MATERNAL NUTRIENT RESTRICTION: CONSEQUENCES ON THE OFFSPRING

by

LINDSEY BROOKE PROCTOR

(Under the Direction of Silvia Giraudo)

ABSTRACT

Objective: To determine the effects of maternal nutrient restriction of dams on two different diets on body weight, food intake, feed efficiency, perirenal fat pads, and body fat percentage of offspring.

Hypothesis: We hypothesize that maternal nutrient restriction with both standard and high-fat chow adversely affects the genetic programming involved in the regulation of food intake of the offspring.

Design: The first experiment aimed to determine the effects of maternal nutrient restriction with standard chow (11% fat) on appetite regulation of offspring. Pregnant mice were fed standard chow either ad libitum throughout gestation or were restricted by 25% during the last trimester. This process was repeated to obtain third generation progeny. The purpose of the second experiment is to examine potential augmented (compared to experiment 1) alterations in the regulation of appetite in offspring in response to a maternal high-fat diet (45% fat) followed by restriction. The same method was utilized with the following change. Once weaned (at 3 weeks of age), each generation of pups was fed high-fat chow for 8 weeks prior to breeding, when females were divided into two groups. Both were fed a high-fat diet until the last trimester, when one was continued on ad libitum food and the other was restricted by 25%.

Subjects: C57BL/6 strain of mice were used.

Measurements: Weekly body weights (grams) and daily food intakes (grams) were measured, and food intake converted into kilocalories (kcal) of each respective diet. Feed efficiency was calculated from food intake and body weight measurements. In the high-fat-fed animals, perirenal fat pads were weighed (grams) and used to estimate body composition with a ratio to final body weight.

Results: The current study did not reveal any *statistically significant* negative alterations in appetite and energy regulation of offspring from two maternal treatments or diets. It did, however, reveal that maternal malnutrition, either from undernutrition (a restricted intake) or overnutrition (HF diet) is likely to have adverse effects in the offspring, including higher average body weights and feed efficiencies, as well as larger perirenal fat pads with a corresponding higher body fat percentage.

INDEX WORDS: fetal programming, maternal treatment, maternal diet, offspring regulation

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CHAPTER 1:

INTRODUCTION

For reasons not yet full understood, the prevalence rates of obesity in the U.S. has increased so rapidly in the last twenty to thirty years that it is now considered an epidemic; specifically, adult and childhood obesity have doubled and tripled, respectively [1-4]. Over 30% of our population is now classified as obese (according to Body Mass Index (BMI) = $\geq$30 kg/m^2) [1], and up to one-third do not even know it [1, 5]. The adverse consequences that result from increased body weight are numerous, but the most common are changes in metabolism (i.e. dyslipidemia, insulin resistance, diabetes mellitus, etc), heart disease, and osteoarthritis, among others [6, 7] The worldwide increase in obesity correlates with higher rates of both chronic diseases and maternal malnutrition, and obesity/overweight is now considered the "largest nutritionally related problem in the world" [8, 9].

Regarding dietary composition, it *appears* that the U.S. has made positive changes with an overall trend of a decrease in percentage of fat intake, from over 40% of energy in 1960 to about 30% in 2000 [10, 11]. With a closer look, though, overall energy intake and dietary fat amount (in grams) has increased, which most likely leads to the decrease in percentage [11]. One possible contributor of these patterns is the increase in availability of HF foods, which is linked to a higher incidence of eating outside the home, where meals are often high in fat and energy [4]. Before the turn of the century, the associations between a HF diet, obesity, and chronic disease were so strong that many health agencies (i.e. American Heart Association) recommended decreasing consumption of total and saturated fat [12-15]. Even still, most people do not meet the current Dietary

Guidelines recommendations' of a diet consisting of 20-35% of calories from total fat and less than 10% from saturated fat [16]. As a consequence, it can be speculated that the excess fat (and overall energy) consumption is stored as fat because body fat is determined by a difference between energy intake and expenditure [4].

A maternal high-fat diet has been suggested to increase the amount of placental nutrient transport, resulting in excess fetal growth [17]. This leads to what authors call a "vicious cycle," meaning that overweight and obese women often consume an energy-rich, HF diet throughout the majority of gestation [18, 19]. Their babies then tend to be larger than normal and have higher risks for becoming overweight or obese adults, as childhood obesity usually carries on to adult obesity; in turn, this generation is more likely to give birth to large babies, and the cycle continues [20-23].

Various studies have shown that gestational manipulations of diet can have detrimental effects on metabolism in offspring and increase future obesity risks [24-32]. These changes seem to persist throughout life in offspring, regardless of post-natal environment or nutrition [33]. Thus, fetal "programming," or adjustments made early in life, carry on into adulthood and are now being associated with the rise in obesity and other health consequences [34]. Similarly, observations from the epidemiologic study of the Dutch Hunger Winter of 1944-1945 support the "thrifty phenotype" hypothesis, which relates malnutrition during gestation to negative metabolic changes in the offspring [8]. There is also strong evidence for maternal dietary restriction to result in offspring with higher mortality, more growth stunting, and developmental delays, as was shown in various species at The Johns Hopkins University [35]. Assuming that maternal malnutrition reduces achievement of optimal fetal growth, the overall impact on various

metabolic outcomes in the offspring can lead to chronic disease states as mentioned above [8].

In addition, the increased risk for future chronic disease is likely to begin in the womb because genes are naturally selected before birth based on the intrauternine environment [4]. For example, women who are overweight or obese during pregnancy tend to give birth to large for gestational age (LGA) babies. These children are then more likely to have negative metabolic consequences, including hyperglycemia and hyperinsulinemia, most likely from a maternal high-fat diet [19, 36]. A maternal high-fat diet has also been linked to an "irreversible" increase in offspring adiposity that is maintained regardless of post-natal nutrition [36]. Given that there is a clear connection between overweight/obesity and the risk for chronic diseases, it is critical to reduce the prevalence of obesity from an early age.

With the large number of obese and overweight Americans, much of the focus today is forced on the *treatment* of the immediate problem. The traditional approach of "lifestyle modification," which involves dietary therapy, incorporation of physical activity, and behavioral modification, is still being advocated, but there have also been many contemporary developments for treatment as of late [37]. These include bariatric surgery, pharmacotherapy, botanical dietary therapy, and even hypnosis and acupuncture [38]. While each have various success rates with meeting weight loss goals, many people then face a lifelong struggle with maintaining their new weight due to the different behaviors required for each as well as physiological mechanisms that oppose the lower body weight (i.e. "set point theory", referred to elsewhere ([39]Weiss) [3]. In fact, research estimates that most of those successful with weight loss tend to regain it in as

little as a few months [3]. Therefore, it seems more logical to direct attention to the causes of obesity in order to develop sufficient *prevention* means. In order to achieve this, though, it is first necessary to understand the basic physiologic mechanisms involved in appetite regulation and food intake.

With that, the hypothalamus has been coined the "most important [brain] area" involved in energy balance, and the role it has in appetite regulation develops *in utero* in humans [17, 40]. Along with appetite, it also is the primary controller of food preferences [41]. Within the brain, neuron maturation begins early in life, continues throughout gestation, and is highly affected by the fetal environment [42]. Importantly, during development the hypothalamus has a high level of "plasiticity," meaning that it is easily affected by the growth environment and adapts accordingly [43]. Even short durations of maternal malnutrition can have a negative impact on the offspring's eating behavior [43, 44]. Therefore, optimal development of the fetal brain is highly depended on the nutritional status of the mother during gestation [45].

Previous research has also shown that maternal diet greatly affects expression of these "feeding-related" peptides in the offspring, especially related to the orexgenic factors [46]. In addition to the effect on the scope of appetite, maternal nutrition also directly affects "offspring's dietary preferences" via modifications that occur in the hypothalamus [47-50]. This provides further evidence that regulation of feeding is developed *in utero* and that maternal malnutrition has life-long effects on both the behaviors and physiological mechanisms that control the act of eating [46].

This research study will focus on two aspects involved in fetal programming, using mice subjects. To begin, we will determine the effect of two different maternal

treatments, control (ad libitum food intake) and restricted food intake (75% of ad libitum food intake during the last trimester of gestation) on the energy balance regulation in offspring in two different experiments. Secondly two different maternal diet compositions will be examined using a standard chow diet and a high-fat diet with each maternal treatment. This will be achieved by measuring weekly body weights, food intakes, feed efficiencies, perirenal fat pad weights, and body fat percentages in three subsequent generations of offspring from each diet and treatment.

REFERENCES:

1. Ogden, C.L., et al., *Prevelance of overweight and obesity in the United States, 1999-2004.* Journal of the American Medical Association, 2006. **295**(13): p. 1549-1555.
2. *Position of the American Dietetic Association: Weight Management.* Journal of the American Dietetic Association, 2002. **102**: p. 1145-1155.
3. James O. Hill, P., M. Helen Thompson, RD, and M. Holly Wyatt, *Weight Maintenance: What's Missing?* Journal of the American Dietetic Association, 2005. **105**(May): p. S64-S66.
4. Harnack, L.J., R.W. Jeffrey, and K.N. Boutelle, *Temporal trends in energy intake in the United States: an ecologic perspective.* Am. J. Clin. Nutr., 2000. **71**: p. 1471-1484.
5. Bray, G., ed. *Who Are the Overweight? Body Composition and Prevalence*. 2nd ed. 2003, Handbooks in Health Care Company: Newtown, PA. 9-34.
6. Cathy A. Nonas, M., RD, CDE, *Clinical Monitoring in Managing Obesity - A Clinical Guide*. 2004.
7. George A. Bray, M. and P. Catherine M. Champagne, RD, FADA, *Beyond Energy Balance: There is More to Obesity than Kilocalories.* Journal of the American Dietetic Association, 2005. **105**(May, Supplement 1): p. S17-23.
8. Stocker, C.J., J.R.S. Arch, and M.A. Cawthorne, *Fetal origins of insulin resistance and obesity.* Proceedings of the Nutrition Society, 2005. **64**: p. 143-151.

9. Willet, W.C., *Dietary fat plays a major role in obesity: no.* Obesity Reviews, 2002. **3**: p. 59-68.

10. Guo, F. and K.L.C. Jen, *High-fat Feeding During Pregnancy and Lactation Affects Offspring Metabolism in Rats.* Physiology & Behavior, 1995. **57**(4): p. 681-686.

11. J.D. Wright, M., et al., *Trends in Intake of Energy and Macronutrients - United States, 1971-2000*, C. National Center for Health Statistics, Editor. 2004, CDC: Atlanta, GA.

12. 1995, *Nutrition and Your Health: Dietary Guidelines for Americans*, U.S.D.o. Agriculture and U.S.D.o.H.a.H. Services, Editors. 1995, USDA DHSS: Washington, D.C.

13. Krauss, R.M., et al., *Dietary Guidelines for healthy American adults. A statement for health professionals from the Nutrition Committee, American Heart Association.* Circulation, 1996. **94**: p. 1795-1800.

14. *American Cancer Society: Dietary guidelines advisory committee guidelines on diet, nutrition, and cancer prevention: reducing the risk of cancer with healthy food choices and physical activity.* CA Cancer J. Clin., 1996. **46**: p. 325-341.

15. Eileen T. Kennedy, D., RD, P. Shanthy A. Bowman, and M. Renee Powell, *Dietary-Fat Intake in the US Population.* Journal of the American College of Nutrition, 1999. **18**(3): p. 207-212.

16. *Dietary Guidelines for Americans 2005*, U.S.D.o.H.a.H. Services and U.S.D.o. Agriculture, Editors. 2005, U.S. Government Printing Office: Washington, D.C.

17. Muhlhausler, B.S., et al., *Increased maternal nutrition alters development of the appetite-regulating network in the brain.* FASEB Journal, 2006. **20**: p. E556-E565.

18. Ehrenberg, H.M., B.M. Mercer, and P.M. Catalano, *The influence of obesity and diabetes on the prevalence of macrosomia.* Am. J. Obstet Gynecol, 2004. **191**: p. 964-968.

19. Jones, H.N., et al., *High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice.* The FASEB Journal, 2009. **23**(January): p. 1-9.

20. Cnattingius, S., et al., *Prepregnancy weight and the risk of adverse pregnancy outcome.* N. Engl. J. Med, 1998. **338**: p. 147-152.

21. Catalano, P.M., *Obesity and pregnancy - the propagation of a viscous cycle?* J. Clin. Endocrinol. Metab., 2003. **88**: p. 3505-3506.

22. Jansson, N., et al., *Maternal hormones linking maternal body mass index and dietary intake to birth weight.* Am. J. Clin. Nutr., 2008. **87**: p. 1743-1749.

23. Serdula, M., et al., *Do obese children become obese adults? A review of the literature.* Prev. Med., 1993. **22**: p. 167-177.

24. Ghusain-Choueiri, A.A. and E.A. Rath, *Effect of carbohydrate source on lipid metabolism in lactating mice and on pup development.* British Journal of Nutrition, 1995. **74**: p. 821-831.

25. Hausman, D.B., H.M. McCloskey, and R.J. Martin, *Maternal fat type influences the growth and fatty acid composition of newborn and weanling rats.* Journal of Nutrition, 1991. **121**: p. 1917-1923.

26. Koski, K.G., F.W. Hill, and L.S. Hurley, *Effect of low carbohydrate diets during pregnancy on parurition and postnatal survival of the newborn rat pup.* Journal of Nutrition, 1986. **116**: p. 1938-1948.

27. Laychock, S.G., S. Vadlamudi, and M.S. Patel, *Neonatal rat dietary carbohydrate affects pancreatic islet insulin secretion in adults and progeny.* American Journal of Physiology, 1995. **32**: p. E739-E744.

28. Oscai, L.B., *Dietary-induced severe obesity: a rat model.* American Journal of Physiology, 1982. **242**: p. R212-R215.

29. Plagemann, A., et al., *Morphological alterations of hypothalamic nuclei due to intrahypothalamic hyperinsulinism in newborn rats.* International Journal of Dev. Neurosci., 1999. **17**: p. 37-44.

30. Koski, K.G. and F.W. Hill, *Effect of low carbohydrate diets during pregnancy on embryognesis and fetal growth and development in rats.* Journal of Nutrition, 1986. **116**.

31. Vadlamudi, S., et al., *Long-term effects on pancreatic function of feeding a HC formula to rats during preweaning period.* Amercan Journal of Physiology, 1993. **265**: p. E565-E571.

32. Vadlamudi, S., S.C. Kalhan, and M.S. Patel, *Persistence of metabolic consequences in the progeny of rats fed a HC formula in their early postnatal life.* American Journal of Physiology, 1995. **32**: p. E731-E738.

33. Roeder, L.M. and J.K. Stephan, *Influence of maternal diet on reproductive performance and on survival of the offspring.* Federation Proc., 1970 (abstr.). **29**(363).

34. Ferezou-Viala, J., et al., *Long-term consequences of maternal high-fat feeding on hypothalamic leptin sensitivity and diet-induced obesity in the offspring.* Am. J. Physiol. Regul. Integr. Comp. Physiol., 2007. **293**(September): p. R1056-R1062.

35. Bacon F. Chow, P.D. and A.A. Rider, *Implications of the Effects of Maternal Diets in Various Species.* J. Anim. Sci., 1973. **36**: p. 167-173.

36. Bayol, S.A., et al., *Offspring from mothers fed a 'junk food' diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females.* J. Physiol, 2008. **586**(13): p. 3219-3230.

37. Anthony N. Fabricaotre, P., *Behavior Therapy and Cognitive-Behavioral Therapy of Obesity: Is There a Difference?* Journal of the American Dietetic Association, 2007. **107**(1): p. 92-99.

38. E. Paul Cherniack, M., *Potential Applications for Alternative Medicine to Treat Obesity in an Aging Population.* Alternative Medicine Review, 2008. **13**(1): p. 34-42.

39. Weiss, S.R. and M. Michael H. Ebert, *Psychological and Behavioral Characteristics of Normal-Weight Bulimics and Normal-Weight Controls.* Psychosomatic Medicine, 1983. **45**(4): p. 293-303.

40. Kozak, R., et al., *Dietary composition during fetal and neonatal life affects neuropeptide Y functioning in adult offspring.* Developmental Brain Research, 2000. **125**: p. 75-82.

41. Mulhausler, B.S., C.L. Adam, and I.C. McMillen, *Maternal nutrition and the programming of obesity.* Organogenesis, 2008. **4**(3): p. 144-152.

42. Altman, J. and G.D. Das, *Autoradiographic and histological studies on postnatal neurogenesis* J. Comp. Neurol., 1966. **126**: p. 337.

43. Keesey, R.E. and M.D. Hirvonen, *Body weight set-points: determination and adjustment.* J. Nutr., 1997. **127**: p. 1875S-1883S.

44. Bellinger, L., D.V. Sculley, and S.C. Langley-Evans, *Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats.* Int. J. Obes. (Lond), 2006. **30**(5): p. 729-738.

45. Terroni, P.L., et al., *Expression of agouti-related peptide, neuropeptide Y, pro-opiomelanocortin and the leptin receptor isoforms in fetal mouse brain from pregnant dams on a protein-restricted diet.* Molecular Brain Research, 2005. **140**(1-2): p. 111-115.

46. Chang, G.-Q., et al., *Maternal High-Fat Diet and Fetal Programming: Increased Proliferation of Hypothalamic Peptide-Producing Neurons That Increase Risk for Overeating and Obesity.* The Journal of Neuroscience, 2008. **28**(46): p. 12107-12119

47. Taylor, P.D. and L. Poston, *Developmental programming of obesity in mammals.* Exp. Physiol, 2007. **92**: p. 287-298.

48. Covasa, M. and R.C. Ritter, *Adaptation to high-fat diet reduces inhibition of gastric emptying by CCK and intestinal oleate.* Am. J. Physiol. Regul. Integr. Comp. Physiol., 2000. **278**: p. R166-R170.

49. McMillen, I.C. and J.S. Robinson, *Developmental origins of the metabolic syndrome: prediction, plasticity, and programming.* Physiol. Rev., 2005. **85**: p. 571-633.

50. Plagemann, A., *Perinatal nutrition and hormone-dependent programming of food intake.* Horm. Res., 2006. **65 (Suppl. 3)**: p. 83-89.

CHAPTER 2

LITERATURE REVIEW

"Obesity correlates with '…a dysfunctional control of appetite…'" ~ Bayol and colleagues [1].

In today's society, obesity is a growing health problem that has become an epidemic over a relatively short period of time. While many are quick to blame genetics, it is likely due to other factors as well, including environment and lifestyle changes. In fact, there are two types of obesity: monogenic and polygenic. The first is *very* rare in humans and is caused by a single gene mutation, while the latter results from several genes and their combined interaction with environment. These genes have been studied in different aspects of weight control, including food intake, energy expenditure, and macronutrient metabolism [2].

Adding to the genetic complexity of obesity, environment comes into play by increasing or decreasing the expression of many genes. Indeed, Clement stated the cause of obesity to be the "interaction between environmental factors (overeating, diet composition changes and/or a reduction in physical activity) and hereditary factors" [1]. Furthermore, gene alleles can have different effects depending on an individual's physical activity level, implying that gene dominance varies among individuals [2, 3]. More specifically, environment, behavior, socio-economic status, and biological makeup are all determinants in obesity risk [1]. The significant increase in obesity occurrence in a relatively short time period of a few decades indicates that it is more likely due to environmental *and* epigenetic causes [4].

With respect to the epigenetic causes, genes are naturally selected *before* birth based on the intrauterine environment, making maternal nutrition important in offspring's future health [5]. In fact, appetite regulation is one of the key systems that is developed in the womb, and Bellinger et al. reported that prenatal diet clearly influences feeding later in life [13]. This implies that suboptimal maternal nutrition will warrant offspring to have impaired control of what should be an innate behavior [6]. More evidence for this reasoning is that the risk factor for many chronic diseases such as cardiovascular disease, diabetes, and obesity is increased by a "suboptimal intrauterine environment" and differ greatly in prevalence depending on the specific region of the world [7-9]. These facts, along with the wide range of body weight differences (30-80%) from genetics, add to the suggestion that there is more to this severe health problem outside of the biology of the human genome [10, 11]. Overweight and obese women are two to three times more likely to give birth to children with high birth weights, and over half of all pregnant American women fit this category [12]. In turn, obese children are much more likely to become obese adults and pass on such characteristics to their children, this issue needs to be addressed in the immediate future to prevent a vicious cycle of obesity [13].

Fetal Programming/Growth:

"Fetal programming describes a process whereby a stimulus in utero establishes a permanent response in the fetus leading to enhanced susceptibility to later disease." ~ James A. Armitage, et al. [14].

Given that gestation is a crucial time for development, maternal health and nutrition play a big role in the future health of the child. As a result, fetal programming has recently become a topic of interest in obesity research and refers to the influence of

the gestational environment on initial growth and detrimental health conditions throughout life [15]. Not only is the actual programming essential, but timing of certain events both pre- and immediately post-birth can have drastic effects on the offspring [3, 16]. The development of eating patterns is directly related to the period of gestation in which "nutrient insult" occurs. For example, a temporary low-protein diet fed to rats during a specific time of gestation produced offspring with very different eating habits than offspring whose mothers had been protein-restricted throughout gestation [17]. In addition this particular study reported variations in macronutrient intake across the timing of protein restriction. Those offspring exposed to early maternal protein restriction tended to choose more carbohydrate and protein compared with restriction in the middle or end of pregnancy. Female offspring from all of the restricted dams also had decreased fat intakes versus control offspring [17]. Even though it is not fully understood, these behavioral changes were permanent, making maternal diet critical in life-long appetite control of offspring [17, 18].

The first stages in the womb are critical for anatomical and physiological development, and deviations from normal circumstances have lasting effects [7]. Cell proliferation happens most frequently during this time, and thus maternal restriction would drastically decrease cell replication. This leads to permanent reductions in offspring achieving both maximal body size and organ cell numbers [16]. Moreover, it has been reported that fetal growth is most often inhibited in *chronic* maternal undernutrition because a small woman has a limited amount of uterine space for the fetus to grow. This is in comparison to acute malnutrition, which must be fairly severe to evoke strong effects [19, 20]. Adding to the difficulty these newborns have from being

small, they also are likely to experience a natural rapid growth period after birth in attempts of achieving normal body size and making up for uterine restrictions. This catch-up growth, which occurs much faster than normal growth, has been linked with negative future health outcomes [21, 22].

In addition, maternal nutrition and metabolism are important during the latter end of gestation to ensure proper development of the offspring's hypothalamus [23]. Maternal nutrition affects first generation offspring, but has also been suggested to carry over to future generations [24].

Many epidemiological studies have shown that the nutritional status of the mother during pregnancy greatly affects the offspring's risk of obesity and is one of the most important factors affecting birth weight in numerous different species [25]. High birth weights, gestational diabetes, and gestational glucose intolerance have all been linked to future obesity in offspring [26-31].

It is not clear as to the exact mechanisms behind every maternal manipulation due to lack of factual evidence, but there are several supported theories [5]. Barker coined the phrase "fetal origin hypothesis" as the alteration of fetal growth resulting from a detrimental event early in gestation [32, 33]. In particular, concerning obesity, it has been called the "thrifty state of metabolism" [34]. This theory states that the metabolism of offspring is directly impacted by the levels of nutritional stress a mother encounters throughout pregnancy [35]. In other words, maternal nutrient restriction increases the possibility of obesity risk in the progeny (offspring) because of fetal programming adjustments. Specifically, selection and/or expression of specific genes leads to the storage of unused calories for a potential future starvation [5, 34]. Similarly, the "thrifty

hypothesis," explains that malnutrition in the mother causes the child's metabolism to adjust to allow for survival later in life [35] While this is beneficial if dietary restriction continues after birth, it is detrimental in an affluent lifestyle of abundant nutrition availablity, as is common in society today [5]. This phenotype is also associated with adult non-insulin dependent diabetes as evidenced in the Dutch population [36]. It is therefore evident that some physiological changes occur in offspring when a mother is faced with a less-than-optimal gestational environment.

The effects of maternal nutritional stress on offspring were directly shown in one unplanned human study, referred to as the Dutch hunger winter of 1944-1945. During this bitterly cold season, the Nazis decreased the amount of food available in Holland from 1500 calories/person/day to 750 calories, and during the worst times down to 450 calories. In 2005, the children of these women were found to have more cases of adult diabetes and obesity compared with others of similar age [37]. It has been established that newborns of lower weight are at much higher risk of being overweight adults than those of normal birth weight [35]. Other such studies report that slowed or delayed growth early in life is linked with insulin resistance (IR) and obesity in adulthood [3]. With respect to the timing of maternal nutrition, a restriction earlier in gestation increased the likelihood of these children (now adults) becoming obese. They displayed elevated BMIs and waist circumference measures versus those where restrictions occurred later during pregnancy [38, 39]. In addition, this famine-associated adult obesity was not dependent on birth weight because differences were not seen among children born to restricted or non-restricted mothers [38]. Conversely, work by Law et al. showed an

inverse relationship between waist-hip ratio and birth weight, implying greater abdominal fat in the low birth weight group as well [40].

A similar study examined the children born to Scottish women between 1948 and 1954 whose mothers had completed diet surveys while pregnant. Maternal body mass index (BMI) was related to fasting insulin concentrations of the children, with a higher maternal BMI leading to greater changes in glucose and insulin metabolism in the children. Specifically, compared with those born or conceived before the Dutch famine, children of the restricted Scottish mothers had higher 2-hour fasting glucose and insulin levels [36]. This could be speculated to contribute to their subsequent pattern of overweight, which was also shown in the well-known Nurses Health Study where lower birth weights were paired with higher BMIs in adulthood [41]. [It is noteworthy to mention that the Scottish population described above has the chance to show biased results as all those involved were from the same area and had not moved away from the location of birth [36].

Beginning with the coining of the term "thrifty genotype" by Neel in 1622, suggestions have been made by numerous researchers as to different ways that this adaptation may occur: a change in insulin signaling that allows an individual to store more and use less energy, or the mitochondrial DNA may require more energy and increase cravings for high-fat foods, leading the individual to be less "thrifty" [5, 42, 43]. It is known that famines negatively influence an organism's ability to reproduce because it is surviving on a relatively small amount of energy [44]. Referring back to the Dutch hunger winter, conception and birth rates were lower during this time, indicating that those women who *did* successfully conceive were more "thrifty" than their counterparts

[44]. Therefore, both low and high birth weights are associated with adult disease risk [29-31].

Maternal Nutrition:

With maternal malnutrition, the fetal brain and central nervous system (CNS) adapt to prevent harmful effects by utilizing the majority of the available nutrients [45], which leaves a minimal amount those left for organ development [46]. Potential developmental mechanisms are still unclear as adverse effects of both under and overnutrition during pregnancy have been described.

With respect to prenatal exposure to undernutrition, results are mixed. Several studies restricted nutrition of rat dams and resulted in pups with various negative outcomes. These included diabetes and hyperphagia, the latter of which was associated with postnatal obesity [47-51]. In relation to metabolic factors, researchers have reported a decreased maternal protein intake in rodents escalates offsprings' genes for lipogenesis [52], and a gestational nutrient restriction in sheep leads to increased deposition of fat [53]. Both of these studies support the notion that fat metabolism is developed before birth, as suggested by Stocker and colleagues. In addition, Stocker and colleagues found that maternal undernutrition of a restricted food intake during gestation followed with giving offspring access to a high-fat diet after birth lead to offspring that demonstrated an escalated obesity level [3]. Additional support for this notion is shown by Vickers et al., who associated hyperphagia, obesity, and increased fat deposition in offspring (of restricted dams) when they were given nutrient-rich diets after birth [18, 54]. In contrast to the above results, other researchers found a 50% maternal dietary restriction during both mating and gestation produced pups with low birth weights. Even with adequate

maternal nutrition during lactation, these pups were still smaller than controls and did not match controls' body weights when fed ad libitum [55, 56]. Comparatively, pups of restricted dams given to unrestricted dams post-birth had similar body weights at weaning as controls, but were 10% smaller after six months. These pups did not have as low of body weights as those from mothers who were restricted during gestation and lactation. Interestingly, even though they were lighter, they had almost equal food intakes, meaning they had enhanced feed efficieny [57]. This study conflicts with others that observed maternal undernutrition in both gestation and/or lactation caused lower food efficiencies and very high food intakes per unit body weight in offspring [58, 59]. The exact mechanism behind this phenomenon is yet to be determined. Roeder and Chow first proposed problems with digestion, absorption, or use of the nutrients [16], Hsueh and Chow later reported a lower level of gastric secretions in the offspring [56], and even later Chow and Rider suggested metabolic changes in the offspring of restricted dams [58]. Lastly, researchers in the United Kingdom found that while maternal nutrient restriction during gestation does affect offspring feeding behavior, it does not have much impact on the deposition of fat or obesity. It is noteworthy that this study used control and low-protein diets during various stages of gestation and lactation, and did not give offspring access to excess food or nutrition after birth [60].

Details of a study on maternal undernutrition in rats provide a strong precedence for our current hypothesis. Results showed that birth weights were unchanged with the different maternal diets. Subcutaneous fat was significantly lower in the offspring of mothers on the low-protein (LP) diet during middle gestation and throughout gestation. Furthermore, food intake was much greater in male offspring of mothers given LP diets

(v. control) during middle gestation. Therefore, the hypothesis that maternal diet influences offsprings' feeding patterns was supported, but the impact on fat distribution and future obesity was less clear [60].

It has been shown that the increased consumption of high-fat, high-sugar foods interferes with appetite control, which has detrimental effects on life-long food intake [61]. When pregnant women eat high-fat foods, specific signals are released and transmitted to the fetus, potentially having a negative influence on fetal brain alterations [62]. Specifically, food high in fat and/or sugar tends to increase hunger and reward signals while simultaneously decreasing satiety signals, both of which "offset normal appetite regulation [63]. This leads to children that overeat and have excess weight gain, even post-weaning [64]. Not surprisingly, this has also been shown in animal models, with offspring of high-fat fed dams preferring high-fat foods more so than their control counterparts [1]. Likewise, pups previously exposed to high-fat diet that were given chow diets had a decreased energy intake versus controls without such exposure, reiterating that palatability is very important in both control of appetite and overall intake [1, 61]. In addition, it has been noted that a diet of high-fat foods may increase consumption due to both a higher palatability and lower satiating effect [65]. However, changing fat composition may not be the only factor altering food intake, as rats that had *always* received high-fat food (no change in diet) also had reduced intake (in grams) but similar energy intakes as controls [66]. It could be that varying amounts dietary fat content in food changes satiety feelings, with highly palatable food increasing the expression of both hunger and satiety signals [4]. Interestingly, though, this type of food seems to simultaneously diminish the actual response to the satiety signals and enhance

the reward systems involved in food intake [63]. Some researchers went as far and suggested that the fairly recent increased availability of high-fat, high-sugar foods is causing an imbalance in appetite regulation [61]. With the importance of maternal diet established, fetal programming may be contributing more to our current obesity epidemic than previously thought.

Another suggestion is that varying maternal nutrition directly affects the offspring's hypothalamus and its role in feeding, particularly concerning "dietary preferences" [4, 67, 68]. It has even been reported that overweight/obese females have a greater preference for high-fat foods compared to their normal weight counterparts [69]. Optimal growth and development of the brain is highly dependent on the nutritional status of the mother during gestation [70]. It is understood that maintaining energy balance requires the cooperation between different areas of the brain [71], but most evidence points to the hypothalamus as a critical regulator of appetite control [72]. An optimal fetal environment is crucial for full hypothalamic development, and the pathways responsible for energy balance are developing as early as the second postnatal week of life [73-75]. *Any* fetal exposure to a maternal protein restriction, though, can alter the offspring's hypothalamic feeding centers, as was shown in rats [76]. Data from rat studies show that the greatest amount of growth occurs at the end of gestation, which corresponds with organ development [60]. Moreover, Keesey & Hirvonen suggest that the "plasticity of the hypothalamus" is very important in setting appetite control during this same optimal growth period [77]. Even a short duration of maternal malnutrition can negatively affect feeding control in offspring throughout life, but the timing of this malnutrition is the primary factor determining the exact consequences [38, 60].

Similarly, fetal programming effects are also seen from maternal overnutrition. This could lead to increased birth weight of offspring as well as disturbances in metabolism [1, 78]. Khan and colleagues propose that offspring of high-fat fed dams have a "predictive adaptive response" in that pups have increased food intakes without a correlating increase in adipocytes [79]. *{Definition: "predictive adaptive response:" ". . .the degree of mismatch between the pre- and postnatal environments is an important determinant of subsequent disease." [14]}.* To be more detailed, this phrase is used to describe the fetal adjustments made in preparation for what is expected in postnatal life. When the prediction is correct (i.e. pre- and post- natal environments correspond), the offspring is normal, but when the expected and actual environments are not similar, negative health consequences result [80-82]. Strong evidence of this phenomenon was seen in a study of pregnant pigs being fed either a control or high-fat "pro-atherogenic" diet. At weaning, offspring were either kept on the same diet as their mother, or switched to the opposite diet. High-fat fed offspring from control-fed sows displayed significantly more fatty streaks around their aortas than those offspring who had had previous exposure *in utero* to the high-fat diet [83].

Along these lines, one study fed pregnant dams a high-fat diet during gestation, then fed pups a high-fat diet, and measured outcomes of body weight and feed efficiency. It was seen that these pups had lower values of both factors compared to controls, implying that the maternal high-fat diet enhanced the offspring's energy expenditure and was "protective against the adverse effects of the high-fat diet" [that was given to pups]. It is noteworthy that when pups of high-fat fed dams were fed control diets post-birth, they had more undesirable effects, including higher body weights, hyperinsulinemia, and

hyperleptinemia, than pups from high-fat fed dams also fed high-fat diets (HF-HF). As stated, the HF-HF offspring displayed normal body weight (compared to controls) and metabolism, either from a programmed defense mechanism or maintenance of diet composition [84] . Two other studies reported similar results: The first restricted dams and then fed offspring a high-calorie diet, leading to increases in symptoms of metabolic syndrome and larger retroperitoneal fat pads [18]. The second utilized a maternal diet of inadequate protein, which in and of itself produced offspring who did not live as long as controls, but the life-span was reduced even further when offspring were given a high-calorie diet [85]. All of this provides further evidence that maternal diet plays an important role in predetermining the offspring's future health and appetite regulation.

Furthermore, Muhlhausler and colleagues used the evidence from previous reports of the relationship between maternal overnutrition and appetite and body fat of offspring [6, 86, 87]. Contrasting the above suggestion of the potential *protection* of maternal diet, they found that birth weights of offspring from controls and overfed dams were similar. Subcutaneous fat was considerably greater in the latter; however, perirenal fat pads were not notably different [6]. On the other hand, a later study in ruminant animals *did* find that maternal overnutrition led to offspring with preserved perirenal fat, even though they were smaller fetuses, meaning an increased ratio of perirenal fat to body weight compared to controls [25]. It was suggested these results could be from an increased glucose supply (from higher maternal glucose levels) to the fetus during the first two trimesters, which provided additional substrate for fetal fat deposition [25, 88, 89]. Likewise, Bayol and colleagues reported potential for the lasting effects of maternal high-fat diet on offspring. Even with switching pups of high-fat fed dams to chow diets, these

animals still had larger perirenal fat pads many weeks later compared to pups of control dams [90]. Therefore, a high-fat diet during gestation and lactation seems to be more detrimental to offspring's fat deposition than soley a high-fat diet given to offspring after birth. In fact, fetal adaptation to maternal malnutrition does not affect overall growth patterns, but does seem to play a role in how the offspring controls body fat accumulation throughout life [38]. It was stated that a maternal high-fat diet is very essential in "irreversibly" changing offspring's metabolism with respect to adipocytes and fat pads [90]. On the other hand, the reverse of these negative effects of maternal high-fat diet on offspring was reported in a different study. Dams were fed control diets during gestation and lactation with offspring then given a high-fat diet. The pups had similar body weights as controls, implying that even with excess nutrition post-weaning, the maternal control diet decreased the offspring's chances of becoming obese [1]. In fact, it has been shown that regardless of genetics and gestational environment, the risk of becoming obese can be somewhat lessened with a proper postnatal diet and lifestyle. These factors seem to "override" some tendencies that may otherwise lead to negative health outcomes [91]. Thus, the exact conclusion of a maternal high-fat diet being more protective or detrimental for the offspring remains unclear.

Using evidence of the relationship between periconceptual maternal BMI and its effects on offspring, Gallou-Kabani and colleagues were one of the few who used an animal model to investigate this connection. They began with high-fat diet-induced obese dams and switched one group to control diets before conception, during gestation, or during lactation. It was determined that with the more balanced control maternal diet during any stage of development, female offspring were not as prone to developing

symptoms of metabolic syndrome (i.e. excess abdominal weight, dyslipidemia, hypertension, obesity, type 2 DM [92]) as their male and high-fat diet (of dams) counterparts [63]. This provides more evidence of the complexity of fetal programming as it relates to not only maternal body weight but maternal diet as well.

Looking at one specific mechanism of the causes and consequences of excess fetal growth, Jones and colleagues were the first to study nutrient transport via the placenta in mice. Dams given a high-fat diet had higher energy intakes with corresponding higher amounts of glucose transfer through the placenta, resulting in larger fetuses [93]. This contrasts other reports of a lack of overweight fetuses from high-fat fed mice, although these offspring did have an expected alteration in metabolism, specifically increased levels of both insulin and leptin [94]. It is worth mentioning that in the former study the high-fat fed dams did not develop obesity or IR. While these mice *did* have higher intakes of fat versus controls, the carbohydrate and energy intakes were comparable, whereas overweight and obese women generally have higher intakes of all three during gestation. Therefore, researchers are still in debate about the relationship between fetal growth and actual dietary fat content of overweight and obese women during gestation [93].

Even though it is still in its infancy of research, maternal overnutrition has been studied in humans, as in the following restrospective cohort. Regarding gestational weight gain recommendations, mothers who exceeded these amounts had children who had almost a 50% higher chance of being overweight. The additional weight gain is likely due to the high-calorie, high-fat diet that many pregnant women are eating today [14]. The above relationship was seen even after adjustments were made for various

factors, including race, age, and prepregnancy BMI of mothers [95]. Authors suggested this could be due to maternal hyperglycemia (from maternal surplus energy and weight gain) leading to hyperinsulinemia in the fetus. This, in turn, produces children with higher birth weights from the growth effects of insulin, and a lower glucose tolerance, both of which increase the child's likelihood of future obesity [30, 96]. A fetal insulin overload has also been associated with excess food intake and weight gain after birth in other studies due to its contribution to neurotrophic pathways in brain development [97-100].

Eating Behavior:

"…energy intake in excess of energy expenditure is the principal pathophysiological disturbance leading to the deposition of excess body fat." ~ Larry A. Tucker & Marshall J. Kano [69].

As mentioned above, eating behavior is complex and involves integration of external and internal stimuli. Woods et al. proposed that exogenous factors like environment and emotion start the eating process, whereas endogenous factors such as Neuropeptide Y are responsible for ending a meal [101]. The authors state this short-term control is based on the "depletion-repletion model" an organism begins to eat when energy levels fall below a certain value and stops when restored to adequate levels [101]. In contrast, long-term eating behavior may be controlled more due to the "lipostatic model," which states that adipocytes give off signals that interact with various other influences to affect eating behavior [102]. More specifically, the act of eating can be broken into two stages. The first is the "appetitive" phase, or finding food, and the second is the "consummatory" phase, or actual eating [103]. While regulation of eating

entails various components at a given time, regulation of body weight occurs over both the short and long term. The former concerns the time between meals and the latter is more complex, both of which include several neuropeptide signals [104].

Just like the body weight and adiposity of offspring, their future eating behavior also has the possibility to be affected by maternal adiposity levels. A prospective study observed 82 infants from mothers with BMIs over the 66th percentile or under the 33rd percentile for age, which correlated with 'high' or 'low' obesity risk. There was no variation in birth weights. However, there was a great difference in eating patterns at three months of age, with the infants in the higher risk group having a much higher suckling rate compared with those in the lower risk group [105, 106]. This could serve as the basis for the 'vigorous eating style' often seen in overweight children [107-113]. Authors noted that the infants may have not only been eating for existing energy needs of the moment, but also for energy to be used up to one year later as well, similar to what is seen in migrating and hibernating animals [105]. On the other hand, hyperphagia was not seen in offspring of high-fat fed dams even though these offspring were heavier than their control counterparts, possibly due to a reduction in basal metabolic rate [114].

Another factor concerning eating behavior is the feeding efficiency of the organism. It has been previously established that both weight gain and feed efficiency (FE) are augmented with a high-fat diet [69, 115-117]. Specifically, rats fed a high-fat diet during the first sixteen weeks of life had greater body weights and drastically reduced caloric intakes versus those fed a control diet. These high-fat fed rats also displayed a much higher FE during this time period, and FE for all animals slowly diminished with time [117]. The same researchers had previously observed that a high-

fat diet led to a lower food intake compared to controls, but the high-fat subjects consumed a significantly greater amount of calories due to the densely concentrated food. Furthermore, the rats fed the high-fat diet gained weight at a faster rate, meaning that they did not compensate for the more calorically-dense food [116]. This information can be interpreted to mean that with the calorically-dense diet that is available in society today, body weights will only continue to increase because of an organism's ability to store unused calories as extra adipose tissue.

Summary:

It is evident from precedence of both research and societal observations that the obesity epidemic in this country is getting worse by the day. While many factors, including genetic and environmental, contribute to this detrimental health issue, focus is now leading toward alterations that may occur *in utero* that may potentially affect the offspring throughout life. Not only does maternal diet composition seem to play an important role, but adequate nutrition during this crucial time of development should not be overlooked. This study will investigate the changes in body weight, food intake, feed efficiency, and adipose tissue deposition that occur across three generations of offspring from dams fed either chow or high-fat diets, given either ad libitum food or a restricted amount.

Hypothesis:

We hypothesize that maternal nutrient restriction with both standard and high-fat chow adversely affects the genetic programming involved in the regulation of food intake of the offspring.

REFERENCES

1. Bayol SA, Farrington SJ, Strickland NC: **A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring**. *British Journal of Nutrition* 2007, **98**:843-851.
2. Clement K: **Symposium on 'Genes, behaviour and environment:' Genetics of human obesity**. *Proceedings of the Nutrition Society* 2005, **64**:133-142.
3. Stocker CJ, Arch JR, Cawthorne MA: **Fetal origins of insulin resistance and obesity**. *Proc Nutr Soc* 2005, **64**:143-151.
4. Taylor PD, Poston L: **Developmental programming of obesity in mammals**. *Exp Physiol* 2007, **92**:287-298.
5. Prentice AM, Rayco-Solon P, Moore SE: **Insights from the developing world: thrifty genotypes and thrifty phenotypes**. *Proceedings of the Nutrition Society* 2005, **64**:163-161.
6. Muhlhausler BS, Adam CL, Findlay PA, Duffield JA, McMillen IC: **Increased maternal nutrition alters development of the appetite-regulating network in the brain**. *FASEB Journal* 2006, **20**:E556-E565.
7. Thapar A, Harold G, Rice F, Ge X, Boivin J, Hay D, Bree Mvd, Lewis A: **Do intrauterine or genetic influences explain the foetal origins of chronic disease? A novel experimental method for disentangling effects**. *BMC Medical Research Methodology* 2007, **7**(25).
8. Barker DJ: **The intrauterine environment and adult cardiovascular disease**. *Ciba Found Symp* 1991, **156**:3-10; discussion 10-16.

9. Barker DJ, Osmond C: **Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales**. *Lancet* 1986, **1**:1077-1081.

10. Bouchard C: **Current understanding of the etiology of obesity: genetic and nongenetic factors**. *American Journal of Clinical Nutrition* 1991, **53**(Supplement):1561S-1565S.

11. Carmelli D, Cardon LR, Fabsitz R: **Clustering of hypertension, diabetes, and obesity in adult male twins: same genes or same environments?** *American Journal of Human Genetics* 1994, **55**:566-573.

12. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM: **Prevelance of overweight and obesity in the United States**. *Journal of the American Medical Association* 1999-2004, **295**:1549-1555.

13. Serdula M, Ivery D, Coates R, Freedman D, Williamson D, Byers T: **Do obese children become obese adults? A review of the literature**. *Prev Med* 1993, **22**:167-177.

14. Armitage JA, Taylor PD, Poston L: **Experimental models of developmental programming: consequences of exposure to an energy rich diet during development**. *J Physiol* 2005, **565**(1):3-8.

15. Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, Rodriguez-Gonzalez GL, Guzman C, Larrea F, Nathanielsz PW: **Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation**. *J Physiol* 2005, **566**:225-236.

16. Lois M. Roeder SD, Bacon F. Chow PD: **Maternal undernutrition and its long-term effects on the offspring**. *The American Journal of Clinical Nutrition* 1972, **25**(August):812-821.

17. Bellinger L, Langley-Evans SC: **Fetal programming of appetite by exposure to a maternal low-protein diet in the rat**. *Clin Sci (Lond)* 2005, **109**:413-420.

18. Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD: **Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition**. *Am J Physiol Endocrinol Metab* 2000, **279**:E83-E87.

19. O'Brien PMS, Wheeler T, Barker DJP (eds.): **Genomic imprinting of fetal growth**. London: RCOG Press; 1999.

20. Susser M: **Maternal weight gain, infant birth weight, and diet: casual sequences**. *American Journal of Clinical Nutrition* 1991, **53**:1384-1396.

21. Forsen T, Turmilehto J, Reunanen A, Osmond C, Barker D: **The fetal and childhood growth of persons who develop type II diabetes**. *Annals of Internal Medicine* 2000, **33**:176-182.

22. Lucas A, Fewtrell MS, Cole TJ: **Fetal origins of adult disease - the hypothesis revisited**. *British Medical Journal* 1999, **319**:245-249.

23. Kozak R, Burlet A, Burlet C, Beck B: **Dietary composition during fetal and neonatal life affects neuropeptide Y functioning in adult offspring**. *Developmental Brain Research* 2000, **125**:75-82.

24. Barker DJ, Clark PM: **Fetal undernutrition and disease later in life**. *Rev Reprod* 1997, **2**:105-112.

25. Matsuzaki M, Milne JS, Aitken RP, Wallace JM: **Overnourishing pregnant adolescent ewes preserves perirenal fat deposition in their growth-restricted fetuses**. *Reproduction, Fertility and Development* 2006, **18**:357-364.

26. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D: **Size at birth, childhood growth, and obesity in adult life**. *Int J Obes Relat Metab Disord* 2001, **25**:735-740.

27. Cetin I, Morpurgo PS, Radaelli T, Taricco E, Cortelazzi D, Bellotti M, Pardi G, Beck-Peccoz P: **Fetal plasma leptin concentrations: relationship with different fetal growth patterns from 19 weeks up to term**. *Pediatr Res* 2000, **48**:646-651.

28. Metzger BE: **Biphasic effects of maternal metabolism on fetal growth: quintessential expression of fuel-mediated teratogenesis**. *Diabetes* 1991, **40**:99-105.

29. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dorner G: **Overweight and obesity in infants of mothers with long-term insulin-dependent diabetes or gestational diabetes.** *Int J Obes Relat Metab Disord* 1997, **21**:451-456.

30. Silverman BL, Rizzo TA, Cho NH, Metzger BE: **Long term effects of the intrauterine environment. The Northwestern University Diabetes in Pregnancy Center**. *Diabetes Care* 1998, **21 (Suppl. 2)**:B142-B149.

31. Silverman BL, Rizzo TA, Green OC, Cho NH, Winter RJ, Ogata ES, Richards GE, Metzger BE: **Long-term prospective evaluation of offspring of diabetic mothers**. *Diabetes* 1991, **40**:121-125.

32. Hales CN, Barker DJ: **Type 2 (non-insulin-dependent) diabetes mellitus: The thrify phenotype hypothesis**. *Diabetologia* 1992, **35**:595-601.

33. Lucas A: **Programming by early nutrition in man**. *Ciba Found Symp* 1991, **156**:38-50.

34. Knackstedt MK, Hamelmann E, Arck PC: **Mothers in Stress: Consequences for the Offspring**. *AJRI* 2005, **54**:63-69.

35. Hales CN, Barker DJ: **The thrifty phenotype hypothesis**. *Br Med Bull* 2001, **60**:5-20.

36. Shiell AW, Campbell DM, Hall MH, Barker DJP: **Diet in late pregnancy and glucose-insulin metabolism of the offspring 40 years later**. *British Journal of Obstetrics and Gynaecology* 2000, **July**(107):890-895.

37. Roseboon TJ, Meulen JHvd, Ravelli AC, Osmond C, Barker DJ, Bleker OP: **Effects of prenatal exposure to the Dutch famine on adult disease in later life: An overview**. *Mol Cell Endocrinol* 2001, **185**:93-98.

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

39. Ravelli G, Stein Z, Susser MW: **Obesity in young men after famine exposure *in utero* and in early infancy**. *N Engl J Med* 1976, **295**:349-353.

40. Law CM, Barker DJP, Osmon C, Fall CH, Simmons SJ: **Early growth and abdominal fatness in adult life**. *J Epidemiol Community Health* 1992, **46**:184-186.

41. Curhan GC, Chertow GM, Willet WC, Spiegelman D, Colditz GA, Manson JE: **Birth Weight and adult hypertension and obesity in women**. *Ciculation* 1996, **94**:1310-1315.

42. Neel JV: **Diabetes mellitus: a 'thrifty' genotype rendered detrimental by progress**. *American Journal of Human Genetics* 1962, **14**:353-362.

43. Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC: **Effects of purifying and adaptive selection on regional variation in human mtDNA**. *Science* 2004, **303**:223-226.

44. Stein Z, Susser M, Saenger G, Marolla F: **Famine and Human Development: The Dutch Hunger Winter of 1944-1945**. New York: Oxford University Press; 1975.

45. Barker DJ: **Intrauterine programming of adult disease**. *Mol Med Today* 1995, **1**:418-423.

46. Widdowson EM, Crabb DE, Milner RD: **Cellular development of some human organs before birth**. *Arch Dis Child* 1972, **47**:652-655.

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

48. Fernandez-Twinn DS, Wayman A, Ekizoglou S, Martin MS, Hales CN, Ozanne SE: **Maternal protein restriction leads to hyperinsulinemia and reduced insulin-signalling protein expression in 21-mo-old female fat offspring**. *Physiol Regul Integr Comp Physiol* 2005, **288**:R368-R373.

49. Ozanne SE, Smith GD, Tikerpae J, Hales CN: **Altered regulation of hepatic glucose output in the male offspring of protein-malnourished rat dams**. *Am J Physiol* 1996, **270**:E559-E564.

50. Zambrano E, Rodriguez-Gonzalez GL, Guzman C, Garcia-Becerra R, Boeck L, Diaz L, Menivar M, Larrea F, Nathanielsz PW: **A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development**. *J Physiol* 2005, **563**:275-284.

51. Rennie KL, Johnson L, Jebb SA: **Behavioural Determinants of obesity**. *Best Pract Res Clin Endocrinol Metab* 2005, **19**:343-358.

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

53. Symonds ME, Mostyn A, Pearce S, Budge H, Stephenson T: **Endocrine and nutritional regulation of fetal adipose tissue development**. *Journal of Endocrinology* 2003, **179**:293-299.

54. Vickers MH, Breier BH, McCarthy D, Gluckman PD: **Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition**. *Am J Physiol Regul Integr Comp Physiol* 2003, **285**:R271-R273.

55. Chow BF, Lee CJ: **Effects of dietary restriction of pregnant rats on body weight gain of the offspring**. *J Nutr* 1964, **82**(10).

56. Hsueh AM, Agustin CE, Chow BF: **Growth of young rats after differential manipulation of maternal diet**. *J Nutr* 1967, **91**(195).

57. Chow BF, Stephan JK: **Influence of maternal diet on reproductive performance and on survival of the offspring**. *Nutr Rept Intern* 1970, **4**(245).

58. Bacon F. Chow PD, Rider AA: **Implications of the Effects of Maternal Diets in Various Species**. *J Anim Sci* 1973, **36**:167-173.

59. Lee CJ, Chow BF: **Protein metabolism in the offspring of underfed mother rats**. *J Nutr* 1965, **87**(439):812-820.

60. Bellinger L, Sculley DV, Langley-Evans SC: **Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats**. *Int J Obes (Lond)* 2006, **30**(5):729-738.

61. Erlanson-Albertsson C: **How palatable food disrupts appetite regulation**. *Basic Clin Pharmacol Toxicol* 2005, **97**:61-73.

62. News B: **Craving for junk food 'inherited'**. In.; 2007.

63. Gallou-Kabani C, Vige A, Gross M-S, Boileau C, Rabes J-P, Fruchart-Najib J, Jais J-P, Junien C: **Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods**. *Am J Physiol Endocrinol Metab* 2007, **292**:1095-1100.

64. Chang G-Q, Gaysinskaya V, Karatayev O, Leibowitz SF: **Maternal High-Fat Diet and Fetal Programming: Increased Proliferation of Hypothalamic Peptide-Producing Neurons That Increase Risk for Overeating and Obesity**. *The Journal of Neuroscience* 2008, **28**(46):12107-12119.

65. Rolls BJ: **The Role of Energy Density in the Overconsumption of Fat**. *J Nutr* 2000, **130**:268S-271S.

66. Khan IY, Dekou V, Douglas G, Jensen R, Hanson MA, Poston L, Taylor PD: **A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring**. *Am J Physiol Endocrinol Metab* 2005, **292**:E1095-E1100.

67. McMillen IC, Robinson JS: **Developmental origins of the metabolic syndrome: prediction, plasticity, and programming**. *Physiol Rev* 2005, **85**:571-633.

68. Plagemann A: **Perinatal nutrition and hormone-dependent programming of food intake**. *Horm Res* 2006, **65 (Suppl. 3)**:83-89.

69. Tucker LA, Kano MJ: **Dietary fat and body fat: A multivariate study of 205 adult females**. *Am J Clin Nutr* 1992, **56**:616-622.

70. Terroni PL, Anthony FW, Hanson MA, Cagampang FRA: **Expression of agouti-related peptide, neuropeptide Y, pro-opiomelanocortin and the leptin receptor isoforms in fetal mouse brain from pregnant dams on a protein-restricted diet**. *Molecular Brain Research* 2005, **140**(1-2):111-115.

71. Schwartz MW, Woods SC, Daniel Porte J, Seely RJ, Baskin DG: **Central nervous system control of food intake**. *Nature* 2000, **404**:661-671.

72. Williams G, Bing C, Cai XJ, Harrold JA, King PJ, Liu XH: **The hypothalamus and the control of energy homeostasis: Different circuits, different purposes**. *Physiology & Behavior* 2001, **74**:683-701.

73. Bouret SG, Draper SJ, Simerly RB: **Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice**. *J Neurosci* 2004, **24**:2797-2805.

74. Grove KL, Allen S, Grayson BE, Smith MS: **Postnatal development of the hypothalamic neuropeptide Y system**. *Neurosci* 2003, **116**:393-406.

75. Rinaman L: **Postnatal development of catecholamine inputs to the paraventricular nucleus of the hypothalamus in rats**. *J Comp Neurol* 2001, **438**:411-422.

76. Plagemann A, Harder T, Rake A, Melchior K, Rohde W, Dornier G: **Hypothalamic nuclei are malformed in weanling offspring of low protein malnourished rat dams**. *J Nutr* 2000, **130**:2582-2589.

77. Keesey RE, Hirvonen MD: **Body weight set-points: determination and adjustment**. *J Nutr* 1997, **127**:1875S-1883S.

78. Catalano PM: **Obesity and pregnancy - the propagation of a viscous cycle?** *J Clin Endocrinol Metab* 2003, **88**:3505-3506.

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

80. Gluckman PD, Hanson MA: **Developmental origins of disease paradigm: a mechanistic and evolutionary perspective**. *Pediatr Res* 2004, **56**:311-327.

81. Gluckman PD, Hanson MA: **The developmental origins of the metabolic syndrome**. *Trends Endocrinol Metab* 2004, **15**:183-187.

82. Gluckman PD, Hanson MA: **Living with the past: evolution, development, and patterns of disease**. *Science* 2004, **305**:1733-1736.

83. Norman JF, LeVeen RF: **Maternal atherogenic diet in swine is protective against early atherosclerosis development in offspring consuming an atherogenic diet post-natally.** *Atherosclerosis* 2001, **157**:41-47.

84. Ferezou-Viala J, Roy A-F, Serougne C, Gripois D, Parquet M, Bailleux V, Gertler A, Delplanque B, Djiane J, Riottot M *et al*: **Long-term consequences of maternal high-fat feeding on hypothalamic leptin sensitivity and diet-induced obesity in the offspring**. *Am J Physiol Regul Integr Comp Physiol* 2007, **293**(September):R1056-R1062.

85. Ozanne SE, Hales CN: **Lifespan: catch-up growth and obesity in male mice**. *Nature* 2004, **427**:411-412.

86. Plagemann A, Harder T, Rake A, Waas T, Melchior K, Ziska T, Rohde W, Dorner G: **Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed wealing rats**. *J Neuroendocrinol* 1999, **11**:541-546.

87. Martin RJ, Hausman GJ, Hausman DB: **Regulation of adipose cell development in utero**. *Proc Soc Exp Biol Metabol* 1998, **219**:200-210.

88. Wallace JM, Bourke DA, Aitken RP, Cruickshank MA: **Switching maternal dietary intake at the end of the first trimester has profound effects on placental development and foetal growth in adolescent ewes carrying singleton fetuses**. *Biol Reprod* 1999, **61**:101-110.

89. Wallace JM, Silva PD, Aitken RP, Cheyne MA: **Maternal endocrine status in relation to pregnancy outcome in rapidly growing adolescent sheep**. *J Endocrinol* 1997, **155**:359-368.

90. Bayol SA, Simbi BH, Bertrand JA, Strickland NC: **Offspring from mothers fed a 'junk food' diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females**. *J Physiol* 2008, **586**(13):3219-3230.

91. Gorski JN, Dunn-Meynell AA, Hartman TG, Levin BE: **Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance**. *Am J Physiol Regul Integr Comp Physiol* 2006, **291**:768-778.

92. Eckel RH, Grundy SM, Zimmet PZ: **The metabolic syndrome**. *Lancet* 2005, **365**:1415-1428.

93. Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, Jansson T: **High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice**. *The FASEB Journal* 2009, **23**(January):1-9.

94. Sameulsson A-M, Matthews PA, Argenton M, Christie MR, McConnell JM, Jansen EHJM, Piersma AH, Ozanne SE, Fernandez-Twinn D, Remacle C *et al*: **Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension and insulin resistance**. *Hypertension* 2008, **51**:383-392.

95. Wrotniak BH, Shults J, Butts S, Stettler N: **Gestational weight gain and risk of overweight in the offspring at age 7 y in a multicenter, multiethnic cohort study**. *Am J Clin Nutr* 2008, **87**(1818-1824).

96. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA: **Maternal gestational diabetes, birth weight, and adolescent obesity**. *Pediatrics* 2003, **111**:e221-e226.

97. Kalra SP, Dube MG, Pu S, Xu B, Hovarth TL, Kalra PS: **Interacting appetite-regulating pathways in the hypothalamic regulation of body weight**. *Endocr Rev* 1999, **20**:68-100.

98. Folli F, Ghidella S, Bonfanti L, Khan CR, Merighi A: **The early intracellular signaling pathway for the insulin/insulin-like growth factor receptor family in the mammalian central nervous system**. *Mol Neurobiol* 1996, **13**:155-183.

99. Torres-Aleman I, Naftolin F, Robbins RJ: **Trophic effects of insulin-like growth factor-I on fetal rat hypothalamic cells in culture**. *Neurosci* 1990, **35**:601-608.

100. Weisenhorn DM, Roback J, Young AN, Wainer BH: **Cellular aspects of trophic actions in the nervous system**. *Int Rev Cytol* 1999, **189**:177-265.

101. Woods SC, Strubbe JH: **The psychobiology of meals**. *Psychonomic Bull Rev* 1994, **1**:141-155.

102. Kennedy GC: *Proc R Soc London B Biol Sci* 1953, **140**:579.

103. Hillebrand JJG, Wied Dd, Adan RAH: **Neuropeptides, food intake, and body weight regulation: a hypothalamic focus**. *Peptides* 2002, **23**:2283-2306.

104. Konturek SJ, Konturek JW, Pawlik T, Brzozowki T: **Brain-gut axis and its role in the control of food intake**. *Journal of Physiology and Pharmacology* 2004, **55**(1):137-154.

105. Stunkard AJ, Berkowitz RI, Schoeller D, Maislin G, Stallings VA: **Predictors of body size in the first 2y of life: a high-risk study of human obesity**. *International Journal of Obesity* 2004, **28**:503-513.

106. Holly R. Hull P, Mary K. Dinger P, Allen W. Knehans P, David M. Thompson P, David A. Fields P: **Impact of maternal body mass index on neonate birthweight and body composition**. *Amercan Journal of Obstetrics & Gynecology* 2008, **198**:416.e411-416.e416.

107. Hall WG, Williams CL: **Suckling isn't feeding, or is it? A search for developmental continuities**. *Adv Study Behav* 1983, **13**:219-254.

108. Raskin LA: **The ontogeny of amphetamine anorexia. A behavioral analysis**. *J Comp Physiol Psychiatry* B.A. Campbell, **95**:425-435.

109. Drabman RS, Hammer D, Jarvie GJ, Horton W: **Developmental trends in eating rates of normal and overweight preschool children**. *Child Dev* 1979, **50**:211-216.

110. Drabman RS, Hammer D, Harvie GJ: **Eating styles of obese and non-obese black and white children in a naturalistic setting**. *Addict Behav* 1977, **2**:83-86.

111. Waxman M, Stunkard AJ: **Caloric intake and expenditure of obese boys**. *J Pediatr* 1980, **96**:187-193.

112. Waxman M: **Fat families and thick description: a naturalistic study of obese girls and their nonobese sisters**. In.: University of Pennslyvania; 1988.

113. Barkeling B, Ikman S, Rossner S: **Eating behaviour in obese and normal weight 11-year-old children**. *Int J Obes Relat Metab Disord* 1992, **16**:335-360.

114. Khan IY, Taylor PD, Dekou V, Seed PT, Lakasing L, D. Graham ea: **Gender-linked hypertension in offspring of lard-fed pregnant rats**. *Hypertension* 2003, **41**:168-175.

115. Hill JO, Lin D, Yakubu F, Peters JC: **Development of dietary obesity in rats: Influence of amount and composition of dietary fat**. *Int J Obes* 1992, **16**:321-333.

116. Jen K-LC: **The effect of high fat diet on the behavioral patterns of male rats**. *Physiol Behav* 1980, **25**:373-381.

117. Jen K-LC: **Effects of diet composition on food intake and carcass composition in rats**. *Physiol Behav* 1988, **42**:551-556.

CHAPTER 3

MATERNAL NUTRIENT RESTRICTION: CONSQUENCES ON THE OFFSPRING[1]

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INTRODUCTION

Due to rising obesity rates, the idea of fetal programming has gained interest as a potential contributor to the epidemic. Specifically, it refers to the role that gestational environment has on initial growth and adverse health effects later in life [1]. The best evidence for this in humans is the well-known epidemiological study of the Dutch Hunger Winter of 1944-1945, which strongly correlated maternal malnutrition with negative metabolic changes in the offspring [2]. In turn, these metabolic alterations can lead to various chronic diseases in adulthood (i.e. obesity, diabetes mellitus, and heart disease [3]) [2]. Therefore, further research into the potential factors involved in the risk for obesity is imperative in order to prevent the current epidemic from increasing.

The hypothalamus has been coined the "most important [brain] area" involved in energy balance, and optimal development of this organ is highly affected by the fetal environment [4, 5]. Due to the high "plasiticity" of the developing hypothalamus, it easily affected by the growth environment and adapts accordingly [6]. This means that even short durations of maternal malnutrition can have a negative impact on the offspring's eating behavior [6, 7]. Concerning growth and maturation, the fetal brain and central nervous system (CNS) respond to maternal malnutrition to prevent harmful effects. This occurs via the utilization of the majority of the nutrients that *are* available [8], leaving very little nutrition for other organ development [9].

Thus far, there is conflicting evidence regarding the effects on the offspring of maternal undernutrition during gestation. The Dutch Hunger Winter children of women who had an extremely low food availability during pregnancy tended to have a higher

prevalence of obesity and diabetes as adults compared to others of similar ages [10]. This provides support for the "thrifty state of metabolism" hypothesis, which relates maternal nutritional stress to the metabolism of the offspring [11, 12] In addition, animal research reports increased diabetes and hyperphagia in offspring of restricted rat dams [lit review 45-49]. Conversely, though, other researchers found that a 50% maternal dietary restriction during both mating and gestation produced pups with low birth weights who never matched body weights in controls even with adequate postnatal nutrition [13, 14].

Maternal overnutrition has also drawn attention as of late with the lack of people who successfully meet the Dietary Guidelines requirements of 20-35% of calories from dietary fat, but again the evidence is not clear [15]. Women who are overweight or obese during pregnancy likely eat a relatively high-fat (HF) diet, and then give birth to large for gestational age (LGA) babies [16, 17]. These children are thus at increased risk of being obese as an adult as childhood obesity usually carries on to adulthood [16]. Such a HF maternal diet is also associated with an "irreversible" increase in offspring adiposity that is maintained regardless of post-natal nutrition [17]. It has been proposed that upon maternal consumption of HF-foods, specific signals are released and transmitted to the fetus, which may have a negative influence on fetal brain development [18]. Specifically, food high in fat and/or sugar tends to increase hunger and reward signals while simultaneously decreasing satiety signals, both of which "offset normal appetite regulation" [19]. On the other hand, one study reported pups of HF-fed dams to have lower body weights than controls, and other researchers found similar birth weights in offspring of both restricted and control dams [4, 20]. Concerning the latter, it is noteworthy that offspring in the HF group had much more subcutaneous fat, but perirenal

fat pads were relatively similar [4]. Thus, there is still much in debate about potential developmental mechanisms of appetite and food regulation as adverse effects of both under and overnutrition during pregnancy have been described.

The purpose of this study was to determine the effect of two different maternal treatments, control (ad libitum food intake) and restricted (75% of ad libitum food intake during the last trimester of gestation) on the energy balance regulation in offspring in two different experiments. Using mice subjects, two different maternal diet compositions were also be examined using a standard chow diet and a high-fat diet with each maternal treatment. This was achieved by measuring weekly body weights, food intakes, feed efficiencies, perirenal fat pad weights, and body fat percentages in three subsequent generations of offspring from each diet and treatment.

Research shows that maternal diet is critical in offspring development, specifically with aspects related to appetite regulation. **The aim of Experiment 1 is to determine if maternal nutrient restriction with standard chow causes an increase in body weight, food intake (grams and/or kilocalories (kcal)), feed efficiency, or adipose tissue deposition in offspring up to the third generation. Results will provide evidence for the adverse effects of maternal malnutrition on offspring.**

In today's society, more people are consuming high-fat diets, which have been strongly associated with increasing rates of obesity [21]. **The aim of Experiment 2 is to determine if a maternal diet of significantly greater fat composition (compared to standard chow) followed by restriction further enhances the effects on body weight, food intake (grams and kcal), feeding efficiency, and adipose tissue deposition in**

offspring up to the third generation. Results will give insight into the effects of increased maternal fat intake on the appetite regulation of future generations.

METHODS

Aim 1 Experiment: Does maternal nutrient restriction with standard chow adversely affect the fetal programming of feeding behavior and subsequent body weight across three generations of offspring?

C57Bl/6 female mice (age 3 months) were bred with C57BL/6 males and fed standard chow (Purina Mouse Diet #5015: 17% protein, 11% fat, 3% fiber, 6.5% ash, 2.5% minerals; 3.73 kcal/gram, 88.1 % Total Digestible Nutrients) during breeding period. Daily body weights (grams) were measured. A 10 gram body weight increase signified the third trimester of gestation and females were separated from males. Standard chow was continuously fed to this point in gestation, upon which they were divided into two groups. One group of females was restricted by 25% of the ad libitum food intake and the other fed ad libitum. Pups were born within the following 7 days. 24 hours post-delivery, litter weight was measured and recorded. F1 pups remained with the mother for three weeks and then were weaned. At this time, individual weights were taken, and mice were placed in individual cages and fed standard chow for 8 weeks. Daily food intakes and weekly body weights were measured, and daily food intakes were combined for a weekly average. At 11 weeks of age, all F1 pups (except for those females kept for subsequent breeding) were killed by decapitation; brains and perirenal fat pads were collected. Brains were dated and stored at -80°F and fat pads were weighed and stored. Randomly selected F1 females (11 weeks old) were bred with normal males to obtain second generation progeny. (Original F0 dams were sacrificed (after pups were

weaned), and brains collected, dated, and stored at -80°F). F1 females were weighed daily (grams) and fed standard chow until increased body weight of 10 grams, when they were separated from the males. The above process was repeated to obtain two more sequential generations, with each having 24 subjects (12 from restricted dams, 12 from non-restricted dams; each with 6 male and six female). Appropriate timing for the breeding of the three generations was achieved by breeding all females at the same time point in the life cycle (11 weeks old). This assures consistency and decreases the potential differences that could occur with various ages of dams. *Refer to Table 1.*

Aim 2 Experiment: Does maternal nutrient restriction combined with high-fat chow adversely affect the fetal programming of feeding behavior and subsequent body weight across three generations of offspring?

The following changes from Experiment 1 were implemented for the protocol of Experiment 2. The F1 progeny of mice were obtained using the same restriction procedure except that pregnant F0 dams were fed a high-fat diet. After weaning (3 weeks old), F1 pups were fed a high-fat diet (Research Diets D12451-20% protein, 35% carbohydrate, 45% fat; 4.73 kcal/gram) for 8 weeks, when males and females were then killed (11 weeks old). Additional F1 females were bred with normal males and fed standard chow until the last trimester, when they were divided into two groups. One group was fed high-fat diet ad libitum and the other high-fat diet of 25% restriction from ad libitum food intake. As before, F2 pups were fed high-fat diet. This process was repeated, resulting in three generations with each having 24 subjects as in Experiment 1. *Refer to Table 1.*

Analysis of weekly body weights, weekly food intakes, feed efficiency, and adipose tissue weight were carried out in the pups of restricted v. nonrestricted dams, and first v. second v. third generations across the 8 weeks post-weaning. Feed efficiency was calculated by dividing body weight gained (grams) by food intake (Kcal). A high feed efficiency implied that the animal was biologically efficient at depositing excess dietary calories as adipose tissue for potential later use, while a lower feed efficiency suggested the animal had to eat the same amount of kcal to obtain body weight equivalent to more efficient animals. Percent body fat was estimated using weight of perirenal fat pads divided by overall body weight at week 11 from each generation progeny of Experiment 2 (high-fat diet). A higher ratio indicated a higher body fat percentage. PowerAnalysis was utilized to determine sample size of n=12. Data from both experiments were pooled together to obtain the following independent variables: generation 1, generation 2, generation 3, ad lib food intake, restricted food intake, chow diet, and high-fat diet. The dependent variables analyzed were: body weight (grams), food intake (grams), food intake (kcal), feed efficiency, and adipose tissue weight (grams).

Statistical Analysis:

Body weight, food intake, feed efficiency, perirenal fat pad weight, and body fat percentage were compared within and between groups using a Repeated Measure ANOVA (SPSS 16.0, Chicago IL). Maternal treatment and diet were used as covariates to determine changes between groups. The differences between specific groups were compared by paired t-tests, if needed. Differences were considered significant if $P<0.05$ and $P<0.01$ for Repeated Measures and t-tests, respectively.

RESULTS:

This study involved the use of 254 mice, spread over 2 experiments (chow diet and high-fat diet) with 3 subsequent generations in each experiment.

Generation 1: (Refer to Tables 2 & 3)

In the first generation progeny, the differences in maternal food availability (control v. restricted intake) and diet composition (chow v. high-fat) led to a significant difference in body weight with an interaction between time and treatment (P=0.016). The offspring from restricted dams had higher average body weights at weaning (11.7g ± 0.042), but afterwards were lighter across all weeks compared to those from controls. There was also a body weight interaction between time and diet (P=0.0001), which showed a consistently higher body weight in the Ch groups compared to the HF groups (C/Ch: 25.2g ± 0.844, R/Ch: 25g ± 0.924 versus C/HF: 25g ± 0.807, R/HF: 23g ± 0.714). At week 11 the R/HF group was the lightest (23g ± 0.714) and the C/Ch was the heaviest (25g ± 0.844).

Regarding food intake and diet comparison, there was significant interaction between time and diet concerning food intake, with the Ch groups consistently consuming more kilocalories (Kcal) across weeks than HF groups (Ch: 113 kcal ± 2.235; HF: 94 kcal ± 1.013; P=0.0001). This was a similar pattern as seen with the body weights of this generation, although not statistically significant here concerning food intake. The Ch-fed animals ate and weighed more compared to the HF-fed, and animals from control dams also ate more than those from restricted dams. However, at weeks 10 and 11, intakes became approximately equal among both HF-fed groups (Week 11: C/HF

96 Kcal, R/HF: 96 Kcal. This likely contributed to the higher body weights observed in the chow groups compared to the high-fat groups.

There were no significant interactions or differences regarding feed efficiencies in the first generation progeny. Over time, though, feed efficiency both decreased and increased with respect to both treatment and diet, and but changes had no discernable pattern.

An increase in feed efficiency would imply that the animals were remarkably thrifty in storing the majority of food Kcal consumed as body weight, deposited either for fat storage or lean tissue, rather than immediately expending this food energy. Conversely, animals with lower feed efficiency expend a greater percentage of food energy consumed; thus, they must eat more food to achieve the same body weights as their more efficient counterparts. Therefore, if two animals with high and low feed efficiencies, respectively, consume the same amount of Kcal, the less efficient animal will have a lower body weight, as the Kcal from food are used immediately for energy rather than being stored as fat for later use.

In this particular study, it appears that all offspring in generation 1 had relatively similar "thriftyness" as all had comparable feed efficiencies. This implies that all animals conserved energy consumed (via body weight gain) for potential future starvation, regardless of diet or maternal treatment. Even with the observed differences in body weights and food intakes (Ch groups were higher in both parameters), similar feed efficiencies suggest that each of the four groups of animals was able to metabolically adapt to the specific diet and/ or maternal treatment and prevent excess weight gain over time. In regard to the HF-fed pups, there seems to be an enhanced change in metabolism

because in order to prevent what would otherwise be inevitable weight gain from the more calorically dense diet, these animals apparently expended the excess energy (Kcal) from food at time of consumption (given their overall lower body weights) versus the ordinary production of fat stores that would have resulted from excess energy intake.

Generation 2: (Refer to Tables 2 & 3)

Concerning body weight in the second progeny, there were no interactions or significant differences among treatments or diets. Interestingly, though, animals from control dams tended to have lower body weights than restricted groups across most weeks. Results were slightly less predictable between the two diets, with the chow group only weighing less in 3 of the 9 measured weeks. It is also interesting to note that at week 11, the C/Ch group average was the heaviest (25.6g ± 0.926) while the R/HF group was the lightest (23g ± 0.850), just as seen in the first generation.

There was a significant interaction for food intake between time and diet in the second generation with the HF-fed animals consuming significantly less Kcal (Ch: 113.1 kcal ± 2.239; HF: 94 kcal ± 0.858; P=0.0001). This matched the heavier average body weights among Ch groups compared to HF groups across time. Over time, though, food intake patterns varied between both treatments and diets. In the offspring from control dams, there was no discernable pattern, although food intake increased, decreased, and increased again with time, while the offspring from restricted dams showed a marked decrease by week 11. As a group, this drastic drop in food consumption among offspring from restricted dams was most likely from the R/HF group data, who ate the least at week 11 (92 kcal ± 1.279) because the R/Ch group had the highest food intake (100 kcal ± 3.368) among all four groups. This is interesting because while eating the least kcal, the

R/HF group did not display the lowest average body weight at week 11; rather, the R/Ch group weighed the least (24g ± 0.817).

A significant interaction between time and diets (P=0.0001) was also found regarding feed efficiency, and further analysis revealed the Ch-fed animals to have an overall lower average across weeks than the HF-fed animals (Ch: 0.013 ± 0.002, HF: 0.018 ± 0.001; P=0.038). It is interesting, though, that a week 11 comparison of the four groups showed the R/Ch group to be the most efficient ($8.175 * 10^{-3} \pm 3.953 * 10^{-3}$), while the C/Ch group was the least efficient ($4.647 * 10^{-3} \pm 1.683 * 10^{-3}$). As in generation one, all groups showed an overall decrease in feed efficiency over time.

Generation 3: (Refer to Tables 2 & 3)

The differences in maternal treatments and diets led to significant differences in body weight with interactions between time and treatment (P=0.049) and time and diet (P=0.0001). A trend was seen within the Ch diet with offspring from control dams consistently weighing less than those from restricted dams, even though this was not the case at weaning. The high incidence of lower body weights in the Ch-fed animals as a group compared to HF-fed animals is likely related to the lower caloric density of the chow diet. This could potentially be explained with the same reasoning of the higher caloric density of the diet, but here the Ch groups may have weighed less due fewer calories consumed.

The C/Ch group initially weighed the most (wean weight: 13.7g ± 0.408), but both the HF groups gained to have similar heaviest average body weights at week 11 (C/HF: 24.8g ± 0.914, R/HF: 24.7g ± 0.797) (*Figure 1*). This difference in weight gain pattern was inversely matched to food intake in that at week 11 both Ch groups (control

and restricted) had higher average intakes (C/Ch: 120 kcal ± 3.588, C/R: 114 kcal ± 5.350) than the HF groups (C/HF: 97 kcal ± 2.254, R/HF: 97 ± 2.251) (*Figure 2*). Within the HF group, though, offspring in the control group had lower body weights up to week 6, after which those from restricted dams weighed less. Concerning the C/Ch group, though, these results directly correlate with their low feed efficiency. This means that these animals had to eat a higher amount of food to achieve a relatively similar body weight as the other groups.

There was also a three-way interaction with food intake between time, treatments, and diets (P=0.006). A second repeated measures was analyzed with a separation by treatment and revealed significance interactions between time and diet in both the control and restricted group (P=0.009, P=0.038, respectively). A t-test, however, then only showed significantly lower intakes among the controls in weeks 6 (P=0.009) and 10 (P=0.006) in, but no significant differences in offspring in the restricted group.

A third repeated measures analysis of feed intake was analyzed with a separation by diet, and this showed significance between time and treatment (P=0.023) in the Ch group. In regard to this group, the animals from restricted dams had lower average food intakes in all weeks except for 5 and 6. Results within the HF diet were more interesting in that the restricted group had slightly lower average food intakes up to week 9, after which food intakes in controls and restricted were almost equal.

When looking at all animals from restricted dams from both studies (chow and high-fat diets), there was a significant three-way interaction between time, treatment, and diet (P=0.019). A separation by diet then revealed a significant difference among

offspring of restricted dams on each diet (P=0.004), with the Ch groups having higher feed efficiencies than the HF groups across all weeks.

In addition, it is interesting that within the control groups, Ch-fed animals tended to have higher feed efficiencies across all weeks compared to HF-fed animals, except for the first and last week, although this was not significant (*Figure 3*). This could indicate that feed efficiency may be an adaptable mechanism able to be programmed *in utero* depending on maternal diet composition and availability. Regarding these findings, it suggests that offspring from Ch-fed dams were more efficient at storing excess calories as body fat compared than their HF counterparts within all controls. A possible implication for this observation is similar to that described above in that the pups from HF-fed dams were programmed *in utero* in response to the maternal HF diet. It matches that both groups of HF offspring were more efficient at week 11 than the either Ch group. Thus, after birth, the HF animals were apparently more metabolically able to expend the excess calories from a HF diet rather than store this energy as body fat, whereas the Ch-fed animals had no such adaptation and kept to the typical body weight gain pattern seen with normal maturation and development. Finally, there were no clear patterns of differences in feed efficiency in any group across all weeks (Graph 3).

Perirenal Fat Pads: (Refer to Tables 4-6)

At week 11, fat pads of the both HF groups were collected and weighed. A one-way ANOVA analysis was completed for these fat pad weights (in grams); however, there was not an interaction between generation and treatment, nor were significant differences found between generations or maternal treatments. Overall average fat pad weights increased with subsequent generations (Generation 1: 0.183g ± 0.015;

Generation 2: 0.216g ± 0.015; Generation 3: 0.230g ± 0.015). When comparing between treatments, it was seen that the control group had overall higher average fat pad weights versus the restricted (Control: 0.213g ± 0.012; Restricted: 0.206g ± 0.013). Specifically, though, the controls had heavier fat pads in the first and second generations, but this was reversed by the third generation with the restricted group having the heaviest fat pads. It is noteworthy that fat pad weight gradually increased with subsequent generations in the restricted group, but within the control group there was no discernable pattern in fat pad weight changes.

Body Fat Percentage: (Refer to Tables 4-6)

A one-way ANOVA analysis was also completed for perirenal fat as a percentage of total body weight [(perirenal fat pad (g)/week 11 body weight (g)) * 100] of both HF groups. Again, though, there was not an interaction between generation and treatment or any statistically significant differences within generation or maternal treatment. When both treatments were combined, there was a slight increase in perirenal fat percentage with subsequent generations, which matches the increase seen above in fat pad weights. The overall comparison of the two treatments revealed a minor increase in perirenal fat percentage in the restricted group versus the controls (Control: 0.800% ± 0.001; Restricted: 0.900% ± 0.001). Although there were not any clear patterns of increase or decrease across generations, results showed highest perirenal fat percentages in the last generation of both treatments (Generation 1 Control: 0.649% v. Generation 3 Control: 0.874%; Generation 1 Restricted: 0.891% v. Generation 3 Restricted: 0.963%).

Thus, the results match that within the HF third generation offspring, the restricted group had lower average body weights (Control: 25g, Restricted: 24.7g) and

higher average fat pad weights (Control: 0.2194g, Restricted: 0.2409g) than controls, resulting in higher perirenal fat percentages in this group as well. This variation could denote that the maternal restriction modified fat metabolism in offspring to increase deposition and overall body fat percentage for future energy needs.

DISCUSSION:

There is strong evidence to support the relatively new idea of fetal programming and its association with the influence of maternal diet on the future health of the offspring [1, 22]. The objective of this study was to determine if two maternal metabolic states and two different maternal diets affected various energy regulation outcomes across three generations of offspring.

Within the first generation progeny, a restricted maternal treatment led to offspring that weighed less than control counterparts, as seen in a study by Bayol and colleagues. They observed a lower body weight in offspring of dams on a HF diet [23]. In our study, offspring from HF-fed dams also displayed lighter body weights than Ch-fed. At week 11, the lowest average body weight was seen in the R/HF group. Offspring of the Ch-fed dams also consumed more kcal than those from HF-fed dams, which contrasts previous findings of rats fed a cafeteria-type diet having higher energy intakes and weight gain than chow-fed [24, 25].

Previous reports of maternal HF diets and the consequential body weights and feed efficiencies in offspring revealed increases in both paramenters [26-29]. In the current study, though, a lower feed efficiency was *expected* in the HF compared to Ch animals based on the lower body weight gain and food intakes, but this was not the overall observation. Lower body weights and feed efficiency in pups of HF-fed dams has

been previously reported as well [20]. Therefore, the present results suggest that animals in the HF groups may have had an enhanced metabolic adaptation in response to the more calorically dense diet. As suggested by other researchers, this could have been due to an increase in energy expenditure, which may have been a mechanism to protect "against the adverse effects of the HF diet…" [20]. This occurrence obviously allowed them to avoid what would have inevitably been weight gain with a similar kcal intake as the Ch groups. In other words, the HF animals both weighed and ate less, probably from the higher satiating diet as well as factors mentioned above, but still showed to be more efficient with calories consumed. Their regulatory mechanisms controlling appetite were apparently adapted to the HF diet and thus, they maintained a consistently lower body weight across time instead of storing the excess energy from the diet as body fat.

In regard to the treatment comparison in the second generation of offspring, body weights tended to be slightly lower in control compared to the restricted animals. Although not significant, a comparison of the two diets revealed that the Ch groups had higher average body weights over most weeks of the study compared to the HF groups, while a comparison of the four individual groups showed the C/Ch group to have the highest body weights at week 11. This correlates with a previous study in which both male and female adult offspring of HF-fed dams had 15% and 5% higher body weights than those from chow-fed dams, respectively [20]. Similar to the outcome in the first generation and previous findings [27], the Ch-fed groups in the second progeny also consumed more kcal ($P=0.0001$) than HF-fed groups, with the R/Ch having the highest average food intake.

Results from similar studies (mentioned above) showed a maternal high-fat diet increased feed efficiency in offspring [20], while the current study showed a lower feed efficiency in both groups of Ch-fed animals. These food intake results correlate with these lower feed efficiencies found in both groups of Ch-fed animals of the current study, meaning that these animals had to eat more to obtain comparable body weights. It is interesting to note that while the C/Ch group had the highest average body weight gains, these animals displayed the lowest average feed efficiency and second lowest food intake. This means that although these animals *should* have eaten more food to obtain comparable body weights, they ate less food and still reached a relatively high body weight. A potential mechanism for this occurrence is the expected metabolic outcome from a typical maternal diet and intake, meaning that programming of body weight is a natural process. With maternal malnutrition, it is hypothesized that genes are selected for the organism to survive future food deprivation. This adaptation is beneficial if, in fact, food is not adequately available later in life, but detrimental when with abundant food availability [22]. Thus, in the current study, natural weight gain in the C/Ch offspring was favored due to normal regulatory mechanisms of appetite and the "set point" theory of achieving normal body weight (Normal body weight of a mouse is 25-30g) [30, 31], as well as sufficient food availability. These animals obviously required less kcal for survival and fat deposition.

In addition, within both Ch groups, offspring from restricted dams may have consumed more kcal than other groups in response to the previous semi-starvation exposure *in utero*. This could have been an attempt at "catch up growth" to achieve similar body weights as controls for an increased chance of survival, which has been

reported by Ong and colleages [32]. By consuming more kcal, these animals may have been compensating for a gestational malnutrition, and subsequent fat deposition by storing more fat from food consumption after birth. In agreement, then, this group (R/Ch) also had the highest average feed efficiency at week 11, meaning these animals deposited all excess kcal (not needed for immediate energy) into storage. This could be similar to the response to maternal restriction described above, in that these pups were compensating for a reduction in gestational nutrients and subsequent fat deposition by storing more fat from food consumption *after* birth.

The final third generation will probably more accurately reflect any long-term effects of maternal treatment differences or dietary composition. Concerning the gains in body weight, offspring from restricted dams on both diets were consistently lighter than those from controls dams on both diets. It is interesting, though, that within all Ch-fed animals, the opposite was seen with the non-restricted group having lighter body weights than restricted offspring. This is likely related to the lower caloric density of the chow diet. Within HF-fed animals, controls were initially lighter but restricted animals gradually displayed heavier body weights to match the pattern in the Ch groups, probably as a result of the continued consumption of the HF diet. Given that a "maternal junk food diet" and/or a nutrient excess during pregnancy increases obesity risk in the offspring [23, 33, 34], it matches here that HF-fed animals were ultimately the heaviest, although not statistically significant.

Offspring from restricted dams in both diet groups also had lower food intakes across time. Previous research, however, showed that maternal dietary restriction led to offspring with hyperphagia, but this evidence was not based on multiple subsequent

generations as was the current study [35]. Therefore, it is speculated that by the third generation, the fetal programming effect of maternal dietary restriction had become stronger and offspring were more respondent to the intrauterine environment. In turn, with sequential progenies of inadequate maternal nutrition exposure, brain regulatory mechanisms of appetite and feeding may have programmed the animals to require less food energy than what is normally expected. Moreover, the maternal HF diet study mentioned above that showed an increased likelihood of offspring being overweight also reported an increased dietary intake among these offspring [23]. These results may differ from the current findings because of the exact macronutrient composition of each diet and the respective satiation consequences.

Regarding feed efficiency, Ch-fed animals were less efficient than HF-fed animals in both treatments. These findings are surprising given the high body weights and food intakes among both Ch groups. It could be similar to the set point theory mentioned above of obtaining normal body weight [31] in that these animals required a higher amount of food to reach comparable body weights as HF animals, and so to achieve their increased body weight, they had to eat even a larger amount of food. This result, though, is more likely a result of the lowest average feed efficiency in the R/Ch group compared to the others at week 11, which lowered average among all Ch animals. This implies that even with a relatively high food intake, these animals were not able to store excess consumption of kcal but rather immediately expended food energy.

In addition, varying digestibility between the two diets may have accounted for some of the differences observed regarding feed efficiency. The standard chow diet provided 3.73 Kcal/gram and had a percentage of Total Digestible Nutrient (TDN) of

88.1, meaning that only 3.26 Kcal/g was available as digestible energy [36]. The HF diet, however, provided 4.73 Kcal/gram with a TDN of 95%, which would only provide 4.49 Kcal/gram as available energy [37]. Therefore, the lower nutrient availability in the standard chow diet may have led to the lower feed efficiencies among these animals. Furthermore, a decreased amount of available energy from food would explain their relatively higher food consumption (in Kcal), which overall may have attributed to their higher body weights as well.

Perirenal fat pad weights in the first two generations of offspring from restricted dams fed a HF diet weighed less. This could have been due to these pups immediately utilizing energy consumed in lieu of the immediate unknown food availability rather than depositing fat, which is likely a regulatory response to the previous deprivation in the womb. In fact, previous research implied that a maternal HF diet raised energy expenditure in offspring [20], which provides support for the lighter fat pads seen in these two generations of the current study. By the third generation, though, the restricted group had heavier fat pads, which may have been the result of a cumulative effect of the maternal HF diet on the metabolic programming of offspring. In other words, the subsequent breedings using the HF diet may have stimulated fetal adaption to the increased density of the diet, which led offspring to deposit more food energy as fat instead of expending it upon consumption. It has been suggested that a maternal HF diet leads to offspring who consume excess calories and consequently display excess weight gain [38]. In addition, another study compared control and overfed ewes and showed an increase in subcutaneous fat in lambs in the overfed group, but no differences with perirenal fat [4]. Finally, Matsuzaki and colleagues examined maternal overnutrition and

found that while offspring were born premature with low birth weights, their perirenal fat pads were preserved [39]. Therefore, the current study had similar findings concerning the heavier fat depots in the third progeny, but are contradict one sheep study reporting heavier perirenal fat pads but lower fetus weights in lambs of overfed ewes [39].

Patterns of perirenal fat percentages were slightly different than with fat pads. The higher percentages within the restricted groups of the first and third generations could be the effect of several components. Concerning the first, it cannot be explained by hyperphagia from deprivation in the womb because these offspring did not have higher food intakes. Therefore, it could have been the higher density of the HF diet potentially providing excess calories at each feeding (i.e. in a relatively short time period), even with a smaller amount of food consumed. This may have promoted the observed increase in fat deposition. The highest average perirenal fat percentage seen in the third generation is likely due to the same mechanisms as those of the fat pad weights. In addition, Mulhasuer and colleagues also referred to studies showing that a gestational abundance of nutrients can have twice the negative effects as once thought. Not only can it can alter appetite regulation in the offspring, it is possible that this maternal diet results in offspring with metabolic abnormalities and adipocytes that encourage fat deposition later in adulthood [4, 40]. Regarding the increase among both treatments seen with the generational pattern, it is likely that both groups achieved gradually metabolic adaptation to their respective maternal treatments so that by the third generation, offspring were more efficient at storing excess energy from the HF diet as body fat, as mentioned above [40]. Other researchers, however, have reported that pups of HF-fed dams who were then fed a HF diet themselves have “normal body weight and metabolic parameters,” from

adaptation and “long-term protection” from the higher density diet [20], which contradicts the current and previous findings.

Although statistical significance was not found, the following observations were noteworthy. Within study 1 (chow diet), average body weights were gradually lower from the second to third generations in animals in the control group, but body weights increased among the restricted group, while food intake in both groups increased. Conversely, body weight increased between these two generations in both treatment groups fed the HF diet. Food intake among both HF groups increased between the second and third generations in both diets as well (the difference in controls was trivial (<1 kcal). Concerning the C/Ch animals, a lower average body weight in the third generation along with a higher food intake (compared to generation two) would *suggest* a correlating decrease in feed efficiency because these animals appeared to have to consume more kcal to maintain or prevent large body weight losses. Results showed, however, that the third generation of C/Ch animals had a slightly *elevated* feed efficiency compared to the second. Similarly, the R/Ch animals also displayed a decrease in body weight with a corresponding increase in food intake between generations two and three, which supports the increase seen in feed efficiency among these animals. This implies that although this group of animals consumed more energy, they were quite efficient at depositing excess energy as body fat.

In regard to the increases seen in both body weight and food intake in the C/HF groups between the second and third generation, it would be *expected* for feed efficiency to be higher in the third generation. This was not the case, though, as animals in generation three were less feed efficient compared to generation two. It could be that

over time the subsequent breedings led these animals to adapt to the HF diet and required more food energy to deposit body fat and grow. Therefore, they ate more to obtain these higher body weights, which led their efficiency to be decreased. The R/HF group showed a slight increase in feed efficiency with body weight and food intake also both increasing. Given that the difference in all three measurements was so small, though, these results could have been due to individual natural responses in the animals or the animals having a relative energy balance to only consume the needed amount of energy. Furthermore, with similar body weights and food intakes in both generations, a drastic change in feed efficiency would not be expected.

Across all generations of both groups (control and restricted), the animals fed the HF diet had consistently lower body weights than the Ch-fed. Overall average food intakes in both HF groups (control and restricted) were lower than in Ch groups across generations, potentially from the increased satiety that comes with higher-density food, leading the animals to consume less. These patterns in body weight and food intake give further evidence for the higher feed efficiencies in the high-fat groups across all weeks (Graph 3). Apparently, even though the groups fed the HF diet consumed less food overall, they were relatively efficient at depositing this food energy consumed as body fat. In other words, a low body weight and low food intake could correlate with a high feed efficiency if the animal stored the small amount of food eaten as fat rather than expending it for immediate energy needs. Moreover, the higher caloric density of the high-fat diet could be a reason for the lower food intakes among the high-fat groups. This does not explain the lower body weights, though, as the high-fat diet was 126% more calorically dense (4.73 kcal/gram) than the chow diet (3.73 kcal/gram), so it would

be expected for these animals to gain just as much if not more weight even with a lower food intake.

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Table 1: Experimental Design

	Experiment 1:		Experiment 2:	
	F0 females + F∅ males *Chow diet*		F0 females + F∅ males *High-fat diet* *(fed standard chow during breeding)*	
Timeline	**Control**	**Restricted**	**Control**	**Restricted**
3 weeks gestation	Females fed ad libitum	Females fed ad libitum until last trimester → 25% restriction	Females fed ad libitum	Females fed ad libitum until last trimester → 25% restriction
3 weeks with dams → wean → monitor for 8 weeks	F1 Progeny	F1 Progeny	F1 Progeny	F1 Progeny
	F1 females (11 weeks old) + F∅ males	F1 females (11 weeks old) + F∅ males	F1 females (11 weeks old) + F∅ males *(fed standard chow during breeding)*	F1 females (11 weeks old) + F∅ males *(fed standard chow during breeding)*
3 weeks gestation	Females fed ad libitum	Females fed ad libitum until last trimester → 25% restriction	Females fed ad libitum	Females fed ad libitum until last trimester → 25% restriction
3 weeks with dams → wean → monitor for 8 weeks	F2 Progeny	F2 Progeny	F2 Progeny	F2 Progeny
	F2 females (11 weeks old) + F∅ males	F2 females (11 weeks old) + F∅ males	F2 females (11 weeks old) + F∅ males *(fed standard chow during breeding)*	F2 females (11 weeks old) + F∅ males *(fed standard chow during breeding)*
3 weeks gestation	Females fed ad libitum	Females fed ad libitum until last trimester → 25% restriction	Females fed ad libitum	Females fed ad libitum until last trimester → 25% restriction
3 weeks with dams → wean → monitor for 8 weeks	F3 Progeny	F3 Progeny	F3 Progeny	F3 Progeny

Table 2: Chow (Ch) diet animals' *average* body weight, food intake, and feed efficiency of each treatment and diet at Week 11

Control/Chow (C/Ch)			
	Body Weight (grams)[1]	**Food Intake (kcal)[2]**	**Feed Efficiency[3]**
Generation 1	25.2 ± 0.844		
Generation 2	25.7 ± 0.927	97 ± 3.528	$4.647 * 10^{-3} \pm 1.683 * 10^{-3}$
Generation 3	24.2 ± 0.666	120 ± 3.588	$4.958 * 10^{-3} \pm 1.832 * 10^{-3}$
Restricted/Chow (R/Ch)			
	Body Weight (grams)[4]	**Food Intake (kcal)[5]**	**Feed Efficiency[6]**
Generation 1	25.0 ± 4.130	100 ± 8.405	$5.179 * 10^{-3} \pm 1.628 * 10^{-3}$
Generation 2	23.7 ± 2.828	100 ± 8.912	$8.175 * 10^{-3} \pm 3.953 * 10^{-3}$
Generation 3	22.8 ± 0.559	114 ± 5.350	$3.756 * 10^{-3} \pm 3.222 * 10^{-3}$

[1, 2, 3] Average BW, FI, and FE of all chow-fed animals from control dams within each generation

[4, 5, 6] Average BW, FI, and FE of all chow-fed animals from control dams within each generation

Table 3: High-fat (HF) diet animals' *average* body weight, food intake, and feed efficiency of each treatment at Week 11

Control/High-fat (C/HF)			
	Body Weight (grams)[1]	**Food Intake (kcal)[2]**	**Feed Efficiency[3]**
Generation 1	24.9 ± 4.115	94.9 ± 1.468	$5.959 * 10^{-3} \pm 9.37 * 10^{-4}$
Generation 2	23.6 ± 0.877	96 ± 1.309	$7.796 * 10^{-3} \pm 9.490 * 10^{-4}$
Generation 3	24.8 ± 0.915	97 ± 2.254	$5.834 * 10^{-3} \pm 1.036 * 10^{-3}$
Restricted/High-fat (R/HF)			
	Body Weight (grams)[4]	**Food Intake (kcal)[5]**	**Feed Efficiency[6]**
Generation 1	22.9 ± 3.349	96.1 ± 2.945	$6.602 * 10^{-3} \pm 1.470 * 10^{-3}$
Generation 2	23.4 ± 0.850	92 ± 1.279	$5.115 * 10^{-3} \pm 5.970 * 10^{-3}$
Generation 3	24.7 ± 0.797	97 ± 2.521	$5.958 * 10^{-3} \pm 1.241 * 10^{-3}$

[1, 2, 3] Average BW, FI, and FE of all high-fat-fed animals from control dams within each generation

[4, 5, 6] Average BW, FI, and FE of all high-fat-fed animals from control dams within each generation

Table 4: Average comparison of perirenal fat pad weight and perirenal fat as a % of body weight of all high-fat-fed animals at week 11

	Perirenal fat pad weight (grams)[1]		
Treatment	**Generation 1**	**Generation 2**	**Generation 3**
Control	0.1924	0.2285	0.2194
Restricted	0.1742	0.2036	0.2409
	Perirenal fat as a % of body weight (perirenal fat (grams)/body weight (grams))[2]		
Control	0.649%	0.969%	0.874%
Restricted	0.891%	0.872%	0.963%

[1, 2] Average of all high-fat-fed animals within each treatment

Table 5: Generation comparison of all high-fat-fed animals at week 11

	Perirenal fat pad weight (grams)[1]	Perirenal fat as a % of body weight (perirenal fat (grams)/body weight (grams))[2]
Generation 1	0.183 ± 0.015	0.800% ± 0.001
Generation 2	0.216 ± 0.015	0.900% ± 0.001
Generation 3	0.230 ± 0.015	0.900% ± 0.001

[1, 2] Average of all high-fat-fed animals within each generation

Table 6: Treatment comparison of all high-fat-fed animals at week 11

	Perirenal fat pad weight (grams)[1]	Perirenal fat as a % of body weight (perirenal fat (grams)/body weight (grams))[2]
Control	0.213 ± 0.012	0.800% ± 0.0001
Restricted	0.206 ± 0.013	0.900% ± 0.0001

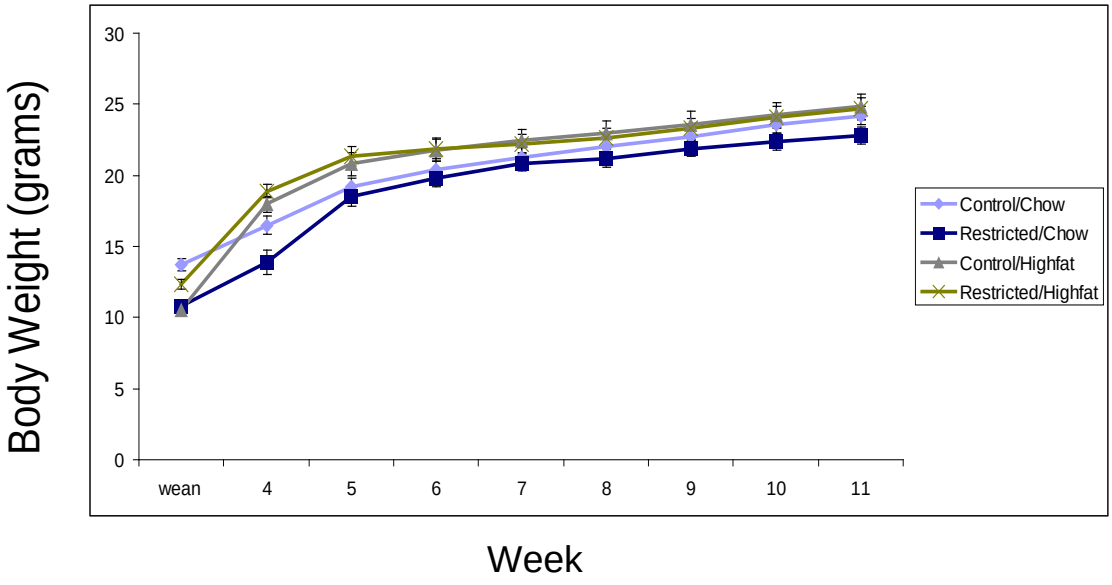

Figure 1: Generation 3 *average* body weight across time

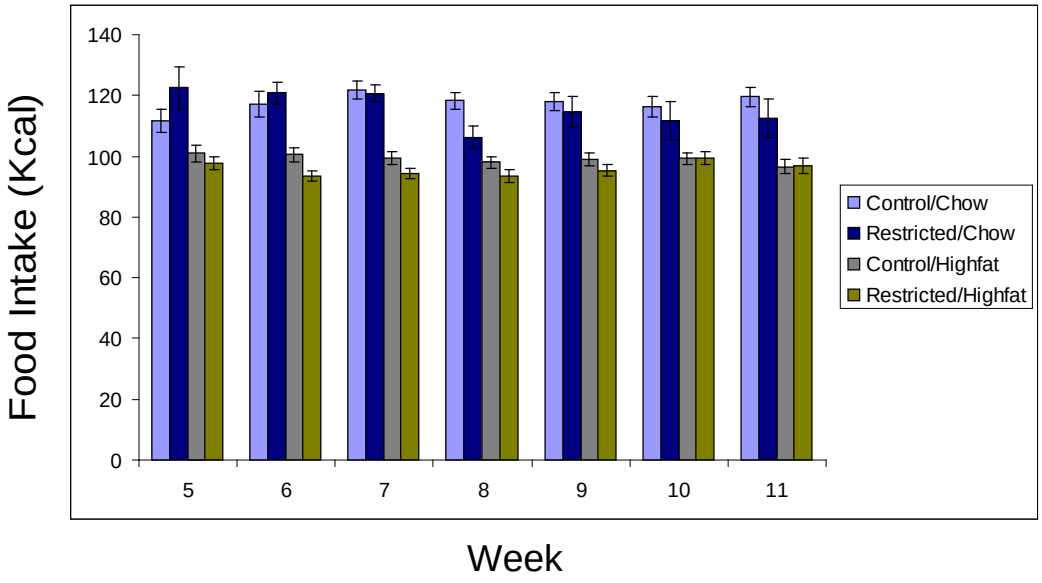

Figure 2: Generation 3 *average* food intake across time

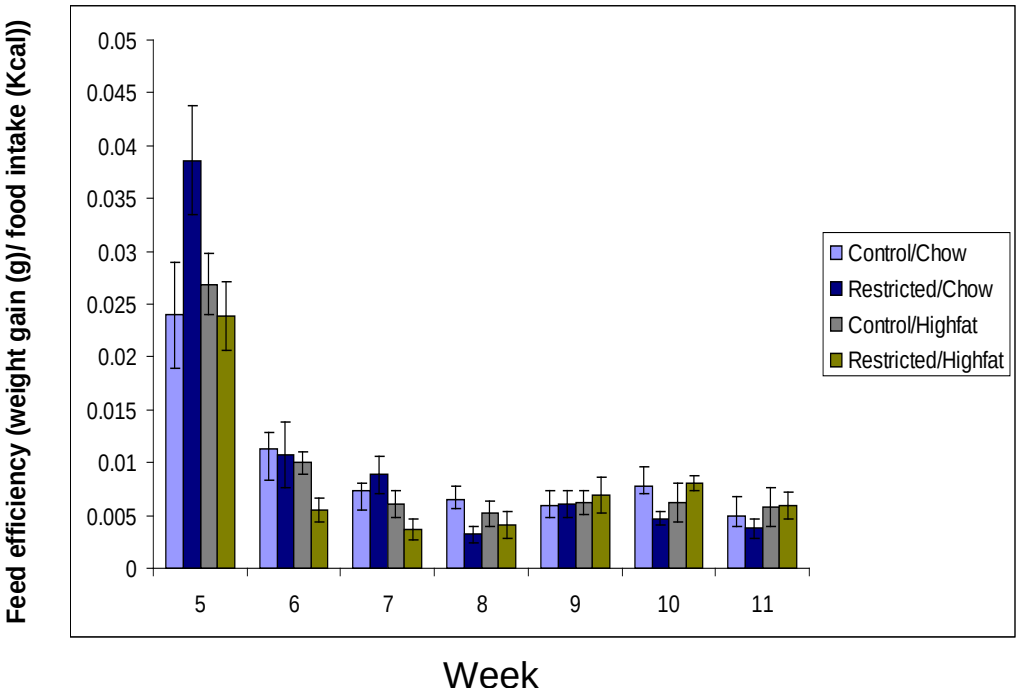

Figure 3: Generation 3 *average* feed efficiency across time

REFERENCES

1. Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, Rodriguez-Gonzalez GL, Guzman C, Larrea F, Nathanielsz PW: **Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation**. *J Physiol* 2005, **566**:225-236.
2. Stocker CJ, Arch JRS, Cawthorne MA: **Fetal origins of insulin resistance and obesity**. *Proceedings of the Nutrition Society* 2005, **64**:143-151.
3. George A. Bray M, Catherine M. Champagne P, RD, FADA: **Beyond Energy Balance: There is More to Obesity than Kilocalories**. *Journal of the American Dietetic Association* 2005, **105**(May, Supplement 1):S17-23.
4. Muhlhausler BS, Adam CL, Findlay PA, Duffield JA, McMillen IC: **Increased maternal nutrition alters development of the appetite-regulating network in the brain**. *FASEB Journal* 2006, **20**:E556-E565.
5. Altman J, Das GD: **Autoradiographic and histological studies on postnatal neurogenesis** *J Comp Neurol* 1966, **126**:337.
6. Keesey RE, Hirvonen MD: **Body weight set-points: determination and adjustment**. *J Nutr* 1997, **127**:1875S-1883S.
7. Bellinger L, Sculley DV, Langley-Evans SC: **Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats**. *Int J Obes (Lond)* 2006, **30**(5):729-738.
8. Barker DJ: **Intrauterine programming of adult disease**. *Mol Med Today* 1995, **1**:418-423.

9. Widdowson EM, Crabb DE, Milner RD: **Cellular development of some human organs before birth**. *Arch Dis Child* 1972, **47**:652-655.

10. Roseboon TJ, Meulen JHvd, Ravelli AC, Osmond C, Barker DJ, Bleker OP: **Effects of prenatal exposure to the Dutch famine on adult disease in later life: An overview**. *Mol Cell Endocrinol* 2001, **185**:93-98.

11. Knackstedt MK, Hamelmann E, Arck PC: **Mothers in Stress: Consequences for the Offspring**. *AJRI* 2005, **54**:63-69.

12. Hales CN, Barker DJ: **The thrifty phenotype hypothesis**. *Br Med Bull* 2001, **60**:5-20.

13. Chow BF, Lee CJ: **Effects of dietary restriction of pregnant rats on body weight gain of the offspring**. *J Nutr* 1964, **82**(10).

14. Hsueh AM, Agustin CE, Chow BF: **Growth of young rats after differential manipulation of maternal diet**. *J Nutr* 1967, **91**(195).

15. **Dietary Guidelines for Americans 2005**. In. Edited by Services USDoHaH, Agriculture USDo, 6th edition edn. Washington, D.C.: U.S. Government Printing Office; 2005.

16. Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, Jansson T: **High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice**. *The FASEB Journal* 2009, **23**(January):1-9.

17. Bayol SA, Simbi BH, Bertrand JA, Strickland NC: **Offspring from mothers fed a 'junk food' diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females**. *J Physiol* 2008, **586**(13):3219-3230.

18. BBC News: **Craving for junk food 'inherited'**. In.; 2007.

19. Gallou-Kabani C, Vige A, Gross M-S, Boileau C, Rabes J-P, Fruchart-Najib J, Jais J-P, Junien C: **Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods**. *Am J Physiol Endocrinol Metab* 2007, **292**:1095-1100.

20. Ferezou-Viala J, Roy A-F, Serougne C, Gripois D, Parquet M, Bailleux V, Gertler A, Delplanque B, Djiane J, Riottot M *et al*: **Long-term consequences of maternal high-fat feeding on hypothalamic leptin sensitivity and diet-induced obesity in the offspring**. *Am J Physiol Regul Integr Comp Physiol* 2007, **293**(September):R1056-R1062.

21. Erlanson-Albertsson C: **How palatable food disrupts appetite regulation**. *Basic Clin Pharmacol Toxicol* 2005, **97**:61-73.

22. Prentice AM, Rayco-Solon P, Moore SE: **Insights from the developing world: thrifty genotypes and thrifty phenotypes**. *Proceedings of the Nutrition Society* 2005, **64**:163-161.

23. Bayol SA, Farrington SJ, Strickland NC: **A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring**. *British Journal of Nutrition* 2007, **98**:843-851.

24. Rolls BA, Gurr MI, Duijvenvoorde PMV, Rolls BJ, Rowe EA: **Lactation in lean and obese rats: Effects of cafeteria feeding and of dietary obesity on milk composition**. *Physiol Behav* 1986, **38**:185-190.

25. Rolls BJ, Rowe EA: **Pregnancy and lactation in the obese rats: Effects on maternal and pup weights**. *Physiol Behav* 1982, **28**:393-400.

26. Hill JO, Lin D, Yakubu F, Peters JC: **Development of dietary obesity in rats: Influence of amount and composition of dietary fat**. *Int J Obes* 1992, **16**:321-333.

27. Jen K-LC: **The effect of high fat diet on the behavioral patterns of male rats**. *Physiol Behav* 1980, **25**:373-381.

28. Jen K-LC: **Effects of diet composition on food intake and carcass composition in rats**. *Physiol Behav* 1988, **42**:551-556.

29. Tucker LA, Kano MJ: **Dietary fat and body fat: A multivariate study of 205 adult females**. *Am J Clin Nutr* 1992, **56**:616-622.

30. Mulhausler BS, Adam CL, McMillen IC: **Maternal nutrition and the programming of obesity**. *Organogenesis* 2008, **4**(3):144-152.

31. Weiss SR, Michael H. Ebert M: **Psychological and Behavioral Characteristics of Normal-Weight Bulimics and Normal-Weight Controls**. *Psychosomatic Medicine* 1983, **45**(4):293-303.

32. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB: **Association between postnatal catch-up growth and obesity in childhood: prospective cohort study**. *Br Med Bull* 2000, **320**:967-971.

33. Silverman BL, Rizzo TA, Cho NH, Metzger BE: **Long term effects of the intrauterine environment. The Northwestern University Diabetes in Pregnancy Center**. *Diabetes Care* 1998, **21 (Suppl. 2)**:B142-B149.

34. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dorner G: **Overweight and obesity in infants of mothers with long-term insulin-dependent diabetes or gestational diabetes.** *Int J Obes Relat Metab Disord* 1997, **21**:451-456.

35. Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD: **Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition**. *Am J Physiol Endocrinol Metab* 2000, **279**:E83-E87.

36. **LabDiet: Your work is worth it** [http://www.labdiet.com/indexlabdiethome.htm]

37. **Diet Induced Obesity (DIO) Diets** [http://www.researchdiets.com/pdf/Data%20Sheets/DIO%20Series.pdf; http://www.researchdiets.com/OSD/DIDM/obesity.htm]

38. Chang G-Q, Gaysinskaya V, Karatayev O, Leibowitz SF: **Maternal High-Fat Diet and Fetal Programming: Increased Proliferation of Hypothalamic Peptide-Producing Neurons That Increase Risk for Overeating and Obesity**. *The Journal of Neuroscience* 2008, **28**(46):12107-12119.

39. Matsuzaki M, Milne JS, Aitken RP, Wallace JM: **Overnourishing pregnant adolescent ewes preserves perirenal fat deposition in their growth-restricted fetuses**. *Reproduction, Fertility and Development* 2006, **18**:357-364.

40. Sameulsson A-M, Matthews PA, Argenton M, Christie MR, McConnell JM, Jansen EHJM, Piersma AH, Ozanne SE, Fernandez-Twinn D, Remacle C *et al*: **Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension and insulin resistance**. *Hypertension* 2008, **51**:383-392.

CHAPTER 4

CONCLUSION

The study described in Chapter 3 was aimed at investigating the concept of fetal programming and its potential effects on energy regulation in three subsequent generations of mice. Using two maternal treatments (control (C) and restricted (R)) and two maternal diets (chow (Ch) and high-fat (HF)), we measured body weights and food intakes across 11 weeks, and fat pad weights at week 11. Feed efficiency and body fat percentages were then calculated.

When comparing all generations, no single group of offspring (C/Ch, R/Ch, C/HF, R/HF) displayed drastically higher average body weight across all weeks. The animals fed the Ch diet, however, weighed more than the HF animals in the first generation, but by the third the HF animals were heavier. This implies a long-term accumulating effect of a HF diet. At week 11, the R/HF group had the lightest average body weights, and the C/Ch had the heaviest in both generations one and two. In generation three, though, it differed with the R/Ch group the lightest and the C/HF group the heaviest. Previous researchers have also reported that a maternal HF diet can negatively affect offspring in numerous subsequent generations concerning body weight, blood glucose, triglycerides [1]. Added to the heavier body weight results seen in the C/HF animals in the current study, implications for human dietary recommendations during pregnancy are to maintain a moderate dietary fat consumption in order to prevent harm in not only the first generation, but many more to come as well.

Even though no clear connection was seen between body weight patterns and maternal treatment or diet, it can be speculated from the higher body weights in the HF

groups that a HF diet during both gestation and after pups are born greatly contributes to an increased chance in offspring obesity. Concerning treatment, though, the consequence of maternal nutrient restriction was evident even with the first generation progeny due to the observation of lighter body weights seen among the offspring of restricted dams in both diet groups. In relation to the impact in humans, this implies that maternal undernutrition has almost immediate detrimental effects with respect to future health consequences of our society. It is speculated that the maternal restricted intake of mothers during gestation not only reduces the vitamin and mineral status of the mothers, but causes the offspring in the womb to receive less nutrients as well. Thus, our study provides additional support for the current dietary guidelines of not exceeding the recommendations for dietary fat intake during both pregnancy and throughout life and reiterates the importance of adequate maternal nutritional intake.

Concerning food intake, the Ch groups ate more kcal than HF groups in all three generations. Within the first generation, average food intakes at week 11 were approximately the same between Ch- and HF-fed animals. Offspring from the HF groups ate the least in generations two and three, with the R/HF group having the lowest intake in generation two and both HF groups having similar low intakes in generation three. This corresponds with the Ch groups eating the most, on average, at week 11. In the second generation, though, the R/Ch group had the lowest intake, and the C/Ch had the highest. Within generation three at week 11, both HF groups had almost equally low intakes, and the C/Ch group ate the most. Therefore, it is probable that a HF diet can lead to a favorable reduction in energy intake, but the high probability of obesity and its adverse health effects (i.e. cardiovascular disease, type 2 diabetes, metabolic syndrome,

etc [2]) from a HF diet outweigh this small benefit. In addition, the lower food intakes observed among HF-fed animals correlated with their lower body weights in the first two generations, but does not explain the higher average body weights of the HF animals in generation three, which is addressed below regarding feed efficiency.

While our study did not show an increased food intake among HF-fed animals, previous research states otherwise. It is known that palatability of food is an important component in the regulatory mechanisms of appetite and subsequent energy intake [3]. In fact, a maternal HF diet has been shown to increase the preference for HF foods in the offspring and can also contribute to more calorically dense milk during lactation [4]. Futhermore, offspring in the former study seemed to have reduced satiety, increased hunger signals, and an increased reward factor from the HF diet [4]. Researchers stated that these "reward centers" in the brain may even have a greater influence on feeding than simple energy balance [5].

Finally, there were only minimal patterns regarding feed efficiencies between treatments and diets. All offspring in the first generation had comparable feed efficiencies, but this implies that those fed the HF diet may have had an enhanced metabolism. If these animals had not adapted to the higher density diet, it is likely they would have shown a greater feed efficiency due to depositing the excess calories as body fat versus expending it, which they apparently did because of their lighter body weights. Within the second and third generations, the HF group had the highest overall feed efficiencies. At week 11 of generation two, the C/Ch group was the least efficient and the R/Ch group was the most. By the third generation, this outcome was slightly different. At week 11, the HF animals were the most efficient on average. Interestingly,

though, the R/Ch group had the lowest efficiency at week 11 and the R/HF group had the highest efficiency. As mentioned above, the HF group had the highest average body weights but lowest food intakes in generation three. With their higher average feed efficiencies, this implies that these animals were remarkably adapted to store *any and all* excess dietary energy as body fat for future use.

The patterns seen regarding perirenal fat pad weights of the HF groups showed the control group to have more perirenal fat than the restricted group, on average. By generation three, though, the restricted animals had the heaviest fat pads, corresponding with both groups' trend of increasing fat pad weights with each generation. Body fat percentages were highest in the restricted groups of the first and last generations in offspring of restricted dams, and the generational pattern corresponded with fat pad weights with a gradual increase in body fat percentage across generations. Therefore, maternal undernutrition (offspring from restricted dams) seems to increase not only individual fat depots (here perirenal fat) in offspring, but also has a negative effect on their overall body composition in that the ratio of perirenal fat pads to body weight was increased compared to offspring from control dams.

In conclusion, the current study did not reveal any *statistically significant* negative alterations in appetite and energy regulation of offspring from two maternal treatments or diets. It did, however, reveal that maternal malnutrition, either from undernutrition (a restricted intake) or overnutrition (HF diet) is likely to have adverse effects in the offspring, including higher average body weights and feed efficiencies, as well as larger perirenal fat pads with a corresponding higher body fat percentage.

It can be speculated that the differences observed in the current study were due to epigenetic alterations, which were likely to be passed on to the subsequent generations. Given that differences were seen through the third generation, a change in genetics or gene expression is more likely than an acute response to the maternal nutrient restriction. This could have been related to a modification in the genes that program for the numerous neuropeptides and/or hormones involved in appetite regulation. Even if analysis of these neuropeptides had been examined in the current study, alterations may have been missed if they occurred at any stage prior to the time of sacrifice, when animals were eleven weeks old at sacrifice, . Therefore, brain analysis would need to be performed at earlier and multiple intervals of life in order to detect any possible changes from the maternal treatment and/or diet. Looking to the future, research could address the potential mechanisms behind the permanent effects of maternal nutritional status, including the both treatment and/or diet on the brain's control of feeding and energy expenditure in offspring.

REFERENCES

1. Nasu, R., et al., *Effect of a High-fat Diet on Diabetic Mother Rats and Their Offspring through Three Generations.* Endocrine Journal, 2007. **54**(4): p. 563-569.
2. Mulhausler, B.S., C.L. Adam, and I.C. McMillen, *Maternal nutrition and the programming of obesity.* Organogenesis, 2008. **4**(3): p. 144-152.
3. Erlanson-Albertsson, C., *How palatable food disrupts appetite regulation.* Basic Clin. Pharmacol. Toxicol., 2005. **97**: p. 61-73.
4. Bayol, S.A., S.J. Farrington, and N.C. Strickland, *A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring.* British Journal of Nutrition, 2007. **98**: p. 843-851.
5. Broberger, C., *Brain regulation of food intake and appetite: molecules and networks.* J. Intern. Med, 2005. **258**: p. 307-327.