher expression of the *Hadhb* gene in the liver, but had no corresponding changes in methylation (Lomba *et al.* 2010). As another example, Rönn *et al.* (2013) analyzed DNA methylation in the adipose tissue of 23 men before and after 6 months of exercise and found methylation changes in 7,663 genes. Of those genes, 197 also had differential expression, 61% of which had an inverse relationship between DNA methylation and gene expression. That study illustrates a common finding in obesity epigenetics studies: while there are methylation differences associated with obesity, many changes in DNA methylation do not cause detectable changes in the expression of nearby genes, and those that do lack a straightforward directional relationship. Much more research needs to be done to characterize the relevant DNA methylation changes in obesity.

So far, candidate-gene studies have revealed DNA methylation differences between obese and lean individuals in a handful of genes in different tissues, such as leptin (*Lep*) and proopiomelanocortin (*Pomc*) in the blood (van Dijk *et al.* 2015). In the liver, C57BL/6J mice with higher body weights were found to have higher dipeptidyl peptidase 4 (*Dpp4*) expression and lower methylation in four intronic CpG sites flanking exon 2 (Baumeier *et al.* 2017). DNA methylation is thought to play a role in regulating the inflammation response, as demonstrated when Malodobra-Mazur *et al.* (2014) overexpressed stearoyl-CoA desaturase 1 (*Scd1*) in mouse

3T3-L1 adipose cells and found DNA methylation differences in the promoters of 4 of 22 inflammatory genes that corresponded with expression differences. These studies have been informative, but are restricted to only genes that seem like good candidates for obesity, thus limiting the identification of other important but unexpected genes.

Genome-wide methylation studies have revealed differentially methylated regions not only in genes associated with obesity, but in genes involved in cell differentiation, the immune system, and transcriptional regulation (van Dijk *et al.* 2015). More broadly, DNA methylation within a tissue is strongly correlated with the developmental age of the tissue ($r = 0.96$); the rate of age-related changes in methylation has been shown to accelerate with increasing Body Mass Index (BMI) in human livers (every 10 BMI units added 2.2 years of DNA methylation age, the expected developmental age of a tissue inferred from its level of DNA methylation) (Horvath *et al.* 2014). To understand how changes in DNA methylation affect gene expression in obesity, however, it is important to consider genetic background. For example, C57BL/6NCrl and BFMI (the Berlin Fat Mouse Inbred line, which develops juvenile obesity) mice on a high-fat diet showed decreased methylation at the melanocortin-4 receptor (*Mc4r*) gene in the brain compared to mice on a standard diet, but expression increased only in the BMFI mice (Widiker *et al.* 2010). Thus, it is useful to investigate obesity epigenetics in different genetic contexts, as I have done here by using a less commonly studied strain of mouse, the SM/J strain. This strain has a strong obesogenic response to an HF diet (Cheverud *et al.* 1999, Ehrich *et al.* 2003, Partridge *et al.* 2014). I took a genome-wide approach to identify genes previously unknown as playing a role in obesity as well as to clarify the regulation of known genes. I synthesized genome-wide expression data with genome-wide methylation data to investigate changes induced by a high-fat diet.

METHODS

Animal Rearing

The inbred SM/J mouse strain from The Jackson Laboratory (Bar Harbor, Maine) was used in this study. The SM/J strain originated from a selective breeding experiment for small size at 60 days of age (MacArthur 1944). Fifteen males and 15 females born in the facility at Loyola University Chicago were bred to produce 56 mice for the study population. The offspring were either weaned onto a low fat (LF) or high fat (HF) diet at 3 weeks of age (the sample size was 16 HF females, 12 LF females, 18 HF males, and 10 LF males). The diets were nearly isocaloric; however, 15% of the calories came from fat in the LF diet (Research Diets D12284), whereas 42% of the calories came from fat in the HF diet (Harlan Teklad diet TD.88137) (Table 3.1). Previous work in the lab by Erich *et al.* (2003) demonstrated that SM/J mice consume the same amount of food whether they are on the HF diet or the LF diet. The mice were fed *ad libitum*, and after weaning each mouse was housed with one other mouse of the same sex and diet in a cage containing a wooden gnawing block (Bio Serve), a red privacy hut (Alt Design), and a 2" x 2" cotton nestlet (Ancare). Procedures followed an approved Institutional Animal Care and Use Committee protocol (Project #1188).

Obesity Phenotype

The mice were weighed weekly from 1-17 weeks of age. They underwent an intraperitoneal glucose tolerance test (IPGTT) at 15 weeks of age. All tests started with a 4-hour fast at 6:00 am, followed by a tail snip to measure the baseline glucose level, and an intraperitoneal injection of glucose (1 mg/g body weight). Glucose measurements were then taken from tail blood at 30, 60, and 120 minutes after injection. At 16 weeks of age, the mice received an intraperitoneal insulin tolerance test (IPITT). The protocol for the IPITT is the same

as for the IPGTT, except that insulin is injected instead of glucose (0.75 mU/g body weight). If the blood glucose levels of a mouse fell below 25 mg/dL, the mouse received a 10% glucose injection and was not included in the IPITT results. For both the IPGTT and IPITT, the glucose values at the 4 different time points were used to calculate the area under the curve (AUC) using the trapezoidal summation method. At 17 weeks of age, the mice were fasted for 4 hours and sacrificed via carbon dioxide asphyxiation between the hours of 10:00 am and 2:00 pm. Blood from a cardiac puncture (0.5 mL) was centrifuged at 4°C, and the serum was sent to Washington University in St. Louis's Core Laboratory for Clinical Studies to measure insulin and leptin, and to the Diabetes Models Phenotyping Core to measure triglycerides, glucose, cholesterol, and free fatty acids. I performed the necropsies on ice and recorded the weights of the liver, heart, reproductive fat pad, kidneys, spleen, brown fat, and skeletal muscle (gastrocnemius). I weighed only the reproductive fat pad rather than all of the fat pads, because it is strongly genetically (h^2 = 0.7-0.9) and phenotypically correlated (r = 0.67-0.82) with the other fat pads (Cheverud *et al.* 2004, Cheverud *et al.* 2011). I flash-froze liver tissue in liquid nitrogen for RNA extraction. I ran a general linear model in SYSTAT (Version 12, Systat Software, San Jose, CA) to analyze differences in obesity phenotypes between the diet treatments. Multivariate tests were performed on the following three groups of traits: weekly weights, diabetes-related traits (week 15 and 16 weight, baseline glucose during the IPGTT, IPGTT AUC, baseline glucose during the IPITT, and IPITT AUC), and necropsy traits (week 17 weight, organ weights, and serum biomarkers), as well as all the associated univariate tests. Differences were interpreted as significant for p-values less than 0.05.

Gene Expression

I extracted RNA from the liver tissue using the Qiagen RNeasy Plus Mini kit and

submitted it to Washington University in St. Louis's GTAC facility for RNA-seq with poly-A selection. A total of 21 libraries were sequenced, each with 2 mice of the same sex and diet pooled together. There were 6 LF female libraries, and 5 of each of the other sex-diet groups. A 1x50 single read sequencing run was done on an Illumina HiSeq 2500 machine (Illumina Inc.). The FastQ files were aligned to the Ensembl release 76 assembly using STAR version 2.0.4b (Dobin *et al.* 2013). The gene counts were then analyzed with the R package edgeR (Robinson *et al.* 2010); differences in library size were accounted for with a TMM normalization, and genes with counts of zero were filtered out. The weighted likelihoods were then calculated using the voom function in the R package Limma based on the mean-variance relationship of each gene and transcript. Generalized linear models were used to test for differential expression. Any gene with a false discovery rate (FDR) adjusted q-value of 0.05 or less was considered differentially expressed. I performed a pathway analysis using the R package GAGE (Luo *et al.* 2009) and visualized the pathways with the R package Pathview (Luo and Brouwer 2013).

I validated the differential expression for 3 genes in the females (*Adam11*, *Lad1*, and *Galnt10*) and 3 in the males (*Adam11*, *Abcg8*, and *Col1a1*), with *Gapdh* as a normalizer (Table 3.11). To do this, I extracted total RNA from the livers of 3 HF and 3 LF mice of each sex using Tri-Reagent (MRC), following the manufacturer's instructions. The concentration and quality of the RNA from each sample was assessed twice with a NanoDrop Spectrophotometer, and only samples with a 260/280 ratio between 1.7-2.1 and a 260/230 ratio between 2.0-2.4 were used. I then immediately reverse transcribed the RNA to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), following the manufacturer's instructions. Primers were selected from the literature, and if none were found I used PrimerBank (https://pga.mgh.harvard.edu/primerbank/) to design the primers. All primers were synthesized

by Thermo Fisher Scientific (the sequences are listed in Table 3.4). I performed RT-qPCR using 10 µL of PowerUp™ SYBR® Green Master Mix (Thermo Fisher), 1 µL of the forward primer, 1 µL of the reverse primer, 4 µL of 20-fold diluted cDNA, and 4 µL of water, with a total volume of 20 µL for each reaction. The RT-qPCR was performed with a StepOnePlus Real-Time PCR System (Applied Biosystems) at the following conditions: 20 seconds at 95°C, followed by 40 cycles of 3 seconds at 95°C and 30 seconds at 60°C. For each of the 3 biological replicates, 3 technical replicates were used, along with a no-template control and a no-reverse-transcriptase control. I did a relative quantification of each gene using *Gapdh* as a reference using the comparative ΔΔCt method.

DNA Methylation

I performed a phenol-chloroform extraction to isolate DNA from the liver tissue. Genome-wide DNA methylation was then assessed with Methylated DNA Immunoprecipitation Sequencing (MeDIP-seq) and Methylation-sensitive Restriction Enzyme Sequencing (MRE-seq), as described by Li *et al.* (2015). MeDIP-seq detects methylated sites while MRE-seq detects unmethylated sites, and when used in combination these two techniques provide a single CpG resolution methylation map that has high concordance with whole-genome bisulfite sequencing at only a fraction of the cost (Stevens *et al.* 2013). Four mice of the same sex and diet treatment were pooled per library, yielding 2 biological replicates per group and a total of 8 methylation libraries. The R package methlyMnM was used to analyze the MRE-seq data in conjunction with the MeDIP-seq data to identify differentially methylated regions (DMRs). I used methylMnM to split the mouse mm9 genome into 500-base-pair windows (for a total of 5,283,825 windows), assess the proportion of methylated CpGs in each window, and then to calculate the novel M&M test statistic to determine if the methylation level was different between the two diet treatments

(Zhang *et al.* 2013). M&M can only test two groups at a time, which yielded 4 pairwise comparisons of the female libraries and 4 of the males. To synthesize the information from all 4 library comparisons per sex, I used Fisher's combined probability test (Fisher 1954). To examine DMRs due to diet in the females, the p-value from the M&M test comparing the first HF-female library with the first LF-female library was combined with the p-value from the M&M test comparing the second HF-female library with the second LF-female library according to the following equation by Fisher:

$$X_{2k}^2 \sim -2\sum_{i=1}^{k} \ln(p_i)$$

In this case, p_i is the p-value from the pairwise M&M test, and k is the number of tests combined (which was 2). I calculated a combined p-value for each 500-base-pair window, corrected for FDR with the Benjamini-Hochberg method, and calculated how many of these windows were differentially methylated based on q-value cutoffs of less than 0.05, 0.01, and 0.001.

For each DMR, I then identified the nearest gene to it, if it fell within a gene, if it fell within a promoter, if it contained a known regulatory element listed in Ensembl (mouse genome assembly GRCm38.p5), and if the gene closest to it was already known to be involved in obesity, diabetes mellitus, or cardiovascular diseases based on Phenopedia's continuously updated list of genes uncovered by genetic association studies in humans (downloaded May 7, 2017). I also classified the DMR as being either in an intergenic region, exon, intron, or promoter. This was done using the full list of introns, exons, and genes downloaded from the NCBI37/mm9 assembly on the UCSC Genome Browser. If the DMR overlapped both an intron and an exon, it was classified as falling within an exon. It was classified as a promoter if it was within 2,000 base pairs upstream of a transcription start site or 600 base pairs downstream of one. To

determine if the DMRs were associated with gene expression, I randomized the DMRs across the genome and calculated how many fell within differentially expressed genes due to chance. To account for the general underrepresentation of DMRs in intergenic regions during the randomization, the percent of DMRs that were allowed to be randomized into intergenic regions was equal to the percent that actually exist in those regions. The overall methylation results highlighted the wide range of genomic regions that diet-induced DMRs are found in, as well as the complex relationship that DMRs have with gene expression.

RESULTS

Obesity phenotype

A full model testing for the effect of sex, diet, and a sex-by-diet interaction for the obesity traits indicated that there was not a significant sex-by-diet effect ($p = 0.13$); however, sex-by-diet was significant on a univariate level for cholesterol, insulin, and glucose AUC during the intraperitoneal insulin tolerance test. The reduced model testing for just sex and diet revealed that sex significantly affected the weekly weights ($p = 8.71 \times 10^{-8}$) (Figure 3.1), the diabetes-related traits ($p = 8.99 \times 10^{-11}$), and the necropsy traits ($p = 1.13 \times 10^{-6}$) (Table 3.2). Regardless of diet, males weighed more than females and had heavier hearts, kidneys, and livers. Males also had higher serum levels of insulin and cholesterol, and had higher area under the curve values for the intraperitoneal glucose and insulin tolerance tests. Sex did not affect the week 1-3 weights, leptin, triglycerides, glucose, free fatty acids, or the weights of the fat pad, spleen, brown fat, or gastrocnemius.

Diet also significantly affected the weekly weights ($p = 1.02 \times 10^{-4}$), diabetes-related traits ($p = 2.06 \times 10^{-3}$), and necropsy traits ($p = 1.78 \times 10^{-14}$). After only one week of being on the diet treatment (4 weeks of age), HF mice weighed significantly more than LF mice, and the

difference became more pronounced with age. The HF mice had reproductive fat pads that were more than 8 times larger than the LF mice. There was an overall increase in all organ weights on an HF diet, including 2.8 times heavier livers, 1.4 times heavier kidneys, 1.8 times heavier spleens, 1.6 times heavier hearts, and 3.5 times more brown fat on average (Figure 3.2).

Diet also significantly affected the response to intraperitoneal glucose and insulin tolerance testing, with HF mice having higher area under the curve values for the glucose tolerance test (1.4 times higher for females and 1.7 times higher for males) as well as for the insulin tolerance test (1.7 times higher for females and 2.3 times higher for males), indicating impaired glucose and insulin signaling. All serum biomarkers except for free fatty acids had higher levels due to an HF diet, particularly in males. HF mice had higher levels of cholesterol (2.2 times higher in females, 3 times higher in males), triglycerides (1.3 times higher in females, 2.1 times higher in males), glucose (1.4 times higher in females, 2 times higher in males), and insulin (6.7 times higher in females, 38 times higher in males) (Figure 3.3). Like insulin resistance, leptin resistance can occur in obesity. That was the case in this study, where compared to LF mice, HF female mice had 20 times more leptin in their serum and HF male mice had 42 times more. Despite these drastically elevated levels of leptin, the mice had a reduced ability to respond to leptin due to having 7 times lower expression of the leptin receptor (*Lepr*) gene (Figure 3.5).

Gene Expression

The multidimensional scaling (MDS) plot indicated that the gene expression libraries clustered primarily by sex (dimension 1) and then by diet (dimension 2) (Figure 3.4). Diet altered the expression of 4,356 genes in the liver, or approximately one-fifth of the genome (Table 3.3). Compared to Phenopedia's list of genes from gene association studies in humans, 419 of the

differentially expressed genes matched the 1,720 known obesity genes, 807 matched the 3,455 known diabetes mellitus genes, and 1,017 matched the 4,540 genes known to be involved in cardiovascular disease. Considerably more differentially expressed genes were detected in males (3,330) than in females (1,750). Of the genes that were differentially expressed, 848 were differentially expressed due to diet only in females and 2,428 were unique to males (Supplementary Tables 3.12-3.13). There were 184 genes with significantly different expression due to a sex-by-diet interaction, which a GO Enrichment analysis showed were enriched for three biological processes: epoxygenase P450 pathway ($p = 2.36x10^{-5}$), oxidation-reduction process ($p = 5.58x10^{-5}$), and response to stilbenoid ($p = 5.21x10^{-3}$). This indicates that the difference between the male and female response to dietary fat may be mediated by sex differences in these pathways.

The GAGE pathway analysis revealed that the ribosome and oxidative phosphorylation pathways were upregulated in males compared to females, whereas the steroid hormone biosynthesis, linoleic acid metabolism, and retinol metabolism were downregulated in males. An HF diet changed the regulation of 7 pathways overall (Table 3.8). This included the downregulation of the oxidative phosphorylation pathway and upregulation of the cytokine-cytokine pathway, indicating that the HF diet reduced mitochondrial function and increased inflammation (Figure 3.6). In females, there were 4 pathways upregulated by an HF diet: cytokine-cytokine receptor interaction, chemokine signaling, cell adhesion molecules, and the natural killer cell mediated cytotoxicity pathways. In males, the cytokine-cytokine receptor interaction pathway was also upregulated by an HF diet, while the ribosome and oxidative phosphorylation pathways were downregulated (Table 3.9). None of the genes in the ribosome pathway had differentially methylated regions, but the other two pathways in males had a

handful of such genes. Numerous GO Biological Processes were upregulated by an HF diet, and none were downregulated. In females, 29 processes were upregulated, nearly all of them related to the immune system. Even more were upregulated in males, with 61 affected processes, again mostly involved in the immune system (Table 3.10).

Methylation

A q-value cutoff of 0.05 revealed tens of thousands of differentially methylated regions (DMRs) due to diet, which encompassed 0.6-0.8% of the nearly 5.3 million 500-base-pair windows in the genome. A cutoff of 0.01 was more discriminating, with less than 0.04% of windows falling below it, allowing us to focus on a few thousand genes with differential methylation (Supplementary Tables 3.14-3.15). The comparison of HF and LF females resulted in 2,356 DMRs ($q < 0.01$), which was more than the 1,539 DMRs between the HF and LF males (Table 3.5). There were even more DMRs due to sex than diet, with HF males and females differing at 3,831 regions and LF males and females differing at 5,632 regions ($q < 0.01$). A greater percentage of DMRs were found on the X chromosome in the between-sex comparisons (2.3-2.8%) than in the within-sex comparisons (0.1-0.3%, $q < 0.01$), consistent with the expectation that the X chromosome is regulated differently in males and females (Cotton *et al.* 2011, El-Maarri *et al.* 2007).

In all, 7,814 genes (38.3% of genes) in the liver had at least one diet-induced DMR ($q < 0.05$) in the females, as did 7,086 genes (34.7%) in the males (Table 3.7). When the DMRs were assigned to one of four categories, 15% fell within promoters, 25% fell within exons, 34% fell within introns, and 31% were in intergenic regions (Table 3.6). Not all of these categories were mutually exclusive, since several DMRs encompassed both exons and promoters. Many of the DMRs were in regulatory regions, including 10-12% in enhancers, 14-16% in promoters, 3-7%

in CTCF transcription factor binding sites, and 34% in promoter flanking regions. Differentially methylated regions were far more likely to be found in these regulatory regions than non-regulatory regions of the genome ($p < 1 \times 10^{-10}$ from a χ^2). Across the genome, only 4% of the windows overlapped enhancers, 2% overlapped CTCF binding sites, and 8% overlapped promoter flanking regions. Thus, a high-fat diet alters not just the methylation of gene promoters, but also enhancers and transcription factor binding sites.

Although only a small percentage of the DMRs fell in differentially expressed genes, it still happened more often than expected by chance ($p = 2.2 \times 10^{-8}$). In the females, 2,170 (5.6% of) DMRs fell within differentially expressed genes, whereas only 1,994 (5.1%) were expected to by chance. In the males, 3,209 (10.2% of) DMRs fell within differentially expressed genes, whereas only 2,992 (9.5%) were expected to by chance. Differential methylation thus is associated with differential gene expression.

DISCUSSION

Both sex and diet had a statistically significant effect on body weight by 4 weeks of age. By 17 weeks of age, mice on a high fat (HF) diet weighed approximately 70% more than mice on a low fat (LF) diet. An HF diet significantly increased the following traits: all body weights and organ weights, baseline glucose levels, and serum levels of cholesterol, triglycerides, glucose, leptin, and insulin. The HF diet did not increase levels of free fatty acids in the serum, but Do *et al.* (2011) found the same trend in HF-fed C57BL/6J mice, despite elevated fatty acid levels in the liver. In our study, HF-fed mice had higher area under the curve (AUC) values for the glucose and insulin tolerance tests, indicating hyperglycemia and insulin resistance.

This strong obesogenic response to an HF diet was driven by changes in DNA methylation and gene expression. Diet significantly altered the expression of 4,356 genes and the

methylation of more than 7,000 genes in the mice. An HF diet had an extensive effect on methylation, with more than one-third of genes in the liver having at least one differentially methylated region (DMR), and one-fifth of genes having more than one DMR in response to diet. The DMRs occurred in regulatory regions such as enhancers, CTCF transcription factor binding sites, other transcription factor binding sites, and promoter flanking regions significantly more often than these regions occur in the genome, supporting the notion that methylation plays an important role in regulating the response to an HF diet. That role is not straightforward, however. The DMRs fell within differentially expressed genes significantly more often than expected by chance; however, 90% of the DMRs were not located in differentially expressed genes. In fact, 41% of the DMRs did not even occur within genes at all, although 17% of those contained enhancers, CTCF binding sites, or other transcription factor binding sites. This is on par with the findings of other studies in the field, such as Rönn *et al.* (2013) who found DMRs in 7,663 genes after 6 months of exercise in men's adipose tissue, but only 197 of those genes had expression changes and just 61% of those had an inverse relationship between methylation and expression. The effect of methylation on gene expression depends on where the methylated region is located in the genome (Parle-McDermott and Ozaki 2011). It will be interesting in the future to correlate the DNA methylation changes with histone modifications, as these two regulatory features work together to modulate gene expression, and we know that an HF diet induces chromatin remodeling (Leung *et al.* 2014).

In the females, 2,170 (5.6%) of DMRs ($q < 0.05$) occurred within genes that were differentially expressed due to diet. This was higher in males, where 3,209 (10.2%) of DMRs occurred within genes that were differentially expressed. The differentially expressed gene with the lowest q-value in the females was ADAM metallopeptidase domain 11 (*Adam11*) (q =

$7.6x10^{-20}$), which also had three adjacent DMRs (q = 0.00029, q = $1.6x10^{-8}$, and q = $3.3x10^{-6}$) encompassing exons 14-18 of the gene. The males had the same three DMRs and exhibited the same pattern as the females of having higher *Adam11* expression as well as higher methylation at the DMRs due to an HF diet (Figure 3.7A). Although the *Adam11* gene is known to be expressed in the mouse liver (MGI Gene Expression Database), its role in obesity and diabetes has not been discussed. It belongs to a family of genes involved in cell signaling, migration, and adhesion, and mice lacking *Adam11* have impaired spatial learning and motor coordination, along with a reduced response to inflammatory pain (Takahashi *et al.* 2006). Perhaps the increased inflammation associated with obesity leads to an increase in inflammation-related pain.

An HF diet also increased expression of the UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (*Galnt10*) gene in the females. There is a DMR in the first intron, which is more heavily methylated by an HF diet (Figure 3.7B). A genome-wide association study found a SNP in the human *Galnt10* gene that is associated with BMI (Monda *et al.* 2013), and another study found a SNP in it associated with physical activity (Ahmad *et al.* 2015). *Galnt10* is known to be expressed in the mouse liver (MGI Gene Expression Database), and the *Galnt10* protein participates in post-translational modification in the Golgi apparatus where it catalyzes the synthesis of mucin-type O-glycosylation. Important mucin-type O-linked glycoproteins include the cytokine interluekin-2 as well as proteins involved in homing leukocytes to inflamed areas (Hang and Bertozzi 2005).

An HF diet also upregulated the expression of the ladinin 1 (*Lad1*) gene in the females and decreased methylation at two adjacent DMRs in its first intron, which is also considered to be a promoter region (Figure 3.7C). *Lad1* is highly expressed in the kidneys and lungs, and is found in lower levels in the liver and spleen (Motoki *et al.* 1997). The protein is a part of the

basement membrane, a thin matrix that holds the epithelium to its connective tissue and is also involved in angiogenesis. In liver fibrosis, the basement membrane increases around the liver vessels, and basement membrane peptides increase in the serum with the severity of liver damage (Walsh *et al.* 2000). This is relevant because the HF mice had visibly fattier livers, in line with the increased risk of non-alcoholic fatty liver disease (NAFLD) due to obesity.

Further epigenetic evidence of liver distress induced by an HF diet is the upregulation of the collagen type I alpha 1 chain (*Col1a1*) gene in HF males, accompanied by increased methylation at a DMR spanning exons 23 and 24 of the gene (Figure 3.7D). The COL1A1 protein is a subunit of type 1 collagen, which is found in many parts of the body such as tendons, bone, and scar tissue. It is the main type of collagen that accumulates in the liver during fibrosis and cirrhosis. When mice with advanced liver fibrosis were administered siRNA to degrade transcripts of *Col1a1*, collagen deposition decreased by half and several other profibrogenic genes became downregulated as well (Calvente *et al.* 2015). Our results support the notion that siRNA or other epigenetic treatments for elevated *Col1a1* levels may help in obesity related liver fibrosis.

The ATP binding cassette subfamily G member 5 (*Abcg5*) gene lies head-to-head with *Abcg8*, and both were expressed more in HF males than in LF males. A DMR close to the start of both genes, located in the first intron of *Abcg5*, had lower methylation due to an HF diet and may be involved in the co-regulation of the genes (Figure 3.7E). They encode proteins forming a heterodimer that facilitates the excretion of cholesterol and other phytosterols into bile. Mutations in either gene are associated with atherosclerosis and sitosterolemia, a condition that leads to cardiovascular disease through the accumulation of sterols in the body (Yu *et al.* 2014). Our findings support previous studies that have identified the upregulation of *Abcg5* and *Abcg8*

in response to insulin resistance and an HF diet (Biddinger *et al.* 2008, Yamazaki *et al.* 2011, Do *et al.* 2011, Yu *et al.* 2014). The upregulation of the heterodimer may have been an attempt to eliminate the excess cholesterol from the body, although even with the upregulation high-fat-fed mice still had 2-3 times as much serum cholesterol as the low-fat-fed mice.

Our gene expression findings mostly support those of other mouse studies, while highlighting differences that can be caused by a different genetic background. For instance, Do *et al.* (2011) compared liver expression of HF- and LF-fed male C57BL/6J mice and found that an HF diet perturbed genes that were enriched for processes involved in immune and inflammatory response. Like us, they found that diet altered biological processes involving the defense response, inflammatory response, and innate immune response. Of the 332 genes they found differentially expressed due to an HF diet, 120 were the same as the ones I identified in the males (they did not study females). These included *Adam11*, *Abcg5*, and *Abcg8*, which I highlighted in my study as being both differentially expressed and differentially methylated due to diet. I found 28 genes in common with Kim *et al.* (2004), who identified 97 differentially expressed genes due to an HF diet in C57BL/6J males. I found more genes in common with Kim *et al.* (2004) and Do *et al.* (2011) than either did with each other (they had *Acox1*, *Cyp3a11*, *Egfr*, *Nsdhl*, *Serpina3g*, and *Sqle* in common), even though they used the same mouse strain, which shows the utility of RNA-seq data over microarray data when comparing across studies. Of the 309 differentially expressed genes that Kirpich *et al.* (2011) identified in male C57BL/6 mice due to an HF diet, I found 124 of the same genes. Kirpich *et al.* (2011) shared 12 genes in common with Kim *et al.* (2004) and 57 genes in common with Do *et al.* (2011), and the only genes found in all three studies were *Nsdhl* and *Sqle* (Figure 3.8). Like Inoue *et al.* (2005) found in C57BL/6Ncrj male mice, I found that *Pparg* and its target gene *Cd36* were both upregulated in the male and female

HF mice, corroborating their conclusion that an HF diet induces liver steatosis by upregulating *Pparg*. Similar to other studies, I found an upregulation of genes in pathways associated with defense, stress, and inflammation responses (Kim *et al.* 2004, Do *et al.* 2011).

I compared my list of differentially expressed genes in the males and females with those found in 9 other strains exposed to an HF diet in the Mouse Phenome Database (MPD, The Jackson Laboratory) from the Shockley *et al.* (2009) study and found between 16 and 27 genes in common with the males in each study and 3 to 15 genes in common with the females (Supplementary Table 3.16). The varied results depending on strain underscore the importance of studying obesity in multiple strains of mice instead of basing conclusions off of one strain. In the males, 13 genes were found to be altered by an HF diet in 3 of the strains (*Aplp2**, *Bach2*, *Cenph**, *Ercc5**, *Fhdc1**, *Kdm5c**, *Nrxn3*, *Pms2**, *Slc8a1**, *Tmem57**, *Vacn**, *Zfp385b*, and *Zfp608**) and 4 genes were altered in 4 strains (*Fgfr2**, *Homer1**, *Kcnma1*, and *Ptprd*) (an asterisk indicates that our SM/J mice constituted one of the strains). In the females, 12 genes were found to be altered by diet in 3 strains (*4933431K23Rik*, *Atp2a2**, *Fam63a**, *Gata6**, *Hmx1*, *Klhl3**, *Osbpl3**, *Pus10*, *Sept8**, *Slc38a2**, *Spty2d1**, and *Syt11**), and one gene was altered in 4 strains (*Fgfr2**). Due to its responsiveness to an HF diet in both males and females of four different mouse strains, *Fgfr2* could be an important therapeutic target in obesity—especially because, along with lower expression in our HF-fed SM/Js, the gene had 2 DMRs in the first intron of the females and 1 DMR in the third intron of the males. *Fgfr2* is important for liver regeneration and its expression is increased in the livers of people with NAFLD (Younossi *et al.* 2005). Additionally, Haworth *et al.* 2014 linked the methylation of this gene to weight when they found that the methylation of three CpGs in the cord blood of newborns was significantly associated with high birth weight.

Replicating differentially methylated regions across studies can be more difficult than replicating gene expression, since methylation can be more variable and fewer studies have investigated it, especially in terms of genome-wide studies. Ge *et al.* (2014) found that *Lep* was less expressed and its promoter was more methylated in the livers of HF-fed female CD-1 mice. I also found a hypermethylated DMR in the *Lep* promoter of the females ($q = 0.02$), but there was no difference in expression. Ge *et al.* (2014) additionally found a hypomethylated *Pparα* promoter, and although I too found a DMR in *Pparα*, mine was hypermethylated by an HF diet, it was located in the second intron, and the gene was not differentially expressed. Yoon *et al.* (2017) identified hypomethylated CpG sites 1.5-kb upstream of the *Casp1* gene in C57BL/6N male mice, but I found no DMRs in that gene. Like them, I did find lower expression of the *Ndufb9* gene in HF-fed males along with a DMR in it, but my DMR was hypomethylated by an HF diet whereas theirs was hypermethylated. As exemplified by this variability across studies, understanding the methylation changes underlying obesity will require much more research in the context of multiple genetic backgrounds.

Males and females respond differently to an HF diet, shown by differences in methylation, gene expression (184 differentially expressed genes had a sex-by-diet interaction), and obesity phenotypes (although there was not a significant sex-by-diet interaction here, males clearly differed due to diet more than females in their serum biomarkers and response to glucose and insulin tolerance testing). The genes with a significant sex-by-diet interaction were enriched for the epoxygenase P450 pathway, oxidation-reduction process, and response to stilbenoid, suggesting sex differences in these pathways mediate the difference between the male and female response to dietary fat. Cytochrome P450 genes are important for homeostasis and encode enzymes involved in metabolizing compounds such as fatty acids and drugs, so sex

differences in this pathway are relevant to pharmaceutical approaches to weight loss. Likewise, a sex-by-diet effect on the response to stilbenoids is interesting because they have been shown to regulate lipids, and Lin *et al.* (2015) found that the stilbenoid TSG prevented non-alcoholic fatty liver disease in HF-fed rats, with results that hinted at a small but inconclusive difference between males and females (Lin *et al.* 2015). Although there were more differentially expressed genes due to diet in males than in females, the opposite was true for DMRs. However, while there were fewer DMRs total in males, more of their DMRs occurred within genes that were differentially expressed due to diet.

This study identified thousands of genes that were differentially expressed and differentially methylated due to a high-fat diet in SM/J mice. Genome-wide studies such as this are essential for developing a better understanding of the relevant epigenetic changes in obesity and identifying new targets for treatments. It is essential that these treatments take sex into consideration, since—from the level of methylation to expression to the obesity phenotypes—males and females responded quite differently to an obesogenic diet.

ACKNOWLEDGEMENTS

I thank the American Heart Association for funding (pre-doctoral fellowship #16PRE26420105). I thank Xiaoyun Xiao and Daofeng Li for performing the methylation sequencing and initial quality control. I also thank the Genome Technology Access Center in the Department of Genetics at Washington University School of Medicine for help with genomic analysis.
The Center is partially supported by NCI Cancer Center Support Grant #P30 CA91842 to the Siteman Cancer Center and by ICTS/CTSA Grant# UL1TR000448 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. This publication is solely the responsibility of the

authors and does not necessarily represent the official view of NCRR or NIH. I also thank the Core Laboratory for Clinical Studies and the Diabetes Models Phenotyping Core (both supported by the Washington University DRC, Grant No. P30 DK020579) at Washington University in St. Louis for performing the biomarker analysis on the serum traits.

LITERATURE CITED

Ahmad, S., W. Zhao, F. Renström, A. Rasheed, M. Samuel, M. Zaidi, N. Shah, N. H. Mallick, K. S. Zaman, M. Ishaq, S. Z. Rasheed, F. U. Memon, B. Hanif, M. Lakhani, F. Ahmed, S. U. Kazmi, P. Frossard, P. W. Franks, and D. Saleheen. 2015. Physical activity, smoking, and genetic predisposition to obesity in people from Pakistan: the PROMIS study. *BMC Med Genet* 16: 114.

Barua, S., S. Kuizon, K. K. Chadman, M. J. Flory, W. Brown, and M. A. Junaid. 2014. Single-base resolution of mouse offspring brain methylome reveals epigenome modifications caused by gestational folic acid. *Epigenetics & Chromatin* 7: 3.

Baumeier, C., S. Saussenthaler, A. Kammel, M. Jähnert, L. Schlüter, D. Hesse, M. Canouil, S. Lobbens, R. Caiazzo, V. Raverdy, F. Pattou, E. Nilsson, J. Pihlajamäki, C. Ling, P. Froguel, A. Schürmann, and R. W. Schwenk. 2017. Hepatic DPP4 DNA methylation associates with fatty liver. *Diabetes* 66(1): 25-35.

Bell, C. G., A. J. Walley, and P. Froguel. 2005. The genetics of human obesity. *Nature Reviews Genetics* 6: 221-234.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)* 57(1): 289-300.

Biddinger, S. B., J. T. Haas, B. B. Yu, O. Bezy, E. Jing, W. Zhang, T. G. Unterman, M. C Carey, and C. R. Kahn. 2008. Hepatic insulin resistance directly promotes formation of cholesterol gallstones. *Nat Med* 14(7): 778-782.

Bird, A. 2002. DNA methylation patterns and epigenetic memory. *Genes & Dev* 16: 6-21.

Bird, A. 2007. Perceptions of epigenetics. *Nature* 447: 396-398.

Bossdorf, O., D. Arcuri, C. L. Richards, M. Pigliucci. 2010. Experimental alteration of DNA methylation affects the phenotypic plasticity of ecologically relevant traits in *Arabidopsis thaliana*. *Evol Ecol* 24: 541-553.

Buchwald, H. 2005. Bariatric surgery for morbid obesity: health implications for patients, health

professionals, and third-party payers. *J Amer Coll Surg* 200: 593-604.

Calvente, C. J., A. Sehgal, Y. Popov, Y. O. Kim, V. Zevallos, U. Sahin, M. Diken, and D. Schuppan. 2015. Specific hepatic delivery of procollagen α1(I) small interfering RNA in lipid-like nanoparticles resolves liver fibrosis. *Hepatology* 62(4): 1285-1297.

Campión, J., F. I. Milagro, and J. A. Martínez. 2009. Individuality and epigenetics in obesity. *Obesity Reviews* 10(4): 383-392.

Cawley, J., and C. Meyerhoefer. 2012. The medical care costs of obesity: an instrumental variables approach. *J Health Econ*. 31: 219-230.

Cheverud, J. M., L. S. Pletscher, T. T. Vaughn, and B. Marshall. 1999. Differential response to dietary fat in large (LG/J) and small (SM/J) inbred mouse strains. *Physiol Genomics* 15(1): 33-39.

Cheverud, J. M., T. H. Ehrich, T. Hrbek, J. P. Kenney, L. S. Pletscher, and C. F. Semenkovich. 2004. Quantitative trait loci for obesity- and diabetes-related traits and their dietary responses to high-fat feeding in LGXSM recombinant inbred mouse strains. *Diabetes* 53(12): 3328-3336.

Cheverud, J. M., H. A. Lawson, G. L. Fawcett, B. Wang, L. S. Pletscher, A. R Fox, T. J. Maxwell, T. H. Ehrich, J. P. Kenney-Hunt, J. B. Wolf, and C. F. Semenkovich. 2011. Diet-dependent genetic and genomic imprinting effects on obesity in mice. *Obesity* 19(1): 160-170.

Cotton, A. M., L. Lam, J. G. Affleck, I. M. Wilson, M. S. Peñaherrera, D. E. McFadden, M. S. Kobor, W. L. Lam, W. P. Robinson, and C. J. Brown. 2011. Chromosome-wide DNA methylation analysis predicts human tissue-specific X inactivation. *Hum Genet* 130(2): 187-201.

Ding, X., D. Zheng, C. Fan, Z. Liu, H. Dong, Y. Lu, and K. Qi. 2015. Genome-wide screen of DNA methylation identifies novel markers in childhood obesity. *Gene* 566(1): 74-83.

Do, G. M., H. Y. Oh, E. Y. Kwon, Y. Y. Cho, S. K. Shin, H. J. Park, S. M. Jeon, E. Kim, C. G. Hur, T. S. Park, M. K. Sung, R. A. McGregor, and M. S. Choi. 2011. Long-term adaptation of global transcription and metabolism in the liver of high-fat diet-fed C57BL/6J mice. *Mol Nutr Food Res* 55 Suppl 2: S173-S185.

Dobin, A., C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, and T. R. Gingeras. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29(1): 15-21.

El-Maarri, O., T. Becker, J. Junen, S. S. Manzoor, A. Diaz-Lacava, R. Schwaab, T. Wienker, and J. Oldenburg. 2007. Gender specific differences in levels of DNA methylation at selected loci from human total blood: a tendency toward higher methylation levels in males. *Hum Genet* 122(5): 505-514.

Ehrich, T. E., J. P. Kenney, T. T. Vaughn, L. S. Pletscher, and J. M. Cheverud. 2003. Diet,

obesity, and hyperglycemia in LG/J and SM/J mice. *Obesity Research* 11(11): 1400-1410.

Ehrich, T.H., T. Hrbek, J. P. Kenney-Hunt, L. S. Pletscher, B. Wang, C. F. Semenkovich, and J. M. Cheverud. 2005. Fine-mapping gene-by-diet interactions on chromosome 13 in a LG/J x SM/J murine model of obesity. *Diabetes* 54(6): 1863-1872.

Emilsson, V., G. Thorleifsson, B. Zhang, A. S. Leonardson, F. Zink, J. Zhu, S. Carlson, A. Helgason, G. B. Walters, S. Gunnarsdottir, M. Mouy, V. Steinthorsdottir, G. H. Eiriksdottir, G. Bjornsdottir, I. Reynisdottir, D. Gudbjartsson, A. Helgadottir, A. Jonasdottir, A. Jonasdottir, U. Styrkarsdottir, S. Gretarsdottir, K. P. Magnusson, H. Stefansson, R. Fossdal, K. Kristjansson, H. G. Gislason, T. Stefansson, B. G. Leifsson, U. Thorsteinsdottir, J. R. Lamb, J. R. Gulcher, M. L. Reitman, A. Kong, E. E. Schadt, and K. Stefansson. 2008. Genetics of gene expression and its effect on disease. *Nature* 452(7186): 423-428.

Finger, J.H., C. M. Smith, T. F. Hayamizu, I. J. McCright, J. Xu, M. Law, D. R. Shaw DR, R. M. Baldarelli, J. S. Beal, O. Blodgett, J. W. Campbell, L. E. Corbani, J. R. Lewis, K. L. Forthofer, P. J. Frost, S. C. Giannatto, L. N. Hutchins, D. B. Miers, H. Motenko, K. R. Stone, J. T. Eppig, J. A. Kadin, J. E. Richardson, and M. Ringwald. 2017. The mouse Gene Expression Database (GXD): 2017 update. *Nucleic Acids Res* 45 (D1): D730-D736.

Finkelstein, E. A, O. A. Khavjou, H. Thompson, J. G. Trogdon, L. Pan, B. Sherry, and W. Dietz. 2012. Obesity and severe obesity forecasts through 2030. *Am J Prev Med* 42(6): 563-570.

Fisher, R. A. 1954. *Statistical Methods for Research Workers*. 12th ed. Oliver & Boyd, Edinburgh, 356 pp.

Fullston, T., E. M. C. O. Teague, N. O. Palmer, M. J. DeBlasio, M. Mitchell, M. Corbett, C. G. Print, J. A. Owens, and M. Lane. 2013. Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. *FASEB Journal* 27(10): 4226-4243.

Ge, Z.-J., S.-M. Luo, F. Lin, Q.-X. Liang, L. Huang, Y.-C. Wei, Y. Hou, Z.-M. Han, H. Schatten, and Q.-Y. Sun. 2014. DNA methylation in oocytes and liver of female mice and their offspring: effects of high-fat-diet-induced obesity. *Environ Health Perspect* 122(2): 159-164.

Grundberg, E., E. Meduri, J. K. Sandling, A. K. Hedman, S. Keildson, A. Buil, S. Busche, W. Yuan, J. Nisbet, M. Sekowska, A. Wilk, A. Barrett, K. S. Small, B. Ge, M. Caron, S.-Y. Shin, and the Multiple Tissue Human Expression Resource Consortium. 2013. Global analysis of DNA methylation variation in adipose tissue from twins reveals links to disease-associated variants in distal regulatory elements. *Am J Hum Genet* 93: 876-890.

Hang, H. C., and C. R. Bertozzi. 2005. The chemistry and biology of mucin-type O-linked glycosylation. *Bioorg Med Chem* 13(17): 5021-5034.

Haworth, K. E., W. E. Farrell, R. D. Emes, K. M. Ismail, W. D. Carroll, E. Hubball, A. Rooney,

A. M. Yates, C. Mein, and A. A. Fryer. 2014. Methylation of the FGFR2 gene is associated with high birth weight centile in humans. *Epigenomics* 6(5): 477-491.

Hill, J. O., and J. C. Peters. 1998. Environmental contributions to the obesity epidemic. *Science* 280(5368): 1371-1374.

Horvath, S., W. Erhart, M. Brosch, O. Ammerpohl, W. von Schönfels, M. Ahrens, N. Heits, J. T. Bell, P. C. Tsai, T. D. Spector, P. Deloukas, R. Siebert, B. Sipos, T. Becker, C. Röcken, C. Schafmayer, and J. Hampe. 2014. Obesity accelerates epigenetic aging of human liver. *Proc Natl Acad Sci USA* 111(43): 15538-15543.

Illingworth, R.S., and A. P. Bird. 2009. CpG islands—'a rough guide.' *FEBS Lett* 583(11): 1713-1720.

Inoue, M., T. Ohtake, W. Motomura, N. Takahashi, Y. Hosoki, S. Miyoshi, Y. Suzuki, H. Saito, Y. Kohgo, and T. Okumura. 2005. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. *Biochem Biophys Res Commun* 336(1): 215-222.

Khalil, C. A. 2014. The emerging role of epigenetics in cardiovascular disease. *Ther Adv Chronic Dis* 5(4): 178-187.

Kim, S., I. Sohn, J. I. Ahn, K. H. Lee, Y. S. Lee, and Y. S. Lee. 2004. Hepatic gene expression profiles in a long-term high-fat diet-induced obesity mouse model. *Gene* 340(1): 99-109.

Kirpich, I. A., L. N. Gobejishvili, M. Bon Homme, S. Waigel, M. Cave, G. Arteel, S. S. Barve, C. J. McClain, and I. V. Deaciuc. 2011. Integrated hepatic transcriptome and proteome analysis of mice with high-fat diet-induced nonalcoholic fatty liver disease. *J Nutr Biochem* 22(1): 38-45.

Lawson, H. A., J. M. Cheverud, and J. B. Wolf. 2013. Genomic imprinting and parent-of-origin effects on complex traits. *Nat Rev Genet* 14(9): 609-617.

Leung, A., B. W. Parks, J. Du, C. Trac, R. Setten, Y. Chen, K. Brown, A. J. Lusis, R. Natarajan, and D. E. Schones. 2014. Open chromatin profiling in mice livers reveals unique chromatin variations induced by high fat diet. *J Biol Chem* 289(34): 23557-23567.

Li, D., B. Zhang, X. Xing, and T. Wang. 2015. Combining MeDIP-seq and MRE-seq to investigate genome-wide CpG methylation. *Methods* 72: 29-40.

Lin, S., T. C. Thomas, L. H. Storlien, and X. F. Huang. 2000. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord* 24(5): 639-646.

Lin, P., J. Lu, Y. Wang, W. Gu, J. Yu, and R. Zhao. 2015. Naturally occurring stilbenoid TSG reverses non-alcoholic fatty liver diseases via gut-liver axis. *PLoS One* 10(10): e0140346.

Lomba, A., F. I. Milagro, D. F. García-Díaz, A. Marti, J. Campión, and J. A. Martínez. 2010.

Obesity induced by a pair-fed high fat sucrose diet: methylation and expression pattern of genes related to energy homeostasis. *Lipids Health Dis* 9: 60.

Luo, W., M. Friedman, K. Shedden, K. Hankenson, and P. Woolf. 2009. GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics* 10: 161.

Luo, W. and C. Brouwer. 2013. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics* 29(14): 1830-1831.

MacArthur, J. 1944. Genetics of body size and related characters. I. Selection of small and large races of the laboratory mouse. *Amer Natur* 78: 142-157.

Malodobra-Mazur, M., A. Dziewulska, K. Kozinski, P. Dobrzyn, K. Kolczynska, J. Janikiewicz, and A. Dobrzyn. 2014. Stearoyl-CoA desaturase regulates inflammatory gene expression by changing DNA methylation level in 3T3 adipocytes. *Int J Biochem Cell Biol* 55: 40-50.

Martinez, J. A. 2000. Body-weight regulation: causes of obesity. *Proc Nutr Soc* 59(3): 337-345.

McAllister, E. J., N. V. Dhurandhar, S. W. Keith, L. J. Aronne, J. Barger, M. Baskin, R. M. Benca, J. Biggio, M. M. Boggiano, J. C. Eisenmann, M. Elobeid, K. R. Fontaine, P. Gluckman, E. C. Hanlon, P. Katzmarzyk,A. Pietrobelli, D. T. Redden, D. M. Ruden, C. Wang, R. A. Waterland, S. M. Wright, and D. B. Allison. 2009. Ten putative contributors to the obesity epidemic. *Crit Rev Food Sci Nutr* 49(10): 868-913.

Monda, K. L., G. K. Chen, K. C. Taylor, C. Palmer, T. L. Edwards, L. A. Lange, *et al*. 2013. A meta-analysis identifies new loci associated with body mass index in individuals of African ancestry. *Nature Genetics* 45(6): 690-696.

Motoki, K., M. Megahed, S. LaForgia, and J. Uitto. 1997. Cloning and chromosomal mapping of mouse ladinin, a novel basement membrane zone component. *Genomics* 39(3): 323-330.

Ogden, C.L., M. D. Carroll, B. K. Kit, K. M. Flegal. 2014. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA* 311(8): 806-814.

O'Rahilly, S., and I. S. Farooqi. 2006. Genetics of obesity. *Philos Trans R Soc Lond B Biol Sci* 361(1471): 1095-1105.

Parle-McDermott, A., and M. Ozaki. 2011. The impact of nutrition on differential methylated regions of the genome. *Adv Nutr* 2: 463-471.

Partridge, C, G. L. Fawcett, B. Wang, C. F. Semenkovich, and J. M. Cheverud. 2014. The effect of dietary fat intake on hepatic gene expression in LG/J and SM/J mice. *BMC Genomics* 15: 99.

Pérusse, L., T. Rankinen, A. Zuberi, Y. C. Chagnon, S. J. Weisnagel, G. Argyropoulos, B. Walts, E. E. Snyder, and C. Bouchard. 2005. The human obesity gene map: the 2004 update. *Obes Res* 13(3): 381-490.

Razin, A., C. Howard. 1991. DNA methylation and gene expression. *Microbiological Reviews* 5(3): 451-458.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1): 139-140.

Rönn, T., P. Volkov, C. Davegårdh, T. Dayeh, E. Hall, A. H. Olsson, E. Nilsson, A. Tornberg, M. Dekker Nitert, K. F. Eriksson, H. A. Jones, L. Groop, and C. Ling. 2013. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. *PLoS Genet* 9(6): e1003572.

Russo, V. E. A., R. A. Martienssen, A. D. Riggs. 1996. Epigenetic mechanisms of gene regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shockley, K. R., D. Witmer, S. L. Burgess-Herbert, B. Paigen, and G. A. Churchill. 2009. Effects of atherogenic diet on hepatic gene expression across mouse strains. *Physiol Genomics* 39(3): 172-182.

Stevens, M., J. B. Cheng, D. Li, M. Xie, C. Hong, C. L. Maire, K. L. Ligon, M. Hirst, M. A. Marra, J. F. Costello, T. Wang. 2013. Estimating absolute methylation levels at single CpG resolution from methylation enrichment and restriction enzyme sequencing methods. *Genome Res* 23(9): 1541-1553.

Strakovsky, R. S., X. Zhang, D. Zhou, and Y. X. Pan. 2011. Gestational high fat diet programs hepatic phosphoenolpyruvate carboxykinase gene expression and histone modification in neonatal offspring rats. *Journal of Physiology* 589: 2707-2717.

Takahashi, E., K. Sagane, T. Nagasu, and J. Kuromitsu. 2006. Altered nociceptive response in ADAM11-deficient mice. *Brain Res* 1097(1): 39-42.

van Dijk, S. J., P. L. Molloy, H. Varinli, J. L. Morrison, B. S. Muhlhausler, and Members of EpiSCOPE. 2015. Epigenetics and human obesity. *Int J Obes* 39(1): 85-97.

Voisin, S., M. S. Almén, G. Y. Zheleznyakova, L. Lundberg, S. Zarei, S. Castillo, F. E. Eriksson, E. K. Nilsson, M. Blüher, Y. Böttcher, P. Kovacs, J. Klovins, M. Rask-Andersen, and H. B. Schiöth. 2015. Many obesity-associated SNPs strongly associate with DNA methylation changes at proximal promoters and enhancers. *Genome Med* 7: 103.

Volkov, P., A. H. Olsson, L. Gillberg, S. W. Jørgensen, C. Brøns, K. F. Eriksson, L. Groop, P. A. Jansson, E. Nilsson, T. Rönn, A. Vaag, and C. Ling. 2016. A genome-wide mQTL analysis in human adipose tissue identifies genetic variants associated with DNA methylation, gene expression and metabolic traits. *PLoS One* 11(6): e0157776.

Walsh, K. M., A. Fletcher, R. N. MacSween, and A. J. Morris. 2000. Basement membrane peptides as markers of liver disease in chronic hepatitis C. *J Hepatol* 32(2): 325-330.

Wang, X., and C. Hai. 2015. Redox modulation of adipocyte differentiation: hypothesis of "Redox Chain" and novel insights into intervention of adipogenesis and obesity. *Free Radic Biol Med* 89: 99-125.

Widiker, S., S. Karst, A. Wagener, and G. A. Brockmann. 2010. High-fat diet leads to a decreased methylation of the Mc4r gene in the obese BFMI and the lean B6 mouse lines. *J Appl Genet* 51: 193-197.

Yates, A., W. Akanni, M. Ridwan Amode, D. Barrell, K. Billis, D. Carvalho-Silva, C. Cummins, P. Clapham, S. Fitzgerald, L. Gil, C. G. Girón, L. Gordon, T. Hourlier, S. E. Hunt, S. H. Janacek, N. Johnson, T. Juettemann, S. Keenan, I. Lavidas, F. J. Martin, T. Maurel, W. McLaren, D. N. Murphy, R. Nag, M. Nuhn, A. Parker, M. Patricio, M. Pignatelli, M. Rahtz, H. S. Riat, D. Sheppard, K. Taylor, A. Thormann, A. Vullo, S. P. Wilder, A. Zadissa, E. Birney, J. Harrow, M. Muffato, E. Perry, M. Ruffier, G. Spudich, S. J. Trevanion, F. Cunningham, B. L. Aken, D. R. Zerbino, and P. Flicek. 2016. Ensembl 2016. *Nucleic Acids Res* 44(Database issue): D710-D716.

Yamazaki, Y., T. Hashizume, H. Morioka, S. Sadamitsu, A. Ikari, M. Miwa, and J. Sugatani. 2011. Diet-induced lipid accumulation in liver enhances ATP-binding cassette transporter g5/g8 expression in bile canaliculi. *Drug Metab Pharmacokinet* 26(5): 442-450.

Yang, L., and G. A. Colditz. 2015. Prevalence of overweight and obesity in the United States, 2007-2012. *JAMA Intern Med* 175(8): 1412-1413.

Yoon, A., S. A. Tammen, S. Park, S. N. Han, and S.-W. Choi. 2017. Genome-wide hepatic DNA methylation changes in high-fat diet-induced obese mice. *Nutr Res Pract* 11(2): 105-113.

Younossi, Z. M., A. Baranova, K. Ziegler, L. Del Giacco, K. Schlauch, T. L. Born, H. Elariny, F. Gorreta, A. VanMeter, A. Younoszai, J. P. Ong, Z. Goodman, and V. Chandhoke. 2005. A genomic and proteomic study of the spectrum of nonalcoholic fatty liver disease. *Hepatology* 42(3): 665-674.

Yu, W., M. Clyne, M. J. Khoury, and M. Gwinn. 2010. Phenopedia and Genopedia: disease-centered and gene-centered views of the evolving knowledge of human genetic associations. *Bioinformatics* 26(1): 145-146.

Yu, X. H., K. Qian, N. Jiang, X. L. Zheng, F. S. Cayabyab, and C. K. Tang. 2014. ABCG5/ABCG8 in cholesterol excretion and atherosclerosis. *Clin Chim Acta* 428: 82-88.

Zhang, B., Y. Zhou, N. Lin, R. F. Lowdon, C. Hong, R. P. Nagarajan, J. B. Cheng, D. Li, M. Stevens, H. J. Lee, X. Xing, J. Zhou, V. Sundaram, G. Elliott, J. Gu, T. Shi, P. Gascard, M. Sigaroudinia, T. D. Tlsty, T. Kadlecek, A. Weiss, H. O'Geen, P. J. Farnham, C. L. Maire, K. L. Ligon, P. A. Madden, A. Tam, R. Moore, M. Hirst, M. A. Marra, B. Zhang, J. F. Costello, and T. Wang. 2013. Functional DNA methylation differences between tissues, cell types, and across individuals discovered using the M&M algorithm. *Genome Res* 23(9): 1522-1540.

Zhang, P., T. Chu, N. Dedousis, B. S. Mantell, I. Sipula, L. Li, K. D. Bunce, P. A. Shaw, L. S.

Katz, J. Zhu, C. Argmann, R. M. O'Doherty, D. G. Peters, and D. K. Scott. 2017. DNA methylation alters transcriptional rates of differentially expressed genes and contributes to pathophysiology in mice fed a high fat diet. *Mol Metab* 6(4): 327-339.

Zhou, D., R. A. Hlady, M. J. Schafer, T. A. White, C. Liu, J. H. Choi, J. D. Miller, L. R. Roberts, N. K. LeBrasseur, and K. D. Robertson. 2017. High fat diet and exercise lead to a disrupted and pathogenic DNA methylome in mouse liver. *Epigenetics* 12(1): 55-69.

Zwamborn, R. A., R. C. Slieker, P. C. Mulder, I. Zoetemelk, L. Verschuren, H. E. Suchiman, K. H. Toet, S. Droog, P. E. Slagboom, T. Kooistra, R. Kleemann, and B. T. Heijmans. 2017. Prolonged high-fat diet induces gradual and fat depot-specific DNA methylation changes in adult mice. *Sci Rep* 7: 43261.

TABLES AND FIGURES

Component	High-Fat Diet	Low-Fat Diet
Energy from fat, %	42	15
Casein, g/kg	195	197
Sugars, g/kg	341	307
Corn starch, g/kg	150	313
Cellulose, g/kg	50	30
Corn oil, g/kg	0	58
Hydrogenated coconut oil, g/kg	0	7
Anhydrous milk fat, g/kg	210	0
Cholesterol, g/kg	1.5	0
Kilojoules per gram	18.95	16.99

Table 3.1. Diet compositions.

Trait	Diet (p-value)	Sex (p-value)	Sex-by-diet (p-value)
Week 1 Wt	0.898	0.629	0.555
Week 2 Wt	0.904	0.684	0.670
Week 3 Wt	0.866	0.543	0.447
Week 4 Wt	**1.84E-3**	**8.74E-3**	0.298
Week 5 Wt	**4.70E-8**	**1.83E-8**	0.284
Week 6 Wt	**1.28E-10**	**1.81E-6**	0.537
Week 7 Wt	**1.93E-12**	**3.18E-6**	0.484
Week 8 Wt	**6.80E-13**	**1.00E-5**	0.180
Week 9 Wt	**1.27E-12**	**1.00E-4**	0.494
Week 10 Wt	**1.13E-12**	**1.60E-4**	0.497
Week 11 Wt	**6.88E-12**	**4.50E-4**	0.446
Week 12 Wt	**1.42E-12**	**2.40E-4**	0.358
Week 13 Wt	**1.06E-13**	**4.60E-4**	0.651
Week 14 Wt	**1.45E-13**	**2.33E-3**	0.501
Week 15 Wt	**7.11E-15**	**8.09E-3**	0.848
Week 16 Wt	**1.24E-13**	**4.93E-3**	0.786
Week 17 Wt	**2.62E-14**	**4.08E-3**	0.702
IPGTT Baseline glucose	**9.15E-7**	**1.67E-2**	0.654
IPGTT AUC	**2.07E-6**	**2.00E-5**	0.194
IPITT Baseline glucose	**1.00E-5**	**1.80E-2**	0.102
IPITT AUC	**1.21E-7**	**2.37E-2**	**0.024**
Leptin	**5.31E-8**	0.944	0.943
Insulin	**4.50E-4**	**0.00163**	**0.002**
Triglycerides	**3.32E-3**	0.885	0.247
Cholesterol	**4.93E-11**	**1.70E-4**	**0.005**
Glucose	**2.00E-4**	0.149	0.063
Free Fatty Acids	0.409	0.440	0.322
Liver (log)	**8.52E-14**	**0.013**	0.897
Fat pad (log)	**8.54E-12**	0.255	0.199
Heart (log)	**1.79E-8**	**0.01558**	0.938
Kidney Avg (log)	**1.95E-9**	**4.98E-16**	0.635
Spleen (log)	**8.28E-8**	0.110	0.056
Brown fat (log)	**2.89E-8**	0.301	0.923
Leg Muscle (log)	**0.01645**	0.612	0.960

Table 3.2. The effect of diet and sex on weekly weights, diabetes traits, and organ weights.

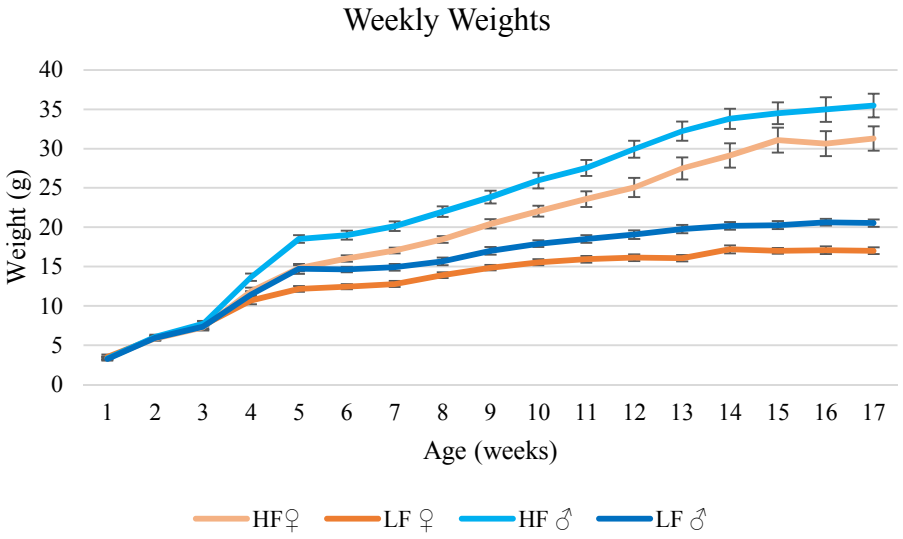


Figure 3.1. The average weight of mice in grams (± one standard error) from 1 to 17 weeks of age. Diet had a statistically significant effect from 4 weeks of age and on. HF = High-fat diet, and LF = Low-fat diet.

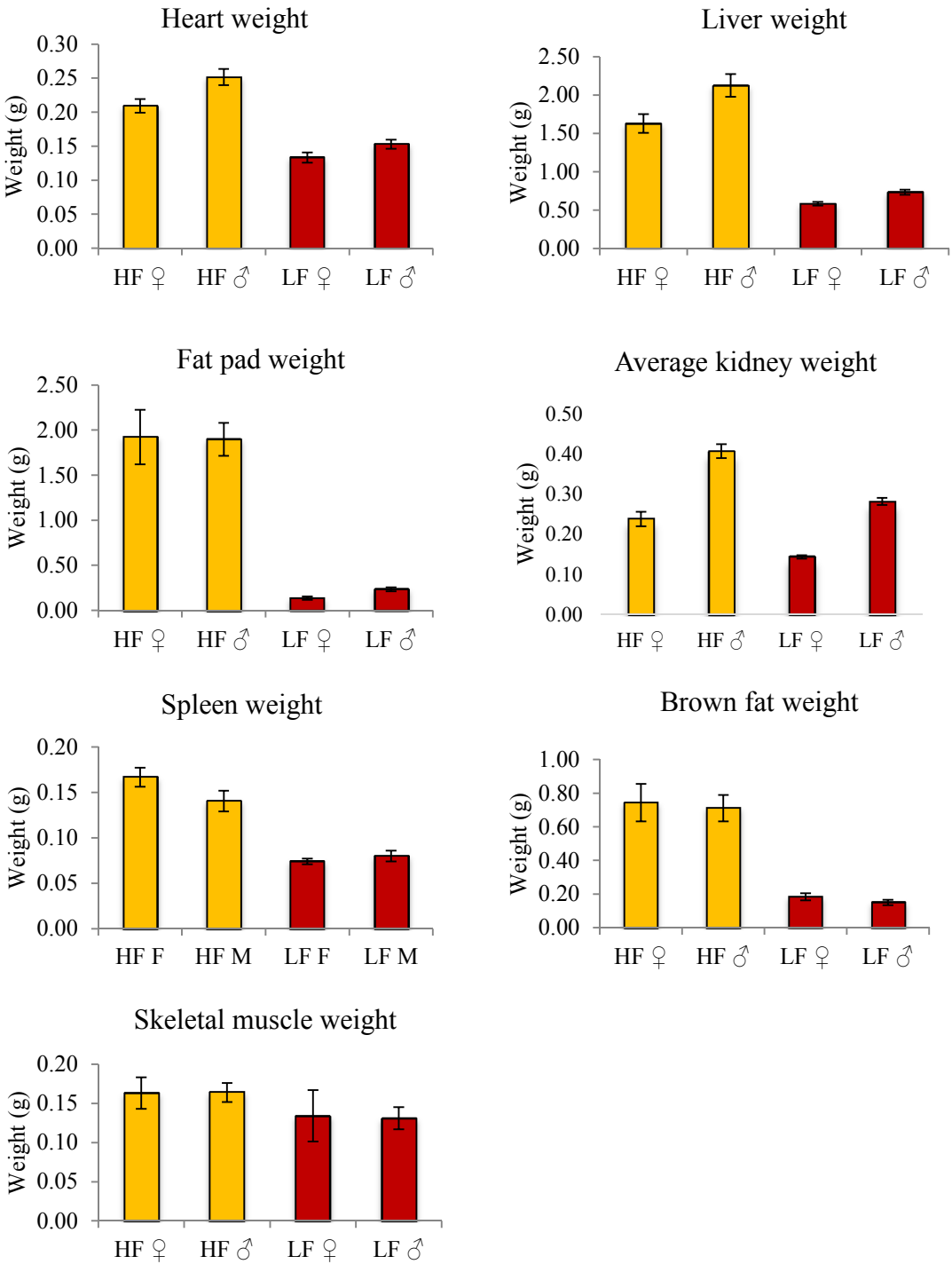


Figure 3.2. The average organ weights for each sex and diet group in grams (± one standard error). All organ weights except were significantly heavier in the high fat mice than the low fat mice. HF = High-fat diet, and LF = Low-fat diet.

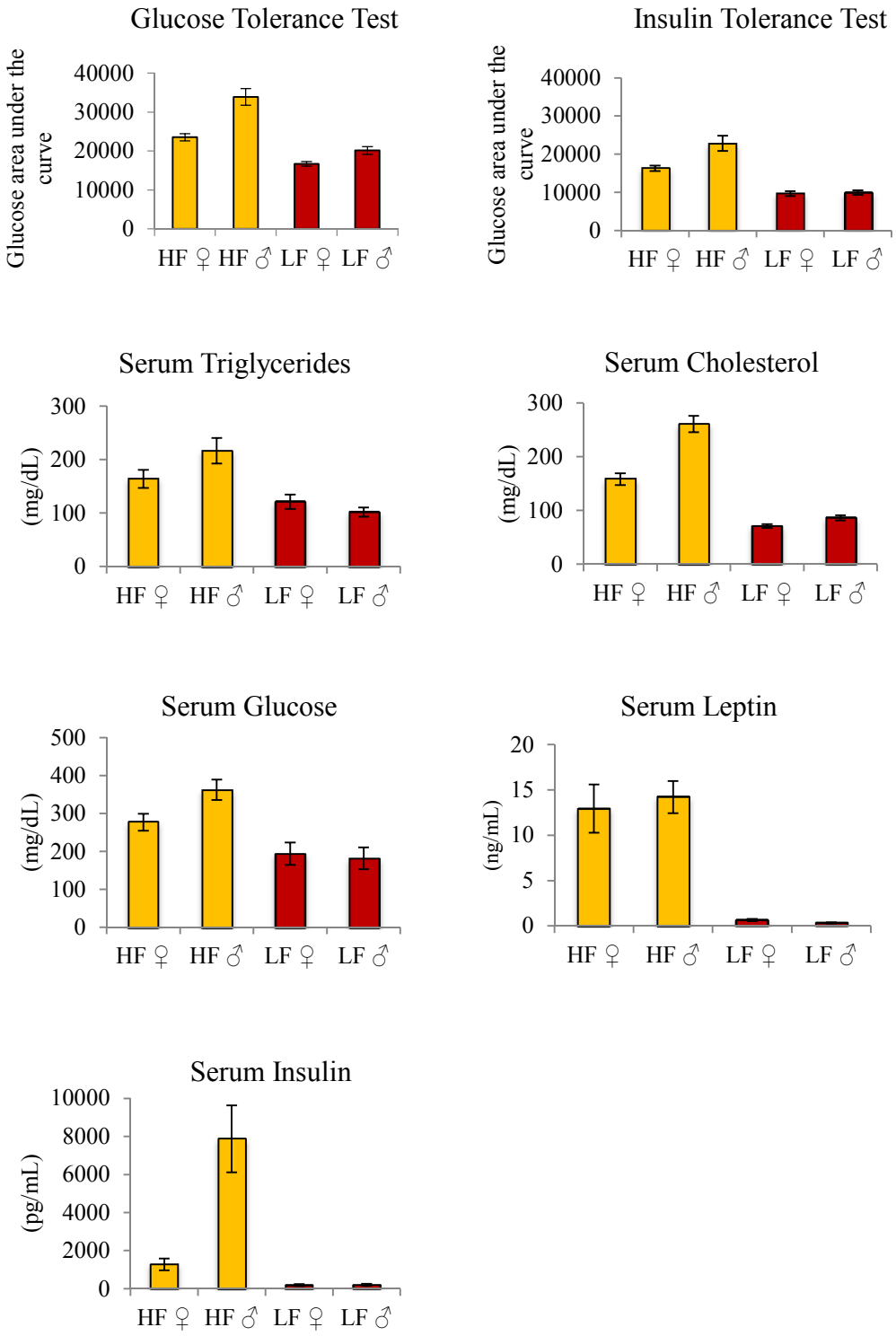


Figure 3.3. High-fat diet mice had an elevated response to glucose tolerance testing and reduced sensitivity to insulin tolerance testing. They also had higher levels of triglycerides, cholesterol, glucose, leptin, and insulin in their serum than low-fat diet mice. Error bars are ± a single standard error, HF = High-fat diet, and LF = Low-fat diet.

Comparison	Differentially expressed genes
All HF vs. LF	4,356
HF vs. LF females	1,750
HF vs. LF males	3,330

Table 3.3. Number of differentially expressed genes due to diet. HF = High-fat diet, and LF = Low-fat diet.

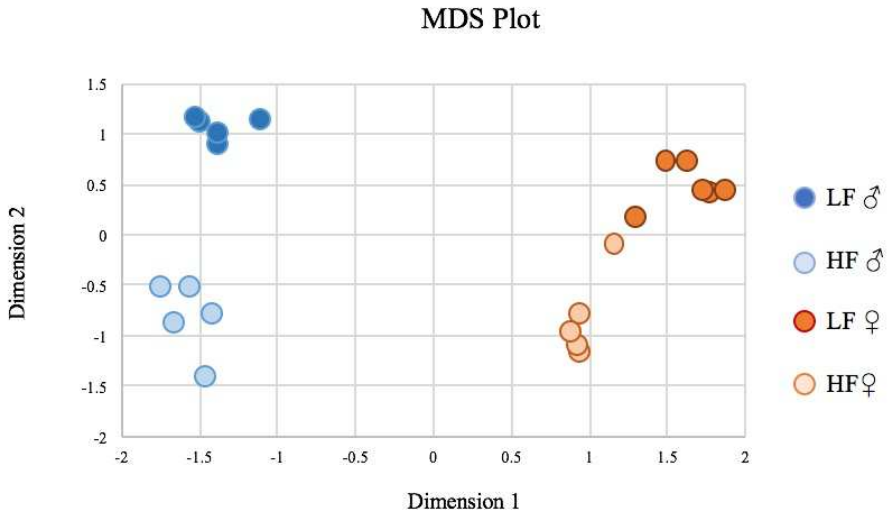


Figure 3.4. The multidimensional scaling plot indicates that gene expression libraries clustered by sex (dimension 1) and then by diet (dimension 2). HF = High-fat diet, and LF = Low-fat diet.

Gene	Forward primer	Reverse primer
Adam11	5'-TGCTGCTGTTACCGCTTCT-3'	5'-TCAGAGCCCTCTGGACTCTCT-3'
Lad1	5'-ATGTCGGTCAGCAGAAAGGAC-3'	5'-CTGTGGTTGAACTCAGGTTGC-3'
Galnt10	5'-TGACCGATGCCGAGAGAGT-3'	5'-AGAGAGCGATTCAGGGAGATT-3'
Abcg8	5'-GTACGTGGGGTGTCCGGGGGTGAG-3'	5'-GCGAGGCTGGTGGAGGGAGATGAG-3'
Col1a1	5'-GCTCCTCTTAGGGGCCACT-3'	5'-CCACGTCTCACCATTGGGG-3'
Gapdh	5'-ACAATGAATACGGCTACAGCAACAG-3'	5'-GGTGGTCCAGGGTTTCTTACTCC-3'

Table 3.4. Primers used for RT-qPCR.

Comparison	Group	<0.05	<0.01	<0.001	DMRs in X (%)
Different diets	LF ♀ vs. HF ♀	38,865	2,356	375	100 (0.3%)
	LF ♂ vs. HF ♂	31,549	1,539	314	17 (0.1%)
Different sexes	HF ♀ vs. HF ♂	36,876	3,831	1,250	1,045 (2.8%)
	LF ♀ vs. LF ♂	44,076	5,632	1,716	994 (2.3%)

Table 3.5. The number of differentially methylated regions (DMRs) due to diet and sex at three different q-value cutoffs. There were thousands of methylation differences due to diet, and even more due to sex. A greater proportion of the DMRs fell on the X-chromosome when comparing across sexes than when comparing across diet treatments. HF = High-fat diet, and LF = Low-fat diet.

Region	Female DMRs	Male DMRs	Whole Genome
Enhancer	237 (10.0%)	180 (11.7%)	3.5%
CTCF Binding Site	157 (6.7%)	55 (3.6%)	1.7%
TF binding site	33 (1.4%)	12 (0.8%)	0.3%
Promoter Flanking Region	795 (33.8%)	522 (33.9%)	8.1%
Promoter	370 (15.7%)	215 (14.0%)	4.5%
Exon	598 (25.4%)	415 (27.0%)	7.5%
Intergenic	748 (31.8%)	471 (30.6%)	58.6%

Table 3.6. The distribution of significant differentially methylated regions (DMRs) ($q < 0.01$) across the genome due to diet. Numbers indicate how many 500 base pair windows overlap each genomic region, with the percent of the total significant DMRs that overlap such regions in parentheses for the female and male mice. The percent of windows across the entire genome that overlap these genomic regions is listed as a comparison, illustrating the overrepresentation of regulatory regions in the DMRs.

	Genes with ≥ 1 DMR in gene body	Genes with > 1 DMR in gene body	Genes with ≥ 1 DMR in promoter
Females	7,814 (38.3%)	3,912 (19.2%)	2,146 (10.5%)
Males	7,086 (34.7%)	3,375 (16.5%)	1,548 (7.6%)

Table 3.7. The number of genes in the mouse liver with: at least one diet-induced differentially methylated region (DMR) within the gene body, more than one DMR in the gene body, and at least one DMR in the promoter, defined as within 2 kb upstream of the transcription start site ($q < 0.05$).

Comparison	Pathway	q-value	Up- or Downregulated
Males vs. females	mmu03010 Ribosome	0.012	up (in males)
	mmu00190 Oxidative phosphorylation	0.024	up
	mmu00140 Steroid hormone biosynthesis	0.052	down
	mmu00591 Linoleic acid metabolism	0.052	down
	mmu00830 Retinol metabolism	0.052	down
High fat vs. low fat diet	mmu04060 Cytokine-cytokine receptor interaction	0.002	up (in high fat)
	mmu04062 Chemokine signaling pathway	0.006	up
	mmu04514 Cell adhesion molecules (CAMs)	0.016	up
	mmu04640 Hematopoietic cell lineage	0.016	up
	mmu04650 Natural killer cell mediated cytotoxicity	0.029	up
	mmu03010 Ribosome	9.74E-06	down
	mmu00190 Oxidative phosphorylation	0.010	down

Table 3.8. The signaling and metabolism pathways up- or downregulated by sex and diet. The ribosome pathway was upregulated in males compared to females, and the cytokine-cytokine and chemokine signaling pathways were upregulated in high-fat mice compared to low-fat mice.

Comparison	Pathway	q-value	Up- or Down-regulated	Genes in Pathway	Genes in the pathway with a DMR
High fat vs. low fat females	mmu04060 Cytokine-cytokine receptor interaction	0.017	up	270	*Acvr1, Bmpr1b, Ccr4, Ccr7, Flt4, Kdr, Lifr, Ngfr, Osm, Pf4, Tnfrsf13b*
	mmu04062 Chemokine signaling pathway	0.017	up	185	*Adcy1, Adcy5, Adcy7, Ccr4, Ccr7, Dock2, Foxo3, Gng7, Grb2, Hck, Jak3, Mapk3, Nfkb1, Pard3, Pf4, Pik3cd, Pik3r2, Pxn, Stat3, Tiam1, Vav2*
	mmu04514 Cell adhesion molecules (CAMs)	0.049	up	145	*Cadm1, Cd6, Cdh15, Cldn18, Cldn2, Itga9, Lrrc4b, Mpzl1, Nrxn3, Ntng1, Pecam1, Spn*
	mmu04650 Natural killer cell mediated cytotoxicity	0.049	up	133	*Cd48, Fyn, Grb2, Lat, Mapk3, Nfatc1, Pik3cd, Pik3r2, Plcg2, Ppp3ca, Sh3bp2, Syk, Vav2, Zap70*
High fat vs. low fat males	mmu04060 Cytokine-cytokine receptor interaction	0.039	up	270	*Acvr1b, Bmp7, Csf1r, Cx3cr1, Flt3, Osm, Pdgfa, Pdgfra, Tgfbr2*
	mmu03010 Ribosome	$3.3x10^{-6}$	down	154	NA
	mmu00190 Oxidative phosphorylation	0.002	down	133	*Atp6v0a1, Lhpp, Ppa1, Ppa2*

Table 3.9. The signaling and metabolism pathways up- or downregulated in female mice and male mice due to a high-fat diet. Any genes in these pathways that have a differentially methylated region are listed. Each of the five significant pathways for females was upregulated by a high-fat diet. In males, a high-fat diet upregulated the cytokine-cytokine pathway and downregulated the ribosome and oxidative phosphorylation pathways.

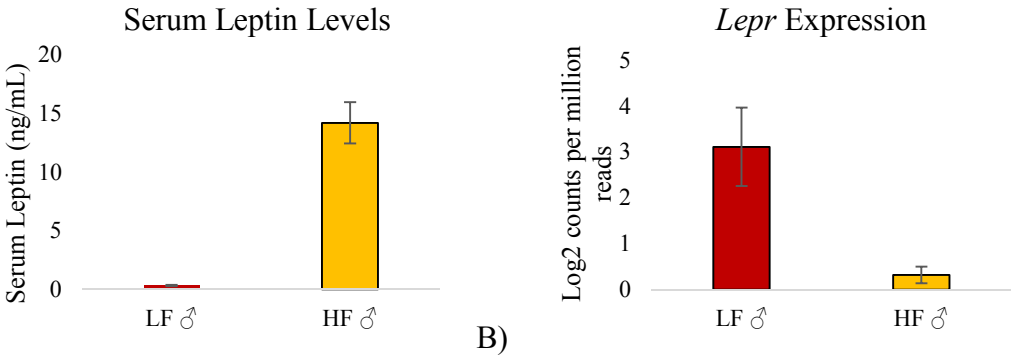
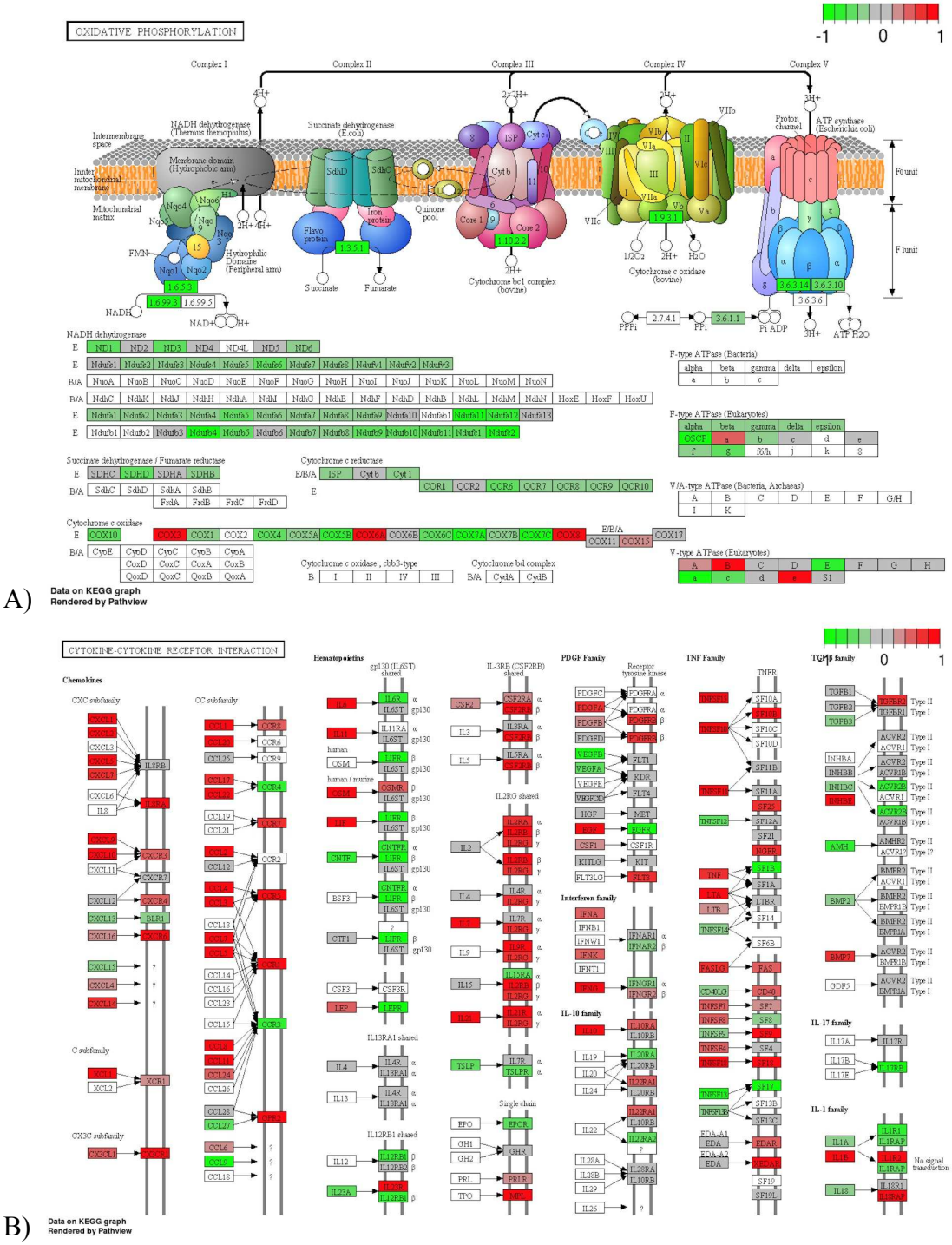


Figure 3.5. (A) A high-fat diet drastically increases leptin levels in male mice, (B) but reduces the expression of the leptin receptor. HF = High-fat diet, and LF = Low-fat diet. Error bars represent ± a single standard error.



A)

B)

Figure 3.6. KEGG pathway diagrams, where red indicates upregulation by a high-fat diet and green indicates downregulation. A) The oxidative phosphorylation pathway is significantly downregulated due to a high-fat diet. B) The cytokine-cytokine pathway is significantly upregulated due to a high-fat diet.

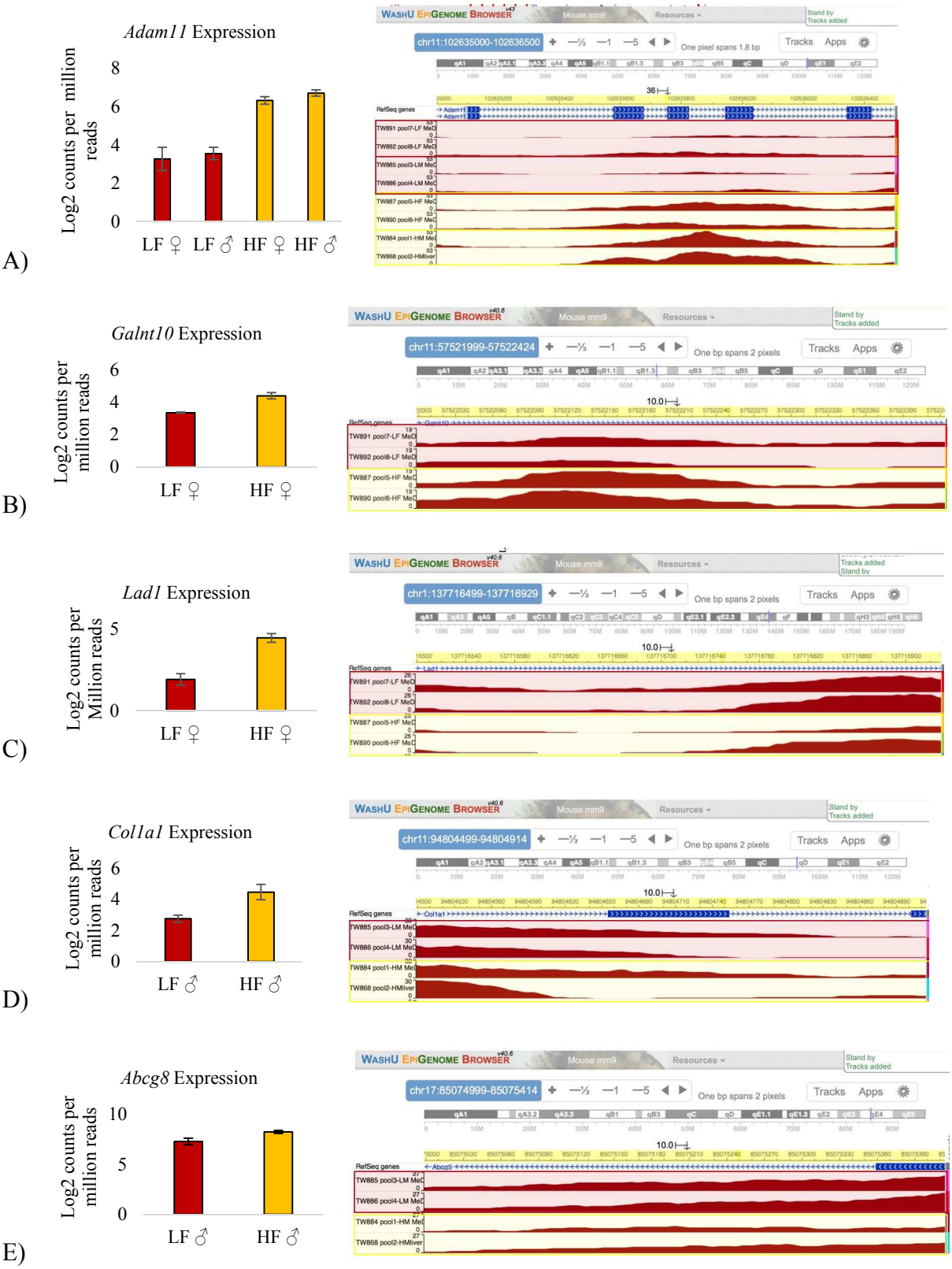


Figure 3.7. Examples of differentially expressed genes that have differentially methylated regions within them. Epigenome Browser screenshots indicate the amount of methylation from MeDIP-seq. HF = High-fat diet, and LF = Low-fat diet. Error bars represent ± a single standard error.

Comparison	GO_ID	q-value	Up or Down-regulated
Sex-by-diet Interaction	GO:0009118 regulation of nucleoside metabolic process	3.75E-02	up
	GO:0030811 regulation of nucleotide catabolic process	3.75E-02	up
	GO:0033121 regulation of purine nucleotide catabolic process	3.75E-02	up
	GO:0046578 regulation of Ras protein signal transduction	3.75E-02	up
	GO:0051056 regulation of small GTPase mediated signal transduction	3.75E-02	up
High-fat vs. low-fat diet	GO:0002684 positive regulation of immune system process	4.95E-05	up
	GO:0043207 response to external biotic stimulus	4.95E-05	up
	GO:0045087 innate immune response	4.95E-05	up
	GO:0051707 response to other organism	4.95E-05	up
	GO:0001816 cytokine production	2.97E-04	up
	GO:0009607 response to biotic stimulus	2.97E-04	up
	GO:0031349 positive regulation of defense response	2.97E-04	up
	GO:0050776 regulation of immune response	2.97E-04	up
	GO:0009617 response to bacterium	2.98E-04	up
	GO:0001817 regulation of cytokine production	3.85E-04	up
	GO:0098542 defense response to other organism	4.12E-04	up
	GO:0002237 response to molecule of bacterial origin	6.30E-04	up
	GO:0060326 cell chemotaxis	1.78E-03	up
	GO:0050900 leukocyte migration	1.88E-03	up
	GO:0002252 immune effector process	2.47E-03	up
	GO:0006935 chemotaxis	2.47E-03	up
	GO:0006954 inflammatory response	2.47E-03	up
	GO:0031347 regulation of defense response	2.47E-03	up
	GO:0032496 response to lipopolysaccharide	2.47E-03	up
	GO:0042330 taxis	2.47E-03	up
	GO:0045088 regulation of innate immune response	2.47E-03	up
	GO:0045321 leukocyte activation	2.47E-03	up
	GO:0050663 cytokine secretion	2.47E-03	up
	GO:0050778 positive regulation of immune response	2.47E-03	up
	GO:0009306 protein secretion	3.43E-03	up
	GO:0046649 lymphocyte activation	3.69E-03	up
	GO:0030595 leukocyte chemotaxis	3.80E-03	up
	GO:0050707 regulation of cytokine secretion	4.43E-03	up
	GO:0034341 response to interferon-gamma	4.53E-03	up
	GO:0042742 defense response to bacterium	4.53E-03	up

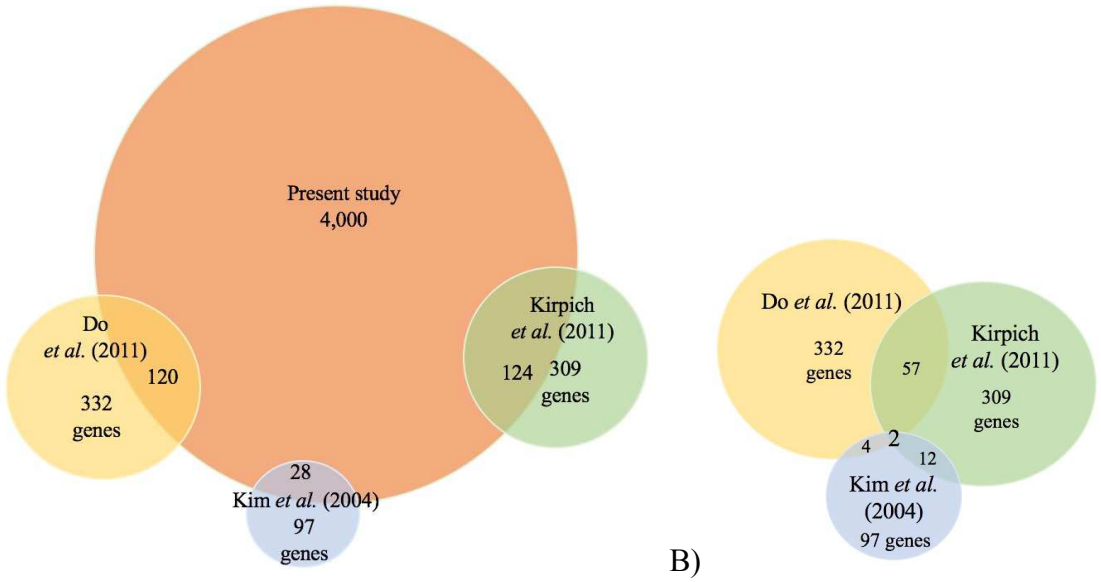
	GO:0000280 nuclear division	4.55E-03	up
	GO:0007067 mitosis	4.55E-03	up
	GO:0042110 T cell activation	4.55E-03	up
	GO:0097529 myeloid leukocyte migration	4.55E-03	up
	GO:0050729 positive regulation of inflammatory response	4.89E-03	up
	GO:0097530 granulocyte migration	6.56E-03	up
	GO:0002521 leukocyte differentiation	6.90E-03	up
	GO:0002757 immune response-activating signal transduction	7.14E-03	up
	GO:0071219 cellular response to molecule of bacterial origin	8.63E-03	up
	GO:0001819 positive regulation of cytokine production	9.12E-03	up
	GO:0032103 positive regulation of response to external stimulus	9.97E-03	up
	GO:0051249 regulation of lymphocyte activation	1.15E-02	up
	GO:0045089 positive regulation of innate immune response	1.18E-02	up
	GO:0050708 regulation of protein secretion	1.18E-02	up
	GO:0071621 granulocyte chemotaxis	1.31E-02	up
	GO:0002764 immune response-regulating signaling pathway	1.37E-02	up
	GO:0002253 activation of immune response	1.47E-02	up
	GO:0071222 cellular response to lipopolysaccharide	1.47E-02	up
	GO:0048285 organelle fission	1.59E-02	up
	GO:0050865 regulation of cell activation	1.59E-02	up
	GO:0071216 cellular response to biotic stimulus	1.59E-02	up
	GO:0072676 lymphocyte migration	1.59E-02	up
	GO:0002696 positive regulation of leukocyte activation	1.73E-02	up
	GO:0030098 lymphocyte differentiation	1.77E-02	up
	GO:0050867 positive regulation of cell activation	1.80E-02	up
	GO:0030593 neutrophil chemotaxis	2.07E-02	up
	GO:0002694 regulation of leukocyte activation	2.08E-02	up
	GO:0071345 cellular response to cytokine stimulus	2.23E-02	up
	GO:0032101 regulation of response to external stimulus	2.34E-02	up
	GO:0050863 regulation of T cell activation	2.34E-02	up
	GO:1990266 neutrophil migration	2.41E-02	up
	GO:0071346 cellular response to interferon-gamma	2.63E-02	up
	GO:0050715 positive regulation of cytokine secretion	2.82E-02	up
	GO:0051251 positive regulation of lymphocyte activation	2.82E-02	up
	GO:0002685 regulation of leukocyte migration	4.36E-02	up
	GO:0032655 regulation of interleukin-12 production	4.36E-02	up
	GO:0050870 positive regulation of T cell activation	4.63E-02	up
	GO:0043900 regulation of multi-organism process	4.64E-02	up

	GO:0034097 response to cytokine	5.18E-02	up
	GO:0002443 leukocyte mediated immunity	5.27E-02	up
	GO:0030217 T cell differentiation	5.27E-02	up
	GO:0002687 positive regulation of leukocyte migration	5.31E-02	up
	GO:0032615 interleukin-12 production	5.45E-02	up
High fat vs. low fat females	GO:0043207 response to external biotic stimulus	1.27E-03	up
	GO:0045087 innate immune response	1.27E-03	up
	GO:0051707 response to other organism	1.27E-03	up
	GO:0002684 positive regulation of immune system process	2.29E-03	up
	GO:0098542 defense response to other organism	3.95E-03	up
	GO:0009607 response to biotic stimulus	6.07E-03	up
	GO:0002252 immune effector process	6.10E-03	up
	GO:0009617 response to bacterium	6.10E-03	up
	GO:0050776 regulation of immune response	6.52E-03	up
	GO:0001816 cytokine production	7.54E-03	up
	GO:0001817 regulation of cytokine production	1.22E-02	up
	GO:0002237 response to molecule of bacterial origin	1.22E-02	up
	GO:0031349 positive regulation of defense response	1.22E-02	up
	GO:0060326 cell chemotaxis	1.35E-02	up
	GO:0042742 defense response to bacterium	1.79E-02	up
	GO:0050900 leukocyte migration	1.79E-02	up
	GO:0009306 protein secretion	1.85E-02	up
	GO:0050663 cytokine secretion	1.90E-02	up
	GO:0030595 leukocyte chemotaxis	2.11E-02	up
	GO:0006954 inflammatory response	2.35E-02	up
	GO:0034341 response to interferon-gamma	2.44E-02	up
	GO:0050778 positive regulation of immune response	2.58E-02	up
	GO:0032496 response to lipopolysaccharide	3.46E-02	up
	GO:0050729 positive regulation of inflammatory response	3.97E-02	up
	GO:0031347 regulation of defense response	4.36E-02	up
	GO:0045088 regulation of innate immune response	4.36E-02	up
	GO:0050707 regulation of cytokine secretion	4.36E-02	up
	GO:0097529 myeloid leukocyte migration	4.36E-02	up
	GO:0097530 granulocyte migration	4.36E-02	up
High-fat vs. low-fat diet males	GO:0002684 positive regulation of immune system process	2.03E-03	up
	GO:0045087 innate immune response	3.38E-03	up
	GO:0043207 response to external biotic stimulus	4.53E-03	up
	GO:0051707 response to other organism	4.53E-03	up
	GO:0001816 cytokine production	5.86E-03	up

	GO:0031349 positive regulation of defense response	5.86E-03	up
	GO:0001817 regulation of cytokine production	6.70E-03	up
	GO:0009617 response to bacterium	6.70E-03	up
	GO:0002237 response to molecule of bacterial origin	6.81E-03	up
	GO:0006935 chemotaxis	6.81E-03	up
	GO:0009607 response to biotic stimulus	6.81E-03	up
	GO:0042330 taxis	6.81E-03	up
	GO:0050776 regulation of immune response	6.81E-03	up
	GO:0045321 leukocyte activation	1.15E-02	up
	GO:0007067 mitosis	1.20E-02	up
	GO:0046649 lymphocyte activation	1.23E-02	up
	GO:0042110 T cell activation	1.55E-02	up
	GO:0032496 response to lipopolysaccharide	1.64E-02	up
	GO:0098542 defense response to other organism	1.64E-02	up
	GO:0031347 regulation of defense response	1.74E-02	up
	GO:0045088 regulation of innate immune response	1.74E-02	up
	GO:0050663 cytokine secretion	1.74E-02	up
	GO:0050900 leukocyte migration	1.74E-02	up
	GO:0002521 leukocyte differentiation	2.09E-02	up
	GO:0050707 regulation of cytokine secretion	2.09E-02	up
	GO:0060326 cell chemotaxis	2.19E-02	up
	GO:0050778 positive regulation of immune response	2.27E-02	up
	GO:0034341 response to interferon-gamma	2.40E-02	up
	GO:0006954 inflammatory response	2.44E-02	up
	GO:0071345 cellular response to cytokine stimulus	2.44E-02	up
	GO:0000280 nuclear division	2.49E-02	up
	GO:0002757 immune response-activating signal transduction	2.81E-02	up
	GO:0030098 lymphocyte differentiation	2.81E-02	up
	GO:0051056 regulation of small GTPase mediated signal transduction	3.30E-02	up
	GO:0051249 regulation of lymphocyte activation	3.30E-02	up
	GO:0030334 regulation of cell migration	3.34E-02	up
	GO:0071219 cellular response to molecule of bacterial origin	3.34E-02	up
	GO:0001819 positive regulation of cytokine production	3.37E-02	up
	GO:0050865 regulation of cell activation	3.37E-02	up
	GO:0097529 myeloid leukocyte migration	3.37E-02	up
	GO:0045089 positive regulation of innate immune response	3.52E-02	up
	GO:0050867 positive regulation of cell activation	3.97E-02	up
	GO:0030595 leukocyte chemotaxis	4.24E-02	up

	GO:0046578 regulation of Ras protein signal transduction	4.36E-02	up
	GO:0071216 cellular response to biotic stimulus	4.36E-02	up
	GO:0032103 positive regulation of response to external stimulus	4.51E-02	up
	GO:0032880 regulation of protein localization	4.51E-02	up
	GO:0050729 positive regulation of inflammatory response	4.51E-02	up
	GO:0071222 cellular response to lipopolysaccharide	4.51E-02	up
	GO:0072676 lymphocyte migration	4.51E-02	up
	GO:2000145 regulation of cell motility	4.51E-02	up
	GO:0097530 granulocyte migration	4.52E-02	up
	GO:0002694 regulation of leukocyte activation	4.56E-02	up
	GO:0002696 positive regulation of leukocyte activation	4.56E-02	up
	GO:0009306 protein secretion	4.56E-02	up
	GO:0002764 immune response-regulating signaling pathway	4.60E-02	up
	GO:0042742 defense response to bacterium	5.01E-02	up
	GO:0034097 response to cytokine	5.09E-02	up
	GO:0002253 activation of immune response	5.24E-02	up
	GO:0030097 hemopoiesis	5.24E-02	up
	GO:0032101 regulation of response to external stimulus	5.24E-02	up

Table 3.10. Significant GO Biological Processes affected by sex and diet.



A) B)

Figure 3.8. A) Venn Diagram illustrating the number of genes whose expression was found to be altered by a high-fat diet in my study of SM/J mice, compared to those found by other researchers using C57BL/6 mice. The present study replicated between 28-40% of the genes found in other studies. B) Only two genes were found by all three studies that used C57BL/6 mice, illustrating the difficulty of replication in studies of how dietary fat alters gene expression.

	Gene	Fold difference of HF vs. LF
Females	*Adam11*	6.57 ± 0.33
	Lad1	3.54 ± 0.37
	Galnt10	4.15 ± 1.80
Males	*Adam11*	7.84 ± 4.10
	Col1a1	9.90 ± 6.08
	Abcg8	11.77 ± 7.30

Table 3.11. The RT-qPCR validation results. Values are presented as high-fat diet expression relative to low-fat diet expression levels. Fold differences were calculated with the ΔΔCT method and reported as a range to include the standard deviation. HF= High-fat diet, LF= Low-fat diet.

CHAPTER 4

Maternal Obesity Alters Offspring Gene Expression, DNA Methylation, and Obesity Risk in Mice

ABSTRACT

In this study, I investigated maternal obesity in inbred SM/J mice by assigning females to a high-fat diet or a low-fat diet at weaning, mating them to low-fat-fed males, then cross-fostering the offspring to low-fat-fed SM/J nurses at birth, and weaning the offspring onto a high fat or low-fat diet. A maternal high-fat diet exacerbated obesity in the high-fat-fed daughters, causing them to weigh more, have heavier livers and reproductive fat pads, and have higher serum levels of leptin as adults, accompanied by gene expression and methylation changes in their livers and hearts (46 differentially expressed genes and 1,700 differentially methylated regions in the liver, 45 differentially expressed genes and 4,103 differentially methylated regions in the heart). RNA sequencing revealed that maternal diet changed the expression of dozens of genes in the liver in both sons and daughters. Maternal obesity particularly affected genes involved in RNA processing, immune response, and mitochondria. It also induced thousands of DNA methylation changes regardless of offspring diet. High-fat-fed offspring had over 7,300 genes in the liver with at least one differentially methylated region due to maternal diet, while low-fat-fed offspring had 9,300 genes with differentially methylated regions. Between one-quarter and one-third of differentially expressed genes contained a differentially methylated region due to maternal diet. An offspring high-fat diet reduced overall variation in DNA methylation, increased body weight and organ weights, increased the lengths and weights of the long bones, decreased insulin sensitivity, and changed the expression of 3,908 genes in the liver. Although the offspring were more affected by their own diet, their maternal diet had epigenetic effects lasting through adulthood, and in the daughters these effects were accompanied by phenotypic changes relevant to obesity and diabetes.

INTRODUCTION

A mother's diet—from its fat and protein content to its richness in methyl donors while DNA methylation is being established in the developing fetus—can directly affect her offspring's epigenome (Wolff *et al.* 1998, Waterland and Jirtle 2003, Dolinoy *et al.* 2006, Oestreich and Moley 2017). Maternal diet is important to study, because half of women giving birth in the United States have pre-pregnancy weights classifying them as overweight or obese (Branum *et al.* 2016). As obesity rates continue rising, it is crucial to investigate individuals' responses to both their own nutritional environment and the environment provided by their mothers to take the next step towards understanding the epigenetic architecture of obesity.

In humans, maternal obesity poses dangers for the mother and her child. Obese women have higher rates of infertility, miscarriage, preeclampsia, gestational diabetes, and cesarean section (Poston *et al*. 2011, Weiss *et al.* 2004). Their babies have a higher risk of stillbirth (Yao *et al.* 2014, Chu *et al. 2*007), neural-tube defects (Poston *et al*. 2011, Rasmussen *et al.* 2008), being born large for gestational age (Sebire *et al.* 2001, Gaudet *et al.* 2014), and being born on average with a higher percentage of body fat, more leptin in their cord blood, and increased levels of the inflammatory cytokine interleukin-6 (Catalano *et al.* 2009). The detrimental health effects of maternal obesity can last throughout life. Maternal obesity raises the rate of childhood obesity (Whitaker 2004, Hillier *et al.* 2007, Yu *et al.* 2013) and is associated with higher levels of insulin, cholesterol, and blood pressure in children (Gaillard *et al.* 2014). Children of obese mothers have also been shown to be at risk of impaired cognitive and executive function (Pugh *et al.* 2015), and 3- to 5-year-olds whose mothers were very severely obese during pregnancy (Body Mass Index ≥ 40 kg/m^2) had an increased risk of neuropsychiatric problems including hyperactivity, aggression, anxiety, and sleep difficulties (Mina *et al.* 2017). The effects of maternal obesity continue into adulthood, increasing the risk of cancer, type 2 diabetes,

cardiovascular disease, and high blood pressure, and raising serum levels of insulin and triglycerides (Hochner *et al.* 2012, Eriksson *et al.* 2014, Stirrat and Reynolds 2014, Galliard 2015).

The effects of maternal obesity in mice are similar to those in humans. C57BL/6J mice exposed to a high-fat diet during gestation had higher levels of obesity, hyperglycemia, hypertension, and insulin resistance as adults, even though they were fed a standard diet after birth (Liang *et al*. 2009). Another study of C57BL/6NCrl mice found that regardless of offspring diet, daughters of high-fat-fed mothers weighed more, had higher cholesterol and blood pressure, and had fattier livers (Elahi *et al.* 2009). Some of the phenotypic changes induced by maternal obesity have been linked to epigenetics and gene expression. For instance, Wankhade *et al.* (2017) found that offspring of high-fat-fed C57BL/6J mothers had larger livers exhibiting inflammation and steatosis, accompanied by higher profibrogenic gene expression and 82 differentially methylated regions. Heart structure and gene expression are also affected by maternal obesity, as exemplified by the findings of Fernandez-Twinn *et al.* (2012) where adult offspring of obese C57BL/6J mouse dams had larger hearts, thicker left ventricles, wider muscle cells, hyperinsulinemia, increased expression of molecular markers indicative of cardiac hypertrophy, and increased expression of genes associated with oxidative distress. Similarly, Blackmore *et al.* (2014) found cardiac dysfunction in the adult sons of obese C57BL/6J mouse dams, despite the sons being fed a low-fat diet. They expressed cardiac genes that normally are expressed only during fetal development, which is a sign of cardiac hypertrophy.

Previous rodent studies have shown that offspring sex affects the response to maternal obesity, with daughters of high-fat-fed mothers having higher blood pressure (Khan *et al.* 2004), higher plasma leptin levels (Bellisario *et al.* 2014), and smaller livers than sons (Miller *et al.*

2014), whereas sons have a more pronounced difference in their transcriptomes (Mischke *et al.* 2013). It is thus important to take offspring sex into account when investigating the phenotypic and epigenetic effects of maternal obesity.

Maternal obesity impacts offspring disease risk through multiple mechanisms across the spectrum of development, from altering the glucose consumption and size of oocytes to disrupting the circadian rhythms and metabolic genes in the hearts and livers of offspring (Jungheim *et al.* 2010, Leary *et al.* 2015, Wang *et al.* 2015). High blood pressure in obese women alters blood vessels, and during pregnancy vasodilatation is impaired in uterine arteries (Roberts *et al.* 2015). On the cellular level, maternal obesity can leave lasting effects on offspring by changing the number, distribution, structure, and function of mitochondria in oocytes (Igosheva *et al.* 2010, Luzzo *et al.* 2012, Grindler and Moley 2013). At the level of the organ, obesity affects offspring prenatally through placental abnormalities, including heavier placental weight, more inflammatory lesions (43% in obese women as opposed to 3.6% in lean women) (Bar *et al.* 2012), lower rates of apoptosis, more muscular vessel walls, higher transport of glucose and amino acids across the placenta (Sferruzzi-Perri *et al.* 2013), and greater inflammatory cytokine expression (Roberts *et al.* 2011). Obesity is an inflammatory disease in addition to a metabolic one (André *et al.* 2014), and exposure to elevated inflammatory cytokines in the womb alters the way the fetal immune system is programmed. Wilson *et al.* (2015) found that the cord blood of infants with obese mothers had fewer CD4 helper T cells and higher levels of the cytokines IFN-α2 and IL-6, which could impair response to vaccination and infection. In rodents, offspring of obese dams have inflammation in their brains (Bilbo and Tsang 2010, Kang *et al.* 2014).

Although obesity can be passed on to offspring via genetic variants, there is growing

evidence that prenatal epigenetic programming plays a substantial role in the transmission of obesity across generations (Oestreich and Moley 2017). A classic example of this phenomenon is the A^{vy} allele at the agouti locus in mice. A^{vy} dams give birth to pups with ectopic agouti expression, which causes obesity. However when a pregnant A^{vy} dam is fed a diet rich in methyl-donors, her offspring are born with increased agouti methylation, restored agouti expression, and they do not develop obesity, thus illustrating an epigenetic route for the enduring effects of maternal diet (Wolff *et al.* 1998, Waterland and Jirtle 2003, Waterland *et al.* 2004). In humans, newborns with obese parents have methylation differences at several imprinted genes in their cord blood (Soubry *et al.* 2015). These methylation changes last at least into childhood, as demonstrated by Guénard *et al.* (2013) in their study of 20 obese mothers who underwent gastric bypass surgery. The children born after their mother's surgery had lower Body Mass Indexes, better cardiometabolic profiles, and methylation differences in more than 5,000 genes in their blood compared to their siblings born before the surgery. Much remains to be learned about the epigenetic changes induced by maternal obesity. Due to cost limitations, most studies only measure methylation in one tissue—even though the epigenetic changes likely vary across tissue types—and methylation is often only assessed in candidate genes, which cannot give a full picture of the extent to which the genome is affected. It is important to uncover the epigenetic dysregulation that offspring experience due to maternal obesity in order to understand how obesity is transmitted *in utero* as well as to identify targets for novel epigenetic therapies. Since the effects of maternal obesity begin even before birth, it is crucial to know which specific epigenetic risk factors a baby is likely to be born with in order to make early intervention possible.

In the last few decades we have seen increasing portion sizes, the rise of the fast food

industry, the birth of fructose-enrichment technology, and the expansion of the food processing industry—which adds large quantities of saturated fats, fructose, and salt to food (Young and Nestle 2002). It is no surprise that obesity rates are skyrocketing, and with as many as 38% of pregnant women classified as obese today (McDonald 2010), it is vital to investigate the epigenetic mechanisms through which a mother's diet can induce obesity in her offspring.

Maternal effects occur when the mother's genotype or phenotype causally influences the offspring phenotype (Wolf and Wade 2009). In the present study of diet, I am concerned with the non-genetic aspect of maternal effects. In the context of evolution, non-genetic maternal effects can be good predictors of the environment that the offspring will experience and thus improve fitness and even help with the colonization of new niches (Maestripieri and Mateo 2009). However, non-genetic maternal effects can also be maladaptive, as in the case of the thrifty-phenotype hypothesis. This posits that exposure to poor nutrition in the prenatal and early postnatal environment results in metabolic syndrome later in life (Hales and Barker 2001).

Here, I investigated the direct effects of a high-fat diet as well as the non-genetic maternal effects of a high-fat diet. I measured the direct effects of diet by assigning offspring to either a high fat or a low-fat diet. I measured the maternal effects of a high-fat diet by comparing groups of offspring that had the same sex and diet but had mothers on different diets (for instance, I investigated if high-fat-fed females who had high-fat-fed mothers were heavier than high-fat-fed females who had low-fat-fed mothers). It can be difficult to disentangle the effects of prenatal and postnatal maternal obesity in humans, so in this study I used a cross-fostering design in mice where offspring of high-fat-fed and low-fat-fed mothers were all fostered at birth to low-fat-fed nurses. Most maternal obesity research in mice focuses on the C57BL/6 strain, but because epigenetic response can be highly dependent on genomic background, I used the less-

studied but highly responsive SM/J strain. While many studies use a candidate-gene approach to assess changes in methylation due to maternal obesity, I took a genome-wide approach in order to identify novel genes involved and gain a more comprehensive picture. I also investigated the DNA methylation in two types of tissue, liver and heart, to better understand the systemic epigenetic effects of maternal obesity. Finally, as another measure of body size I measured the weights and lengths of the long bones. It has been suggested that obesity affects bone metabolism through leptin and inflammatory cytokines (Ducy *et al.* 2000, Cao 2011), but it is not known if exposure to a high-fat diet *in utero* affects the bones of adult offspring.

METHODS

Animal Rearing

The animals used in this experiment were derived from inbred SM/J mice obtained from The Jackson Laboratory (Bar Harbor, Maine). This strain originated from a selective breeding experiment for small size at 60 days of age (MacArthur 1944), and has an extreme obesogenic response to dietary fat (Cheverud *et al.* 1999, Ehrich *et al.* 2003, Partridge *et al.* 2014). The parental generation was born in our facility at Loyola University Chicago and was weaned onto a high fat (HF) diet or a low fat (LF) diet at 3 weeks of age. The diets are nearly isocaloric, but the HF diet has three times as much fat. In the LF diet, fat accounted for 15% of the calories (Research Diets D12284), whereas 42% of the calories came from fat in the HF diet (Harlan Teklad diet TD.88137) (Table 4.1). Erich *et al.* (2003) showed that food consumption does not differ due to the HF or LF diet in SM/J mice. At 10 weeks of age, 12 HF diet females and 14 LF diet females were mated with LF diet males to create an F_1 generation. Males were removed from the cage when abdominal palpation revealed the female to be pregnant. To control for the postnatal effect of maternal diet, I cross-fostered all offspring to an LF-fed SM/J nurse within 24

hours of birth. At three weeks of age, half of the offspring were weaned onto an LF diet and the other half were weaned onto an HF diet to produce 4 diet treatment groups: HF-HF, LF-HF, HF-LF, and LF-LF (where the first diet listed is the maternal diet and the second is the offspring diet) (Figure 4.1). There were 10 male and 10 female offspring in each of the diet treatment groups, and they were housed in same-sex pairs of mice on the same diet. Each cage had a privacy hut (Alt Design), a cotton nestlet (Ancare), a wooden gnawing block (Bio Serve), and food and water provided *ad libitum* in a 12-h light, 12-h dark cycle. Procedures were performed under an approved Institutional Animal Care and Use Committee protocol (Project #1188).

Obesity Phenotypes

The mice were weighed weekly for 17 weeks. They were housed in pairs until 13 weeks of age, after which they were housed individually. When the mice were 14 weeks old, 20 pellets of food were weighed and placed into their food rack. Food consumption was measured by weighing the food remaining in the cage 24, 48, 96, and 168 hours later. Previous research shows that SM/J mice initially lose weight after being moved to single housing (Ehrich *et al.* 2003), so I measured food consumption after the mice had one week to adjust to being housed singly. When the mice were 15 weeks old, they underwent an intraperitoneal glucose tolerance test (IPGTT). All IPGTTs were performed at 10:00 AM, after the mice had been fasted for 4 hours. I measured their baseline glucose levels with a glucometer (Ascensia Bayer Breeze 2) using blood from a tail snip, then intraperitoneally injected a 10% glucose solution (0.01 mL/g body weight). I measured the glucose levels again at 30, 60, and 120 minutes after the injection. When the mice were 16 weeks old, they underwent intraperitoneal insulin tolerance testing (IPITT). The IPITT protocol is similar to the IPGTT protocol, except rather than receiving a glucose injection, the mice were injected with a 0.1% insulin solution (0.75 mU/g body weight). If a mouse's blood

glucose levels dropped under 25 mg/dL, it was injected with a 10% glucose solution and was not included in the IPITT results. After the IPGTT and IPITT, the area under the curve (AUC) was calculated using the blood glucose levels at the 4 different time points via the trapezoidal summation method for each mouse. When the mice were 17 weeks old, they were fasted for four hours and then sacrificed by carbon dioxide asphyxiation between 10:00 am and 2:00 pm. A cardiac puncture was immediately performed to draw blood, and the serum was submitted to Washington University in St. Louis's Core Laboratory for Clinical Studies to measure leptin and insulin, and to the Diabetes Models Phenotyping Core to measure triglycerides, cholesterol, free fatty acids, and glucose. After the blood draw, I necropsied the mice on ice and recorded the weights of the liver, heart, reproductive fat pad, kidneys, spleen, brown fat, and skeletal muscle. I chose to weigh only the reproductive fat pad instead of all of the fat pads, since it is the largest and is strongly phenotypically ($r = 0.67$-0.82) and genetically correlated ($h^2 = 0.7$-0.9) with the other fat pads (Cheverud *et al.* 2004, Cheverud *et al.* 2011). I submerged tissue from the liver, heart, pancreas, reproductive fat pad, and hypothalamus in RNAlater and then stored the samples in a -80C freezer. The mouse bones were cleaned with dermestid beetles, and then the long bones (radius, ulna, femur, and tibia) were weighed and their lengths were double-measured with calipers (Table 4.3 and Figure 4.2), based on landmarks used by Kenney-Hunt *et al.* (2008). These skeletal dimensions provide an alternate measure of body size in addition to body weight, which is strongly influenced by obesity. The repeatabilities of the osteological measurements were all above 0.92 (Table 4.3).

For each obesity trait, differences between the diet treatments were analyzed by running a general linear model in SYSTAT (Version 12, Systat Software, San Jose, CA). A full model was run to test for the effect of sex, maternal diet, offspring diet, nurse ID, and the two-, three-, and

four-way interactions of those variables. A reduced model tested only the effects of maternal diet, offspring diet, sex, offspring-diet-by-sex, and offspring-diet-by-maternal diet terms. Multivariate tests were performed on three groups of traits: weekly weights, diabetes-related traits (week 15 and 16 weight, baseline glucose during IPGTT, IPGTT AUC, baseline glucose during IPITT, IPITT AUC, and food consumption), and necropsy traits (week 17 weight, serum biomarkers, and organ weights), in addition to all of the associated univariate tests. Differences were interpreted as significant for p-values less than 0.05.

Gene Expression

I used the Qiagen RNeasy Plus Mini kit to extract RNA from the liver tissue of the 80 F_1 mice, and submitted it for RNA-seq with poly-A selection at the GTAC facility at Washington University in St. Louis. Quality control indicated that 74 of the 80 samples were of high enough quality for sequencing. A total of 37 libraries were sequenced, each with two mice of the same sex, maternal diet, and offspring diet pooled together. One exception was a HF-HF daughter library, which had 3 mice pooled together due to insufficient quantities of RNA. A 1x50 single read sequencing run was done on an Illumina HiSeq machine. The FastQ files were aligned to the Ensembl release 76 assembly using STAR version 2.0.4b (Dobin *et al.* 2013). The transcript and gene counts were imported to the R package edgeR (Robinson *et al.* 2010), a TMM normalization was performed to account for differences in library size, and genes with counts of zero were filtered out. The voom function in the R package Limma was then used to calculate the weighted likelihoods based on the mean-variance relationship of each gene. Differential expression was then tested with generalized linear models. Any gene with an unadjusted p-value of less than 0.05 and a log fold change (logFC) with an absolute value greater than 2 was considered differentially expressed. Two of the male libraries were determined to be outliers

based on inspection of the MDS plots, and they were not included in the rest of the analysis. The remaining 35 libraries were analyzed, which were: 4 libraries of HF-HF daughters, 5 LF-HF daughters, 5 HF-LF daughters, 4 LF-LF daughters, 5 HF-HF sons, 4 LF-HF sons, 4 HF-LF sons, and 4 libraries of LF-LF sons. The R package WGCNA (Langfelder and Horvath 2008) was used to build a tree from the libraries and then identify modules of genes with highly correlated expression levels. For each module that was significantly associated with at least one diabetes trait, I summarized the module based on its top ten significant Gene Ontology (GO) terms. I also used the R package GAGE (Luo *et al.* 2009) to identify pathways that were perturbed in a single direction or generally dysregulated due to maternal diet, and visualized those pathways using the R package Pathview (Luo and Brouwer 2013).

I validated the differential expression of three genes with RT-qPCR in the HF daughters (*Mpo*, *Anxa2*, and *Chrna4*), using *Gapdh* as a normalizer. I extracted total RNA from the liver tissue of 3 HF-HF and 3 LF-HF daughters with Tri-Reagent (MRC), following the manufacturer's instructions. The concentration and quality of the RNA from each sample was assessed twice with a NanoDrop Spectrophotometer, and I found that all samples had a 260/280 ratio between 1.7-2.1 and a 260/230 ratio between 2.0-2.4. I used the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) to reverse transcribe the RNA to cDNA. I chose primers for qPCR from the literature and PrimerBank (https://pga.mgh.harvard.edu/primerbank/), and those primers were then synthesized by Thermo Fisher Scientific (Table 4.6). I used a reaction volume of 20 µL for the RT-qPCR, with 10 µL of PowerUp™ SYBR® Green Master Mix (Thermo Fisher), 1 µL of the forward primer, 1 µL of the reverse primer, 4 µL of 20-fold diluted cDNA, and 4 µL of water. For each of the 3 biological replicates, I used 3 technical replicates, a no-template control, and a no-reverse-transcriptase control. A StepOnePlus Real-

Time PCR System (Applied Biosystems) was used to perform the RT-qPCR under these conditions: 20 seconds at 95°C and then 40 cycles of 3 seconds at 95°C and 30 seconds at 60°C. I then used the comparative ΔΔCt method to perform a relative quantification of each of the three genes compared to *Gapdh* (Table 4.13).

DNA Methylation

I extracted DNA from the liver and heart tissue using a phenol-chloroform extraction. DNA methylation across the genome was then measured with Methylated DNA Immunoprecipitation Sequencing (MeDIP-seq) and Methylation-sensitive Restriction Enzyme Sequencing (MRE-seq) as previously described in detail by Li *et al.* (2015). MeDIP-seq reveals methylated sites whereas MRE-seq reveals unmethylated sites, and when used together they provide a genome-wide methylation map at single CpG resolution. This technique has high concordance with whole genome bisulfite sequencing at a fraction of the cost (Stevens *et al.* 2013). For the liver tissue, 4 mice of the same sex and from the same maternal diet and offspring diet treatment group were pooled per library, for 2 biological replicates per group and a total of 16 liver libraries. For the heart tissue, only the HF-HF and LF-HF daughters were analyzed, for a total of 4 heart libraries. I combined the MRE-seq and MeDIP-seq data using the R package methylMnM to find differentially methylated regions (DMRs) due to maternal diet. MethlyMnM works by calculating the proportion of methylated CpGs in each window, and then determining the probability that the methylation level is statistically different between the two diet treatment groups. This is accomplished by performing a hypothesis test for each window based on the novel M&M test statistic (Zhang *et al.* 2013). For this analysis, I removed blacklist sites and set the window size to 500 base pairs, which split the genome into 5,283,825 windows. Since there were two biological replicates per group and M&M can only do pairwise comparisons, I used

Fisher's combined probability test to compare offspring on the same diet who had mothers on different diets (Fisher 1954). For example, the p-value from the M&M test comparing the HF-HF-daughter-1 vs. LF-HF-daughter-1 libraries was combined with the p-value from the M&M test comparing the HF-HF-daughter-2 vs. LF-HF-daughter-2 libraries.

$$X_{2k}^2 \sim -2\sum_{i=1}^{k} \ln(p_i)$$

P_i is the p-value from the M&M test, and since I combined p-values from $k = 2$ tests, X has a chi-squared distribution with 4 degrees of freedom. I then corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR), and the resulting corrected combined q-values were used to determine which windows were differentially methylated. Since setting a significance threshold is somewhat arbitrary, I determined how many windows had a q-value below 0.05, 0.01, and 0.001.

For each DMR, I identified the gene it was closest to, any genes or Ensembl regulatory elements it fell within (mouse genome assembly GRCm38.p5), if it fell within a promoter, and if the gene closest to it was previously known to be involved in diabetes mellitus, obesity, or cardiovascular diseases according to Phenopedia's continuously updated list of genetic association studies (retrieved May 7, 2017) (Tables 19-23). I classified each DMR as being located in an intergenic region, intron, exon, or promoter by using the list of introns, exons, and genes in the NCBI37/mm9 assembly of the UCSC Genome Browser. If a DMR overlapped an exon as well as an intron, it was classified as falling within an exon. A DMR was labeled as being in a promoter if it was between 2,000 base pairs upstream of a transcription start site and 600 base pairs downstream of one. To establish if the DMRs were significantly associated with gene expression, I randomized the location of the DMRs across the genome and determined how

many fell within differentially expressed genes by chance. To account for the observed underrepresentation of DMRs in intergenic regions, the percent of DMRs that were randomized into intergenic regions equaled the percent that actually exist in intergenic regions.

RESULTS

Obesity Phenotypes

When the dams were mated at 10 weeks of age to produce the F_1 offspring, the LF dams weighed on average 15.3 grams and the HF dams weighed on average 21.7 grams. I consider this 40% increase in weight of the HF dams to signify obesity. The general linear model showed that the weekly weights, diabetes-related traits, and necropsy traits were all significantly affected by maternal diet ($p = 0.001$), offspring diet ($p = 4.17 \times 10^{-16}$), sex ($p = 2.36 \times 10^{-13}$), and an offspring-diet-by-sex interaction ($p = 3.0 \times 10^{-5}$). Due to the strong offspring-diet-by-sex interaction, sons and daughters were analyzed separately. There was a significant offspring-diet-by-maternal-diet interaction ($p = 0.009$) for the necropsy traits only. Also, there was a borderline significant interaction of maternal diet, offspring diet, and sex for the diabetes-related traits ($p = 0.07$), and a borderline significant sex-by-maternal-diet interaction for the weekly weights ($p = 0.07$).

Unsurprisingly, the offspring's diet had a major effect on the obesity traits. Mice on a high fat (HF) diet had heavier body weights within one week of being weaned onto the diet (4 weeks old), a greater response to glucose tolerance testing, a reduced response to insulin tolerance testing, heavier organs, and higher serum levels of leptin, insulin, triglycerides, glucose, and free fatty acids. HF mice also had longer, heavier long bones than mice on a low fat (LF) diet (Table 4.3 and Table 4.4), consistent with the notion that obesity alters bone metabolism (Cao 2011).

The direct effect of diet was much more extensive than the maternal effect of diet. Maternal diet affected the obesity traits of the daughters, but not the sons. HF-fed daughters weighed more in adulthood (Figure 4.3), had higher serum levels of leptin (Figure 4.4), consumed more food at 14 weeks of age (Figure 4.9A), and had even heavier reproductive fat pads and brown fat if they had HF mothers rather than LF mothers (Figure 4.4). LF-fed daughters of HF mothers had the highest levels of free fatty acids in their serum, and a maternal HF diet actually lowered the triglyceride and glucose levels in the daughters on either diet (Figure 4.4).

Gene Expression

The multidimensional scaling plot indicated that the gene expression libraries clustered by sex (dimension 1) and offspring diet (dimension 2), without any discernable patterns in dimensions 3 or 4 (Figure 4.5). Offspring diet caused expression differences in 3,908 genes in the liver. Of note is the considerable downregulation of the leptin receptor (*Lepr*) gene by an HF diet. Offspring on an HF diet had far more leptin in their serum (HF daughters had over 5 times more leptin than LF daughters, and HF sons had over 3 times more than LF sons) (Figure 4.6A). Despite having more of this satiety hormone, HF offspring actually ate more than LF offspring at 14 weeks of age (Figure 4.6B). This resistance to leptin appears to be mediated in part by the substantial reduction in the expression of *Lepr*, which was 8 times lower in the HF daughters than the LF daughters and 12 times lower in the HF sons than the LF sons (Figure 4.6C).

Maternal diet did not have as widespread an effect on expression as offspring diet, but it significantly altered the expression of dozens of genes. When comparing offspring of the same sex and diet who differed only in maternal diet, I found that maternal diet altered 46 genes in the HF-fed daughters' livers, 45 genes in the HF-fed daughters' hearts, 70 genes in the LF-fed

daughters' livers, 22 genes in the HF-fed sons' livers, and 434 genes in the livers of the LF-fed sons (Table 4.5 and Tables 4.14-4.18). The GAGE pathway analysis revealed many signaling and metabolism pathways that were downregulated due to a maternal HF diet (Table 4.6). More pathways were downregulated in the LF-fed offspring, with 28 downregulated pathways in the LF-fed daughters and 146 in the LF-fed sons, compared to 4 in the HF daughters' livers (and 21 in their hearts) and 51 in the HF sons' livers. A maternal HF diet downregulated the ribosome, spliceosome, oxidative phosphorylation, and RNA transport pathways in the livers of all four of the offspring diet-sex group comparisons. The GO Biological Processes affected by maternal diet showed a similar trend of more being dysregulated in the LF-fed offspring, with 284 processes altered in the LF-fed daughters and 2,660 in the LF-fed sons, compared to 31 in the HF-fed daughters' livers (151 in their hearts) and 625 in the HF-fed sons' livers.

The top GO terms affected by maternal diet in the liver involved RNA splicing and processing, non-coding RNA processing, immune response, and protein catabolic processes (Table 4.7). In the HF-fed daughters' heart tissue, maternal HF diet downregulated biosynthetic and metabolic pathways (Table 4.7). An offspring HF diet significantly changed the regulation of 28 KEGG disease pathways in the liver, including downregulating the non-alcoholic fatty liver disease (NAFLD) (Figure 4.7), Alzheimer's disease (Figure 4.8), Parkinson's disease, and Huntington's disease pathways. A maternal HF diet further downregulated these four pathways in each of the offspring diet-sex group comparisons.

The weighted gene co-expression network analysis (WGCNA) provided more insight into the relationship of the gene expression with the obesity phenotypes (Table 4.9). The phenogram built from clustering the expression libraries had cophenetic correlations all above 0.85, indicating that it closely represented the relationships between the libraries. Setting the minimum

module size to 40 genes yielded 29 modules of co-expressed genes, ranging from 34 to 1,785 genes per module. Four of these modules were significantly associated with the diabetes-related traits (week 15 and week 16 weight, glucose tolerance, insulin tolerance, serum glucose and insulin levels, and food consumption) (Figure 4.8). The black module was negatively correlated with the diabetes traits and contained 637 genes, which were significantly enriched for immune system function. The yellow module was also negatively correlated with the diabetes traits, and it contained 932 genes that were enriched for terms involving oxidation reduction and arachidonic acid (which is an inflammatory intermediate and a vasodilator). The magenta module was positively correlated with the diabetes traits and contained 312 genes that were enriched for mitochondrial and ribosomal processes, suggesting that the genes in this module were regulating the diet-induced changes in metabolism and gene expression. The turquoise module had 1,785 genes and was positively correlated with the diabetes traits, with enrichment for respiratory chain and mitochondrial processes. Overall, the GO term enrichment showed that the modules tended to involve the immune system and mitochondria, indicating that the disruption of these processes lead to the development of the diabetes traits I measured.

Maternal HF diet disrupted gene expression not only in the offspring's livers, but in their hearts as well. Comparing the HF-fed daughters of HF mothers with HF-fed daughters of LF mothers revealed 45 differentially expressed genes due to maternal diet (Table 4.15). The gene expression libraries clustered by maternal diet, and the cophenetic correlations of the phenogram were all above 0.93 (Figure 4.10). The 21 upregulated genes due to maternal HF diet were primarily pseudogenes and non-coding RNAs. Most of the 24 downregulated genes were ones previously reported to be involved in obesity and cardiovascular diseases, in addition to 6 cytochrome P450 genes.

Methylation

There were tens of thousands of differentially methylated regions (DMRs) due to maternal diet that fell below a q-value cutoff of 0.05 (0.7-1.1% of the 5.3 million windows) (Table 4.10). A q-value cutoff of 0.01 encompassed less than 0.2% of the windows, and highlighted several thousand regions affected by maternal diet (Supplementary Tables 4.19-4.23). Less than 300 windows fell below the q-value cutoff of 0.001 in the HF-fed offspring, whereas 10 times as many windows did in the LF-fed offspring. A higher percentage of DMRs were found on the X-chromosome for between-sex comparisons (1.9-3.0%) than within-sex comparisons (0.1-0.5%). The daughters had a greater proportion of DMRs on the X-chromosome (0.2% and 0.5%) due to maternal diet than the sons (0.1%).

An offspring HF diet reduced the overall variation in methylation. When comparing offspring that had the same diet but different maternal diets, the LF-fed offspring had more maternal diet DMRs than the HF-fed offspring. For instance, when HF-fed daughters of HF mothers were compared to HF-fed daughters of LF mothers, they had 1,701 DMRs ($q < 0.01$) due to maternal diet. However, when LF-fed daughters with different maternal diets were compared, they had 9,550 DMRs—far more than the HF-fed daughters had. The same trend was seen in the sons, where the HF-fed sons had 2,262 DMRs due to maternal diet and the LF-fed sons had 8,737 DMRs. The pattern continued when comparing mice of the same maternal diet and offspring diet who were different sexes. HF-fed sons of HF mothers compared to HF-fed daughters of HF mothers had 5,031 DMRs, and similarly HF-fed sons and daughters of LF mothers had 5,610 DMRs. This was far fewer than the LF-fed offspring; when LF-fed sons of HF mothers were compared to LF-fed daughters of HF mothers, they had 14,571 DMRs, and LF-fed sons and daughters of LF mothers had 10,201 DMRs. Thus, methylation patterns of males

and females were more similar to each other if they were both on an HF diet than both on an LF diet. Overall, being on an HF diet reduced the number of DMRs in comparisons of mice of different sexes and also in comparisons of mice with different maternal diets. This indicates that an offspring HF diet constrains variation in methylation patterns in the liver.

In the HF offspring, over 7,300 genes (36% of genes) in the liver had at least one DMR due to maternal diet ($q < 0.05$), whereas LF offspring had 9,300 genes (46% of genes) with a DMR (Table 4.11). About 14% of the maternal diet DMRs fell within promoters, 23% within exons, 35% in intergenic regions, and the rest in introns (Table 4.12). When Ensembl regulatory elements were included in the classification scheme, it became clear that the DMRs were disproportionately found in regulatory regions. Between 10 and 20% of DMRs overlapped enhancers (whereas only 3.5% of the 5.3 million windows genome-wide overlapped enhancers). Similarly, 5-10% of DMRs overlapped CTCF Binding sites (compared to 1.7% of windows genome-wide), 0.6-1.3% overlapped other transcription factor binding sites (compared to 0.3% of windows genome-wide), and 31-50% overlapped promoter flanking regions (compared to 8.1% of windows genome-wide). Clearly, a maternal HF diet preferentially disrupted the methylation of promoters and other regulatory elements across the entire genome.

In HF-fed daughters, 23 (0.067%) of the maternal obesity DMRs ($q < 0.05$) were located in differentially expressed genes, although 45 DMRs (0.13%) would have been expected to fall in differentially expressed genes due to chance. In the LF-fed daughters, 80 (0.15% of) DMRs fell within differentially expressed genes, whereas 97 (0.18%) would have been expected due to chance. Thus, in the daughters, DMRs fell within differentially expressed genes less often than expected due to chance ($p = 0.0002$). The opposite was true in the sons. The HF-fed sons had 22 (0.05%) of their DMRs in differentially expressed genes, while 17 (0.04%) were expected to be

there by chance. The LF-fed sons had 297 (0.52%) of their DMRs in differentially expressed genes, while 208 (0.36%) would have been expected to be there by chance. In the sons, DMRs fell within differentially expressed genes more often than expected ($p = 3.2 \times 10^{-10}$). To look at it another way, 23.9% of differentially expressed genes in the HF-fed daughters had a DMR, as did 37% of genes in the LF-fed daughters, 36.4% of genes in the HF-fed sons, and 23.2% of genes in the LF-fed sons. Thus, one-quarter to one-third of differentially expressed genes contained a DMR due to maternal diet.

DISCUSSION

Independent of maternal diet, an offspring high fat (HF) diet induced a vast array of changes in the SM/J mice: it increased body and organ weights; reduced sensitivity to insulin; increased the serum levels of leptin, insulin, triglycerides, glucose, and free fatty acids; increased the lengths and weights of the long bones; and changed the expression of 3,908 genes in the liver. An HF diet drastically reduced the expression of the leptin receptor (*Lepr*) gene, which may explain why the HF offspring consumed more food despite having 3-5 times more of the satiety hormone leptin in their serum. An HF diet had a larger effect on the males than the females in terms of inducing even more differences in gene expression (1,662 differentially expressed genes in males, 1,224 in females), causing a greater response to intraperitoneal glucose tolerance testing and a more severely impaired response to intraperitoneal insulin tolerance testing, and even further increasing the weights of the liver and kidneys. Meanwhile, an HF diet had a larger effect on the reproductive fat pad weight in the females. There were 1,062 genes differentially expressed in the males that were not differentially expressed in the females, and 602 genes differentially expressed in the females but not the males (637 genes were differentially expressed in both sexes). These sex differences underscore the importance of including both

males and females in obesity studies.

A maternal HF diet changed the expression of dozens of genes in the offspring's livers, induced methylation differences in thousands of genes (36-46% of genes in the liver had at least one differentially methylated region due to maternal diet), and in the daughters it affected the adult body weights, organ weights, and serum biomarkers. Compared to HF-fed daughters of LF mothers, HF-fed daughters of HF mothers weighed more, had heavier fat pads and livers, and had higher levels of leptin in their serum in adulthood. LF-fed daughters were also affected by maternal diet. Compared to LF-fed daughters of LF mothers, LF-fed daughters of HF mothers had significantly higher levels of free fatty acids in their serum. Interestingly, a maternal HF diet actually lowered the triglyceride and glucose levels in the daughters on either diet. This is similar to the finding of Ashino *et al.* (2012) that adult male Swiss mice had higher levels of triglycerides in the liver but lower levels in the serum due to maternal HF diet, possibly due to inadequate export of triglycerides from the liver.

The effect of maternal diet depended on the sex of the offspring. When males and females were analyzed together, there was a significant maternal-diet-by-offspring diet-interaction for the necropsy traits, but not for the weekly weights or diabetes-related traits. When the sexes were analyzed separately, there was no maternal-diet-by-offspring-diet interaction in the sons, however in the daughters there was a significant interaction effect for the weekly weights and necropsy traits. On a univariate level, the interaction significantly affected: the serum levels of leptin, insulin, free fatty acids, and glucose; weight after 10 weeks of age; and the weights of the liver, reproductive fat pad, kidney, and brown fat. The genes that were differentially expressed due to maternal diet were almost entirely different in the sons and daughters (only 6 genes overlapped). Three genes that were disrupted in the same direction in the

LF-fed sons and daughters due to a maternal HF diet were cyclin B2 (*Ccnb2*), cytokine-like 1 (*Cytl1*), and small nucleolar RNA, H/ACA box 78 (*Snora78*). *Ccnb2* was downregulated by a maternal HF diet and regulates the cell cycle, with elevated levels associated with various cancers (Park *et al.* 2007, Soria *et al.* 2000, Shubbar *et al.* 2013). *Cytl1* was upregulated by a maternal HF diet and is a cytokine-like protein involved in chondrogenesis and cartilage homeostasis (Kim *et al.* 2007, Jeon *et al.* 2011), and it has been linked to neuroblastoma (Wen *et al.* 2012). *Snora78* was also upregulated by a maternal HF diet and may be linked to tumorigenesis, since increased expression of this gene has been linked to lower survival rates from lung cancer (Gao *et al.* 2015). Although only the daughters showed phenotypic differences in the obesity and diabetes traits due to maternal diet, gene expression and methylation were affected in all offspring.

The non-alcoholic fatty liver disease (NAFLD) pathway was downregulated by an offspring HF diet and also downregulated by a maternal HF diet. The parts of the pathway that were especially downregulated by a maternal HF diet were the mitochondrial respiratory chain complexes. Reduced activity of the respiratory chain complexes are one of the mitochondrial abnormalities associated with NAFLD, in addition to impaired mitochondrial β-oxidation, lesions, increased mitochondrial size, and decreased mitochondrial number (Pessayre and Fromenty 2005, Wei *et al.* 2008). These results support findings in other mouse studies that maternal obesity can program NALFD in offspring. For instance, C57BL/6J mice fed an obesogenic diet had more severe liver injury if their mothers had also been on obesogenic diets, and this appeared to be mediated by immune dysfunction (Mouralidarane *et al.* 2013) and disrupted circadian rhythms (Mouralidarane *et al.* 2015). This was accompanied by differential expression and promoter hypermethylation of the biological clock genes *Bmal-1* and *Per2* in the

liver (Mouralidarane *et al.* 2015). Our LF-fed sons had a DMR in an intron of the *Per2* gene, but the expression was not altered. Bruce *et al.* (2009) also found that an HF maternal diet led to NAFLD regardless of offspring diet, along with impaired mitochondrial metabolism in the liver and upregulation of pathways involved in lipogenesis, oxidative stress, and inflammation.

It has been demonstrated that NAFLD is associated with Alzheimer's disease in mice (Kim *et al.* 2016), and thus it is not surprising that the Alzheimer's disease pathway was also downregulated by both an offspring and a maternal HF diet in this study. The parts of the pathway particularly downregulated by a maternal HF diet were the mitochondrial respiratory chain complexes and the SERCA Ca(2+)-ATPase intracellular pumps. Disrupted SERCA activity and calcium homeostasis can lead to Alzheimer's disease (Satoh *et al.* 2011). There was also a modest change in the amyloid precursor protein (*App*) gene. APP is cleaved to produce amyloid beta (Aβ) peptides, which can form plaques in the brain in Alzheimer's disease. Aβ is not only produced in the brain, but also in the liver, and it can be transported into the brain by low-density lipoprotein receptors (Sutcliffe *et al.* 2011, Wildsmith *et al.* 2013). It has been shown that treating mice with a drug that cannot cross the blood-brain barrier lowered Aβ in both the blood and the brain (Sutcliffe *et al.* 2011), indicating that Alzheimer's disease may start with a peripheral excess of Aβ that enters the brain. In our study, *App* was slightly overexpressed in the daughters due to a maternal HF diet (1.21 times higher in LF-fed daughters, $p = 0.015$, and 1.15 times higher in HF-fed daughters, $p = 0.062$). It would be interesting in the future to determine if this increase in *App* by maternal obesity raises levels of Aβ in the blood, predisposing the offspring to developing amyloid plaques. Reduced levels of the important antioxidant glutathione have been implicated as a cause for the oxidative stress in Alzheimer's disease (Saharan and Mandal 2014), and it has been suggested that therapeutically increasing

glutathione levels could treat the disease (Pocernich and Butterfield 2012). A maternal HF diet significantly downregulated glutathione metabolism in our mice, which may have been an early indicator of a reduced ability to respond to oxidative stress and could have predisposed the offspring to neurological impairment. It is known that obesity raises the risk of Alzheimer's disease (Kivipelto *et al.* 2005, Profenno *et al.* 2010, Moser and Pike 2016, Pugazhenthi *et al.* 2017), but the results in our study suggest that a maternal HF diet may also elevate that risk. This is plausible given that a maternal HF diet has already been shown to alter the brains of offspring (Ross *et al.* 2007, Bilbo and Tsang 2010, Kang *et al.* 2014).

In all offspring, a maternal HF diet downregulated the ribosome, spliceosome, oxidative phosphorylation, and RNA transport pathways, indicating that maternal diet has an extensive effect on the offspring transcriptome. Other studies have found that a high maternal BMI downregulates genes involved in mitochondrial and lipid metabolism in the cord blood of infants (Costa *et al.* 2011), maternal obesity in sheep downregulates AMPK signaling pathways in offspring skeletal muscle (Zhu *et al.* 2016), and maternal obesity downregulates mitochondrial pathways in the skeletal muscle of male rat offspring, including the oxidative-phosphorylation and electron transport pathways (Latouche *et al.* 2014). In our LF-fed offspring, a maternal HF diet also downregulated mitochondrial pathways such as oxidative-phosphorylation, in addition to several key metabolic pathways. In the daughters, these included the pyruvate, pyrimidine, purine, carbon, glyoxylate and dicarboxylate, and glutathione metabolism pathways. In the sons, these same pathways were downregulated by a maternal HF diet, in addition to the inositol phosphate, starch and sucrose, fatty acid, cysteine and methionine, ascorbate and aldarate, sphingolipid, and fructose and mannose metabolism pathways. The LF-fed sons also had numerous signaling pathways downregulated by a maternal HF diet, including insulin,

adipocytokine, prolactin, p53, PPAR, toll-like receptor, Wnt, AMPK, and TGF-beta. The TGF-beta signaling pathway is an important regulator of glucose and energy homeostasis (Yadav *et al.* 2011), and the AMPK signaling pathway is associated with insulin resistance and lipogenesis in the liver (Zeng *et al.* 2014). Wnt signaling modulates liver metabolism, particularly through the β-catenin (*Ctnnb1*) gene (Liu *et al.* 2011), which had a maternal diet DMR 2.5 kb upstream of the transcription start site in the LF-fed sons in this study. Maternal diet altered the expression and methylation of genes across the genome in the sons, and although these did not translate to changes in the obesity and diabetes traits I measured, it is certainly possible that it affected other traits or that differences would have been detectable after 17 weeks of age.

The weighted gene co-expression network analysis revealed several modules of highly co-expressed genes that were directly linked to the diabetes-related traits in the offspring. The two modules that were negatively associated with the diabetes-related traits contained genes that were significantly enriched for immune system function, oxidation reduction, and arachidonic acid metabolism. The two modules that were positively correlated with the diabetes traits were enriched for mitochondrial, respiratory, and ribosomal processes. Together, these modules indicate that the diabetes-related traits were being regulated by diet-induced changes in the expression of genes involved in inflammation and mitochondria.

In addition to its effects in the liver, a maternal HF diet had an even larger effect on the methylation and gene expression in the hearts of HF-fed daughters. There were 4,103 differentially methylated regions in the heart and 45 differentially expressed genes due to maternal diet in the daughters, including 6 cytochrome P450 genes. Cytochrome P450 genes are important for homeostasis, and encode enzymes involved in metabolizing endogenous compounds such as fatty acids, steroids, and drugs. A strong link between cytochrome P450

enzymes and heart failure has been reported (Zordoky and El-Kadi 2008). Many cytochrome P450 enzymes have been found in the heart, with altered levels during cardiac hypertrophy and heart failure. None of the P450 genes identified in the present study are on Phenopedia's list of genes associated with cardiovascular diseases, but one (*Cyp2c44*) was identified as protective against pulmonary hypertension in female mice. Joshi *et al.* (2016) found that *Cyp2c44*-knockout mice exposed to hypoxia had more ventricular hypertrophy and higher left ventricular and arterial stiffness in their hearts. Due to the role that cytochrome P450 genes play in homeostasis and drug metabolism, the genes I identified (*Cyp4a12a*, *Cyp2c67*, *Cyp2c54*, *Cyp2c50*, *Cyp2c44*, and *Cyp2f2*) should be further investigated in the context of maternal obesity and response to pharmaceutical treatments for metabolic syndrome and heart disease.

The set of genes differentially expressed in the heart and the liver did not overlap at all, underscoring the importance of investigating multiple tissues to understand the full scope of the effects of a maternal high-fat diet. Three of the 46 differentially expressed genes in the livers of the HF daughters (*Mpo*, *Ltf*, *Erbb4*) had been previously identified by genetic association studies in humans to be associated with obesity, diabetes, and cardiovascular diseases. The other genes included 13 predicted genes, 3 sulfotransferase genes (*Sult2a3*, *Sult3a2*, *Sult3a1*), 2 genes involved in antimicrobial activity and the inflammation pathway (*Camp*, *Ltf*), 2 transcriptional regulators (*Lmo1*, *Mybl2*), and 2 glycoproteins involved in the immune system (*Cd177*, *Igkv4-74*).

More DMRs were found on the X-chromosome in between-sex comparisons than within-sex comparisons, which was to be expected because sex has a substantial effect on X-chromosome methylation (El-Maarri *et al.* 2007, Cotton *et al.* 2011, Pai *et al.* 2011, Li *et al.* 2012, Numata *et al*. 2012). More DMRs were located on the X-chromosome in the daughters

than in the sons, which may be a result of X-chromosome inactivation, as this is known to be maintained by DNA methylation (Goto and Monk 1998, Sharp *et al.* 2011). The LF-fed offspring had more genes and pathways disrupted by maternal diet than HF-fed offspring. Likewise, the LF-fed offspring had tens of thousands more methylation differences due to maternal diet than HF-fed offspring, including on the X-chromosome. These trends indicate that the direct effect of an offspring HF diet may be so strong that it dampens the effect that a maternal HF diet has on gene expression and methylation.

Many of the genes that were differentially expressed did not have DMRs (63-77%), and many of the DMRs were located within genes that were not differentially expressed (90%). In the sons, maternal diet DMRs were found in differentially expressed genes more often than expected by chance, but in the daughters the trend was reversed. These results indicate that—while DNA methylation is linked to differential expression in some genes—overall, DNA methylation within genes is likely not the primary driver of differential expression in response to a maternal HF diet. Instead, the DMRs located outside of the gene bodies are likely to play a larger role. The maternal diet DMRs fell within regulatory regions far more often than expected by chance, with 10-20% overlapping enhancers, 5-10% overlapping CTCF Binding sites, and 31-50% overlapping promoter flanking regions.

Nevertheless, there were a handful of genes that exhibited both differential methylation and differential expression that are relevant to the obesity traits measured in this study. For instance, compared to HF-fed daughters of LF mothers, HF-fed daughters of HF mothers had a more highly methylated region in the promoter of the apelin (*Apln*) gene in the liver, which corresponded to higher expression of the gene (Figure 4.11A). *Apln* activates signaling pathways involved in angiogenesis, insulin, and cardiovascular function (Castan-Laurell *et al.*

2011). Altered expression levels of *Apln* in the placenta have been linked to preeclampsia (Inuzuka *et al.* 2013, Liang *et al.* 2016), and SNPs in *Apln* are associated with Body Mass Index in Chinese women (Liao *et al.* 2011) as well as obesity and insulin resistance in Egyptian women (Aboouf *et al.* 2015). *Apln* is secreted by adipocytes and its expression is increased by the inflammatory cytokine TNFα (Castan-Laurell *et al.* 2011), which may cause the increased angiogenesis that occurs in adipose tissue in obesity (Hu *et al.* 2016). Although *Apln* expression in the liver is understudied, Yokomori *et al.* (2012) found that the *Apln* gene and protein were over-expressed in human cirrhotic liver tissue—and the expression increased with the progression of the cirrhosis. Thus, the overexpression of *Apln* due to maternal diet in the HF-fed daughters may indicate that liver damage is worsened by a maternal HF diet.

Another gene that was both differentially methylated and differentially expressed due to maternal diet in the HF daughters was myeloperoxidase (*Mpo*), which is a hemoprotein released by white blood cells during inflammation. Its products can create oxidative stress, and *Mpo* knockout mice are protected from HF diet-induced weight gain and insulin resistance (Wang *et al.* 2014). It has been suggested that *Mpo* links inflammation, oxidative stress, and cardiovascular disease (Stenvinkel *et al.* 2006). *Mpo* activity is higher in the livers of obese patients suffering from nonalcoholic steatohepatitis (Rensen *et al.* 2009) and in the leukocytes of people with chronic kidney disease (Sela *et al.* 2005). In the present study, there was also a direct effect of diet on *Mpo* expression, with HF-fed offspring having much higher *Mpo* expression in their livers that LF-fed offspring (Figure 4.12A). In the daughters, this was exacerbated by a maternal HF diet. Compared to HF-fed daughters of LF mothers, HF-fed daughters of HF mothers had lower methylation in the eighth exon of the *Mpo* gene ($q = 0.02$), which may have contributed to the difference in expression (Figure 4.11B). The high *Mpo* expression in the HF-

fed offspring increased even further with a maternal HF diet in females, indicating that they were under more severe oxidative stress.

In addition to increasing oxidative stress, obesity raises the levels of inflammatory cytokines (Keaney *et al.* 2003, Furukawa *et al.* 2004, Fain 2006, Fernández-Sánchez *et al.* 2011). It is thought that the exposure to inflammatory cytokines in the womb can predispose offspring to metabolic disease (Hauguel-de Mouzon and Guerre-Millo 2006, Madan *et al*. 2009, Zhu *et al.* 2010, Bilbo and Tsang 2010). Elevated cytokines are known to activate the signal transducer and activator of transcription 1 (*Stat1*) gene (Gao 2005). There was a direct effect of offspring diet on *Stat1* in the present study, with the HF-fed sons and daughters having higher *Stat1* expression than the LF-fed mice (Figure 4.12B). This is important because higher levels of *Stat1* are associated with liver injury and inflammation as well as the suppression of liver regeneration (Gao 2005). Maternal diet also affected *Stat1* in HF-fed sons, but in a protective way by decreasing *Stat1* expression. Compared to HF-fed sons of LF mothers, HF-fed sons of HF mothers had decreased methylation at a DMR less than 2 kb from the *Stat1* transcription start site, in an Ensembl-defined promoter flanking region (Figure 4.12C). A maternal HF diet somewhat offset the increase in *Stat1* expression caused by an HF diet in the sons, which illustrates that the gene expression changes caused by maternal obesity are not always the same as those caused by an individual's obesity. Examples such as this show that it is important to study the effects of maternal obesity separately from offspring obesity.

One advantage to taking a whole-genome approach versus a candidate-gene approach in this study is that it enabled us to identify genes that were not well known to be involved in maternal obesity but that were nonetheless important. For instance, I found that in HF-fed daughters, a maternal HF diet decreased methylation and upregulated the expression of the

cholinergic receptor nicotinic alpha 4 subunit (*Chrna4*) gene, a nicotinic acetylcholine receptor well known for its involvement in nicotine sensitivity and epilepsy but not obesity. However, *Chrna4* has been shown to be downregulated in rat livers during liver regeneration (Xu *et al.* 2009), and in a study of the effect of nicotine metabolic genetic variants on obesity, Zhu *et al.* (2014) found that SNPs in the Chrna gene family were associated with abdominal obesity in American Indians. Our finding that *Chrna4* was differentially expressed and differentially methylated in the HF-fed daughters due to a maternal diet suggests that the Chrna gene family should be studied further in the context of obesity, as epigenetic variation in addition to genetic variation may mediate its health effects across generations.

Our study highlighted another gene meriting further investigation of its role in obesity, annexin A2 (*Anxa2*). This gene is a phospholipid-binding protein that is over-expressed in some tumors and in the blood of people with osteoporosis (Deng *et al.* 2011). *Anxa2* is also involved in cholesterol uptake in the intestine, and although it is unclear what this gene does in the liver, its expression is known to be higher in the livers of diabetic sand rats (Levy *et al.* 2010) and the livers of HF-fed C57BL/6J mice (Do *et al.* 2011). In the present study, a maternal HF diet led to over-expression of *Anxa2* in the HF-fed daughters, as well as hypermethylation of a DMR in its first intron (Figure 4.11D). Further research is required to understand the implications of *Anxa2* overexpression resulting from an HF diet.

In the LF-fed daughters, a maternal HF diet altered the levels of triglycerides, glucose, and free fatty acids in the serum. However, their differential expression of the alkaline phosphatase, liver/bone/kidney (*Alpl*) gene suggests that another useful serum maker to measure could have been alkaline phosphatase levels. Bone-specific alkaline phosphatase promotes bone mineralization, and increased methylation of the gene in cord blood at birth is associated with

shorter stature in childhood (Relton *et al.* 2012). Higher serum levels of the bone or the liver isoform can indicate obstructive liver disease (Saraç and Saygili 2007). Furthermore, higher serum levels of bone-specific alkaline phosphatase are associated with reduced insulin sensitivity and higher levels of insulin and high-density lipoprotein cholesterol (Cheung *et al.* 2013), and elevated levels increase the risk of heart attack and mortality in patients with stents (Park *et al.* 2013). A maternal HF diet decreased the methylation at a DMR in the first intron of *Alpl* and increased its expression in the LF-fed daughters (Figure 4.11E). The elevated *Alpl* expression suggests that in the future it may be useful to measure serum levels of alkaline phosphatase when studying the effects of maternal obesity.

In this study, I identified dozens of genes that were differentially expressed due to maternal diet, along with thousands of differentially methylated regions. Many of the differentially expressed genes have been found by previous studies, while many others are novel in their involvement in obesity. In the future, it will be important to incorporate other epigenetic factors such as histone modification into the analysis in order to gain a fuller understanding of the epigenetic changes induced by maternal obesity.

ACKNOWLEDGEMENTS

I thank the American Heart Association for funding. I also thank the Xiaoyun Xing and Daofeng Li for performing the methylation sequencing and initial quality control, and the Genome Technology Access Center in the Department of Genetics at Washington University School of Medicine for help with genomic analysis. The Center is partially supported by NCI Cancer Center Support Grant #P30 CA91842 to the Siteman Cancer Center and by ICTS/CTSA Grant# UL1TR000448 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap

for Medical Research. This publication is solely the responsibility of the authors and does not necessarily represent the official view of NCRR or NIH. I also thank the Core Laboratory for Clinical Studies and the Diabetes Models Phenotyping Core (both supported by the Washington University DRC, Grant No. P30 DK020579) at Washington University in St. Louis for performing the biomarker analysis on the serum traits.

LITERATURE CITED

Aboouf, M. A., N. M. Hamdy, A. I. Amin, and H. O. El-Mesallamy. 2015. Genotype screening of APLN rs3115757 variant in Egyptian women population reveals an association with obesity and insulin resistance. *Diabetes Res Clin Pract* 109(1): 40-47.

André, C., A. L. Dinel, G. Ferreira, S. Layé, and N. Castanon. 2014. Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: focus on brain indoleamine 2,3-dioxygenase activation. *Brain Behav Immun* 41: 10-21.

Ashino, N. G., K. N. Saito, F. D. Souza, F. S. Nakutz, E. A. Roman, L. A. Velloso, A. S. Torsoni, and M. A. Torsoni. 2012. Maternal high-fat feeding through pregnancy and lactation predisposes mouse offspring to molecular insulin resistance and fatty liver. *J Nutr Biochem* 23(4): 341-348.

Bar, J., L. Schreiber, E. Saruhanov, A. Ben-Haroush, A. Golan, and M. Kovo. 2012. Placental histopathological findings in obese and nonobese women with complicated and uncomplicated pregnancies. *Arch Gynecol Obstet* 286(6): 1343-1347.

Bellisario, V., A. Berry, S. Capoccia, C. Raggi, P. Panetta, I. Branchi, G. Piccaro, M. Giorgio, P. G. Pelicci, and F. Cirulli. 2014. Gender-dependent resiliency to stressful and metabolic challenges following prenatal exposure to high-fat diet in the p66Shc−/− mouse. *Front Behav Neurosci* 8: 285.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)* 57(1): 289-300.

Bilbo, S. D., and V. Tsang. 2014. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. *FASEB J* 24(6): 2104-2115.

Blackmore, H. L., Y. Niu, D. S. Fernandez-Twinn, J. L. Tarry-Adkins, D. A. Giussani, and S. E.

Ozanne. 2014. Maternal diet-induced obesity programs cardiovascular dysfunction in adult male mouse offspring independent of current body weight. *Endocrinology* 155(10): 3970-3980.

Branum, A. M., S. E. Kirmeyer, and E. C. Gregory. 2016. Prepregnancy body mass index by maternal characteristics and state: data from the birth certificate, 2014. Natl Vital Stat Rep 65(6): 1-11.

Bruce, K. D., F. R. Cagampang, M. Argenton, J. Zhang, P. L. Ethirajan, G. C. Burdge, A. C. Bateman, G. F. Clough, L. Poston, M. A. Hanson, J. M. McConnell, and C. D. Byrne. 2009. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. *Hepatology* 50(6): 1796-1808.

Cao, J. J. 2011. Effects of obesity on bone metabolism. *J Orthop Surg Res* 6: 30.

Catalano, P. M., L. Presley, J. Minium, and S. Hauguel-de Mouzon. 2009. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care* 32(6): 1076-1080.

Castan-Laurell, I., C. Dray, C. Attané, T. Duparc, C. Knauf, and P. Valet. 2011. Apelin, diabetes, and obesity. *Endocrine* 40(1): 1-9.

Cheung, C. L., K. C. Tan, K. S. Lam, and B. M. Cheung. 2013. The relationship between glucose metabolism, metabolic syndrome, and bone-specific alkaline phosphatase: a structural equation modeling approach. *J Clin Endocrinol Metab* 98(9): 3856-3863.

Cheverud, J. M., L. S. Pletscher, T. T. Vaughn, and B. Marshall. 1999. Differential response to dietary fat in large (LG/J) and small (SM/J) inbred mouse strains. *Physiol Genomics* 15(1): 33-39.

Cheverud, J. M., T. H. Ehrich, J. P. Kenney, L. S. Pletscher, and C. F. Semenkovich. 2004. Genetic evidence for discordance between obesity- and diabetes-related traits in the LGXSM recombinant inbred mouse strains. *Diabetes* 53(10): 2700-2708.

Cheverud, J. M., H. A. Lawson, G. L. Fawcett, B. Wang, L. S. Pletscher, A. R Fox, T. J. Maxwell, T. H. Ehrich, J. P. Kenney-Hunt, J. B. Wolf, and C. F. Semenkovich. 2011. Diet-dependent genetic and genomic imprinting effects on obesity in mice. *Obesity* 19(1): 160-170.

Chu, S. Y., S. Y. Kim, J. Lau, C. H. Schmid, P. M. Dietz, W. M. Callaghan, and K. M. Curtis. 2007. Maternal obesity and risk of stillbirth: a metaanalysis. *Am J Obstet Gynecol* 197(3): 223-228.

Costa, S. M., E. Isganaitis, T. J. Matthews, K. Hughes, G. Daher, J. M. Dreyfuss, G. A. da Silva, and M. E. Patti. 2016. Maternal obesity programs mitochondrial and lipid metabolism gene expression in infant umbilical vein endothelial cells. *Int J Obes* 40(11): 1627-1634.

Cotton, A. M., L. Lam, J. G. Affleck, I. M. Wilson, M. S. Peñaherrera, D. E. McFadden, M. S. Kobor, W. L. Lam, W. P. Robinson, and C. J. Brown. 2011. Chromosome-wide DNA

methylation analysis predicts human tissue-specific X inactivation. *Hum Genet* 130(2): 187-201.

Deng, F.-Y., S.-F. Lei, Y. Zhang, Y.-L. Zhang, Y.-P. Zheng, L.-S. Zhang, R. Pan, L. Wang, Q. Tian, H. Shen, M. Zhao, Y. W. Lundberg, Y.-Z. Liu, C. J. Papasian, and H.-W. Deng. 2011. Peripheral blood monocyte-expressed ANXA2 gene is involved in pathogenesis of osteoporosis in humans. *Molecular & Cellular Proteomics* 10: M111.011700.

Do, G. M., H. Y. Oh, E. Y. Kwon, Y. Y. Cho, S. K. Shin, H. J. Park, S. M. Jeon, E. Kim, C. G. Hur, T. S. Park, M. K. Sung, R. A. McGregor, and M. S. Choi. 2011. Long-term adaptation of global transcription and metabolism in the liver of high-fat diet-fed C57BL/6J mice. *Mol Nutr Food Res* 55 Suppl 2: S173-S185.

Dobin, A., C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, and T. R. Gingeras. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29(1): 15-21.

Dolinoy, D. C., J. R. Weidman, R. A. Waterland, and R. L. Jirtle. 2006. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environmental Health Perspectives* 114(4): 567-572.

Ducy, P., M. Amling, S. Takeda, M. Priemel, A. F. Schilling, F. T. Beil, J. Shen, C. Vinson, J. M. Rueger, and G. Karsenty. 2000. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell* 100(2): 197-207.

Ehrich, T. E., J. P. Kenney, T. T. Vaughn, L. S. Pletscher, and J. M. Cheverud. 2003. Diet, obesity, and hyperglycemia in LG/J and SM/J mice. *Obesity Research* 11(11): 1400-1410.

Elahi, M. M., F. R. Cagampang, D. Mukhtara, F. W. Anthony, S. K. Ohri, and M. A. Hanson. 2009. Long-term maternal high-fat feeding from weaning through pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids and fatty liver in mice. *British Journal of Nutrition* 102(4): 514-519.

El-Maarri, O., T. Becker, J. Junen, S. S. Manzoor, A. Diaz-Lacava, R. Schwaab, T. Wienker, and J. Oldenburg. 2007. Gender specific differences in levels of DNA methylation at selected loci from human total blood: a tendency toward higher methylation levels in males. *Hum Genet* 122(5): 505-514.

Eriksson, J. G., S. Sandboge, M. K. Salonen, E. Kajantie, and C. Osmond. 2014. Long-term consequences of maternal overweight in pregnancy on offspring later health: findings from the Helsinki Birth Cohort Study. *Ann Med* 46(6): 434-438.

Fain, J. N. 2006. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam Horm* 74: 443-477.

Fernández-Sánchez, A., E. Madrigal-Santillán, M. Bautista, J. Esquivel-Soto, Á. Morales-González, C. Esquivel-Chirino, I. Durante-Montiel, G. Sánchez-Rivera, C. Valadez-Vega, and J.

A. Morales-González. 2011. Inflammation, oxidative stress, and obesity. *Int J Mol Sci* 12(5): 3117-3132.

Fernandez-Twinn, D. S., H. L. Blackmore, L. Siggens, D. A. Giussani, C. M. Cross, R. Foo, and S. E. Ozanne. 2012. The programming of cardiac hypertrophy in the offspring by maternal obesity is associated with hyperinsulinemia, AKT, ERK, and mTOR activation. *Endocrinology* 153(12): 5961-5971.

Fisher, R. A. 1954. *Statistical Methods for Research Workers*. 12th ed. Oliver & Boyd, Edinburgh, 356 pp.

Furukawa, S., T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda, and I. Shimomura. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 114(12): 1752-1761.

Gaillard, R. 2015. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. *Eur J Epidemiol* 30: 1141-1152.

Gaillard, R., E. A. Steegers, L. Duijts, J. F. Felix, A. Hofman, O. H. Franco, and V. W. V. Jaddoe. 2014. Childhood cardiometabolic outcomes of maternal obesity during pregnancy: the Generation R Study. *Hypertension* 63(4): 683-691.

Gao, B. 2005. Cytokines, STATs and liver disease. *Cell Mol Immunol* 2(2): 92-100.

Gao, L., J. Ma, K. Mannoor, M. A. Guarnera, A. Shetty, M. Zhan, L. Xing, S. A. Stass, and F. Jiang. 2015. Genome-wide small nucleolar RNA expression analysis of lung cancer by next-generation deep sequencing. *Int J Cancer* 136(6): E623-E629.

Gaudet, L., Z. M. Ferraro, S. W. Wen, and M. Walker. 2014. Maternal obesity and occurrence of fetal macrosomia: a systematic review and meta-analysis. *Biomed Res Int* 2014: 640291.

Goto, T., and M. Monk. 1998. Regulation of X-chromosome inactivation in development in mice and humans. *Microbiol Mol Biol Rev* 62(2): 362-378.

Grindler, N. M., and K. H. Moley. 2013. Maternal obesity, infertility and mitochondrial dysfunction: potential mechanisms emerging from mouse model systems. *Mol Hum Reprod* 19(8): 486-494.

Guénard, F., Y. Deshaies, K. Cianflone, J. G. Kral, P. Marceau, and M.-C. Vohl. 2013. Differential methylation in glucoregulatory genes of offspring born before vs. after maternal gastrointestinal bypass surgery. *PNAS* 110(28): 11439-11444.

Hales, C. N., and D. J. Barker. 2001. The thrifty phenotype hypothesis. *Br Med Bull* 60: 5-20.

Hauguel-de Mouzon, S., and M. Guerre-Millo. 2006. The placenta cytokine network and inflammatory signals. *Placenta* 27(8): 794-798.

Hillier, T. A., K. L. Pedula, M. M. Schmidt, J. A. Mullen, M.-A. Charles, and D. J. Pettitt. 2007. Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care* 30(9): 2287-2292.

Hochner, H., Y. Friedlander, R. Calderon-Margalit, V. Meiner, Y. Sagy, M. Avgil-Tsadok, A. Burger, B. Savitsky, D. S. Siscovick, and O. Manor. 2012. Associations of maternal prepregnancy body mass index and gestational weight gain with adult offspring cardiometabolic risk factors: the Jerusalem Perinatal Family Follow-up Study. *Circulation* 125(11): 1381-1389.

Hu, H., L. He, L. Li, and L. Chen. 2016. Apelin/APJ system as a therapeutic target in diabetes and its complications. *Mol Genet Metab* 119(1-2): 20-27.

Igosheva, N., A. Y. Abramov, L. Poston, J. J. Eckert, T. P. Fleming, M. R. Duchen, and J. McConnell. 2010. Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One* 5(4): e10074.

Inuzuka, H., H. Nishizawa, A. Inagaki, M. Suzuki, S. Ota, H. Miyamura, J. Miyazaki, T. Sekiya, H. Kurahashi, and Y. Udagawa. 2013. Decreased expression of apelin in placentas from severe pre-eclampsia patients. *Hypertens Pregnancy* 32(4): 410-421.

Jeon, J., H. Oh, G. Lee, J. H. Ryu, J. Rhee, J. H. Kim, K. H. Chung, W. K. Song, C. H. Chun, and J. S. Chun. 2011. Cytokine-like 1 knock-out mice (Cytl1-/-) show normal cartilage and bone development but exhibit augmented osteoarthritic cartilage destruction. *J Biol Chem* 286(31): 27206-27213.

Joshi, S. R., A. Lakhkar, V. Dhagia, A. L. Zias, V. Soldatos, K. Oshima, H. Jiang, K. Gotlinger, J. H. Capdevila, M. L. Schwartzman, I. F. McMurtry, and S. A. Gupte. 2016. Cyp2c44 gene disruption exacerbated pulmonary hypertension and heart failure in female but not male mice *Pulm Circ* 6(3): 360-368.

Jungheim, E. S., E. L. Schoeller, K. L. Marquard, E. D. Louden, J. E. Schaffer, and K. H. Moley. 2010. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. *Endocrinology* 151(8): 4039-4046.

Kang, S. S., A. Kurti, D. A. Fair, and J. D. Fryer. 2014. Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring. *J Neuroinflammation* 11: 156.

Keaney, J. F. Jr, M. G. Larson, R. S. Vasan, P. W. Wilson, I. Lipinska, D. Corey, J. M. Massaro, P. Sutherland, J. A. Vita, and E. J. Benjamin; Framingham Study. 2003. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol* 23(3): 434-439.

Kenney-Hunt, J. P., B. Wang, E. A. Norgard, G. Fawcett, D. Falk, L. S. Pletscher, J. P. Jarvis, C. Roseman, J. Wolf, and J. M. Cheverud. 2008. Pleiotropic patterns of quantitative trait loci for 70

murine skeletal traits. *Genetics* 178(4): 2275-2288.

Khan, I, V. Dekou, M. Hanson, L. Poston, and P. Taylor. 2004. Predictive adaptive responses to maternal high-fat diet prevent endothelial dysfunction but not hypertension in adult rat offspring. *Circulation* 110(9): 1097-1102.

Kim, D.-G., A. Krenz, L. E. Toussaint, K. J. Maurer, S.-A. Robinson, A. Yan, L. Torres, and M. S. Bynoe. 2016. Non-alcoholic fatty liver disease induces signs of Alzheimer's disease (AD) in wild-type mice and accelerates pathological signs of AD in an AD model. *J Neuroinflammation* 13: 1.

Kim, J. S., Z. Y. Ryoo, and J. S. Chun. 2007. Cytokine-like 1 (Cytl1) regulates the chondrogenesis of mesenchymal cells. *J Biol Chem* 282(40): 29359-29367.

Kivipelto, M., T. Ngandu, L. Fratiglioni, M. Viitanen, I. Kåreholt, B. Winblad, E. L. Helkala, J. Tuomilehto, H. Soininen, and A. Nissinen. 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch Neurol* 62(10): 1556-1560.

Latouche, C., S. E. Heywood, S. L. Henry, M. Ziemann, R. Lazarus, A. El-Osta, J. A. Armitage, and B. A. Kingwell. 2014. Maternal overnutrition programs changes in the expression of skeletal muscle genes that are associated with insulin resistance and defects of oxidative phosphorylation in adult male rat offspring. *J Nutr* 144(3): 237-244.

Leary, C., H. J. Leese, and R. G. Sturmey. 2015. Human embryos from overweight and obese women display phenotypic and metabolic abnormalities. *Hum. Reprod* 30(1): 122-132.

Levy, E., G. Lalonde, E. Delvin, M. Elchebly, L. P. Précourt, N. G. Seidah, S. Spahis, R. Rabasa-Lhoret, and E. Ziv. 2010. Intestinal and hepatic cholesterol carriers in diabetic *Psammomys obesus*. *Endocrinology* 151(3): 958-970.

Li, D., B. Zhang, X. Xing, and T. Wang. 2015. Combining MeDIP-seq and MRE-seq to investigate genome-wide CpG methylation. *Methods* 72: 29-40.

Li, M., H. Wu, Z. Luo, Y. Xia, J. Guan, T. Wang, Y. Gu, L. Chen, K. Zhang, J. Ma, Y. Liu, Z. Zhong, J. Nie, S. Zhou, Z. Mu, X. Wang, J. Qu, L. Jing, H. Wang, S. Huang, N. Yi, Z. Wang, D. Xi, J. Wang, G. Yin, L. Wang, N. Li, Z. Jiang, Q. Lang, H. Xiao, A. Jiang, Li Zhu, Y. Jiang, G. Tang, M. Mai, S. Shuai, N. Li, K. Li, J. Wang, X. Zhang, Y. Li, H. Chen, X. Gao, G. S. Plastow, S. Beck, H. Yang, J. Wang, J. Wang, X. Li, and R. Li. 2012. An atlas of DNA methylomes in porcine adipose and muscle tissues. *Nat Commun* 3: 850.

Liang, C., M. E. Oest, and M. R. Prater. 2009. Intrauterine exposure to high saturated fat diet elevates risk of adult-onset chronic diseases in C57BL/6 mice. *Birth Defects Res B Dev Reprod Toxicol* 86(5): 377-384.

Liang, M., J. Niu, L. Zhang, H. Deng, J. Ma, W. Zhou, D. Duan, Y. Zhou, H. Xu, and L. Chen. 2016. Gene expression profiling reveals different molecular patterns in G-protein coupled

receptor signaling pathways between early- and late-onset preeclampsia. *Placenta* 40: 52-59.

Liao, Y. C., W. W. Chou, Y. N. Li, S. C. Chuang, W. Y. Lin, B. V. Lakkakula, M. L. Yu, and S. H. Juo. 2011. Apelin gene polymorphism influences apelin expression and obesity phenotypes in Chinese women. *Am J Clin Nutr* 94(3): 921-928.

Lin, S., T. C. Thomas, L. H. Storlien, and X. F. Huang. 2000. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord* 24(5): 639-646.

Liu, H., M. M. Fergusson, J. J. Wu, I. I. Rovira, J. Liu, O. Gavrilova, T. Lu, J. Bao, D. Han, M. N. Sack, and T. Finkel. 2011. Wnt signaling regulates hepatic metabolism. *Sci Signal* 4(158): ra6.

Luo, W., M. Friedman, K. Shedden, K. Hankenson, and P. Woolf. 2009. GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics* 10: 161.

Luo, W. and C. Brouwer. 2013. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics* 29(14): 1830-1831.

Luzzo, K. M., Q. Wang, S. H. Purcell, M. Chi, P. T. Jimenez, N. Grindler, T. Schedl, and K. H. Moley. 2012. High fat diet induced developmental defects in the mouse: oocyte meiotic aneuploidy and fetal growth retardation/brain defects. *PLoS One* 7(11): e49217.

MacArthur, J. 1944. Genetics of body size and related characters. I. Selection of small and large races of the laboratory mouse. *Amer Natur* 78: 142-157.

Madan, J. C., J. M. Davis, W. Y. Craig, M. Collins, W. Allan, R. Quinn, and O. Dammann. 2009. Maternal obesity and markers of inflammation in pregnancy. *Cytokine* 47(1): 61-64.

Maestripieri, D. and J. M. Mateo (eds). 2009. Maternal Effects in Mammals. Chicago: The University of Chicago Press.

McDonald, S. D., Z. Han, S. Mulla, and J. Beyene. 2010. Overweight and obesity in mothers and risk of preterm birth and low birth weight infants: systematic review and meta-analyses. *BMJ* 341: c3428.

Miller, C., S. Krishna, L. Zhoumeng, M. A. Della-Fera, D. Harn, C. de la Serre, C. Baile, and N. Filipov. 2014. Early sex differences in hepatic metabolic signaling in offspring of obese female mice. *FASEB* 28(1): Supplement 1033.11.

Mina, T. H., M. Lahti, A. J. Drake, K. Räikkönen, H. Minnis, F. C. Denison, J. E. Norman, and R. M. Reynolds. 2017. Prenatal exposure to very severe maternal obesity is associated with adverse neuropsychiatric outcomes in children. *Psychol Med* 47(2): 353-362.

Mischke, M., M. G. M. Pruis, M. V. Boekschoten, A. K. Groen, A. R. Fitri, B. J. M. van de

Heijning, H. J. Verkade, M. Müller, T. Plösch, and W. T. Steegenga. 2013. Maternal western-style high fat diet induces sex-specific physiological and molecular changes in two-week-old mouse offspring. *PLoS ONE* 8(11): e78623.

Moser, V. A., and C. J. Pike. 2016. Obesity and sex interact in the regulation of Alzheimer's disease. *Neurosci Biobehav Rev* 67: 102-118.

Mouralidarane, A., J. Soeda, C. Visconti-Pugmire, A. M. Samuelsson, J. Pombo, X. Maragkoudaki, A. Butt, R. Saraswati, M. Novelli, G. Fusai, L. Poston, P. D. Taylor, and J. A. Oben. 2013. Maternal obesity programs offspring nonalcoholic fatty liver disease by innate immune dysfunction in mice. *Hepatology* 58: 128-138.

Mouralidarane, A., J. Soeda, D. Sugden, A. Bocianowska, R. Carter, S. Ray, R. Saraswati, P. Cordero, M. Novelli, G. Fusai, M. Vinciguerra, L. Poston, P. D. Taylor, and J. A. Oben. 2015. Maternal obesity programs offspring non-alcoholic fatty liver disease through disruption of 24-h rhythms in mice. *Int J Obes (Lond)* 39(9): 1339-1348.

Numata, S., T. Ye, T. M. Hyde, X. Guitart-Navarro, R. Tao, M. Wininger, C. Colantuoni, D. R. Weinberger, J. E. Kleinman, and B. K. Lipska. 2012. DNA methylation signatures in development and aging of the human prefrontal cortex. *Am J Hum Genet* 90(2):260-72.

Oestreich, A. K., and K. H. Moley. 2017. Developmental and transmittable origins of obesity-associated health disorders. *Trends Genet* 33(6): 399-407.

Pai, A. A., J. T. Bell, J. C. Marioni, J. K. Pritchard, and Y. Gilad. 2011. A genome-wide study of DNA methylation patterns and gene expression levels in multiple human and chimpanzee tissues. *PLoS Genet* 7(2): e1001316.

Park, J. B., D. Y. Kang, H. M. Yang, H. J. Cho, K. W. Park, H. Y. Lee, H. J. Kang, B. K. Koo, and H. S. Kim. 2013. Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. *Eur Heart J* 34(12): 920-931.

Park, S.-H. G.-R. Yu, W.-H. Kim, W.-S. Moon, J.-H. Kim, and D.-G. Kim. 2007. NF-Y–Dependent Cyclin B2 expression in colorectal adenocarcinoma. *Clinical Cancer Research* 13(3): 858-867.

Partridge, C, G. L. Fawcett, B. Wang, C. F. Semenkovich, and J. M. Cheverud. 2014. The effect of dietary fat intake on hepatic gene expression in LG/J and SM/J mice. *BMC Genomics* 15: 99.

Pessayre, D., and B. Fromenty. 2005. NASH: a mitochondrial disease. *J Hepatol* 42: 928-940.

Pocernich, C. B., and D. A. Butterfield. 2012. Elevation of glutathione as a therapeutic strategy in Alzheimer disease. *Biochim Biophys Acta* 1822(5): 625-630.

Poston, L., L. F. Harthoorn, and E. M. van der Beek. 2011. Obesity in pregnancy: implications

for the mother and lifelong health of the child: a consensus statement. *Pediatric Research* 69: 175-180.

Profenno, L. A., A. P. Porsteinsson, and S. V. Faraone. 2010. Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. *Biol Psychiatry* 67(6): 505-512.

Pugazhenthi, S., L. Qin, and P. H. Reddy. 2017. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim Biophys Acta* 1863(5): 1037-1045.

Pugh, S. J., G. A. Richardson, J. A. Hutcheon, K. P. Himes, M. M. Brooks, N. L. Day, and L. M. Bodnar. 2015. Maternal obesity and excessive gestational weight gain are associated with components of child cognition. *J Nutr* 145(11): 2562-2569.

Rasmussen SA, Chu SY, Kim SY, Schmid CH, Lau J. 2008. Maternal obesity and risk of neural tube defects: a metaanalysis. *Am J Obstet Gynecol* 198(6): 611-619.

Relton, C. L., A. Groom, B. St Pourcain, A. E. Sayers, D. C. Swan, N. D. Embleton, M. S. Pearce, S. M. Ring, K. Northstone, J. H. Tobias, J. Trakalo, A. R. Ness, S. O. Shaheen, and G. Davey Smith. 2012. DNA methylation patterns in cord blood DNA and body size in childhood. *PLoS One* 7(3): e31821.

Roberts, K. A., S. C. Riley, R. M. Reynolds, S. Barr, M. Evans, A. Statham, K. Hor, H. N. Jabbour, J. E. Norman, and F. C. Denison. 2011. Placental structure and inflammation in pregnancies associated with obesity. *Placenta* 32(3): 247-254.

Roberts, V. H., A. E. Frias, and K. L. Grove. 2015. Impact of maternal obesity on fetal programming of cardiovascular disease. *Physiology* 30(3): 224-231.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1): 139-140.

Ross, M. G., M. Desai, O. Khorram, R. A. McKnight, R. H. Lane, and J. Torday. 2007. Gestational programming of offspring obesity: a potential contributor to Alzheimer's disease. *Curr Alzheimer Res* 4(2): 213-217.

Saharan, S., and P. K. Mandal. 2014. The emerging role of glutathione in Alzheimer's disease. *J Alzheimers Dis* 40(3): 519-529.

Saraç, F., and F. Saygili. 2007. Causes of high bone alkaline phosphatase. *Biotechnology & Biotechnological Equipment* 21(2): 194-197.

Satoh, K., T. Matsu-Ura, M. Enomoto, H. Nakamura, T. Michikawa, and K. Mikoshiba. 2011. Highly cooperative dependence of sarco/endoplasmic reticulum calcium ATPase SERCA2a pump activity on cytosolic calcium in living cells. *J Biol Chem* 2011 286(23): 20591-20599.

Sebire, N. J., M. Jolly, J. P. Harris, J. Wadsworth, M. Joffe, R. W. Beard, L. Regan, and S.

Robinson. 2001. Maternal obesity and pregnancy outcome: a study of 287 213 pregnancies in London. *International Journal of Obesity & Related Metabolic Disorders* 25(8): 1175-1182.

Sela, S., R. Shurtz-Swirski, M. Cohen-Mazor, R. Mazor, J. Chezar, G. Shapiro, K. Hassan, G. Shkolnik, R. Geron, and B. Kristal. 2005. Primed peripheral polymorphonuclear leukocyte: a culprit underlying chronic low-grade inflammation and systemic oxidative stress in chronic kidney disease. *J Am Soc Nephrol* 16: 2431-2438.

Sferruzzi-Perri, A. N., O. R. Vaughan, M. Haro, W. G. Cooper, B. Musial, M. Charalambous, D. Pestana, S. Ayyar, A. C. Ferguson-Smith, G. J. Burton, M. Constancia, and A. L. Fowden. 2013. An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB J* 27(10): 3928-3937.

Sharp, A. J., E. Stathaki, E. Migliavacca, M. Brahmachary, S. B. Montgomery, Y. Dupre, and S. E. Antonarakis. 2011. DNA methylation profiles of human active and inactive X chromosomes. *Genome Res* 21(10): 1592-1600.

Shubbar, E., A. Kovács, S. Hajizadeh, T. Z. Parris, S. Nemes, K. Gunnarsdóttir, Z. Einbeigi, P. Karlsson, and K. Helou. 2013. Elevated cyclin B2 expression in invasive breast carcinoma is associated with unfavorable clinical outcome. *BMC Cancer* 13:1.

Soria, J. C., S. J. Jang, F. R. Khuri, K. Hassan, D. Liu, W. K. Hong, and L. Mao. 2000. Overexpression of cyclin B1 in early-stage non-small cell lung cancer and its clinical implication. *Cancer Res* 60(15): 4000-4004.

Soubry, A., S. K. Murphy, F. Wang, Z. Huang, A. C. Vidal, B. F. Fuemmeler, J. Kurtzberg, A. Murtha, R. L. Jirtle, J. M. Schildkraut, and C. Hoyo. 2015. Newborns of obese parents have altered DNA methylation patterns at imprinted genes. *International Journal of Obesity* 39: 650-657.

Soubry, A., L. Guo, Z. Huang, C. Hoyo, S. Romanus, T. Price, and S. K. Murphy. 2016. Obesity-related DNA methylation at imprinted genes in human sperm: Results from the TIEGER study. *Clinical Epigenetics* 8: 51.

Stenvinkel, P., E. Rodríguez-Ayala, Z. A. Massy, A. R. Qureshi, P. Barany P, B. Fellström, O. Heimburger, B. Lindholm, A. Alvestrand. 2006. Statin treatment and diabetes affect myeloperoxidase activity in maintenance hemodialysis patients. *Clin J Am Soc Nephrol* 1(2): 281-287.

Stevens, M., J. B. Cheng, D. Li, M. Xie, C. Hong, C. L. Maire, K. L. Ligon, M. Hirst, M. A. Marra, J. F. Costello, T. Wang. 2013. Estimating absolute methylation levels at single CpG resolution from methylation enrichment and restriction enzyme sequencing methods. *Genome Res* 23(9): 1541-1553.

Stirrat, L. I., and R. M. Reynolds. 2014. Effects of maternal obesity on early and long-term outcomes for offspring. *Research and Reports in Neonatology* 4: 43-53.

Sullivan, E. L., E. K. Nousen, and K. A. Chamlou. 2014. Maternal high fat diet consumption during the perinatal period programs offspring behavior. *Physiol Behav* 123: 236-242.

Sutcliffe, J. G., P. B. Hedlund, E. A. Thomas, F. E. Bloom, and B. S. Hilbush. 2011. Peripheral reduction of β-amyloid is sufficient to reduce brain β-amyloid: implications for Alzheimer's disease. *J Neurosci Res* 89(6): 808-814.

Wang, D., S. Chen, M. Liu, and C. Liu. 2015. Maternal obesity disrupts circadian rhythms of clock and metabolic genes in the offspring heart and liver. *Chronobiol Int* 32(5): 615-626.

Wang, Q., Z. Xie, W. Zhang, J. Zhou, Y. Wu, M. Zhang, H. Zhu, and M. H. Zou. 2014. Myeloperoxidase deletion prevents high-fat diet-induced obesity and insulin resistance. *Diabetes* 63(12): 4172-4185.

Wankhade, U. D., Y. Zhong, P. Kang, M. Alfaro, S. V. Chintapalli, K. M. Thakali, and K. Shankar. 2017. Enhanced offspring predisposition to steatohepatitis with maternal high-fat diet is associated with epigenetic and microbiome alterations. *PLoS One* 12(4): e0175675.

Waterland, R. A., and R. L. Jirtle. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23(15): 5293-5300.

Waterland R. A., M. Travisano, K. G. Tahiliani, M. T. Rached, S. Mirza. 2008. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes (Lond)* 32(9): 1373-1379.

Wei, Y., R. S. Rector, J. P. Thyfault, and J. A. Ibdah. 2008. Nonalcoholic fatty liver disease and mitochondrial dysfunction. *World J Gastroenterol* 14(2): 193-199.

Weiss, J. L., F. D. Malone, D. Emig, R. H. Ball, D. A. Nyberg, C. H. Comstock, G. Saade, K. Eddleman, S. M. Carter, S. D. Craigo, S. R. Carr, and M. E. D'Alton; FASTER Research Consortium. 2004. Obesity, obstetric complications and cesarean delivery rate—a population-based screening study. *Am J Obstet Gynecol* 190(4): 1091-1097.

Wen, M., H. Wang, X. Zhang, J. Long, Z. Lv, Q. Kong, and Y. An. 2012. Cytokine-like 1 is involved in the growth and metastasis of neuroblastoma cells. *Int J Oncol* 41(4): 1419-1424.

Whitaker, R. C. 2004. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics* 114(1): e29-e36.

Wildsmith, K. R., M. Holley, J. C. Savage, R. Skerrett, and G. E. Landreth. 2013. Evidence for impaired amyloid β clearance in Alzheimer's disease. *Alzheimers Res Ther* 5(4): 33.

Wilson, R. M., N. E. Marshall, D. R. Jeske, J. Q. Purnell, K. Thornburg, and I. Messaoudi. 2015. Maternal obesity alters immune cell frequencies and responses in umbilical cord blood samples. *Pediatr Allergy Immunol* 26(4): 344-351.

Wolf, J. B., and M. J. Wade. 2009. What are maternal effects (and what are they not)? *Philos Trans R Soc Lond B Biol Sci* 364(1520):1107-1115.

Wolff, G. L., R. L. Kodell, S. R. Moore, and C. A. Cooney. 1998. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J* 12: 949-57.

Xu, C. S., H. Y. Shao, and B. Du. 2009. Study on correlation of signal molecule genes and their receptor-associated genes with rat liver regeneration. *Genome* 52(6): 505-523.

Yadav, H., C. Quijano, A. K. Kamaraju, O. Gavrilova, R. Malek, W. Chen, P. Zerfas, D. Zhigang, E. C. Wright, C. Stuelten, P. Sun, S. Lonning, M. Skarulis, A. E. Sumner, T. Finkel, and S. G. Rane. 2011. Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling. *Cell Metab* 14(1): 67-79.

Yates, A., W. Akanni, M. Ridwan Amode, D. Barrell, K. Billis, D. Carvalho-Silva, C. Cummins, P. Clapham, S. Fitzgerald, L. Gil, C. G. Girón, L. Gordon, T. Hourlier, S. E. Hunt, S. H. Janacek, N. Johnson, T. Juettemann, S. Keenan, I. Lavidas, F. J. Martin, T. Maurel, W. McLaren, D. N. Murphy, R. Nag, M. Nuhn, A. Parker, M. Patricio, M. Pignatelli, M. Rahtz, H. S. Riat, D. Sheppard, K. Taylor, A. Thormann, A. Vullo, S. P. Wilder, A. Zadissa, E. Birney, J. Harrow, M. Muffato, E. Perry, M. Ruffier, G. Spudich, S. J. Trevanion, F. Cunningham, B. L. Aken, D. R. Zerbino, and P. Flicek. 2016. Ensembl 2016. *Nucleic Acids Res* 44(Database issue): D710–D716.

Yao, R., C. V. Ananth, B. Y. Park, L. Pereira, L. A. Plante. Perinatal Research Consortium. 2014. Obesity and the risk of stillbirth: a population-based cohort study. *Am J Obstet Gynecol* 210(5): 457.e1-457.e9.

Yu, Z., S. Han, J. Zhu, X. Sun, C. Ji, and X. Guo. 2013. Pre-pregnancy body mass index in relation to infant birth weight and offspring overweight/obesity: a systematic review and meta-analysis. *PLoS One* 8(4): e61627.

Yu, W., M. Clyne, M. J. Khoury, and M. Gwinn. 2010. Phenopedia and Genopedia: disease-centered and gene-centered views of the evolving knowledge of human genetic associations. *Bioinformatics* 26(1): 145-146.

Yokomori, H., M. Oda, K. Yoshimura, and T. Hibi. 2012. Enhanced expressions of apelin on proliferative hepatic arterial capillaries in human cirrhotic liver. *Hepatol Res* 42(5): 508-514.

Young L. R., and M. Nestle. 2002. The contribution of expanding portion sizes to the US obesity epidemic. American Journal of Public Health 92(2): 246-249.

Zeng, L., W. J. Tang, J. J. Yin, and B. J. Zhou. 2014 Signal transductions and nonalcoholic fatty liver: a mini-review. *Int J Clin Exp Med* 7(7): 1624-1631.

Zhang, B., Y. Zhou, N. Lin, R. F. Lowdon, C. Hong, R. P. Nagarajan, J. B. Cheng, D. Li, M. Stevens, H. J. Lee, X. Xing, J. Zhou, V. Sundaram, G. Elliott, J. Gu, T. Shi, P. Gascard, M.

Sigaroudinia, T. D. Tlsty, T. Kadlecek, A. Weiss, H. O'Geen, P. J. Farnham, C. L. Maire, K. L. Ligon, P. A. Madden, A. Tam, R. Moore, M. Hirst, M. A. Marra, B. Zhang, J. F. Costello, and T. Wang. 2013. Functional DNA methylation differences between tissues, cell types, and across individuals discovered using the M&M algorithm. *Genome Res* 23(9): 1522-1540.

Zhu, M. J., M. Du, P. W. Nathanielsz, and S. P. Ford. 2010. Maternal obesity up-regulates inflammatory signaling pathways and enhances cytokine expression in the mid-gestation sheep placenta. *Placenta* 31(5): 387-391.

Zhu, Y., J. Yang, F. Yeh, S. A. Cole, K. Haack, E. T. Lee, B. V. Howard, and J. Zhao. 2014. Joint association of nicotinic acetylcholine receptor variants with abdominal obesity in American Indians: the Strong Heart Family Study. *PLoS One* 9(7): e102220.

Zhu, M. J., B. Han, J. Tong, C. Ma, J. M. Kimzey, K. R. Underwood, Y. Xiao, B. W. Hess, S. P. Ford, P. W. Nathanielsz, and M. Du. 2016. AMP-activated protein kinase signalling pathways are down regulated and skeletal muscle development impaired in fetuses of obese, over-nourished sheep. *J Physiol* 586(10): 2651-2664.

Zordoky, B. N., and A. O. El-Kadi. 2008. Modulation of cardiac and hepatic cytochrome P450 enzymes during heart failure. *Curr Drug Metab* 9(2): 122-128.

TABLES AND FIGURES

Table 4.1. Diet Content

Component	**High-fat diet**	**Low-fat diet**
Energy from fat, %	42	15
Casein, g/kg	195	197
Sugars, g/kg	341	307
Corn starch, g/kg	150	313
Cellulose, g/kg	50	30
Corn oil, g/kg	0	58
Hydrogenated coconut oil, g/kg	0	7
Anhydrous milk fat, g/kg	210	0
Cholesterol, g/kg	1.5	0
Kilojoules per gram	18.95	16.99

Figure 4.1. Experiment Overview

	Diet treatment group			
Generation	1	2	3	4
F_0	HF	LF	LF	HF
	↓	↓	↓	↓
F_1	HF	HF	LF	LF

HF = High-fat diet LF = Low-fat diet

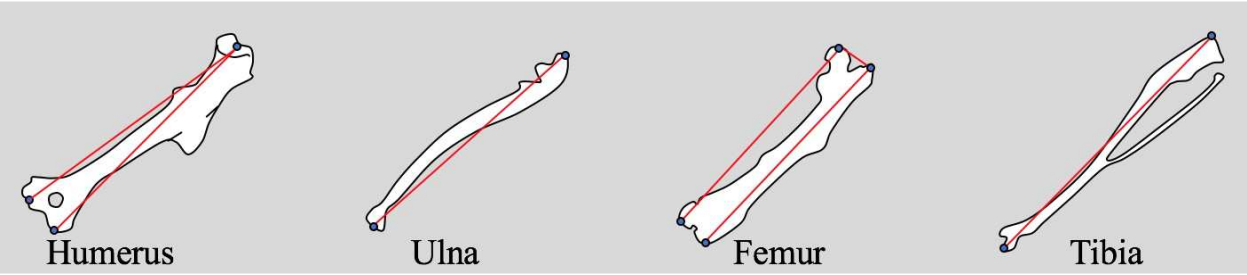


Figure 4.2. Diagram of the long bone lengths that were measured with calipers. A description of the measurements can be found in Table 4.2.

Trait	HF-HF ♀ avg	LF-HF ♀ avg	HF-LF♀ avg	LF-LF ♀ avg	Mat Diet p-value	Mat Diet*Off Diet p-value
Week 9 weight (g)	17.44 ± 0.40	15.91 ± 0.55	12.05 ± 0.34	10.88 ± 1.02	**0.041**	0.781
Week 11 weight (g)	21.86±1.09	18.41±0.68	13.01±0.35	12.84±0.52	**0.016**	**0.028**
Week 12 weight (g)	23.69±1.32	19.86±0.91	12.25±0.97	13.19±0.37	0.138	**0.017**
Week 13 weight (g)	25.96±1.45	19.38±1.12	13.66±0.39	13.67±0.47	**0.002**	**0.002**
Week 14 weight (g)	27.11±1.58	22.33±1.09	13.85±0.35	14.02±0.46	**0.028**	**0.019**
Week 15 weight (g)	29.63±1.67	23.32±1.12	14.35±0.46	14.58±0.45	**0.007**	**0.004**
Week 17 weight (g)	30.73 ± 1.64	25.66 ± 1.11	14.53 ± 0.43	15.62 ± 0.37	0.062	**0.005**
Liver weight (g)	1.38 ± 0.22	0.87 ± 0.13	0.40 ± 0.03	0.53 ± 0.02	0.231	**0.034**
Fat pad weight (g)	1.42 ± 0.15	0.91 ± 0.12	0.10 ± 0.02	0.15 ± 0.03	**0.018**	**0.005**
Kidney weight (g)	0.19 ± 0.02	0.18 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.606	**0.042**
Brown fat weight (g)	0.66 ± 0.08	0.32 ± 0.04	0.10 ± 0.01	0.11 ± 0.02	**0.001**	**0.001**
Serum leptin (ng/mL)	26.82 ± 5.57	12.94 ± 1.30	0.84 ± 0.18	2.82 ± 0.64	**0.050**	**0.011**
Serum insulin (pg/mL)	2184.30 ± 488.34	1120.67 ± 225.60	177.50 ± 31.77	348.10 ± 55.51	0.111	**0.030**
Serum glucose (mg/dL)	288.44 ± 15.84	361.44 ± 20.41	144.60 ± 18.06	301.77 ± 17.17	**1.99E-07**	**0.024**
Serum free fatty acids (mM)	1.42 ± 0.08	1.47 ± 0.09	2.06 ± 0.25	1.41 ± 0.05	**0.045**	**0.019**
Serum triglycerides (mg/dL)	121.48 ± 7.62	206.02 ± 31.48	97.76 ± 5.23	145.26 ± 12.08	**3.00E-04**	0.268

Table 4.2. The effect of maternal diet and a maternal-diet-by-offspring-diet interaction on high-fat-fed daughters. A high-fat maternal diet increased the body weights of high-fat-fed daughters in adulthood, led to heavier reproductive fat pads and brown fat, and increased serum levels of leptin. However, a high-fat maternal diet actually decreased levels of free glucose and triglycerides in the daughters. HF = High-fat diet, LF = Low-fat diet, Mat = Maternal, Off = Offspring.

Measurement	Description	Repeatability	Sex	Offspring diet
Humerus Length 1	Humeral head to outermost edge of the trochlea	0.9984	**2.42E-13**	**4.37E-07**
Humerus Length 2	Humeral head to outermost edge of the capitulum	0.9995	**1.32E-13**	**1.16E-07**
Humerus Weight	Weight of humerus	0.9861	**8.07E-10**	**2.85E-10**
Ulna Length	Topmost edge of olecranon to tip of styloid process	0.9954	**2.63E-14**	**8.06E-07**
Ulna Weight	Weight of ulna	0.9861	0.06	0.98
Femur Length 1	Head of femur to median condyle	0.9993	**2.90E-04**	**3.76E-07**
Femur Length 2	Tip of the greater trochanter to tip of lateral condyle	0.9992	**7.00E-05**	**1.74E-07**
Femur Condyle Width	Tip of medial condyle to tip of lateral condyle	0.9202	**6.70E-07**	0.42
Femur Weight	Weight of femur	0.9963	**2.07E-07**	**2.51E-12**
Tibia Length	Tip of medial condyle to the lateral edge of the medial malleolus	0.9988	**2.42E-07**	**7.30E-09**
Tibia and Fibula Weight	Weight of tibia and fibula	0.9907	**1.96E-09**	**2.42E-12**

Table 4.3. Description of bone length measurements. Maternal diet did not affect the long-bone lengths or weights, but offspring diet and sex had a significant effect. All repeatabilities were above 0.92. HF = High-fat diet, LF = Low-fat diet.

Diet	Humerus Length 1	Humerus Length 2	Humerus Weight	Ulna Length	Ulna Weight	Femur Length 1
HF ♀	10.697 ± 1.04	10.778 ± 1.13	0.015 ± 0.79	12.497 ± 0.98	0.011 ± 0.14	13.911 ± 1.63
LF ♀	10.394 ± 1.04	10.463 ± 1.13	0.014 ± 0.79	12.249 ± 0.98	0.010 ± 0.14	13.253 ± 1.63
HF ♂	11.465 ± 1.74	11.521 ± 1.81	0.018 ± 2.37	13.197 ± 1.71	0.013 ± 0.14	14.196 ± 1.16
LF ♂	10.957 ± 1.74	11.013 ± 1.81	0.015 ± 2.37	12.766 ± 1.71	0.014 ± 0.14	13.729 ± 1.16

Diet	Femur Length 2	Femur Condyle Width	Femur Weight	Tibia Length	Tibia and Fibula Weight	Sample Size
HF ♀	14.260 ± 1.67	2.275 ± 0.07	0.030 ± 1.58	15.657 ± 1.64	0.026 ± 1.92	16
LF ♀	13.536 ± 1.67	2.271 ± 0.07	0.025 ± 1.58	15.045 ± 1.64	0.022 ± 1.92	19
HF ♂	14.615 ± 1.22	2.363 ± 0.38	0.035 ± 2.21	16.187 ± 1.64	0.030 ± 2.41	14
LF ♂	14.086 ± 1.22	2.344 ± 0.38	0.028 ± 2.21	15.576 ± 1.64	0.025 ± 2.41	18

Table 4.4. High-fat mice had longer, heavier bones than low-fat mice, and males had longer, heavier bones than females. Maternal diet did not affect the bone measurements. HF = High-fat diet, LF = Low-fat diet, averages are reported ± one standard deviation unit.

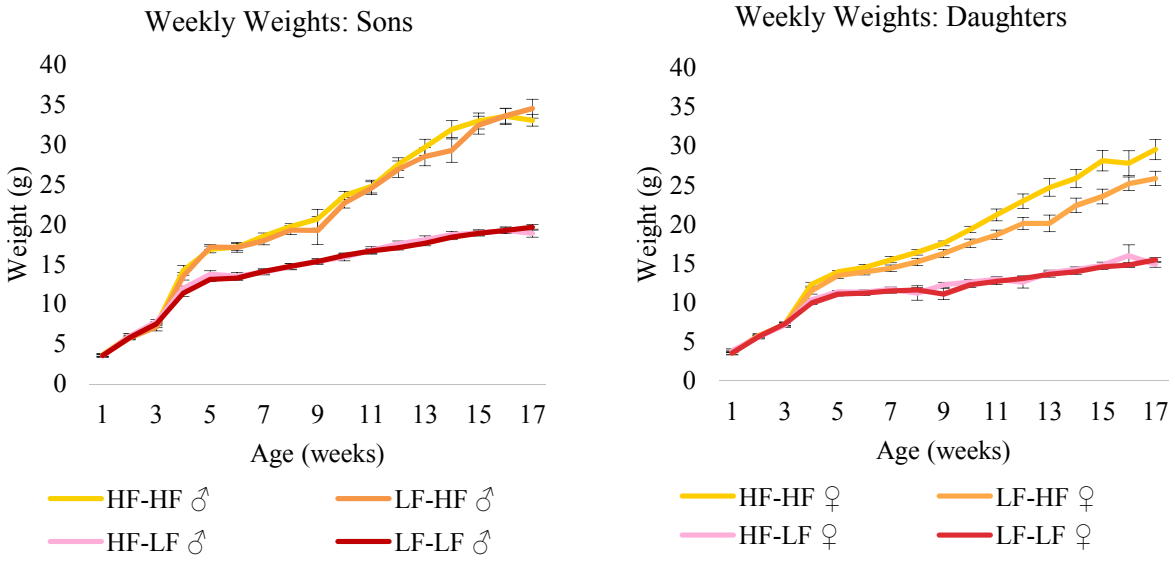


Figure 4.3. Weekly weights of offspring. High-fat diet offspring weigh more than low-fat diet offspring by 4 weeks of age (1 week after being weaned onto the diet). Maternal diet does not affect the sons, but it does affect the daughters. High fat daughters weigh even more if their mothers were also on a high-fat diet. HF = High-fat diet, LF = Low-fat diet.

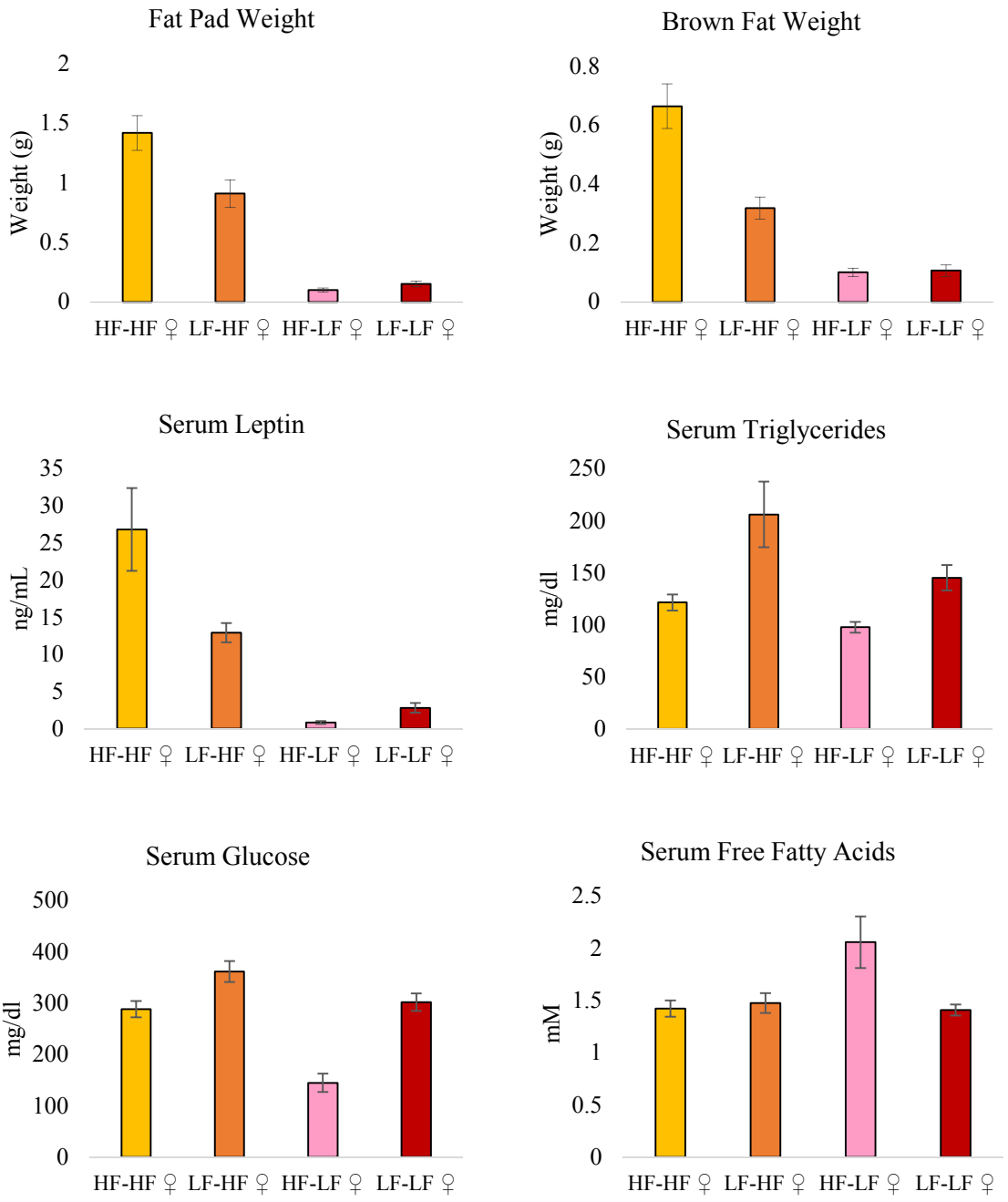
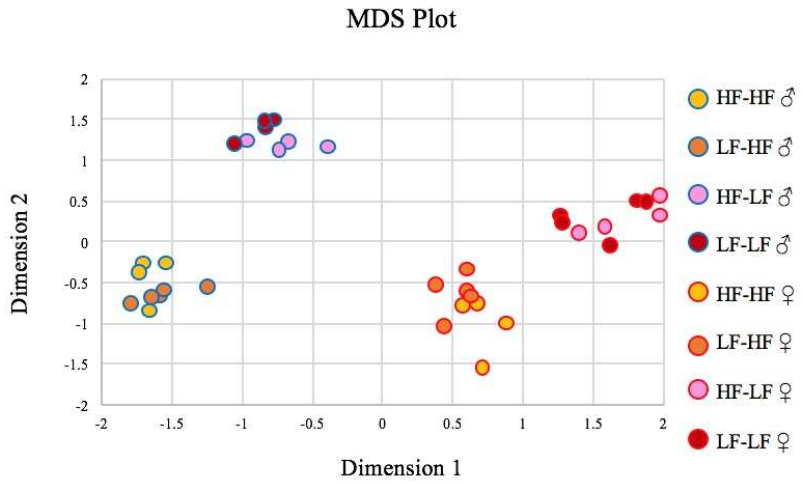
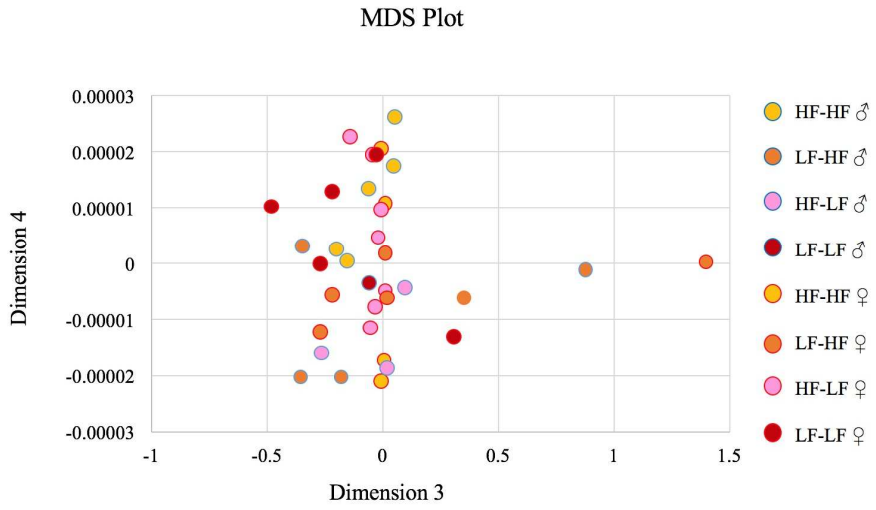


Figure 4.4. Traits in female offspring affected by maternal diet (± SE). Each bar represents the average of 10 offspring. HF = High-fat diet, LF = Low-fat diet.



A)

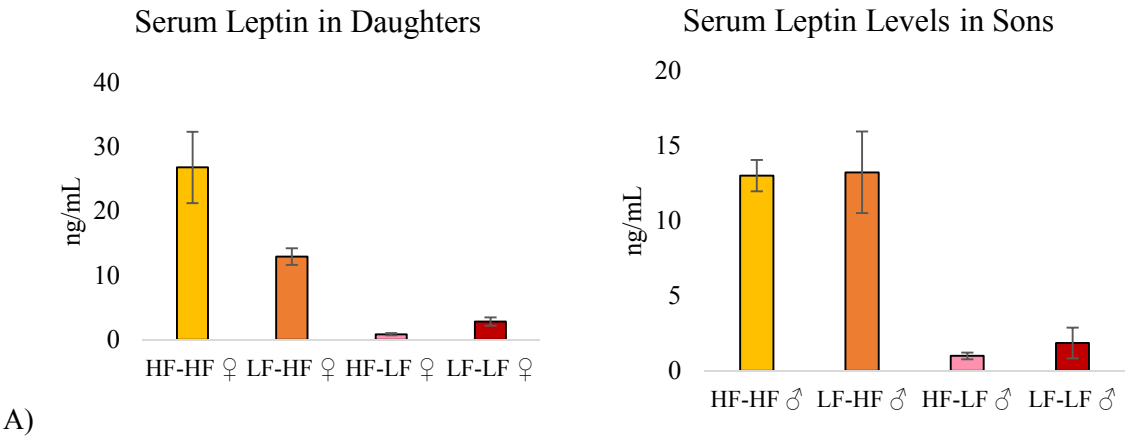


B)

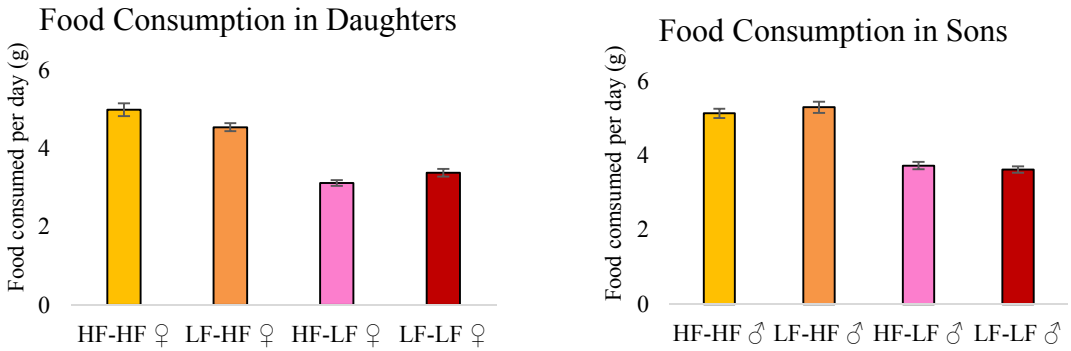
Figure 4.5. (A) The multidimensional scaling plot indicates that gene expression libraries clustered by sex (dimension 1) and offspring diet (dimension 2), but not maternal diet. (B) There were no discernable patterns in dimensions 3 or 4. HF = High-fat diet, and LF = Low-fat diet.

Diet Group	Differentially Expressed Genes
All HF vs. LF	3,908
HF vs. LF daughters	1,224
HF vs. LF sons	1,662
HF-HF vs. LF-HF daughters	46
HF-HF vs. LF-HF sons	22
HF-LF vs. LF-LF daughters	70
HF-LF vs. LF-LF sons	434

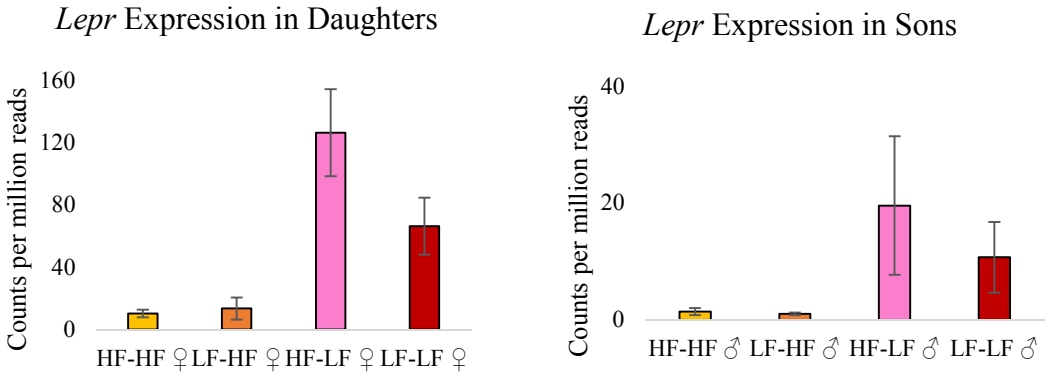
Table 4.5. Number of differentially expressed genes due to offspring diet and maternal diet, $p < 0.05$, absolute value of the logFC > 2. Maternal diet affected the expression of dozens of genes in the offspring, especially the sons. HF = High-fat diet, LF = Low-fat diet.



A)



B)



C)

Figure 4.6. (A) A high fat offspring diet increased the amount of serum leptin, but (B) high fat mice still consumed more food as adults. (C) This could be in part due to the drastically reduced expression of the leptin receptor. Maternal high-fat diet further increased the serum leptin levels in high-fat-fed daughters.

Diet Comparison	KEGG_ID	Mean LogFC	FDR
HF-HF ♀ vs. LF-HF ♀ liver	mmu03010 Ribosome	-6.12	3.85E-07
	mmu03040 Spliceosome	-5.45	8.20E-06
	mmu00190 Oxidative phosphorylation	-3.90	4.36E-03
	mmu03013 RNA transport	-3.22	3.81E-02
HF-HF ♂ vs. LF-HF ♂	mmu03010 Ribosome	-6.16	3.71E-07
	mmu03040 Spliceosome	-5.33	1.25E-05
	mmu00190 Oxidative phosphorylation	-5.27	1.25E-05
	mmu04210 Apoptosis	-5.01	2.82E-05
	mmu04145 Phagosome	-4.92	2.82E-05
	mmu04144 Endocytosis	-4.80	3.63E-05
	mmu04141 Protein processing in endoplasmic reticulum	-4.69	6.72E-05
	mmu04612 Antigen processing and presentation	-4.26	4.70E-04
	mmu03050 Proteasome	-4.29	7.68E-04
	mmu03008 Ribosome biogenesis in eukaryotes	-3.88	1.76E-03
	mmu04623 Cytosolic DNA-sensing pathway	-3.74	2.96E-03
	mmu04668 TNF signaling pathway	-3.63	2.96E-03
	mmu00230 Purine metabolism	-3.58	2.96E-03
	mmu00240 Pyrimidine metabolism	-3.60	2.96E-03
	mmu04062 Chemokine signaling pathway	-3.56	2.96E-03
	mmu03013 RNA transport	-3.33	5.56E-03
	mmu04620 Toll-like receptor signaling pathway	-3.34	5.56E-03
	mmu04622 RIG-I-like receptor signaling pathway	-3.36	5.56E-03
	mmu04060 Cytokine-cytokine receptor interaction	-3.29	5.56E-03
	mmu04120 Ubiquitin mediated proteolysis	-3.31	5.56E-03
	mmu04650 Natural killer cell mediated cytotoxicity	-3.31	5.56E-03
	mmu04064 NF-kappa B signaling pathway	-3.30	5.67E-03
	mmu04380 Osteoclast differentiation	-3.21	6.79E-03
	mmu04722 Neurotrophin signaling pathway	-3.00	1.29E-02
	mmu04666 Fc gamma R-mediated phagocytosis	-2.93	1.69E-02
	mmu04540 Gap junction	-2.88	1.83E-02
	mmu04142 Lysosome	-2.86	1.83E-02
	mmu03030 DNA replication	-2.94	1.86E-02
	mmu04662 B cell receptor signaling pathway	-2.85	1.91E-02
	mmu04915 Estrogen signaling pathway	-2.72	2.45E-02
	mmu04110 Cell cycle	-2.71	2.45E-02
	mmu00051 Fructose and mannose metabolism	-2.69	3.00E-02

	mmu04514 Cell adhesion molecules (CAMs)	-2.60	3.00E-02
	mmu04151 PI3K-Akt signaling pathway	-2.58	3.00E-02
	mmu00520 Amino sugar and nucleotide sugar metabolism	-2.64	3.00E-02
	mmu04621 NOD-like receptor signaling pathway	-2.58	3.21E-02
	mmu00010 Glycolysis / Gluconeogenesis	-2.55	3.38E-02
	mmu01200 Carbon metabolism	-2.52	3.38E-02
	mmu04390 Hippo signaling pathway	-2.50	3.43E-02
	mmu04130 SNARE interactions in vesicular transport	-2.53	3.73E-02
	mmu03015 mRNA surveillance pathway	-2.45	3.73E-02
	mmu04360 Axon guidance	-2.44	3.73E-02
	mmu04071 Sphingolipid signaling pathway	-2.44	3.73E-02
	mmu03420 Nucleotide excision repair	-2.47	3.95E-02
	mmu03410 Base excision repair	-2.46	4.06E-02
	mmu03020 RNA polymerase	-2.47	4.15E-02
	mmu04066 HIF-1 signaling pathway	-2.36	4.34E-02
	mmu04510 Focal adhesion	-2.31	4.61E-02
	mmu04910 Insulin signaling pathway	-2.31	4.65E-02
	mmu04015 Rap1 signaling pathway	-2.29	4.67E-02
	mmu04611 Platelet activation	-2.28	4.76E-02
HF-LF ♀ vs. LF-LF ♀	mmu04110 Cell cycle	-4.55	5.58E-04
	mmu00190 Oxidative phosphorylation	-4.53	5.58E-04
	mmu03040 Spliceosome	-4.32	8.71E-04
	mmu04120 Ubiquitin mediated proteolysis	-4.10	1.38E-03
	mmu03030 DNA replication	-4.26	1.38E-03
	mmu00240 Pyrimidine metabolism	-4.04	1.38E-03
	mmu03050 Proteasome	-4.12	1.92E-03
	mmu04141 Protein processing in endoplasmic reticulum	-3.82	2.14E-03
	mmu01200 Carbon metabolism	-3.62	4.16E-03
	mmu00480 Glutathione metabolism	-3.43	8.85E-03
	mmu03420 Nucleotide excision repair	-3.47	8.94E-03
	mmu04623 Cytosolic DNA-sensing pathway	-3.39	8.94E-03
	mmu04144 Endocytosis	-3.06	1.79E-02
	mmu03010 Ribosome	-3.04	1.79E-02
	mmu00630 Glyoxylate and dicarboxylate metabolism	-3.17	1.79E-02
	mmu00970 Aminoacyl-tRNA biosynthesis	-3.10	1.79E-02
	mmu03460 Fanconi anemia pathway	-2.98	2.27E-02
	mmu00620 Pyruvate metabolism	-2.93	2.49E-02
	mmu03013 RNA transport	-2.85	2.49E-02

	mmu03410 Base excision repair	-2.92	2.49E-02
	mmu00230 Purine metabolism	-2.76	2.98E-02
	mmu03440 Homologous recombination	-2.81	3.32E-02
	mmu04130 SNARE interactions in vesicular transport	-2.76	3.85E-02
	mmu04114 Oocyte meiosis	-2.57	4.65E-02
	mmu04210 Apoptosis	-2.52	4.94E-02
	mmu03060 Protein export	-2.63	4.94E-02
	mmu03008 Ribosome biogenesis in eukaryotes	-2.53	4.94E-02
	mmu00520 Amino sugar and nucleotide sugar metabolism	-2.51	5.07E-02
HF-LF ♂ vs. LF-LF ♂	mmu04144 Endocytosis	-7.17	3.07E-10
	mmu04151 PI3K-Akt signaling pathway	-7.03	3.07E-10
	mmu04110 Cell cycle	-7.00	1.30E-09
	mmu04120 Ubiquitin mediated proteolysis	-6.27	6.91E-08
	mmu04380 Osteoclast differentiation	-6.01	1.48E-07
	mmu04210 Apoptosis	-5.99	1.48E-07
	mmu04062 Chemokine signaling pathway	-5.86	1.66E-07
	mmu04015 Rap1 signaling pathway	-5.81	1.66E-07
	mmu04662 B cell receptor signaling pathway	-5.79	8.33E-07
	mmu04360 Axon guidance	-5.47	9.57E-07
	mmu03010 Ribosome	-5.36	2.44E-06
	mmu04620 Toll-like receptor signaling pathway	-5.40	2.44E-06
	mmu03460 Fanconi anemia pathway	-5.63	2.62E-06
	mmu04722 Neurotrophin signaling pathway	-5.28	2.62E-06
	mmu04510 Focal adhesion	-5.16	2.78E-06
	mmu03040 Spliceosome	-5.24	3.02E-06
	mmu04010 MAPK signaling pathway	-5.06	3.68E-06
	mmu04141 Protein processing in endoplasmic reticulum	-5.14	3.68E-06
	mmu04666 Fc gamma R-mediated phagocytosis	-5.13	6.51E-06
	mmu00230 Purine metabolism	-4.95	6.59E-06
	mmu04650 Natural killer cell mediated cytotoxicity	-4.93	9.13E-06
	mmu04145 Phagosome	-4.79	1.17E-05
	mmu04064 NF-kappa B signaling pathway	-4.88	1.17E-05
	mmu04150 mTOR signaling pathway	-4.70	1.90E-05
	mmu04060 Cytokine-cytokine receptor interaction	-4.63	2.03E-05
	mmu00240 Pyrimidine metabolism	-4.73	2.07E-05
	mmu04152 AMPK signaling pathway	-4.67	2.17E-05
	mmu04068 FoxO signaling pathway	-4.63	2.22E-05
	mmu03013 RNA transport	-4.56	2.81E-05

	mmu04910 Insulin signaling pathway	-4.57	2.81E-05
	mmu04668 TNF signaling pathway	-4.58	2.81E-05
	mmu04142 Lysosome	-4.53	3.49E-05
	mmu04070 Phosphatidylinositol signaling system	-4.55	3.49E-05
	mmu04014 Ras signaling pathway	-4.44	3.51E-05
	mmu04623 Cytosolic DNA-sensing pathway	-4.76	3.51E-05
	mmu04066 HIF-1 signaling pathway	-4.49	3.67E-05
	mmu03008 Ribosome biogenesis in eukaryotes	-4.56	3.67E-05
	mmu04610 Complement and coagulation cascades	-4.49	4.27E-05
	mmu04390 Hippo signaling pathway	-4.39	4.31E-05
	mmu04114 Oocyte meiosis	-4.40	4.40E-05
	mmu04611 Platelet activation	-4.24	8.45E-05
	mmu04919 Thyroid hormone signaling pathway	-4.20	9.90E-05
	mmu04071 Sphingolipid signaling pathway	-4.19	1.02E-04
	mmu04660 T cell receptor signaling pathway	-4.17	1.13E-04
	mmu00562 Inositol phosphate metabolism	-4.25	1.13E-04
	mmu04810 Regulation of actin cytoskeleton	-4.10	1.13E-04
	mmu03410 Base excision repair	-4.43	1.14E-04
	mmu04115 p53 signaling pathway	-4.14	1.34E-04
	mmu04514 Cell adhesion molecules (CAMs)	-4.04	1.45E-04
	mmu03030 DNA replication	-4.42	1.53E-04
	mmu04310 Wnt signaling pathway	-4.01	1.63E-04
	mmu04012 ErbB signaling pathway	-4.06	1.63E-04
	mmu04914 Progesterone-mediated oocyte maturation	-4.03	1.65E-04
	mmu03420 Nucleotide excision repair	-4.27	1.73E-04
	mmu00310 Lysine degradation	-4.18	1.90E-04
	mmu04146 Peroxisome	-3.98	2.14E-04
	mmu04550 Signaling pathways regulating pluripotency of stem cells	-3.77	3.73E-04
	mmu04640 Hematopoietic cell lineage	-3.78	4.02E-04
	mmu04740 Olfactory transduction	4.74	4.08E-04
	mmu04915 Estrogen signaling pathway	-3.77	4.11E-04
	mmu03018 RNA degradation	-3.78	4.36E-04
	mmu01200 Carbon metabolism	-3.74	4.36E-04
	mmu03440 Homologous recombination	-3.91	4.82E-04
	mmu04340 Hedgehog signaling pathway	-3.80	5.54E-04
	mmu04922 Glucagon signaling pathway	-3.61	6.85E-04
	mmu00480 Glutathione metabolism	-3.63	8.52E-04
	mmu04350 TGF-beta signaling pathway	-3.49	1.01E-03

	mmu00860 Porphyrin and chlorophyll metabolism	-3.60	1.06E-03
	mmu04540 Gap junction	-3.47	1.06E-03
	mmu00510 N-Glycan biosynthesis	-3.62	1.08E-03
	mmu04520 Adherens junction	-3.44	1.16E-03
	mmu00071 Fatty acid degradation	-3.45	1.35E-03
	mmu04512 ECM-receptor interaction	-3.36	1.41E-03
	mmu04621 NOD-like receptor signaling pathway	-3.38	1.42E-03
	mmu00983 Drug metabolism - other enzymes	-3.43	1.42E-03
	mmu04130 SNARE interactions in vesicular transport	-3.56	1.46E-03
	mmu00190 Oxidative phosphorylation	-3.26	1.74E-03
	mmu03050 Proteasome	-3.35	2.17E-03
	mmu00532 Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan sulfate	-3.46	2.22E-03
	mmu04330 Notch signaling pathway	-3.21	2.50E-03
	mmu00970 Aminoacyl-tRNA biosynthesis	-3.26	2.60E-03
	mmu00500 Starch and sucrose metabolism	-3.18	2.77E-03
	mmu04670 Leukocyte transendothelial migration	-3.09	2.88E-03
	mmu00980 Metabolism of xenobiotics by cytochrome P450	-3.12	2.88E-03
	mmu04370 VEGF signaling pathway	-3.10	3.14E-03
	mmu03015 mRNA surveillance pathway	-3.06	3.22E-03
	mmu04622 RIG-I-like receptor signaling pathway	-3.08	3.39E-03
	mmu03430 Mismatch repair	-3.29	3.76E-03
	mmu01212 Fatty acid metabolism	-2.98	4.47E-03
	mmu03320 PPAR signaling pathway	-2.94	4.47E-03
	mmu03020 RNA polymerase	-3.11	4.65E-03
	mmu00563 Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	-3.14	4.67E-03
	mmu04022 cGMP-PKG signaling pathway	-2.82	5.74E-03
	mmu03022 Basal transcription factors	-2.90	5.74E-03
	mmu04270 Vascular smooth muscle contraction	-2.80	6.09E-03
	mmu04728 Dopaminergic synapse	-2.74	7.17E-03
	mmu04630 Jak-STAT signaling pathway	-2.72	7.41E-03
	mmu04912 GnRH signaling pathway	-2.74	7.41E-03
	mmu04916 Melanogenesis	-2.72	7.51E-03
	mmu00010 Glycolysis / Gluconeogenesis	-2.74	7.60E-03
	mmu04974 Protein digestion and absorption	-2.69	8.15E-03
	mmu04612 Antigen processing and presentation	-2.67	8.65E-03
	mmu04024 cAMP signaling pathway	-2.64	8.86E-03

	mmu00620 Pyruvate metabolism	-2.73	9.20E-03
	mmu00564 Glycerophospholipid metabolism	-2.61	9.86E-03
	mmu00040 Pentose and glucuronate interconversions	-2.66	1.01E-02
	mmu01230 Biosynthesis of amino acids	-2.61	1.01E-02
	mmu00640 Propanoate metabolism	-2.67	1.13E-02
	mmu04921 Oxytocin signaling pathway	-2.52	1.18E-02
	mmu00270 Cysteine and methionine metabolism	-2.55	1.24E-02
	mmu04725 Cholinergic synapse	-2.49	1.29E-02
	mmu00280 Valine, leucine and isoleucine degradation	-2.47	1.41E-02
	mmu04020 Calcium signaling pathway	-2.43	1.43E-02
	mmu04710 Circadian rhythm	-2.49	1.47E-02
	mmu04976 Bile secretion	-2.44	1.47E-02
	mmu04920 Adipocytokine signaling pathway	-2.42	1.51E-02
	mmu00520 Amino sugar and nucleotide sugar metabolism	-2.43	1.56E-02
	mmu04962 Vasopressin-regulated water reabsorption	-2.40	1.68E-02
	mmu00053 Ascorbate and aldarate metabolism	-2.44	1.69E-02
	mmu00982 Drug metabolism - cytochrome P450	-2.35	1.77E-02
	mmu04750 Inflammatory mediator regulation of TRP channels	-2.30	1.91E-02
	mmu00514 Other types of O-glycan biosynthesis	-2.35	1.97E-02
	mmu00600 Sphingolipid metabolism	-2.32	1.97E-02
	mmu04917 Prolactin signaling pathway	-2.28	2.03E-02
	mmu04672 Intestinal immune network for IgA production	-2.27	2.15E-02
	mmu00900 Terpenoid backbone biosynthesis	-2.28	2.48E-02
	mmu03450 Non-homologous end-joining	-2.40	2.51E-02
	mmu04664 Fc epsilon RI signaling pathway	-2.18	2.54E-02
	mmu00051 Fructose and mannose metabolism	-2.19	2.61E-02
	mmu04970 Salivary secretion	-2.16	2.61E-02
	mmu00100 Steroid biosynthesis	-2.24	2.76E-02
	mmu00770 Pantothenate and CoA biosynthesis	-2.27	2.80E-02
	mmu00260 Glycine, serine and threonine metabolism	-2.13	2.92E-02
	mmu04730 Long-term depression	-2.05	3.30E-02
	mmu00561 Glycerolipid metabolism	-2.05	3.30E-02
	mmu00450 Selenocompound metabolism	-2.17	3.32E-02
	mmu00630 Glyoxylate and dicarboxylate metabolism	-2.09	3.32E-02
	mmu02010 ABC transporters	-2.05	3.33E-02
	mmu00830 Retinol metabolism	-2.03	3.33E-02
	mmu04140 Regulation of autophagy	-2.08	3.33E-02

	mmu00020 Citrate cycle (TCA cycle)	-2.06	3.46E-02
	mmu04918 Thyroid hormone synthesis	-1.98	3.65E-02
	mmu01040 Biosynthesis of unsaturated fatty acids	-1.99	3.94E-02
	mmu00670 One carbon pool by folate	-2.03	3.94E-02
	mmu04713 Circadian entrainment	-1.85	4.73E-02
	mmu04961 Endocrine and other factor-regulated calcium reabsorption	-1.86	4.80E-02
HF-HF ♀ vs. LF-HF ♀ heart	mmu04610 Complement and coagulation cascades	-6.80	3.61E-08
	mmu00830 Retinol metabolism	-6.39	1.96E-07
	mmu00140 Steroid hormone biosynthesis	-5.98	1.08E-06
	mmu03320 PPAR signaling pathway	-4.54	3.16E-04
	mmu00983 Drug metabolism - other enzymes	-4.12	1.93E-03
	mmu01230 Biosynthesis of amino acids	-3.80	3.67E-03
	mmu00590 Arachidonic acid metabolism	-3.76	3.93E-03
	mmu00053 Ascorbate and aldarate metabolism	-3.95	4.48E-03
	mmu00591 Linoleic acid metabolism	-3.51	9.72E-03
	mmu00260 Glycine, serine and threonine metabolism	-3.37	1.21E-02
	mmu00980 Metabolism of xenobiotics by cytochrome P450	-3.31	1.21E-02
	mmu01200 Carbon metabolism	-3.24	1.21E-02
	mmu00010 Glycolysis / Gluconeogenesis	-3.24	1.21E-02
	mmu04151 PI3K-Akt signaling pathway	-3.04	1.63E-02
	mmu00040 Pentose and glucuronate interconversions	-3.17	1.63E-02
	mmu00982 Drug metabolism - cytochrome P450	-3.07	1.68E-02
	mmu00860 Porphyrin and chlorophyll metabolism	-2.99	2.25E-02
	mmu00360 Phenylalanine metabolism	-2.91	3.41E-02
	mmu04918 Thyroid hormone synthesis	-2.62	5.04E-02
	mmu00120 Primary bile acid biosynthesis	-2.76	5.04E-02

Table 4.6. Significantly downregulated signaling and metabolism pathways due to maternal diet. A negative logFC value indicates the pathway was downregulated in mice with high-fat-fed mothers.

Comparison	GO Term	Mean LogFC	FDR
HF-HF ♀ vs. LF-HF ♀	GO:0022613 ribonucleoprotein complex biogenesis	-6.83	7.68E-08
	GO:0016071 mRNA metabolic process	-6.57	8.43E-08
	GO:0008380 RNA splicing	-6.62	8.43E-08
	GO:0034470 ncRNA processing	-6.47	2.19E-07
	GO:0006397 mRNA processing	-6.13	8.27E-07
	GO:0034660 ncRNA metabolic process	-6.11	8.50E-07
	GO:0042254 ribosome biogenesis	-5.58	2.28E-05
	GO:0006412 translation	-5.37	3.55E-05
	GO:0000377 RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	-5.27	6.72E-05
	GO:0000398 mRNA splicing, via spliceosome	-5.27	6.72E-05
HF-HF ♂ vs. LF-HF ♂	GO:0045087 innate immune response	-7.39	9.16E-10
	GO:0050776 regulation of immune response	-6.98	7.56E-09
	GO:0022613 ribonucleoprotein complex biogenesis	-6.88	1.76E-08
	GO:0098542 defense response to other organism	-6.72	1.85E-08
	GO:0043043 peptide biosynthetic process	-6.72	1.85E-08
	GO:0043900 regulation of multi-organism process	-6.67	2.17E-08
	GO:0034660 ncRNA metabolic process	-6.69	2.17E-08
	GO:0006412 translation	-6.57	2.97E-08
	GO:0050778 positive regulation of immune response	-6.57	2.97E-08
	GO:0034470 ncRNA processing	-6.59	4.22E-08
HF-LF ♀ vs. LF-LF ♀	GO:0007067 mitotic nuclear division	-8.65	9.02E-14
	GO:0000280 nuclear division	-7.93	7.91E-12
	GO:0043632 modification-dependent macromolecule catabolic process	-6.90	8.47E-09
	GO:0019941 modification-dependent protein catabolic process	-6.78	1.48E-08
	GO:0006511 ubiquitin-dependent protein catabolic process	-6.72	1.76E-08
	GO:0006281 DNA repair	-6.69	1.90E-08
	GO:0007059 chromosome segregation	-6.73	1.97E-08
	GO:0034660 ncRNA metabolic process	-5.72	5.72E-06
	GO:0010498 proteasomal protein catabolic process	-5.66	5.95E-06
	GO:0034470 ncRNA processing	-5.72	5.95E-06
HF-LF ♂ vs. LF-LF ♂	GO:0006281 DNA repair	-11.77	1.80E-25
	GO:0007067 mitotic nuclear division	-11.62	2.50E-25
	GO:0051345 positive regulation of hydrolase activity	-11.19	7.00E-24
	GO:0000280 nuclear division	-10.62	4.22E-22
	GO:0043632 modification-dependent macromolecule catabolic process	-10.66	6.32E-22

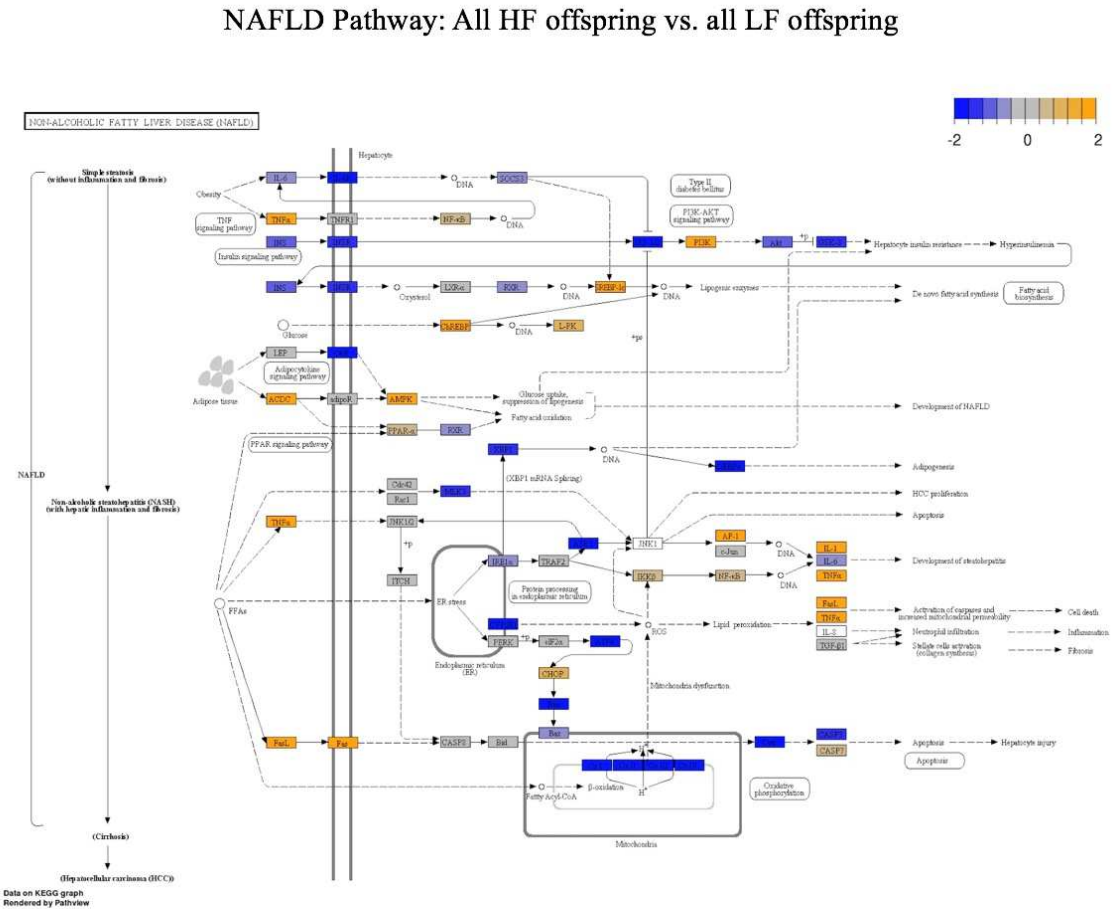
	GO:0019941 modification-dependent protein catabolic process	-10.55	1.46E-21
	GO:0006511 ubiquitin-dependent protein catabolic process	-10.46	3.04E-21
	GO:0050776 regulation of immune response	-10.16	1.64E-20
	GO:0031329 regulation of cellular catabolic process	-10.18	2.17E-20
	GO:0034109 homotypic cell-cell adhesion	-9.95	1.24E-19
HF-HF ♀ vs. LF-HF ♀ heart	GO:0006631 fatty acid metabolic process	-6.18	3.11E-06
	GO:0044283 small molecule biosynthetic process	-5.81	1.18E-05
	GO:0016053 organic acid biosynthetic process	-5.61	2.32E-05
	GO:0046394 carboxylic acid biosynthetic process	-5.61	2.32E-05
	GO:0042738 exogenous drug catabolic process	-6.06	3.97E-05
	GO:0006520 cellular amino acid metabolic process	-5.42	3.97E-05
	GO:0042737 drug catabolic process	-5.85	6.30E-05
	GO:0008202 steroid metabolic process	-5.24	7.91E-05
	GO:1901605 alpha-amino acid metabolic process	-5.24	7.91E-05
	GO:0071466 cellular response to xenobiotic stimulus	-5.40	8.12E-05

Table 4.7. Top 10 significant GO Biological Processes affected by maternal diet. A negative logFC value indicates that the process was downregulated in mice with high-fat-fed mothers.

Gene	**Forward primer**	**Reverse primer**
Mpo	5'-GGCCTCCCAGGATACAATGC-3'	5'-ACACCGCCCATCCAGATGTC-3'
Chrna4	5'-CTAGCAGCCACATAGAGACCC-3'	5'-GACAAGCCAAAGCGGACAAG-3'
Anxa2	5'-ATGTCTACTGTCCACGAAATCCT-3'	5'-CGAAGTTGGTGTAGGGTTTGACT-3'
Gapdh	5'-ACAATGAATACGGCTACAGCAACAG-3’	5’-GGTGGTCCAGGGTTTCTTACTCC-3’

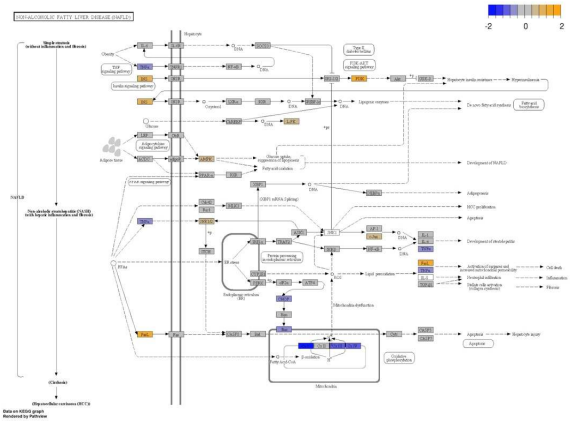
Table 4.8. Primers used for RT-qPCR.

A)



B)

HF-HF ♀ vs. LF-HF ♀



HF-LF ♀ vs. LF-LF ♀

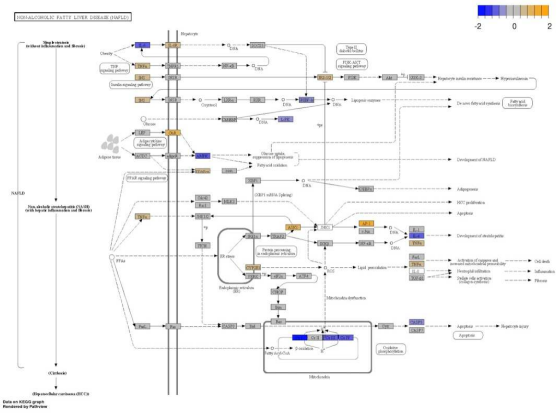
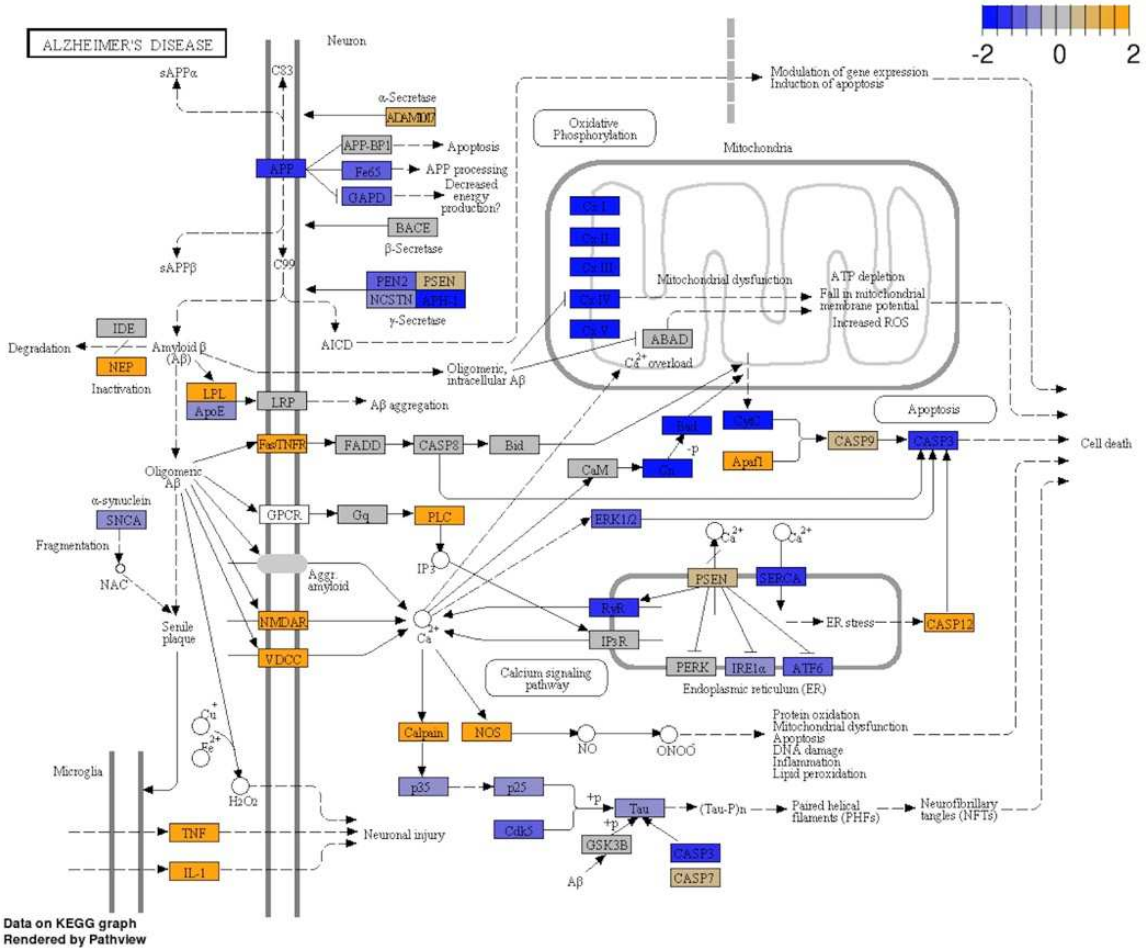


Figure 4.7. Non-alcoholic fatty liver disease (NAFLD) pathway diagrams, where orange indicates upregulation by a high-fat diet and blue indicates downregulation. (A) The NAFLD pathway is significantly downregulated due to an offspring high-fat diet. (B) It is also downregulated by a maternal high-fat diet. HF = High-fat diet, LF = Low-fat diet.

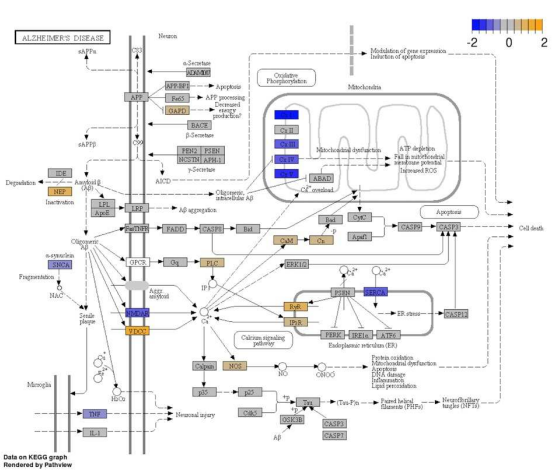
A)

Alzheimer's Disease Pathway: All HF offspring vs. all LF offspring



B)

HF-HF ♀ vs. LF-HF ♀



HF-LF ♀ vs. LF-LF ♀

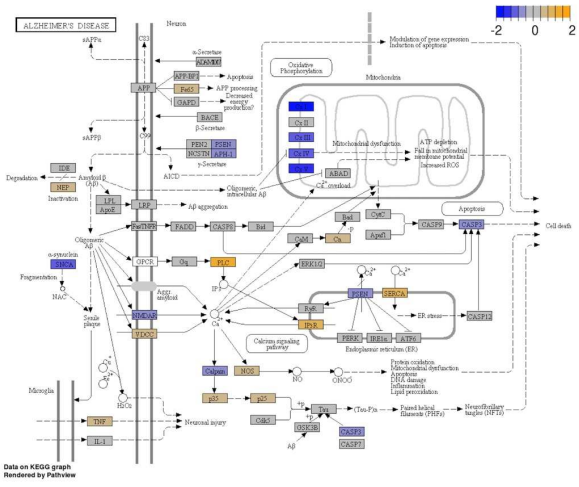


Figure 4.8. Alzheimer's disease pathway diagrams, where orange indicates upregulation by a high-fat diet and blue indicates downregulation. (A) The Alzheimer's disease pathway is significantly downregulated due to an offspring high-fat diet. (B) It is also downregulated by maternal high-fat diet. HF = High-fat diet, LF = Low-fat diet.

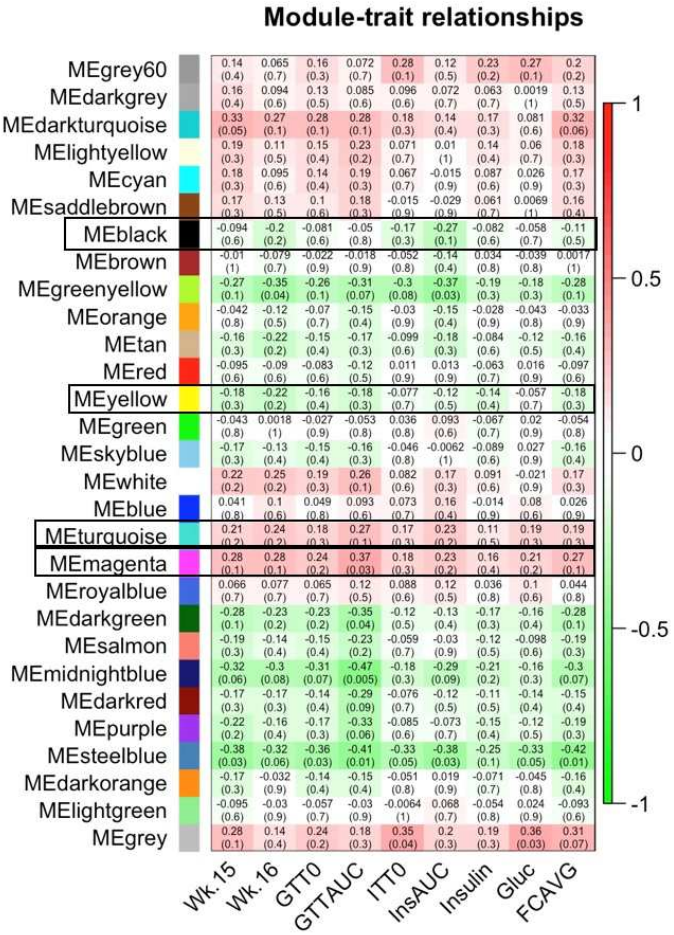


Figure 4.9. The WGCNA analysis revealed 29 modules of co-expressed genes in the offspring. Four of these modules (black, yellow, turquoise, and magenta) were significantly correlated with the diabetes-related traits: week 15 weight, week 16 weight, baseline glucose at week 15, area under the curve for the intraperitoneal glucose tolerance test, baseline glucose at week 16, area under the curve for the intraperitoneal insulin tolerance test, serum insulin and glucose, and average food consumed per day at 14 weeks of age.

Module (# genes)	Enrichment p-value	# Module Genes in GO Term	GO ID	GO Ont	GO Name
Black (637)	3.29E-19	145	GO:0002376	BP	immune system process
	9.95E-17	93	GO:0006955	BP	immune response
	2.83E-11	88	GO:0002682	BP	regulation of immune system process
	7.04E-09	83	GO:0006952	BP	defense response
	4.02E-08	59	GO:0016337	BP	single organismal cell-cell adhesion
	4.24E-07	53	GO:0046649	BP	lymphocyte activation
	5.17E-07	60	GO:0098609	BP	cell-cell adhesion
	7.73E-07	34	GO:0009897	CC	external side of plasma membrane
	1.26E-06	63	GO:0001775	BP	cell activation
	1.51E-06	58	GO:0009986	CC	cell surface
Magenta (312)	1.39E-05	16	GO:0005840	CC	ribosome
	1.75E-05	13	GO:0005761	CC	mitochondrial ribosome
	3.00E-04	12	GO:0044391	CC	ribosomal subunit
	0.003	17	GO:0005759	CC	mitochondrial matrix
	0.045	26	GO:0030529	CC	intracellular ribonucleoprotein complex
	0.046	12	GO:0003735	MF	structural constituent of ribosome
	0.355	6	GO:0005763	CC	mitochondrial small ribosomal subunit
	0.691	6	GO:0005762	CC	mitochondrial large ribosomal subunit
	0.691	6	GO:0015935	CC	small ribosomal subunit
	1	20	GO:0006412	BP	translation
Turquoise (1,785)	2.16E-12	33	GO:0070469	CC	respiratory chain
	1.02E-11	31	GO:0005746	CC	mitochondrial respiratory chain
	3.73E-08	41	GO:0005840	CC	ribosome
	1.31E-06	20	GO:0005747	CC	mitochondrial respiratory chain complex I
	1.31E-06	20	GO:0045271	CC	respiratory chain complex I
	7.96E-06	28	GO:0005761	CC	mitochondrial ribosome
	1.10E-04	14	GO:0003954	MF	NADH dehydrogenase activity
	1.47E-04	213	GO:0005739	CC	mitochondrion
	1.49E-04	16	GO:0005763	CC	mitochondrial small ribosomal subunit
	2.25E-04	35	GO:0003735	MF	structural constituent of ribosome
	4.41E-04	12	GO:0070330	MF	aromatase activity

Yellow (932)	8.36E-04	16	GO:0008395	MF	steroid hydroxylase activity
	0.001	22	GO:0004497	MF	monooxygenase activity
	0.001	12	GO:0016712	MF	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen
	0.002	12	GO:0008392	MF	arachidonic acid epoxygenase activity
	0.002	10	GO:0019373	BP	epoxygenase P450 pathway
	0.005	12	GO:0008391	MF	arachidonic acid monooxygenase activity
	0.010	13	GO:0019369	BP	arachidonic acid metabolic process
	0.032	26	GO:0016705	MF	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen
	0.108	20	GO:0004866	MF	endopeptidase inhibitor activity

Table 4.9. Modules from WGCNA.

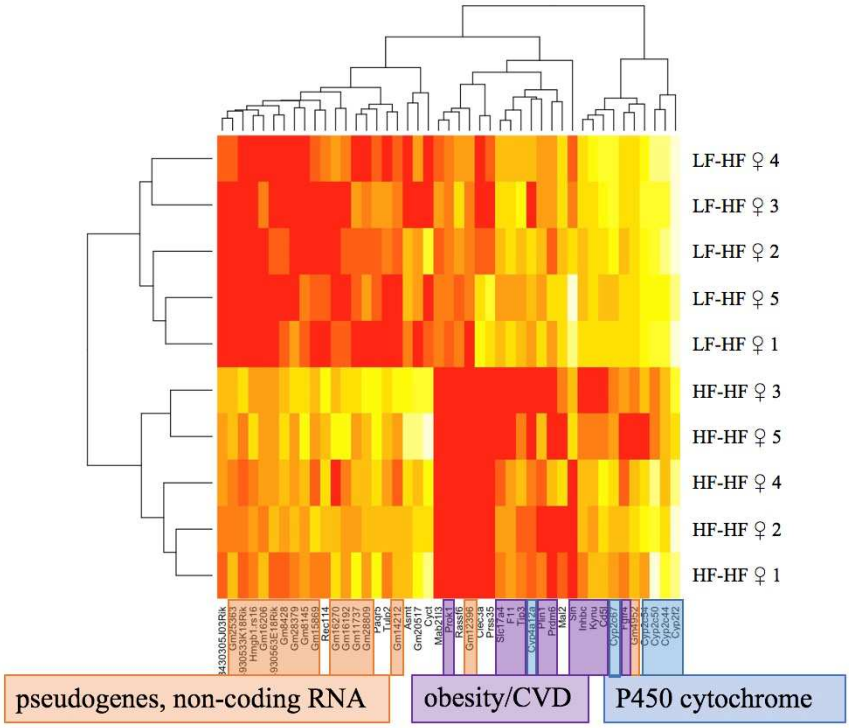


Figure 4.10. Clustering diagram of the heart gene expression libraries in the high-fat diet daughters. The 21 upregulated genes due to a maternal high-fat diet are mostly pseudogenes and non-coding RNAs. The 24 downregulated genes due to a maternal high-fat diet are primarily p450 cytochromes and genes already known to be involved in obesity and cardiovascular diseases. HF= High-fat diet, LF = Low-fat diet, first diet listed is maternal diet and second diet (after the hyphen) is the offspring diet.

Comparison	Group	p<0.05	p<0.01	p<0.001	DMRs in X (%)
Different diets	HF-HF ♀ vs. LF-HF ♀	34,844	1,701	232	79 (0.2%)
	HF-LF ♀ vs. LF-LF ♀	55,014	9,550	2,566	284 (0.5%)
	HF-HF ♂ vs. LF-HF ♂	40,437	2,262	258	28 (0.1%)
	HF-LF ♂ vs. LF-LF ♂	57,374	8,737	1,505	75 (0.1%)
Different sexes	HF-HF ♀ vs. HF-HF ♂	41,340	5,031	1,679	1,219 (3.0%)
	HF-LF ♀ vs. HF-LF ♂	66,447	14,571	4,533	1,278 (1.9%)
	LF-HF ♀ vs. LF-HF ♂	40,766	5,610	1,728	1,074 (2.6%)
	LF-LF ♀ vs. LF-LF ♂	54,304	10,201	3,048	1,185 (2.2%)

Table 4.10. Distribution of DMRs. Thousands of DMRs in the liver were associated with maternal diet. Low-fat offspring had more DMRs than high-fat offspring. There were more than twice as many DMRs on the X-chromosome when comparing between sexes than within sexes. HF= High-fat diet, LF = Low-fat diet, first diet listed is maternal diet and second diet (after the hyphen) is the offspring diet.

	Genes with ≥1 DMR in gene body	Genes with >1 DMR in gene body	Genes with ≥1 DMR in promoter
HF-HF ♀ vs. LF-HF ♀	7,367 (36.1%)	3,554 (17.4%)	1,878 (9.2%)
HF-LF ♀ vs. LF-LF ♀	9,358 (45.8%)	5,347 (26.2%)	2,724 (13.4%)
HF-HF ♂ vs. LF-HF ♂	7,980 (39.1%)	4,031 (19.8%)	2,213 (10.8%)
HF-LF ♂ vs. LF-LF ♂	9,369 (45.9%)	5,260 (25.8%)	3,254 (16.0%)

Table 4.11. Number of genes in the mouse liver with at least one differentially methylated region (DMR) due to maternal diet within the gene body, more than one DMR in the gene body, and at least one DMR in the promoter region (within 2 kb upstream of the transcription start site ($p < 0.05$). HF = High-fat diet, LF = Low-fat diet, first diet listed is maternal diet and second diet (after the hyphen) is the offspring diet.

Region	High Fat Daughters	High Fat Sons	Low Fat Daughters	Low Fat Sons	Whole Genome
Enhancer	180 (10.6%)	259 (20.3%)	1,038 (10.9%)	1,024 (11.7%)	3.5%
CTCF Binding Site	102 (6.0%)	129 (10.1%)	450 (4.7%)	614 (7.0%)	1.7%
TF binding site	16 (0.9%)	16 (1.3%)	62 (0.6%)	93 (1.1%)	0.3%
Promoter Flanking Region	549 (32.3%)	637 (49.9%)	3,625 (38.0%)	2,729 (31.2%)	8.1%
Promoter	251 (14.7%)	311 (13.8%)	1,366 (14.3%)	1,393 (15.9%)	4.5%
Exon	395 (23.2%)	528 (23.4%)	2,651 (27.8%)	1,958 (22.4%)	7.5%
Intergenic	616 (36.2%)	807 (35.7%)	2,870 (30.0%)	3,037 (34.8%)	58.6%

Table 4.12. Distribution of significant differentially methylated regions (DMRs) ($p < 0.01$) across the genome due to maternal diet. Values indicate the number of 500 base-pair windows overlapping each genomic region, with the percent of the total significant DMRs overlapping these regions in parentheses for the sons and daughters. As a comparison, the percentage of windows across the whole genome that overlap these genomic regions is listed, demonstrating how overrepresented these regulatory regions are in the DMRs.

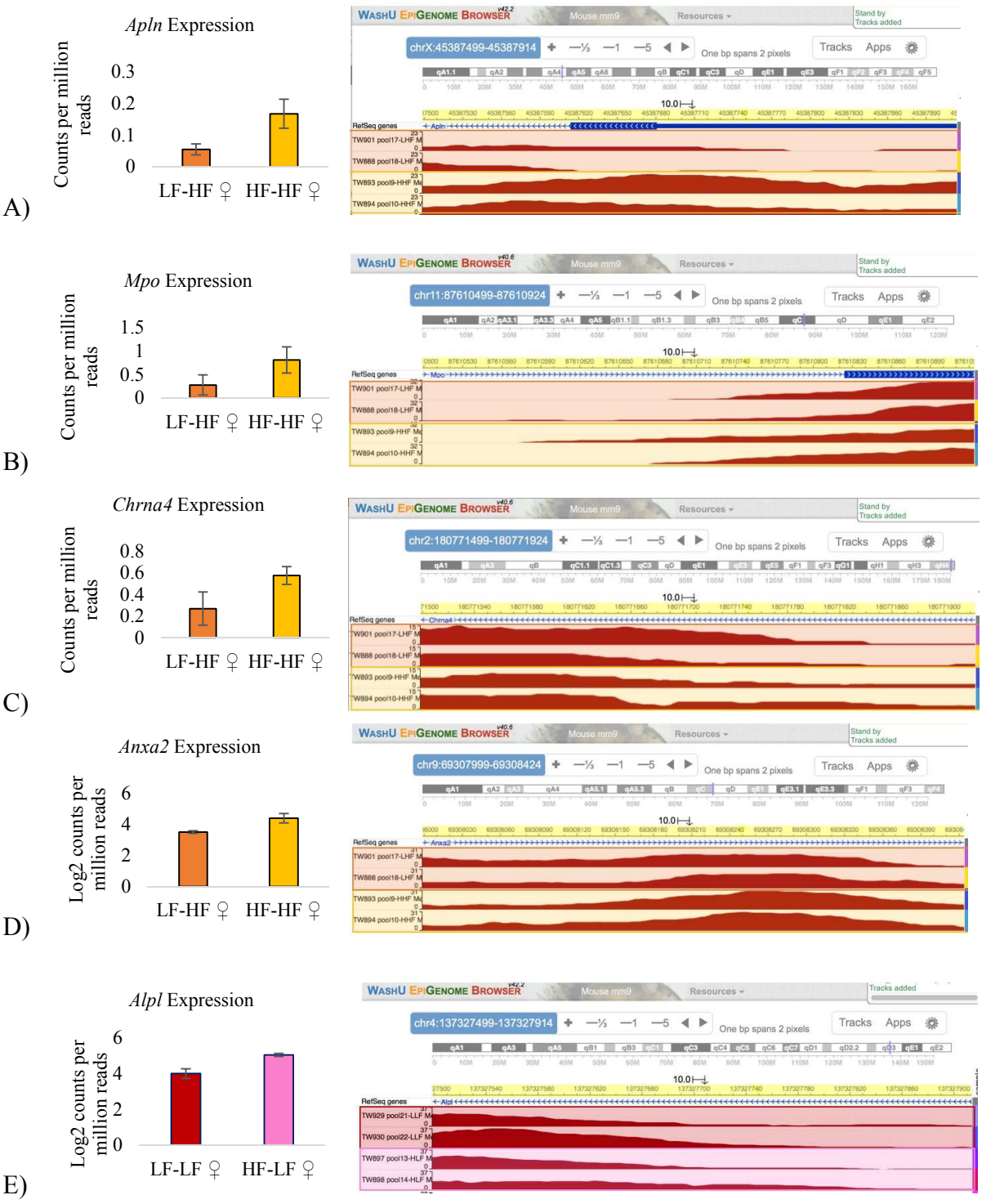
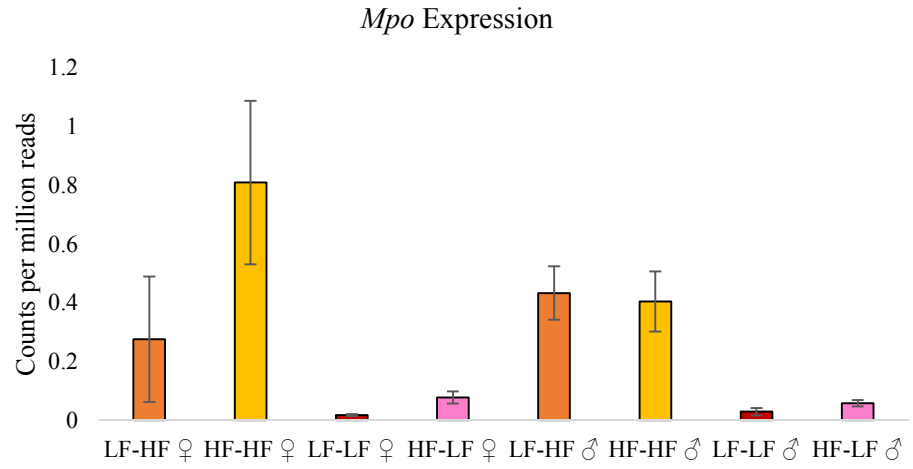
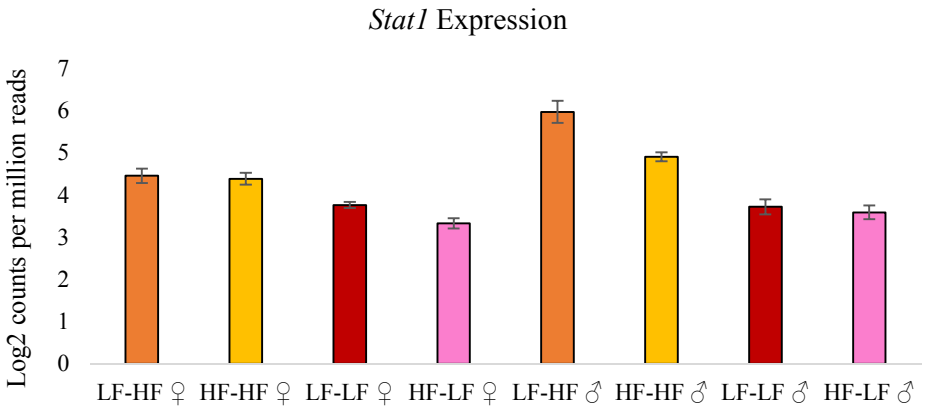


Figure 4.11. Examples of differentially expressed genes that have differentially methylated regions within them due to maternal diet. The WashU Epigenome Browser screenshots indicate amount of methylation from MeDIP-seq. HF = High-fat diet, LF = Low-fat diet, and error bars represent ± the standard error.

A)



B)



C)

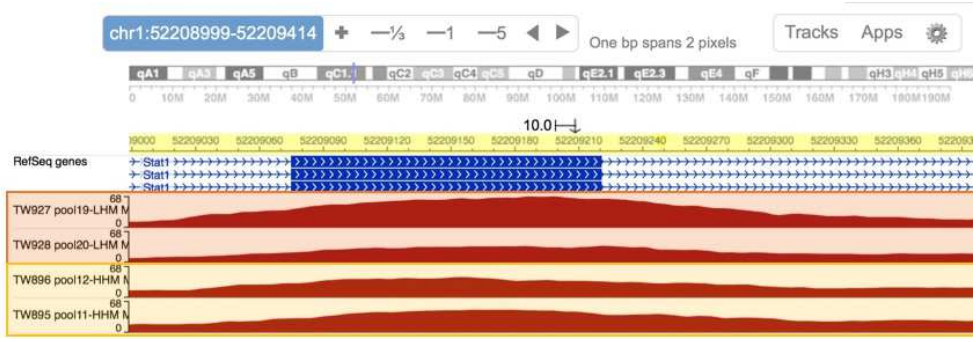


Figure 4.12. (A) Expression of the *Mpo* gene is much higher in high fat offspring than in low fat offspring. (B) *Stat1* expression is also higher in offspring on a high-fat diet than those on a low-fat diet. (C) Compared to high fat sons of low fat mothers, high fat sons of high fat mothers have reduced methylation of the *Stat1* gene. HF = High-fat diet, LF = Low-fat diet, and error bars represent ± the standard error.

Gene	Fold Difference of HF-HF vs. LF-HF daughters
Anxa2	2.85 ± 1.21
Chrna4	5.84 ± 2.09
Mpo	12.96 ± 4.70

Table 4.13. RT-qPCR validation results. Values are presented as HF-HF expression relative to LF-HF expression levels. Fold differences were calculated with the ΔΔCT method and reported as a range to include the standard deviation. HF= High Fat, LF= Low Fat.

Gene Name	**logFC**	**P-value**	**Expression HF-HF**	**Expression LF-HF**	**SE HF-HF**	**SE LF-HF**	**Known Disease Involvement**
Lmo1	3.065	3.24E-04	-0.34	-3.38	0.86	0.77	
Mpo	2.790	2.47E-04	-0.65	-3.41	0.62	1.01	Obesity, Diabetes, CVD
Sult2a3	2.783	8.23E-03	1.78	-1.21	1.03	0.74	
Lgr6	2.739	3.05E-04	-2.79	-5.56	0.41	0.53	
Ngp	2.686	5.02E-03	0.19	-2.42	0.88	0.94	
Sult3a2	2.655	4.99E-05	4.07	1.29	0.73	0.30	
Gm5210	2.504	2.80E-04	-2.95	-5.39	0.33	0.27	
Srpk3	-2.482	6.75E-05	-4.61	-2.14	0.53	0.29	
A4gnt	2.471	1.79E-02	-0.20	-2.55	0.70	0.69	
Slc9b1	2.457	3.54E-04	-3.51	-6.03	0.30	0.20	
Camp	2.455	7.65E-03	-2.57	-4.89	1.05	0.83	
Gm15540	-2.355	4.87E-04	-5.19	-2.93	0.22	0.21	
Gm17022	-2.344	3.16E-04	-5.00	-2.69	0.40	0.28	
Aplnr	2.329	2.17E-04	-1.81	-4.07	0.17	0.66	
Ltf	2.329	2.54E-02	-1.05	-3.30	0.76	0.95	Obesity, Diabetes, CVD
Gm26937	2.312	2.15E-03	-3.42	-5.71	0.78	0.17	
Adam1a	-2.292	6.94E-05	-5.00	-2.71	0.40	0.32	
Kat6b-ps2	2.288	1.01E-04	-2.09	-4.46	0.43	0.48	
Gm17229	2.274	2.64E-03	-3.32	-5.71	0.30	0.39	
Sult3a1	2.269	2.39E-05	6.69	4.32	0.55	0.43	
Cd177	2.251	1.12E-03	-1.91	-4.09	0.42	0.69	
Gm16731	-2.244	8.13E-04	-4.61	-2.28	0.68	0.11	
Il13ra2	-2.242	2.14E-04	-5.19	-3.06	0.22	0.41	
Igkv4-74	2.236	4.11E-03	-2.29	-4.61	0.67	0.58	
2610507I01Rik	-2.229	7.10E-04	-4.79	-2.52	0.36	0.21	
Gm23388	2.210	1.22E-03	-2.41	-4.61	0.18	0.51	
Pla2g4f	2.196	5.01E-03	-0.94	-3.12	0.93	0.78	
Hao2	2.143	1.05E-04	3.06	0.81	0.60	0.28	
Gm23935	2.141	3.89E-04	9.02	6.85	0.13	0.19	
Ltk	-2.140	2.18E-04	-4.79	-2.50	0.59	0.14	

Gm15344	-2.135	3.30E-03	-5.19	-2.76	0.48	0.62	
Mcmdc2	2.110	2.42E-03	-1.98	-3.99	0.14	0.72	
Pcdh11x	2.107	1.71E-03	-1.91	-4.05	0.25	0.55	
Cap2	2.107	8.12E-03	-3.46	-5.71	0.93	0.40	
5430416N02Rik	-2.103	3.30E-04	-3.90	-1.82	0.86	0.21	
Gm4956	-2.102	4.36E-04	-2.32	-0.12	0.28	0.42	
Gm4419	-2.081	3.13E-03	-5.58	-3.43	0.18	0.31	
Mybl2	-2.070	2.61E-04	-4.61	-2.60	0.45	0.35	
mt-Rnr2	2.054	3.45E-04	11.83	9.75	0.18	0.34	
Tmem167-ps1	2.043	1.81E-03	-1.62	-3.72	0.65	0.51	
Gm20125	2.038	2.44E-03	-2.93	-4.83	0.32	0.38	
Mir6236	2.035	1.04E-03	10.84	8.78	0.14	0.26	
Gm28323	-2.030	1.65E-03	-5.19	-3.17	0.22	0.37	
Gm16172	-2.027	4.64E-03	-4.61	-2.62	0.45	0.37	
Chrna4	2.027	2.33E-02	-0.84	-2.73	0.21	0.75	
Erbb4	2.006	6.11E-06	0.52	-1.49	0.46	0.15	Obesity, Diabetes, CVD

Table 4.14. Differentially expressed genes due to maternal diet for high-fat-fed daughters (liver).

Gene Name	logFC	P-value	Expression HF-HF	Expression LF-HF	SE HF-HF	SE LF-HF	Known Disease Involvement
Hmgb1-rs16	2.510	2.38E-06	-2.89	-5.40	0.17	0.06	
Rassf6	-2.807	3.17E-06	-5.48	-2.67	0.04	0.33	
Mab21l3	-2.106	2.30E-05	-5.48	-3.37	0.04	0.20	
Prok1	-2.106	2.55E-05	-5.48	-3.37	0.04	0.21	Diabetes, CVD
Paqr5	2.436	3.70E-05	-1.54	-3.98	0.18	0.41	
Gm25363	2.292	4.23E-05	-2.79	-5.08	0.14	0.31	
Gm16206	2.190	1.05E-04	-2.89	-5.08	0.19	0.32	
4930533K18Rik	2.029	1.52E-04	-3.37	-5.40	0.35	0.06	
Gm8145	2.371	2.49E-04	-2.25	-4.62	0.18	0.52	
B430305J03Rik	1.974	2.96E-04	-3.11	-5.08	0.22	0.31	
Mal2	-2.699	3.38E-04	-4.03	-1.32	0.76	0.19	
Rec114	2.483	3.40E-04	-2.13	-4.62	0.35	0.51	
4930563E18Rik	2.101	3.89E-04	-2.98	-5.08	0.33	0.32	
Gm8428	2.132	4.43E-04	-2.64	-4.77	0.33	0.36	
Gm20517	2.438	7.29E-04	-1.21	-3.64	0.21	0.75	
Gm28379	2.240	7.67E-04	-2.60	-4.84	0.26	0.53	
Slc17a4	-2.333	8.04E-04	-4.06	-1.74	0.67	0.21	CVD
Gm16192	1.973	8.09E-04	-2.35	-4.30	0.24	0.43	
Tjp3	-1.984	8.43E-04	-3.75	-1.76	0.55	0.09	
Cyct	3.146	1.16E-03	-0.75	-3.89	0.26	1.15	
Gm11737	2.112	1.25E-03	-2.18	-4.30	0.34	0.50	
Plin1	-2.198	1.32E-03	-4.14	-1.95	0.58	0.33	Obesity, Diabetes
Cyp2c54	-2.332	1.40E-03	-2.55	-0.25	0.83	0.21	
Gm12396	-1.959	1.47E-03	-5.48	-3.52	0.04	0.56	
Kynu	-2.334	1.47E-03	-3.26	-0.94	0.67	0.36	CVD
Cyp2c67	-2.077	1.59E-03	-2.61	-0.58	0.47	0.39	
Cyp2f2	-1.983	1.62E-03	-0.25	1.66	0.57	0.36	
Fgfr4	-2.121	1.67E-03	-3.49	-1.37	0.62	0.30	CVD
Gm14212	1.971	1.93E-03	-2.48	-4.45	0.44	0.38	
Cyp2c44	-2.494	2.09E-03	-1.83	0.61	0.48	0.52	
Gm28809	1.984	2.36E-03	-2.21	-4.20	0.23	0.59	
Gm15869	1.977	2.37E-03	-2.78	-4.77	0.43	0.42	
Tulp2	1.962	2.52E-03	-2.51	-4.47	0.22	0.57	
F11	-2.163	3.06E-03	-3.72	-1.58	0.76	0.27	Diabetes, CVD
Inhbc	-1.986	3.81E-03	-3.32	-1.34	0.69	0.29	Obesity, CVD
Prss35	-2.715	3.87E-03	-5.48	-2.76	0.04	1.04	
Asmt	2.037	5.86E-03	-1.48	-3.50	0.21	0.83	
Gm4952	-1.961	6.47E-03	-3.02	-1.06	0.77	0.30	

Prdm6	-2.170	6.66E-03	-4.79	-2.61	0.71	0.45	CVD
Cd5l	-1.972	1.34E-02	-2.81	-0.87	0.86	0.40	CVD
Clec3a	-2.255	1.41E-02	-5.48	-3.22	0.04	1.07	
Gm16270	2.006	1.46E-02	-2.54	-4.52	0.73	0.54	
Cyp4a12a	-2.099	1.62E-02	-4.38	-2.26	0.50	0.82	
Cyp2c50	-2.063	1.65E-02	-1.62	0.36	0.97	0.44	
Sln	-3.061	2.38E-02	-4.15	-1.05	0.78	1.21	Diabetes, CVD

Table 4.15. Differentially expressed genes due to maternal diet for high-fat-fed daughters (heart).

Gene Name	logFC	P-value	Expression HF-LF	Expression LF-LF	SE HF-LF	SE LF-LF	Known Disease Involvement
Dmbt1	3.529	4.21E-02	1.04	-2.09	0.97	0.96	
Gm11454	-3.475	2.98E-07	-4.41	-0.96	0.44	0.53	Obesity, Diabetes, CVD
Sftpa1	3.256	8.08E-05	-1.42	-4.54	0.67	0.51	
Gucy2e	3.108	1.10E-05	-1.83	-5.15	0.21	0.58	
Hmmr	-3.081	2.36E-04	-4.04	-0.82	0.63	0.54	
Cdc20	-3.054	4.90E-05	-2.94	0.14	0.52	0.23	
Pdk4	3.023	3.63E-05	5.37	2.40	0.53	0.18	
Rpl3l	2.940	1.57E-05	-2.54	-5.55	0.52	0.35	
Arhgap8	2.920	2.60E-06	-2.90	-5.95	0.37	0.31	
Zfp811	2.835	4.81E-05	-3.01	-5.95	0.34	0.31	
Lockd	-2.772	4.49E-06	-5.20	-2.34	0.23	0.36	
Trpv3	2.768	1.11E-04	-3.12	-5.95	0.25	0.31	
Ppp1r3g	2.720	2.84E-04	5.68	3.03	0.55	0.76	
Vpreb3	2.707	2.22E-05	1.18	-1.54	0.29	0.56	Obesity, CVD
Cytl1	2.706	3.66E-04	-2.33	-5.15	0.25	0.54	Obesity, Diabetes, CVD
Gm28644	2.659	3.76E-04	-3.17	-5.95	0.33	0.31	
Dsg1a	2.633	6.76E-06	-2.28	-4.97	0.17	0.57	
Cdkn3	-2.617	7.59E-06	-4.73	-2.08	0.47	0.39	
Gcsam	2.614	4.63E-05	-3.24	-5.95	0.22	0.31	
Ccnb2	-2.611	3.54E-03	-3.32	-0.48	0.73	0.64	
Gngt1	-2.608	1.90E-05	-4.24	-1.62	0.31	0.27	
Cenpf	-2.575	2.30E-04	-3.63	-0.98	0.56	0.33	
Gm10032	2.571	6.68E-04	-3.29	-5.95	0.58	0.31	CVD
Kifc1	-2.559	5.06E-05	-4.88	-2.28	0.41	0.39	
Rapgef4os2	2.465	2.52E-04	-0.77	-3.02	0.12	0.67	
Gm10787	2.414	2.94E-04	-2.53	-5.15	0.51	0.58	
Fam69b	2.362	9.33E-05	-2.67	-4.97	0.27	0.31	Diabetes, CVD
Mmrn1	2.361	3.67E-04	-2.48	-4.97	0.47	0.64	
Gm11832	-2.309	3.48E-06	-2.66	-0.38	0.39	0.42	
Egfros	2.306	2.35E-02	-1.39	-3.39	0.58	1.38	
Hapln1	-2.294	2.15E-03	-1.43	0.60	0.18	0.82	
Gm26744	2.271	9.07E-04	-2.77	-5.37	0.30	0.78	
Ppbp	2.256	1.88E-03	-1.75	-4.06	0.31	0.87	
Pnpla5	-2.246	2.55E-06	-1.56	0.71	0.48	0.34	
Ckap2	-2.244	1.12E-03	-3.57	-1.13	0.66	0.55	
Sytl3	2.239	1.31E-03	-3.63	-5.95	0.16	0.31	
Spdye4c	2.223	4.18E-04	-3.63	-5.95	0.50	0.31	
Rmi2	-2.210	1.67E-03	-4.73	-2.61	0.39	0.25	

Rasd1	2.206	4.73E-04	-0.95	-3.18	0.47	0.25	
Osbpl3	2.202	3.70E-03	1.95	-0.08	0.44	0.64	
Snora78	2.202	2.48E-03	-2.84	-4.97	0.38	0.29	
A530013C23Rik	2.201	6.79E-04	-1.05	-3.37	0.57	0.73	
Atp2b2	-2.194	4.42E-06	0.47	2.71	0.34	0.27	Obesity, CVD
Gm12186	2.194	1.19E-03	-3.68	-5.95	0.30	0.31	Obesity, Diabetes, CVD
Meox1	-2.193	1.76E-04	-3.80	-1.57	0.47	0.11	
4930509G22Rik	2.185	5.85E-04	-2.91	-4.97	0.19	0.29	
Dgki	2.179	2.28E-04	-3.68	-5.95	0.24	0.31	
Gm11914	2.125	1.95E-03	-3.17	-5.24	0.24	0.56	
Klhl32	2.124	1.36E-03	-3.01	-5.02	0.26	0.77	
Gm12353	2.119	3.98E-03	-3.39	-5.55	0.29	0.35	
Gm20404	2.110	4.73E-03	-3.17	-5.55	0.34	0.61	
Gm12735	2.103	1.87E-03	-2.99	-4.97	0.34	0.31	
Acot5	2.100	2.16E-04	1.55	-0.55	0.19	0.50	
Gm17597	2.095	6.66E-04	-2.80	-4.79	0.33	0.37	
Gm15785	2.091	5.56E-04	-3.78	-5.95	0.16	0.31	
Arntl	-2.076	3.40E-04	-0.31	1.82	0.36	0.49	
Pnpla3	-2.067	3.22E-04	1.98	4.04	0.17	0.59	
Ighv3-1	2.066	9.25E-04	-3.85	-5.95	0.57	0.31	
Gm13855	-2.062	8.22E-06	-2.02	-0.03	0.33	0.24	
Apitd1	-2.058	4.20E-04	-3.56	-1.42	0.46	0.21	
Gm16291	2.043	6.41E-04	-0.45	-2.31	0.12	0.69	
2310040G07Rik	2.031	6.28E-03	-3.49	-5.55	0.25	0.32	
Arhgef39	-2.029	8.15E-04	-4.24	-2.08	0.63	0.38	
Lrrtm3	2.028	2.18E-03	-3.49	-5.55	0.21	0.35	
Sag	2.023	4.54E-04	-3.85	-5.95	0.47	0.31	
Gm11205	2.018	3.84E-03	-3.49	-5.55	0.25	0.35	
Ephx4	2.014	2.63E-03	-3.85	-5.95	0.45	0.31	
Gm6114	-2.006	2.40E-03	-5.20	-3.11	0.40	0.21	
Scn3b	2.005	4.95E-03	-3.39	-5.37	0.15	0.46	
Unc79	2.005	2.39E-04	1.97	-0.06	0.30	0.65	

Table 4.16. Differentially expressed genes due to maternal diet for low-fat-fed daughters.

Gene Name	logFC	P-value	Expression HF-HF	Expression LF-HF	SE HF-HF	SE LF-HF	Known Disease Involvement
Myh6	2.679	0.0420	-3.23	-6.05	1.37	0.32	Diabetes, CVD
Unc79	2.590	0.0166	-1.38	-3.79	0.41	1.04	
BC043934	2.468	0.0004	-3.00	-5.34	0.28	0.47	
Scarna13	2.450	0.0009	-2.77	-5.07	0.22	0.73	
Hsd17b1	2.445	0.0002	-1.86	-4.20	0.29	0.79	Obesity, Diabetes
Mb	2.336	0.0200	-3.63	-6.05	1.13	0.32	
D830044D21Rik	2.327	0.0014	-3.33	-5.65	0.24	0.22	
E230001N04Rik	2.309	0.0025	-1.98	-4.20	0.41	0.55	
Slc22a29	-2.225	0.0007	-5.45	-3.35	0.10	0.71	
Myl2	2.191	0.0073	-3.78	-6.05	1.04	0.32	Obesity, Diabetes, CVD
Ryr2	2.161	0.0048	-3.49	-5.65	0.67	0.22	Diabetes, CVD
Slc22a27	-2.153	0.0463	-3.72	-1.74	0.86	0.66	
Kcne3	-2.136	0.0013	-4.82	-2.56	0.71	0.15	Diabetes, CVD
Tssk4	2.128	0.0023	-2.29	-4.39	0.27	0.43	
Gm12168	-2.114	0.0002	-4.35	-2.25	0.45	0.19	
A430093F15Rik	2.098	0.0112	-2.81	-4.76	0.29	0.50	
4933406C10Rik	2.092	0.0025	-3.34	-5.34	0.46	0.47	
Gprasp2	-2.092	0.0009	-4.99	-2.91	0.42	0.34	
Wfdc3	2.083	0.0021	-3.08	-5.07	0.24	0.28	
Pnck	2.054	0.0081	-3.11	-5.07	0.27	0.28	
Rcor2	2.046	0.0062	-2.99	-5.25	0.35	0.65	
Omp	-2.034	0.0001	-4.99	-2.91	0.44	0.32	Diabetes, CVD

Table 4.17. Differentially expressed genes due to maternal diet for high-fat-fed sons.

Gene Name	logFC	P-value	Expression HF-LF	Expression LF-LF	SE HF-LF	SE LF-LF	Known Disease Involvement
Mb	5.527	2.72E-07	-1.33	-6.56	1.50	0.26	
Myh6	4.375	2.53E-03	-1.30	-5.19	1.95	0.38	Diabetes, CVD
Myl2	4.082	1.39E-05	-2.61	-6.56	1.19	0.26	Obesity, Diabetes, CVD
Tnni3	3.769	3.51E-05	-2.47	-6.16	0.89	0.43	Obesity, Diabetes, CVD
Xirp2	3.560	2.10E-06	-2.95	-6.56	1.02	0.26	
Ckmt2	3.364	5.83E-07	-3.08	-6.56	1.00	0.26	
Actn2	3.297	2.53E-06	-2.85	-6.16	1.05	0.43	Obesity, CVD
Eef1a2	3.225	1.72E-04	-2.85	-5.98	1.05	0.42	
Myh7	3.201	6.17E-05	-3.21	-6.56	0.86	0.26	CVD
Gm15473	3.097	1.08E-07	-3.42	-6.56	0.52	0.26	
Mybpc3	3.042	2.22E-03	-2.41	-5.19	1.30	0.38	Diabetes, CVD
Gm11991	3.007	4.05E-06	-3.21	-6.16	0.31	0.26	
Tcap	2.984	4.07E-04	-2.87	-5.77	1.05	0.29	CVD
Cox8b	2.963	3.96E-05	-3.21	-6.16	0.61	0.26	
Myoz2	2.856	6.74E-06	-3.53	-6.56	0.70	0.26	CVD
Actc1	2.856	4.72E-02	-1.57	-3.99	1.79	0.21	Diabetes, CVD
Gm6881	2.838	9.48E-05	-3.42	-6.16	0.65	0.26	
Mfap5	2.819	6.85E-04	-3.32	-6.16	0.61	0.26	CVD
Ckm	2.758	1.01E-04	-3.30	-6.16	0.82	0.26	Diabetes, CVD
Dlk2	2.751	4.33E-05	-3.02	-5.77	0.27	0.38	
Gm24187	2.746	1.10E-03	-3.30	-6.16	0.58	0.50	
Dpysl5	2.741	3.87E-04	-3.48	-6.16	0.72	0.43	
Rad54l	-2.741	1.00E-06	-4.97	-2.08	0.18	0.41	CVD
Gm25381	2.724	8.85E-07	-3.79	-6.56	0.35	0.26	
Gm7719	2.724	8.47E-05	-3.79	-6.56	0.35	0.26	
Gm5533	2.705	7.00E-07	-4.00	-6.56	0.76	0.26	
Adprhl1	2.698	1.60E-05	-3.69	-6.56	0.57	0.26	
Gm29155	2.693	5.24E-04	-3.39	-6.16	0.18	0.50	
BC100451	2.686	3.09E-04	-3.48	-6.16	0.72	0.50	
Fabp3	2.685	1.11E-03	-3.01	-5.77	0.62	0.51	Obesity, Diabetes, CVD
Gm9167	2.683	1.89E-03	-2.72	-5.37	0.30	0.23	
Nppb	2.678	8.22E-07	-3.71	-6.56	0.67	0.26	Obesity, Diabetes, CVD
Hsd11b2	2.675	1.80E-03	-2.99	-5.50	0.53	0.88	Diabetes, CVD
Ryr2	2.669	2.09E-03	-2.89	-5.58	1.05	0.63	Diabetes, CVD
Gm23628	2.625	1.27E-04	-3.88	-6.56	0.65	0.26	
Aox4	2.601	3.48E-07	-4.00	-6.56	0.65	0.26	
Krt222	2.595	1.16E-03	-2.90	-5.40	0.39	0.48	

Gm17059	2.585	5.81E-05	-3.81	-6.56	0.49	0.26	
Gm12717	2.582	2.63E-04	-3.39	-5.86	0.18	0.54	
Gm7803	2.569	9.97E-06	-4.00	-6.56	0.63	0.26	
Cox6a2	2.565	3.00E-03	-1.94	-4.39	0.70	0.62	
Gm6430	2.542	9.61E-05	-4.00	-6.56	0.59	0.26	
Slc9b1	2.542	9.96E-05	-4.00	-6.56	0.59	0.26	
Gm14276	-2.530	1.98E-06	-4.97	-2.36	0.18	0.31	
Sgcg	2.527	2.13E-05	-3.88	-6.56	0.52	0.26	Diabetes
Platr9	2.521	1.26E-04	-4.00	-6.56	0.57	0.26	
Gm11127	2.504	1.97E-03	-3.48	-5.86	0.62	0.54	
Gm11954	2.502	2.79E-07	-4.18	-6.56	0.64	0.26	
Gm13773	2.502	2.80E-07	-4.18	-6.56	0.64	0.26	
4933402J07Rik	2.502	2.08E-06	-4.18	-6.56	0.64	0.26	
Gm28155	2.502	1.56E-05	-4.18	-6.56	0.64	0.26	
Adcy8	2.502	2.59E-05	-4.18	-6.56	0.64	0.26	
Kcnb2	2.502	1.43E-04	-4.18	-6.56	0.64	0.26	CVD
Trim63	2.496	1.12E-04	-3.39	-5.86	0.53	0.54	CVD
Gm13094	2.483	6.88E-04	-3.60	-6.16	0.41	0.43	
4930451E10Rik	2.483	2.96E-04	-3.60	-6.16	0.41	0.43	
Sult6b2	2.479	9.28E-06	-4.18	-6.56	0.89	0.26	
Ankrd1	2.467	2.23E-04	-1.28	-3.70	0.51	0.38	CVD
H2-Ob	-2.463	3.74E-05	-4.58	-2.26	0.33	0.45	
Figf	-2.456	1.30E-04	-4.27	-1.96	0.62	0.45	Obesity, Diabetes
Gm13472	2.423	1.23E-04	-3.81	-6.16	0.71	0.26	
Ppp1r14c	2.417	2.11E-05	-4.00	-6.56	0.42	0.26	CVD
Tlx2	2.417	7.77E-05	-4.00	-6.56	0.42	0.26	
Gm13827	2.417	1.02E-04	-4.00	-6.56	0.42	0.26	
Gm2541	2.417	5.45E-06	-4.00	-6.56	0.42	0.26	
Abra	2.417	1.03E-04	-4.00	-6.56	0.42	0.26	
Gramd2	2.417	5.66E-04	-4.00	-6.56	0.42	0.26	
Fbxo15	2.414	2.54E-03	-3.21	-5.77	0.17	0.54	
Hist1h2ba	2.406	1.10E-04	-3.88	-6.16	0.76	0.26	
Chst4	2.401	2.26E-03	-3.60	-5.86	0.56	0.54	
Ccnb2	-2.392	8.32E-03	-3.60	-1.06	0.79	0.57	
Gm15946	2.390	5.81E-07	-4.18	-6.56	0.50	0.26	
Rps29-ps	2.390	4.26E-06	-4.18	-6.56	0.50	0.26	
Csmd2	2.390	1.97E-05	-4.18	-6.56	0.50	0.26	Diabetes, CVD
Tmem198	2.390	7.97E-05	-4.18	-6.56	0.50	0.26	
Gm11295	2.390	2.62E-04	-4.18	-6.56	0.50	0.26	
Mcpt8	2.389	3.47E-06	-4.18	-6.56	0.50	0.26	

Gm14323	2.389	4.31E-06	-4.18	-6.56	0.50	0.26	
Sh2d6	2.389	5.51E-05	-4.18	-6.56	0.50	0.26	
1700105P06Rik	2.389	6.03E-05	-4.18	-6.56	0.50	0.26	
4930578C19Rik	2.389	1.03E-04	-4.18	-6.56	0.50	0.26	
Gm7363	2.389	2.91E-04	-4.18	-6.56	0.50	0.26	
Btn1a1	2.366	1.15E-05	-4.18	-6.56	0.48	0.26	
Gm15157	2.366	2.07E-05	-4.18	-6.56	0.48	0.26	
Gm26448	2.366	3.57E-05	-4.18	-6.56	0.48	0.26	
Gm13842	2.366	9.15E-05	-4.18	-6.56	0.48	0.26	
Gm11646	2.366	1.01E-04	-4.18	-6.56	0.48	0.26	
Gm15992	2.366	1.63E-04	-4.18	-6.56	0.48	0.26	
Scarna3b	2.366	1.67E-04	-4.18	-6.56	0.48	0.26	
Gm13000	2.366	1.72E-06	-4.18	-6.56	0.48	0.26	
Gm12321	2.366	2.53E-06	-4.18	-6.56	0.48	0.26	
Muc5b	2.366	1.80E-05	-4.18	-6.56	0.48	0.26	
Gm13809	2.366	2.10E-05	-4.18	-6.56	0.48	0.26	
Cfap52	2.366	3.39E-05	-4.18	-6.56	0.48	0.26	
Gm11760	2.358	1.19E-04	-3.79	-6.16	0.52	0.50	
Hist1h2bb	2.358	3.38E-04	-3.79	-6.16	0.52	0.50	
Gm10284	2.349	2.91E-03	-3.08	-5.46	0.42	0.73	
Pcdhb6	2.341	1.17E-03	-3.79	-5.98	0.52	0.42	
Gm18284	2.336	4.10E-03	-3.21	-5.46	0.17	0.53	
Rpl21-ps12	2.310	4.80E-03	-3.21	-5.77	0.17	0.69	
Gm5871	2.299	5.61E-05	-3.79	-6.16	0.31	0.43	
Cdh19	2.294	1.43E-02	-2.74	-5.19	0.59	0.82	
Trbv13-1	2.292	4.62E-03	-3.71	-5.98	0.77	0.42	
1700034E13Rik	2.288	2.99E-06	-4.39	-6.56	0.71	0.26	
4930570D08Rik	2.288	2.99E-06	-4.39	-6.56	0.71	0.26	
1700018B08Rik	2.288	7.61E-06	-4.39	-6.56	0.71	0.26	
Fscn3	2.288	3.71E-05	-4.39	-6.56	0.71	0.26	
Ighv1-18	2.288	9.48E-05	-4.39	-6.56	0.71	0.26	
1700013G24Rik	2.288	1.11E-04	-4.39	-6.56	0.71	0.26	
Sftpc	2.288	1.28E-04	-4.39	-6.56	0.71	0.26	
Malrd1	2.288	2.10E-04	-4.39	-6.56	0.71	0.26	
Hrc	2.285	5.98E-03	-3.02	-5.58	0.95	0.78	CVD
Gm9144	2.279	1.12E-02	-3.23	-5.40	0.70	0.57	
Frmpd4	2.277	5.45E-05	-3.81	-5.98	0.71	0.42	Diabetes, CVD
Gm13620	2.270	1.86E-03	-4.00	-6.16	0.65	0.26	
1700048M11Rik	2.265	3.16E-03	-3.79	-6.16	0.73	0.50	
Txlnb	2.258	2.19E-03	-2.16	-4.30	0.36	0.43	

Tnni3k	2.253	1.89E-07	-4.18	-6.56	0.28	0.26	Obesity, Diabetes, CVD
Foxd3	2.253	1.89E-07	-4.18	-6.56	0.28	0.26	Diabetes, CVD
Gm15812	2.253	1.89E-07	-4.18	-6.56	0.28	0.26	
Nppa	2.253	2.61E-07	-4.18	-6.56	0.28	0.26	Obesity, Diabetes, CVD
Gm12400	2.253	2.34E-06	-4.18	-6.56	0.28	0.26	
Gm22980	2.253	2.44E-06	-4.18	-6.56	0.28	0.26	
Olfr520	2.253	5.02E-06	-4.18	-6.56	0.28	0.26	
C230012O17Rik	2.253	1.35E-05	-4.18	-6.56	0.28	0.26	
Gm25732	2.253	1.55E-05	-4.18	-6.56	0.28	0.26	
Hist1h2ad	2.253	1.86E-05	-4.18	-6.56	0.28	0.26	
Gm14051	2.253	2.13E-05	-4.18	-6.56	0.28	0.26	
Gm13937	2.253	3.58E-05	-4.18	-6.56	0.28	0.26	
Psd2	2.253	3.83E-05	-4.18	-6.56	0.28	0.26	
Gm11639	2.253	4.02E-05	-4.18	-6.56	0.28	0.26	
Gm12098	2.253	4.83E-05	-4.18	-6.56	0.28	0.26	
Scube2	2.253	7.91E-05	-4.18	-6.56	0.28	0.26	CVD
Gm26517	2.253	2.12E-04	-4.18	-6.56	0.28	0.26	
Gm22455	2.253	2.60E-04	-4.18	-6.56	0.28	0.26	
Ccdc85a	2.253	3.38E-04	-4.18	-6.56	0.28	0.26	Obesity
Mir1960	2.253	3.48E-04	-4.18	-6.56	0.28	0.26	
Gm13816	2.253	3.51E-04	-4.18	-6.56	0.28	0.26	
H2-Ke6	2.250	3.77E-03	-3.88	-5.86	0.84	0.54	
Enthd1	2.250	2.80E-06	-4.39	-6.56	0.69	0.26	
1700042G07Rik	2.250	2.80E-06	-4.39	-6.56	0.69	0.26	
C1ql2	2.250	9.63E-06	-4.39	-6.56	0.69	0.26	
Rps19-ps9	2.250	3.80E-05	-4.39	-6.56	0.69	0.26	
Gm22311	2.250	3.98E-05	-4.39	-6.56	0.69	0.26	
Gm9378	2.250	1.30E-04	-4.39	-6.56	0.69	0.26	
Myl3	2.249	1.51E-02	-2.45	-4.48	1.29	0.23	Diabetes, CVD
Gm3086	2.247	3.99E-04	-3.81	-5.98	0.68	0.42	
Gm12356	2.238	3.80E-04	-4.00	-6.16	0.63	0.26	
Cd200r3	2.238	1.22E-03	-4.00	-6.16	0.63	0.26	
Lrrc2	2.234	4.33E-03	-3.39	-5.77	0.55	0.86	
Camp	2.233	2.26E-02	-3.48	-5.58	0.62	0.38	
Acod1	-2.232	1.07E-02	-4.27	-2.25	0.62	0.59	
Gm16365	2.213	2.90E-03	-3.39	-5.46	0.18	0.47	
En2	2.212	1.25E-04	-4.00	-6.16	0.59	0.26	
Ms4a4c	-2.211	8.98E-04	-3.60	-1.63	0.64	0.37	
Serpina3a	2.211	6.98E-03	-2.59	-4.88	0.29	0.65	
E530011L22Rik	2.210	5.26E-03	-3.60	-5.86	0.56	0.77	

Gm3809	2.197	7.25E-03	-3.21	-5.28	0.17	0.54	
Gm13612	2.191	3.13E-03	-3.21	-5.37	0.31	0.76	
Igf2os	2.189	9.25E-04	-4.00	-6.16	0.57	0.26	
Fgf13	2.177	1.99E-03	-3.60	-5.58	0.63	0.38	Diabetes, CVD
Gm13511	2.175	9.99E-05	-4.27	-6.56	0.62	0.26	
Gm13009	2.175	1.44E-04	-4.27	-6.56	0.62	0.26	
Gm14425	2.175	8.54E-04	-4.27	-6.56	0.62	0.26	
Fmr1nb	2.175	1.40E-03	-4.27	-6.56	0.62	0.26	
Bmp8b	2.174	7.19E-03	-3.48	-5.58	0.57	0.44	Obesity
Gm23130	2.164	1.50E-03	-4.00	-6.16	0.63	0.43	
Wdr72	2.164	4.44E-03	-3.60	-5.77	0.46	0.38	Diabetes
Syngr4	2.156	1.65E-03	-3.08	-5.19	0.25	0.34	
Gm8508	2.152	3.32E-03	-2.55	-4.72	0.53	0.47	
Gm13181	2.150	7.90E-03	-3.08	-5.19	0.58	0.25	
A530020G20Rik	-2.148	8.59E-03	-4.27	-2.13	0.62	0.36	
Ttn	2.147	2.85E-02	-1.55	-3.39	1.28	0.44	Diabetes, CVD
Gm14017	2.142	4.39E-04	-4.00	-6.16	0.65	0.50	
Gm7221	-2.141	4.43E-05	-4.39	-2.28	0.50	0.20	
Gm11951	2.138	5.50E-04	-4.00	-6.16	0.59	0.43	
Rps2-ps13	2.138	6.50E-04	-4.00	-6.16	0.59	0.43	
Shcbp1	-2.137	1.17E-03	-3.32	-1.20	0.59	0.26	
Mirlet7c-2	2.135	1.93E-03	-4.00	-5.86	0.76	0.54	
Gm8444	2.125	8.81E-04	-3.81	-6.16	0.49	0.50	
Gm12525	2.125	8.23E-05	-4.00	-5.98	0.65	0.42	
Akap3	2.122	7.43E-03	-3.60	-5.46	0.98	0.53	
Olfr726	2.119	1.65E-03	-3.79	-5.77	0.52	0.29	
Fabp5l2	2.117	9.43E-04	-3.57	-5.77	0.63	0.29	
Gm15353	2.116	4.32E-03	-4.00	-6.16	0.57	0.43	
Tmem40	2.116	9.02E-03	-2.90	-5.19	0.32	0.65	
Gm15798	2.110	2.32E-03	-4.00	-6.16	0.63	0.50	
Gm17276	2.098	8.77E-03	-2.34	-4.39	0.41	0.60	
Adam24	2.098	5.06E-04	-4.18	-6.16	0.64	0.43	
Gm9009	2.098	5.33E-04	-4.18	-6.16	0.64	0.43	
Pcdha11	2.098	8.48E-04	-4.18	-6.16	0.64	0.43	
Gm8392	2.098	9.47E-04	-4.18	-6.16	0.64	0.43	
Iqgap3	-2.092	8.80E-03	-3.71	-1.68	0.77	0.29	
Gm6793	2.091	1.01E-02	-3.60	-5.58	0.63	0.53	
Traf3ip3	-2.087	3.77E-04	-4.27	-2.28	0.62	0.18	
Smpx	2.086	3.43E-05	-4.00	-6.16	0.42	0.26	
Gm11620	2.086	3.13E-03	-4.00	-6.16	0.42	0.26	

Myom2	2.086	3.72E-04	-4.00	-6.16	0.42	0.26	
Cytl1	2.084	2.20E-02	-2.54	-4.70	0.78	1.00	
Dnah5	-2.083	1.42E-03	-4.27	-2.34	0.62	0.47	CVD
Slc6a19	2.083	3.00E-03	-4.00	-6.16	0.59	0.50	CVD
Gm24407	2.077	3.27E-03	-3.88	-5.77	0.84	0.38	
Gm8773	2.076	2.01E-06	-4.58	-6.56	0.53	0.26	
Emx1	2.076	2.33E-06	-4.58	-6.56	0.53	0.26	
Gm16351	2.076	2.33E-06	-4.58	-6.56	0.53	0.26	
Prss21	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Padi1	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Piwil1	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Tgif2-ps2	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Spag16	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	Obesity, Diabetes
Zdhhc25	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Ap3b2	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Rnu12	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Gm15067	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Gm13126	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Gm13050	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Rps19-ps14	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Gm5942	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Gm4991	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Gm13715	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Gm15873	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
4933406K04Rik	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Ccdc42os	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Gm22518	2.076	2.35E-06	-4.58	-6.56	0.53	0.26	
Cdk19os	2.076	3.67E-06	-4.58	-6.56	0.53	0.26	
Gm16229	2.076	4.96E-06	-4.58	-6.56	0.53	0.26	
Gm22092	2.076	6.44E-06	-4.58	-6.56	0.53	0.26	
Gm8419	2.076	6.91E-06	-4.58	-6.56	0.53	0.26	
Gm13915	2.076	7.74E-06	-4.58	-6.56	0.53	0.26	
Gm26004	2.076	8.54E-06	-4.58	-6.56	0.53	0.26	
Mir7659	2.076	8.87E-06	-4.58	-6.56	0.53	0.26	
Gm17752	2.076	9.43E-06	-4.58	-6.56	0.53	0.26	
Gdap1l1	2.076	9.71E-06	-4.58	-6.56	0.53	0.26	
Gm17189	2.076	9.76E-06	-4.58	-6.56	0.53	0.26	
2900072N19Rik	2.076	1.01E-05	-4.58	-6.56	0.53	0.26	
Gm16578	2.076	1.01E-05	-4.58	-6.56	0.53	0.26	
Cfap44	2.076	1.02E-05	-4.58	-6.56	0.53	0.26	

Gm3943	2.076	1.07E-05	-4.58	-6.56	0.53	0.26	
Slfn5os	2.076	1.27E-05	-4.58	-6.56	0.53	0.26	
Gm22613	2.076	1.30E-05	-4.58	-6.56	0.53	0.26	
1700030M09Rik	2.076	1.47E-05	-4.58	-6.56	0.53	0.26	
Gm12269	2.076	1.51E-05	-4.58	-6.56	0.53	0.26	
Slc5a5	2.076	1.60E-05	-4.58	-6.56	0.53	0.26	
Gm3617	2.076	1.68E-05	-4.58	-6.56	0.53	0.26	
Gm11479	2.076	1.80E-05	-4.58	-6.56	0.53	0.26	
Gm15432	2.076	1.80E-05	-4.58	-6.56	0.53	0.26	
Gm10689	2.076	2.17E-05	-4.58	-6.56	0.53	0.26	
Gm26465	2.076	2.71E-05	-4.58	-6.56	0.53	0.26	
Gm24693	2.076	3.04E-05	-4.58	-6.56	0.53	0.26	
Gm13703	2.076	3.08E-05	-4.58	-6.56	0.53	0.26	
Il5ra	2.076	4.17E-05	-4.58	-6.56	0.53	0.26	Obesity, Diabetes, CVD
Ighv1-59	2.076	4.31E-05	-4.58	-6.56	0.53	0.26	
Zfp92	2.076	4.87E-05	-4.58	-6.56	0.53	0.26	
Gm27514	2.076	4.98E-05	-4.58	-6.56	0.53	0.26	
Clec2l	2.076	4.99E-05	-4.58	-6.56	0.53	0.26	
Gm6181	2.076	5.21E-05	-4.58	-6.56	0.53	0.26	
Gm5321	2.076	5.80E-05	-4.58	-6.56	0.53	0.26	
Gm14094	2.076	6.74E-05	-4.58	-6.56	0.53	0.26	
Ighv1-80	2.076	6.83E-05	-4.58	-6.56	0.53	0.26	
Aire	2.076	8.50E-05	-4.58	-6.56	0.53	0.26	Obesity, Diabetes
Gm17199	2.076	1.49E-04	-4.58	-6.56	0.53	0.26	
Sox5os5	2.076	1.61E-04	-4.58	-6.56	0.53	0.26	
Hmgb1-ps6	2.076	1.92E-04	-4.58	-6.56	0.53	0.26	
Gm12407	2.076	2.01E-04	-4.58	-6.56	0.53	0.26	
Ces2f	2.076	2.06E-04	-4.58	-6.56	0.53	0.26	
Vwa5b2	2.076	2.65E-04	-4.58	-6.56	0.53	0.26	
Tspan1	2.076	3.00E-04	-4.58	-6.56	0.53	0.26	Diabetes
Mir6921	2.076	3.59E-04	-4.58	-6.56	0.53	0.26	
Gm4943	2.076	5.01E-04	-4.58	-6.56	0.53	0.26	
Stac	2.076	4.78E-03	-4.58	-6.56	0.53	0.26	
Gm23851	2.074	1.67E-03	-3.79	-5.77	0.52	0.38	
Ccdc13	2.071	1.68E-06	-4.39	-6.56	0.50	0.26	
Gm1335	2.071	1.72E-06	-4.39	-6.56	0.50	0.26	
Gm23640	2.071	1.72E-06	-4.39	-6.56	0.50	0.26	
Gm12998	2.071	4.25E-06	-4.39	-6.56	0.50	0.26	
Gal3st3	2.071	1.64E-05	-4.39	-6.56	0.50	0.26	
Gm14226	2.071	1.64E-05	-4.39	-6.56	0.50	0.26	

Gm29200	2.071	2.13E-05	-4.39	-6.56	0.50	0.26	
Gm2976	2.071	3.45E-05	-4.39	-6.56	0.50	0.26	
Gm15763	2.071	4.53E-05	-4.39	-6.56	0.50	0.26	
Tecrl	2.071	5.21E-05	-4.39	-6.56	0.50	0.26	Diabetes, CVD
Gm13523	2.071	6.44E-05	-4.39	-6.56	0.50	0.26	
Gm26685	2.071	7.37E-05	-4.39	-6.56	0.50	0.26	
Gm15483	2.071	1.95E-04	-4.39	-6.56	0.50	0.26	
Fscn2	2.071	2.44E-04	-4.39	-6.56	0.50	0.26	
Gm24714	2.071	2.58E-04	-4.39	-6.56	0.50	0.26	
Gm26706	2.071	4.01E-04	-4.39	-6.56	0.50	0.26	
Gm16725	2.071	4.32E-04	-4.39	-6.56	0.50	0.26	
1700061E17Rik	2.071	4.45E-04	-4.39	-6.56	0.50	0.26	
Gm15965	2.071	7.07E-04	-4.39	-6.56	0.50	0.26	
Gm26477	2.071	1.72E-06	-4.39	-6.56	0.50	0.26	
Gm8597	2.071	1.72E-06	-4.39	-6.56	0.50	0.26	
Plppr4	2.071	9.26E-06	-4.39	-6.56	0.50	0.26	
Gja3	2.071	1.14E-05	-4.39	-6.56	0.50	0.26	
Gm25057	2.071	1.31E-05	-4.39	-6.56	0.50	0.26	
Gm24146	2.071	1.86E-05	-4.39	-6.56	0.50	0.26	
Mir6392	2.071	2.35E-04	-4.39	-6.56	0.50	0.26	
Eya1	2.071	2.54E-04	-4.39	-6.56	0.50	0.26	
Igkv5-45	2.071	2.80E-04	-4.39	-6.56	0.50	0.26	CVD
Btnl10	2.071	4.78E-04	-4.39	-6.56	0.50	0.26	
Igkv3-7	2.067	3.61E-03	-3.88	-6.16	0.52	0.50	
Gm24105	2.066	8.59E-03	-3.39	-5.40	0.65	0.57	
C4bp-ps1	2.065	2.40E-02	-2.62	-4.79	0.47	0.70	
Arxes2	2.062	5.74E-03	-4.00	-6.16	0.65	0.63	
Gm12470	2.059	1.85E-05	-4.18	-6.16	0.50	0.26	
Ccdc113	2.059	5.62E-05	-4.18	-6.16	0.50	0.26	
Gm11682	2.059	1.40E-04	-4.18	-6.16	0.50	0.26	
Gm6564	2.059	1.46E-04	-4.18	-6.16	0.50	0.26	
Gm17798	2.059	5.81E-04	-4.18	-6.16	0.50	0.26	
Gm5445	2.059	2.22E-03	-4.18	-6.16	0.50	0.26	
Gm5575	2.058	1.64E-04	-4.18	-6.16	0.50	0.26	
Mypn	2.058	1.53E-02	-2.90	-4.88	0.39	0.36	
Atg4a-ps	2.058	1.81E-03	-3.79	-5.98	0.31	0.66	
Gm2735	2.048	1.57E-06	-4.58	-6.56	0.51	0.26	
Gm25881	2.048	1.58E-06	-4.58	-6.56	0.51	0.26	
Gm15714	2.048	2.16E-06	-4.58	-6.56	0.51	0.26	
Dmrtc2	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	

Tmbim7	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Vsx2	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Odf3l1	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Cypt3	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Hdhd1a	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
4930469G21Rik	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Grm5	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	Diabetes, CVD
Mrgprg	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Olfr1029	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Nkx6-3	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm22772	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm15176	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm5395	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm15580	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
H2af-ps	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm15168	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm16330	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
4930557F10Rik	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm15721	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm15668	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
4930401O12Rik	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm9719	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm15849	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Tmem207	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Vmn2r-ps19	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm26783	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
1700034K08Rik	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
1700025F24Rik	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Gm29395	2.048	2.19E-06	-4.58	-6.56	0.51	0.26	
Rps13-ps5	2.048	2.48E-06	-4.58	-6.56	0.51	0.26	
Gm11774	2.048	2.74E-06	-4.58	-6.56	0.51	0.26	
Gm13921	2.048	3.23E-06	-4.58	-6.56	0.51	0.26	
Gm8659	2.048	3.95E-06	-4.58	-6.56	0.51	0.26	
Gm13414	2.048	3.95E-06	-4.58	-6.56	0.51	0.26	
Vmn1r79	2.048	3.95E-06	-4.58	-6.56	0.51	0.26	
Gm14106	2.048	4.25E-06	-4.58	-6.56	0.51	0.26	
Gm13294	2.048	6.22E-06	-4.58	-6.56	0.51	0.26	
Mir6939	2.048	6.68E-06	-4.58	-6.56	0.51	0.26	
Haglr	2.048	6.80E-06	-4.58	-6.56	0.51	0.26	
Gssos1	2.048	6.95E-06	-4.58	-6.56	0.51	0.26	

Gm10087	2.048	8.28E-06	-4.58	-6.56	0.51	0.26	
Hmgb1-rs18	2.048	9.94E-06	-4.58	-6.56	0.51	0.26	
Gm5105	2.048	1.05E-05	-4.58	-6.56	0.51	0.26	
Gm26928	2.048	1.10E-05	-4.58	-6.56	0.51	0.26	
Rsph14	2.048	1.12E-05	-4.58	-6.56	0.51	0.26	
Fbxo39	2.048	1.12E-05	-4.58	-6.56	0.51	0.26	
Slc16a14	2.048	1.21E-05	-4.58	-6.56	0.51	0.26	
Slco6c1	2.048	1.28E-05	-4.58	-6.56	0.51	0.26	
Gm25687	2.048	1.28E-05	-4.58	-6.56	0.51	0.26	
A430108G06Rik	2.048	1.35E-05	-4.58	-6.56	0.51	0.26	
Gm136	2.048	1.41E-05	-4.58	-6.56	0.51	0.26	
Gm26614	2.048	1.47E-05	-4.58	-6.56	0.51	0.26	
Gm14769	2.048	1.51E-05	-4.58	-6.56	0.51	0.26	
Synpr	2.048	1.59E-05	-4.58	-6.56	0.51	0.26	
Slc36a3os	2.048	1.66E-05	-4.58	-6.56	0.51	0.26	
Gm5451	2.048	1.67E-05	-4.58	-6.56	0.51	0.26	
Gm10800	2.048	1.72E-05	-4.58	-6.56	0.51	0.26	
Apobec4	2.048	2.23E-05	-4.58	-6.56	0.51	0.26	
Gm14170	2.048	2.32E-05	-4.58	-6.56	0.51	0.26	
Gm8129	2.048	3.12E-05	-4.58	-6.56	0.51	0.26	
Gm13584	2.048	3.13E-05	-4.58	-6.56	0.51	0.26	
Tnnt3	2.048	3.31E-05	-4.58	-6.56	0.51	0.26	Diabetes, CVD
Snord111	2.048	3.83E-05	-4.58	-6.56	0.51	0.26	
BC016579	2.048	4.92E-05	-4.58	-6.56	0.51	0.26	
Gm8338	2.048	4.97E-05	-4.58	-6.56	0.51	0.26	
Gm27193	2.048	6.33E-05	-4.58	-6.56	0.51	0.26	
Gm16265	2.048	7.46E-05	-4.58	-6.56	0.51	0.26	
Gm12808	2.048	7.67E-05	-4.58	-6.56	0.51	0.26	
Gm5396	2.048	7.97E-05	-4.58	-6.56	0.51	0.26	
Olfr755-ps1	2.048	9.73E-05	-4.58	-6.56	0.51	0.26	
Gm25235	2.048	9.84E-05	-4.58	-6.56	0.51	0.26	
Gm22067	2.048	1.01E-04	-4.58	-6.56	0.51	0.26	
Gm11438	2.048	1.12E-04	-4.58	-6.56	0.51	0.26	
Ctsj	2.048	1.25E-04	-4.58	-6.56	0.51	0.26	
Gm17828	2.048	1.60E-04	-4.58	-6.56	0.51	0.26	
Gm28586	2.048	1.65E-04	-4.58	-6.56	0.51	0.26	
Dhx58os	2.048	1.88E-04	-4.58	-6.56	0.51	0.26	
Zfp385c	2.048	1.92E-04	-4.58	-6.56	0.51	0.26	
Ptchd4	2.048	2.06E-04	-4.58	-6.56	0.51	0.26	
Gm12260	2.048	2.19E-04	-4.58	-6.56	0.51	0.26	

Gm5759	2.048	2.46E-04	-4.58	-6.56	0.51	0.26	
Gm2670	2.048	2.94E-04	-4.58	-6.56	0.51	0.26	
Tarm1	2.048	3.70E-04	-4.58	-6.56	0.51	0.26	
Gm5844	2.048	3.80E-04	-4.58	-6.56	0.51	0.26	
Atp5k-ps2	2.048	4.47E-04	-4.58	-6.56	0.51	0.26	
E330017L17Rik	2.048	4.51E-04	-4.58	-6.56	0.51	0.26	
Mir7212	2.048	8.00E-04	-4.58	-6.56	0.51	0.26	
Gm17039	2.048	8.06E-04	-4.58	-6.56	0.51	0.26	
Crybb1	2.048	8.98E-04	-4.58	-6.56	0.51	0.26	
Col28a1	2.048	9.34E-04	-4.58	-6.56	0.51	0.26	Diabetes, CVD
Gm12774	2.048	1.37E-03	-4.58	-6.56	0.51	0.26	
4930520O04Rik	2.048	1.39E-03	-4.58	-6.56	0.51	0.26	
Gm29375	2.048	2.43E-03	-4.58	-6.56	0.51	0.26	
Gm8822	-2.048	5.42E-05	-4.97	-2.82	0.18	0.29	
Trdn	2.046	1.23E-03	-3.81	-6.16	0.49	0.63	Diabetes, CVD
Tg	2.044	2.41E-03	-4.00	-5.98	0.57	0.42	Obesity, Diabetes
1700021N21Rik	2.044	3.58E-03	-4.00	-5.98	0.57	0.42	
Snora78	2.044	1.44E-02	-2.62	-4.72	0.49	0.68	
Gm11945	2.043	1.22E-03	-4.18	-6.16	0.64	0.50	
Slc28a3	2.035	7.05E-04	-4.18	-6.16	0.48	0.26	CVD
Gm6139	2.035	2.23E-03	-4.18	-6.16	0.48	0.26	
Gm26669	2.035	4.38E-03	-4.18	-6.16	0.48	0.26	
Gm9294	2.035	7.20E-04	-4.18	-6.16	0.48	0.26	
Rsph4a	2.035	4.07E-03	-4.18	-6.16	0.48	0.26	CVD
Gm13449	-2.033	7.05E-04	-4.39	-2.32	0.50	0.32	
Spata31d1b	2.026	1.37E-04	-4.18	-5.98	0.64	0.42	
4933400F21Rik	2.026	3.45E-03	-4.18	-5.98	0.64	0.42	
Gm8151	2.025	7.59E-05	-3.79	-5.77	0.35	0.29	
Ap3s1-ps2	2.025	2.51E-02	-2.79	-4.76	0.82	0.49	
Gm15372	2.015	2.99E-02	-2.74	-4.64	0.41	1.17	
Gm13784	2.012	1.44E-03	-4.00	-6.16	0.42	0.43	
Gm13680	2.011	8.69E-03	-3.39	-5.40	0.18	0.70	
Gm20695	2.008	8.58E-03	-3.60	-5.58	0.56	0.61	
Gm29488	2.005	4.86E-03	-3.79	-5.77	0.31	0.29	
Gm14848	2.003	2.13E-03	-4.00	-6.16	0.59	0.63	
Acta1	2.003	5.14E-03	-3.48	-5.77	0.69	0.54	Diabetes, CVD

Table 4.18. Differentially expressed genes due to maternal diet for low-fat-fed sons.

CHATPER 5

Conclusion of the Dissertation

The principal goal of this research was to uncover the gene expression and methylation changes that drive the physiological response to dietary fat in two generations of mice. I assigned inbred SM/J mice to a low-fat diet or a high-fat diet and measured their body and organ weights, their glucose and insulin tolerance, and biomarkers in their serum. I studied the effect of prenatal maternal diet by mating SM/J females on either diet with low-fat-fed males, fostering all of their offspring to low-fat-fed SM/J nurses, and weaning their offspring onto low fat or high-fat diets at three weeks of age. I measured the same traits in the offspring as in the parents, as well as additional behavior and bone phenotypes. To quantify expression and epigenetic profiles, I extracted RNA from the liver tissue for RNA-sequencing and DNA for MeDIP- and MRE-sequencing. I then identified the changes that maternal obesity induced in gene expression and methylation, which has helped to provide a better understanding of the epigenetic architecture of obesity. I found that although offspring diet had a more substantial effect on obesity, gene expression, and methylation patterns in SM/J mice, maternal diet affected all three of these trait categories as well. Based on genes that had differences in both expression and methylation, I also discussed numerous candidate loci for researchers to follow-up on in other strains of mice and in humans in studies of epigenetic therapies for obesity.

One mechanism through which non-genetic maternal effects can persist in offspring is via epigenetics. The epigenetic factor I chose to investigate here was DNA methylation, because it is sensitive to prenatal programming and has been shown to affect offspring behavior and health. In a ground-breaking study, Meaney and Szyf (2005) showed that differences in maternal care in rats changed the methylation of the offspring glucocorticoid receptor promoter in the hippocampus. The altered methylation state lasted for life, as did the altered stress response. DNA methylation has also been shown to be responsive to maternal diet, as exemplified by the

research revealing that supplementing dietary folate—an important methyl donor—reduced offspring obesity and methylation of the A^{vy} locus in mice (Wolff *et al.* 1998, Waterland and Jirtle 2003, Waterland *et al.* 2004).

In Chapter 4, I found that a maternal high-fat diet altered the methylation of more than 7,300 genes in the liver tissue of the offspring. Although fewer than 1% of the differentially methylated regions fell within differentially expressed genes, they did occur within gene regulatory regions far more often than expected by chance alone, in line with previous knowledge of methylation's role in gene regulation. The offspring were more affected by the direct effect of their own diets than the effect of maternal diet. While maternal diet induced changes in dozens of genes, offspring diet induced changes in thousands of genes. On a phenotypic level, maternal diet induced changes in some of the traits of only the female offspring, whereas offspring diet induced changes in virtually every trait measured in both sexes. The results of Chapter 2 supported this trend, where the only behavioral trait that maternal diet affected was where the offspring built their nests, as opposed to the wide range of traits affected by offspring diet: a high-fat diet reduced nest quality, reduced activity levels, and increased anxiety. Other studies have revealed that a maternal high-fat diet increases anxiety in mice, so the fact that I found no effect in Chapter 2 suggests that the lactation environment rather than the prenatal environment mediates this phenomenon. Another noteworthy finding in Chapter 2 was that the significant weight gain induced by the high-fat diet was evident several weeks before the reduction in activity levels was observed. This suggests that weight gain can lead to inactivity, not only that inactivity leads to obesity. The lower activity levels may have exacerbated the weight gain from the high-fat diet.

In Chapter 3, I found that a high-fat diet altered the expression of 4,356 genes in the liver and dysregulated several pathways. The genes in the cytokine-cytokine receptor interaction pathway were more highly expressed due to a high-fat diet, and 11 of those genes also had differentially methylated regions in the females, as did 9 genes in the males. The upregulation of the cytokine pathway is one of many lines of evidence that a high-fat diet increased inflammation. Of the dozens of GO Biological Processes that were upregulated by a high-fat diet, nearly all of them involved the immune system. *Galnt10* was upregulated by a high-fat diet in females, which may indicate that more mucin-type O-glycosylation was occurring due to increased production of inflammatory proteins. The *Adam11* gene, not previously discussed with regard to obesity, was strongly upregulated in mice on a high-fat diet. The gene also had 3 consecutive DMRs spanning 5 exons that were more methylated in high-fat-fed mice. Since knocking out this gene in mice reduces the response to inflammatory pain, my findings indicate that further studies of the role *Adam11* plays in obesity-associated inflammation are merited.

Diet-induced obesity is a state of chronic inflammation, which can cause liver fibrosis (Calvente *et al.* 2015). The livers of the high fat mice were 2.8 times heavier than the low-fat mice, and they had a yellow discoloration from fat buildup. This was accompanied by altered methylation and increased expression of genes such as *Col1a1*—which encodes the type of collagen that comprises scar tissue and accumulates in the liver during fibrosis—and *Lad1*, a component of the basement membrane, which overgrows in the liver during fibrosis. The livers of high-fat mice showed an epigenetic response to the high cholesterol induced by the diet by reducing the methylation and raising the expression of the *Abcg5* and *Abcg8* genes, which encode a heterodimer responsible for eliminating cholesterol from the body. Despite this

upregulation, the high-fat-fed mice still had 2-3 times more serum cholesterol than the low-fat-fed mice.

When I compared my gene expression results to those of other high-fat diet studies, I found that the *Fgfr2* gene was differentially expressed in the males and females of three other strains in addition to SM/J. *Fgfr2* is upregulated in the livers of people with non-alcoholic fatty liver disease (NAFLD) (Younossi *et al.* 2005), and its methylation in cord blood has been linked to birth weight in newborns (Haworth *et al.* 2014). Due to these previous studies, as well as the sensitivity of *Fgfr2* to a high-fat diet in both sexes of multiple mouse strains, and the fact that I found DMRs in both sexes of the gene, *Fgfr2* could be an important therapeutic target in obesity.

In Chapter 4, I showed that a maternal high-fat diet downregulated the ribosome, spliceosome, oxidative phosphorylation, and RNA transport pathways regardless of offspring diet. Notable disease pathways downregulated by a maternal high-fat diet included the NAFLD and Alzheimer's disease pathways. Although by 17 weeks of age, changes in the obesity traits were detected only in the daughters, it would be interesting in the future to test the memories and cognitive function of older mice, knowing that the Alzheimer's pathway was downregulated in both sexes by a maternal high-fat diet. The weighted gene co-expression network analysis revealed several modules of highly co-expressed genes that were directly associated with the diabetes-related traits in the offspring. The two modules that were negatively associated with the traits were enriched for genes involved in immune system function, oxidation reduction, and arachidonic acid, whereas the two modules that were positively associated with the diabetes traits were enriched for mitochondrial, respiratory, and ribosomal processes. This indicates that the diabetes-related traits were associated with an altered metabolism and immune response. A maternal high-fat diet appeared to exacerbate the liver distress caused by an offspring high-fat

diet, since genes such as *Apln* and *Mpo* were upregulated by a maternal high-fat diet in the high fat daughters. However, *Stat1*, a gene that was upregulated by an offspring high-fat diet and is associated with liver injury, was actually decreased by a maternal high-fat diet in sons. This indicates that the gene expression changes induced by maternal obesity are not always the same as those induced by an individual's obesity. By examining the gene expression in both the liver and heart tissue in the high fat daughters, I found that each tissue had a completely different set of differentially expressed genes, highlighting the importance of studying multiple tissues to grasp the full extent of the effects of maternal obesity. Six of the genes that were differentially expressed in the heart were cytochrome P450 genes, which, due to their role in drug metabolism and the involvement of other cytochrome P450 genes in heart disease, should be studied further as possible targets for treating metabolic syndrome.

When considering Chapters 3 and 4 together, their results are highly consistent and several epigenetic trends emerge. First of all, more DMRs were located on the X-chromosome in between-sex comparisons than within-sex comparisons. Only 1% of DMRs were on the X-chromosome in the F_0 males, 1.1% in the F_1 high fat males, and 1.0% in the F_1 low fat males. Females had more DMRs on the X-chromosome than males, with 3.5% of DMRs located on the X chromosome in the F_0 females, 3.9% in the F_1 high fat females, and 3.4% in the F_1 low fat females. Thus, it was clear in both generations is that females have more than 3 times as many DMRs on the X-chromosome as males, potentially due to the X-chromosome inactivation that happens in females. Comparing differences between sexes revealed that 5.7-7.0% of DMRs fell on the X-chromosome in the F_0 mice, and 4.3-6.5% of DMRs did in the F_1 mice, showing unsurprisingly that X-chromosome methylation patterns are more different between males and females than within sexes. Another interesting trend that arose in both generations of mice was

that comparisons of high fat mice with each other yielded 1.4 times fewer DMRs than comparisons of low fat mice with each other. This indicates that a high-fat diet reduced variation in methylation. In light of the behavioral results from Chapter 2, where high fat mice spent more time sleeping and less time walking and climbing, it is possible that the reduction in methylation variation was caused by the overall reduction in activity levels caused by a high-fat diet.

In Chapter 3, I compared my gene expression findings with those of other dietary fat studies in mice. I found between 28-40% of the same genes in my study as researchers did in C57BL/6 mice. When comparing my results from Chapters 3 and 4, 50% of the same genes were differentially expressed due to diet in both sets of mice. Although there was a higher repeatability of differential expression in the two generations of mice I studied, this also highlights the wide variability in gene expression even in mice of the same strain housed in the same facility exposed to the same diets. Replication of experimental results is always important in science, but it is especially important to keep this in mind when interpreting results from gene expression and epigenetic studies.

In addition to contributing to the field of obesity research, my findings also have implications for the field of evolutionary biology. Epigenetics is involved in phenotypic plasticity by mediating the response to different environments through regulating pathways and stabilizing phenotypic variation in certain directions. Although I know that epigenetics is involved in phenotypic plasticity, in most cases we have not elucidated the mechanism yet. In my study system, it is clear that DNA methylation changes substantially in response to different dietary environments. The phenotypic plasticity I observed in the genetically identical SM/Js was mediated by changes in methylation and gene expression. Epigenetics is important to the field of evolution beyond just its role in phenotypic plasticity. For instance, epigenetic inheritance may

facilitate or enhance the processes of genetic assimilation and genetic accommodation (Jablonka and Raz 2009). Additionally, a few heritable epialleles have been discovered, such as paramutation in maize and the *AxinFu* allele in mice, where the epigenetic state of the allele gets faithfully passed on across generations. Epigenetic variation can also help populations adapt and diverge even in the absence of sufficient genetic variation (Flatscher *et al*. 2012). Small populations tend to have much lower genetic variation and are at a higher risk of extinction if the environment changes suddenly. However, if they have heritable epialleles that confer phenotypic variation, small populations may be better equipped to adapt to changing environmental conditions—even without genetic variation.

Epigenetics may further affect evolution through its impact on mutation and recombination rates. In condensed chromatin, the rates of mutation, transposition, and recombination are lower compared to those in open chromatin (Belyaev and Borodin 1982; Jablonka and Lamb 1995). Additionally, methylated cytosines spontaneously deaminate to thymines over evolutionary time. The CpG-to-TpG mutation rate is 50 times higher than any other transitional change, which causes CpGs to occur at only 21% of the expected frequency in the genome (Xia *et al.* 2012). Gene promoters that stay hypermethylated persistently across generations due to epigenetic inheritance or a consistent environmental effect may have higher mutation rates, which could drive faster evolutionary change by accelerating the decay of a gene's promoter region. Hypothetically, if a gene that protects against obesity is turned off due to hypermethylation from a maternal high-fat diet over many generations, the CpGs may deaminate to TpGs and the promoter could eventually decay. Furthermore, epigenetics may be involved in establishing and maintaining reproductive isolation, as in the case of *Peromyscus polionotus* and *P. maniculatus,* where reproductive isolation between these two species has been shown to be

driven by epigenetic mechanisms (Vrana *et al*. 2000). However, before we fully understand the extent of the effect that epigenetics has on evolution, more research must be conducted on the epigenetic changes that accompany environmental changes. My dissertation research on the DNA methylation changes that accompany a high-fat diet and a prenatal environment of maternal obesity contribute in a small way to that knowledge.

Overall, this dissertation has identified thousands of genes and methylated genomic regions in the liver that are altered by a high-fat diet. It supports the fetal origins of adult disease hypothesis, which posits that the environment that an individual experiences during early development affects adult risk of type 2 diabetes, cardiac disease, and hypertension (Hales *et al.* 1991, Barker *et al.* 2002). A prenatal maternal high-fat diet altered the gene expression and methylation patterns in the offspring, and increased the body weights, leptin levels, and weights of the liver and fat pad in the high-fat-fed daughters. Knowing that these particular traits are affected in females, along with the accompanying changes in gene expression and methylation, will inform health interventions and treatments in the future.

LITERATURE CITED

Barker, D. J., J. G. Eriksson, T. Forsén, and C. Osmond. 2002. Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol* 31(6): 1235-1239.

Belyaev, D. K., and P. M. Borodin. 1982. The influence of stress on variation and its role in evolution. *Biologisches Zentralblatt* 100: 705-714.

Calvente, C. J., A. Sehgal, Y. Popov, Y. O. Kim, V. Zevallos, U. Sahin, M. Diken, and D. Schuppan. 2015. Specific hepatic delivery of procollagen α1(I) small interfering RNA in lipid-like nanoparticles resolves liver fibrosis. *Hepatology* 62(4): 1285-1297.

Flatscher, R., B. Frajman, P. Schonswetter, and O. Paun. 2012. Environmental heterogeneity and phenotypic divergence: can heritable epigenetic variation aid speciation? *Genetics Research International* 2012: 1-9.

Hales, C. N., D. J. Barker, P. M. Clark, L. J. Cox, C. Fall, C. Osmond, and P. D. Winter. 1991. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 303(6809): 1019-1022.

Haworth, K. E., W. E. Farrell, R. D. Emes, K. M. Ismail, W. D. Carroll, E. Hubball, A. Rooney, A. M. Yates, C. Mein, and A. A. Fryer. 2014. Methylation of the FGFR2 gene is associated with high birth weight centile in humans. *Epigenomics* 6(5): 477-491.

Jablonka E., and Lamb M. J. 1995. *Epigenetic Inheritance and Evolution: The Lamarckian Dimension*. Oxford (UK): Oxford University Press.

Jablonka, E., and G. Raz. 2009. Transgenerational epigenetic inheritance: Prevalence, mechanisms, and implications for the study of heredity and evolution. *The Quarterly Review of Biology* 84(2): 131-176.

Meaney, M. J., and M. Szyf. 2005. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin Neurosci* 7(2): 103-123.

Vrana, P. B., J. A. Fossella, P. Matteson, T. del Rio, M. J. O'Neill, and S. M. Tilghman. 2000. Genetic and epigenetic incompatibilities underlie hybrid dysgenesis in *Peromyscus*. *Nat Genet* 25(1): 120-124.

Waterland, R. A., and R. L. Jirtle. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23(15): 5293-5300.

Waterland R. A., M. Travisano, K. G. Tahiliani, M. T. Rached, S. Mirza. 2008. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes (Lond)* 32(9): 1373-1379.

Wolff, G. L., R. L. Kodell, S. R. Moore, and C. A. Cooney. 1998. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J* 12: 949-957.

Xia, J., L. Han, and Z. Zhao. 2012. Investigating the relationship of DNA methylation with mutation rate and allele frequency in the human genome. *BMC Genomics* 13(Suppl 8): S7.

Younossi, Z. M., A. Baranova, K. Ziegler, L. Del Giacco, K. Schlauch, T. L. Born, H. Elariny, F. Gorreta, A. VanMeter, A. Younoszai, J. P. Ong, Z. Goodman, and V. Chandhoke. 2005. A genomic and proteomic study of the spectrum of nonalcoholic fatty liver disease. *Hepatology* 42(3): 665-674.