The developmental environment and metabolism in the house mouse (*Mus musculus*)

by

Aubrey Erin Sirman

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 2nd, 2014

Approved by

Jack Feminella, Chair, Professor of Biological Sciences
Wendy Hood, Assistant Professor of Biological Sciences
Arthur Appel, Professor of Entomology
Haruka Wada, Assistant Professor of Biological Sciences
Julie Richmond, Assistant Professor of Biological Sciences

Abstract

The environment under which an individual develops plays an important role in determining its physiological phenotype. Maternal diet is one of several variables that can have lasting impacts on offspring phenotype. It's been suggested that these changes can be set during early development can last through adulthood, ultimately affecting an individual's ability to function maximally in their adult environment. The environmental matching hypothesis predicts that an individual's fitness is highest under conditions in which the developmental and adult environments are matched. Accordingly, when those environments are mismatched individuals will have reduced fitness. This response may be important for shaping an individual's life history strategy. However, there have been few empirical tests of this hypothesis with respect to diet and fitness, particularly in wild populations. In this study I investigated the environmental matching hypothesis in a natural population of house mice (*Mus musculus*). I experimentally manipulated dietary protein levels and monitored individuals into adulthood. We placed the parental (F_0 generation) on either a high (H, 20% protein) or low (L, 10% protein) and the offspring of these mice were then kept on a similar diet (HH, LL) or given the alternate diet (HL, LH). I also measured several reproductive variables including age at first reproduction, reproductive status, and reproductive effort. In addition, I measured resting metabolic rate (RMR) at 30 days (weaning) and 75 days. I also measured circulating levels of the metabolic hormone IGF-1 and IGF-1 gene expression at 30 days and at approximately 1 year.

Under the environmental matching hypothesis I predicted 1) individuals matched with their developmental environment to have greater reproductive success than individuals that are mismatched and 2) the developmental environment can alter aspects of an individual's physiology that persist into adulthood. Dietary treatment had a significant effect on age at first reproduction, with mismatched individuals reproducing significantly earlier than matched individuals. This is in contrast to predictions made by the environmental matching hypothesis. We found no differences between treatment and reproductive status or reproductive effort. However, there was a trend that suggested LH individuals had greater reproductive effort than other treatment groups. There was no difference in RMR between treatment groups at 30 days. It was not until 75 days that metabolic adjustments became apparent. At 75 days, matched individuals exhibited similar RMR while there were significant differences in RMR between mismatched groups. Concentrations of IGF-1 at 30 days did not differ between treatment groups. However, LH individuals had significantly lower concentrations of IGF-1 at adulthood. There were no differences in IGF-1 expression among treatment groups at 30 days or adulthood, although a trend suggested that adults had greater expression levels than juveniles. Interestingly, when we compared IGF-1 levels to reproductive effort, individuals with lower levels of IGF-1 had greater reproductive effort. This result may be due to relative age of animals at the time of sample collection. The results of this study suggest that reproductive success in the house mouse is not determined by matching the metabolic phenotype to the maternal diet.

Acknowledgments

I owe a great deal of gratitude to several people have contributed to this thesis. I am grateful for my advisor Wendy Hood for providing valuable insights and direction to my work. I am also thankful for Julie Richmond and Arthur Appel as both contributed not only their labs and equipment but their time to help me complete my research project. Perhaps, most importantly I am appreciative of our team of undergraduates that aided in husbandry and monitoring of our mouse populations. Without their help, this project would never have been possible. I would like to thank the lab members of the Hood and Hill labs as they provided valuable feedback and assistance for my project, especially Molly Staley, who guided me through several molecular techniques. Finally, I would like to thank my friends and family for their continued support as well as Joseph Hurd whose unwavering confidence in my abilities was a continuous source of inspiration.

Style manual or journal used: Physiological and Biochemical Zoology (PBZ)

Computer software used: Microsoft Word

Table of Contents

List of Tables

CHAPTER TWO

List of Figures

CHAPTER ONE

CHAPTER TWO

CHAPTER ONE

The effects of early nutrition on resting metabolic rate and age at first reproduction in the house mouse (*Mus musculus*)

INTRODUCTION

A central component of life history theory predicts that individuals have a finite energy budget from which they allocate energy to support the costs of self-maintenance, growth, and reproductive activities (Stearns 1992). The size of the energy resource pool in an individual and the amount of available energy partitioned to life-history demands are thought to play an important role in determining individual differences in reproductive performance and longevity. Resting metabolic rate (RMR) reflects the cost of maintaining basic physiological functions necessary for survival (McNab 2002, Speakman 2008). These costs can vary by as much as 3-fold between individuals within any given population (Burton et al. 2011 and references therein). However, the factors that contribute to individual variation in RMR and how they impact individual fitness are poorly understood. The relationship between fitness and RMR remains ambiguous as high RMR has been correlated with improved fitness under some environmental conditions and reduced fitness under others. In response, Burton et al (2011) suggested that optimal RMR likely varies with environment and the environment that an individual experiences during development may play a key role in matching an offspring's metabolism to its environment (i.e. the environmental matching hypothesis, Monaghan 2008).

Because of its low heritability (Nilsson et al 2009, Labocha et al 2004), high variance among individuals (Speakman et al. 1994, Speakman 2000) it is likely that RMR is largely influenced by the conditions an individual experiences early in life. In mammals, the mother's phenotype, as well as the environment can influence the conditions and resources she provides for her young during gestation and lactation. A mother's diet is among one of several environmental variables that can impact offspring phenotype. Maternal diet has been shown to impact both RMR and the metabolic variables that contribute to an individual's RMR. For example, Ayala-Moreno et al (2013) showed that, relative to the young of ad lib fed mothers, the offspring of food-restricted rat mothers display reduced RMR roughly 2 months after weaning. Changes to an individual's physiology are relatively plastic and can occur within short periods of time. For example, caloric restricted lab mice showed changes in fatty acid composition of phospholipids from several organs including the brain, liver and kidneys after just one month (Faulks et al. 2006), indicating that long term dietary conditions are likely to impact metabolic rate by altering an individual's physiology. In laboratory mice (*Mus musculus*), the alimentary organs, including organs of the gut and liver, explained up to 71% of the variation seen in metabolic rate (Speakman and McQueenie 1996). However, studies selecting for low and high intake mice suggest that the liver as whole is responsible for most of the variation seen in metabolic rate (Selman et al. 2001).

The impact of the maternal diet and offspring metabolic rate on offspring fitness is less well studied however; both metabolic rate and nutrient intake have been shown to contribute to individual variation in performance. High metabolic rates are correlated with increased growth rates (Metcalfe et al. 1995, Mathot et al. 2009, Criscuolo et al. 2008) and increased reproductive performance in birds (Chastel et al. 2003). Nutrition prior to and during reproduction can

influence growth rates and age at first reproduction in small mammals (McAdam and Millar 1999, Dobson and Kjelgaard 1985, Boutin 1990). For example, cotton rats (*Sigmodon hispidus*) consuming high protein dicots reached first estrous sooner than comparably sized cotton rats consuming low protein monocots (Cameron and Eshelman 1996). Yet when poor nutrition is associated with reduced growth rate followed by a period of compensatory growth, a delay in onset of first reproduction is common (Morgan and Metcalfe 2001, Oli et al. 2002). Consequently, individuals that experience compensatory growth may incur costs that are not evident until later in life (Metcalfe and Monaghan 2001).

The timing of first reproduction determines how quickly early maturing genotypes are introduced into a population (Oli 2003). Therefore, any phenotypic variants associated with an early age at first reproduction, such as those with a favorable metabolic rate, are likely to incur a fitness advantage. In this study, we investigated the effects of the early nutritional environment on metabolic rate and the onset of first reproduction in the house mouse (*Mus musculus*). We selected house mice for this investigation because of their short generation times and, under conditions designed to mimic the wild, house mice display increased sensitivity to intrinsic and extrinsic stressors over their laboratory counterparts (Meagher et al. 2000, Ruff et al. 2013). Laboratory mice have been shown to display metabolic plasticity in both glucose and lipid metabolism in response to maternal nutrition (Zambrano et al. 2005, Gluckman et al. 2007). To investigate the effect of nutrition on metabolism, we manipulated the protein intake of breeding females. We chose to manipulate protein intake for this investigation because the effects of maternal protein intake on offspring metabolism are more consistent than other dietary manipulations (McMillen and Robinson 2005). Similar diets have been shown to impact the metabolic phenotype (Reeves et al. 1993) and reproductive performance of rodents (Guzmán et

al. 2006, Zambrano et al. 2005). Lastly, variation in protein intake by mice is ecologically relevant, with the protein intakes of wild mice varying from low protein for mice that consume a diet that is entirely grain (~10-13% protein for corn and wheat) to high protein (~20% protein) for mice that consume mostly insects (Bomford 1987, Smith et al 2002, Tann et al 1991, (Robbins 1993).

To investigate the role of nutrition during early development on RMR and the onset of breeding, we manipulated diet in a population of wild-derived house mice. The parental generation of mice (F_0) was given *ad lib* access to a high (20%, H) or low protein (10%, L) diet. At weaning, the F_1 offspring were either maintained on the same diet (HH, LL) or switched to the alternate diet (HL, LH). Under the thrifty phenotype hypothesis, the environment experienced in early life may stimulate a developmental trajectory that permanently alters metabolic processes such as RMR. The environmental matching hypothesis predicts that those individuals matched with their developmental environment should have increased fitness relative to those that are mismatched. Therefore, alterations made to an individual's physiology during development will ultimately reflect differences in seen in fitness. We predicted that individuals that are matched with their maternal environment (HH and LL) should reproduce earlier than those that are mismatched (HL and LH).

MATERIALS AND METHODS:

Animal Husbandry

All mice were 9 generation removed from the wild at the onset of the experiment and were obtained from established breeding colonies maintained by W. Potts, University of Utah. We maintained mice in enclosures designed to mimic ambient conditions, home range sizes, and

population densities of mice living in natural demes within a barn or similar structure (Ilmonen et al 2007). Each side of the ten 1.4 m x 3.5 m enclosure is lined with 3.5 m aluminum flashing to prevent escapes. The enclosures were divided between 2 adjacent buildings that are secure, keeping predators out, but exposing mice to ambient temperatures through hardware-cloth windows. We maintained no more than 10 breeding age mice in an enclosure. When animals first arrived, we kept them segregated by sex for 8 weeks and then placed males and females in mixed sex groups of 3 males and 7 females. Deviation from a 1:1 sex ratio was employed to reduce male aggression. Further, we put mice on their experimental diets within one-week of their arrival.

I allowed females and males to breed and compete for mates as they would in a natural population. Pups were born between late December 2012 and March 2013 for the F_1 generation. No more than two males and two females per litter. If two individuals of the same sex were collected, then we placed them on different diets. Female and male siblings of the same litter were never placed in the same enclosure. A pit tag was inserted subcutaneously in all mice (HPT8, Biomark, Inc) to allow for identification with handheld reader. In addition, I punched (1-2 mm) the ears of each mouse in a unique pattern to provide a secondary form of identification.

Diet

I randomly assigned mice to a 20% (high) or 10% (low) protein custom diet prepared by TestDiet (Purina Animal Nutrition, St Louis, MO). The 10 % and 20% protein diets were isocaloric, providing 3.9 kJ of energy per kg. Cornstarch was used to compensate for the energy deficit in the 10% protein diet. The diets were otherwise similar, including the concentration of fat, fiber, minerals, and vitamins that each diet provided.

Husbandry

Breeding of individuals was monitored in each enclosure daily. We entered each enclosure, identified all adult females using the handheld pit tag reader, and counted the number of young that each female was rearing from birth until weaning. I removed F1 juveniles at 4 weeks and either euthanized or retained them to examine the impact of maternal diet on offspring RMR and age at first reproduction. I moved retained young into a juvenile enclosure and provided them with a high or low protein diet that was similar (HH, LL) or dissimilar (HL, LH) to their mothers.

Sexual maturity in the house mouse generally occurs between 6 and 8 weeks (Whittingham and Wood 1983). However, this can be delayed up to 12 weeks depending on a number of factors including environmental conditions or the presence or absence of other mice (Lantham and Mason 2004). Males generally reach sexual maturity sooner than females, but this can be highly variable (Singleton and Krebs 2007, Miller et al. 2002). Most females reach sexual maturity at a greater mass than males (Singleton and Krebs 2007). Outbred and inbred strains of wild house mice, ancestors of those used in this experiment, were reported to reach sexual maturity at 45 days (Meagher et al. 2000). In this experiment we ensured that males in each enclosure were older or the same age as the oldest female in each enclosure at the time breeding populations was established.

Metabolic Rate

I measured RMR in juvenile mice at 30 days old, just prior to the diet switch, and again at 75 days. I trapped individuals from the breeding enclosures and fasted them for 8 hours prior to metabolic rate measurements. I carried out metabolic rate measurements using a closed Sable

Systems TR-3 respirometry system (Sable systems, Henderson, NV, USA). In this system, incoming air is drawn through a Li-Cor6251 CO_2 analyzer (LI-COR Inc. Lincoln, NE, USA) and scrubbed of CO_2 and H_2O using a Drierite® - Ascarite®-Drierite® tube before O_2 analysis. The CO_2 and H_2O free air will then flow through the system at a known rate of 750ml/min. We analyzed oxygen consumption of the mice with an Oxilla II O_2 Analyzer (Sable Systems, Henderson, NV, USA) that was calibrated (zeroed and spanned) regularly. We used 100ppm Certified CO_2 (Airgas South, Theodore, AL USA) to span. To zero, we used 0ppm CO_2 generated by passing incoming air through the FT-IR Whatman purge-gas generator and a Drierite® - Ascarite®-Drierite® tube.

Mice were kept in small cages and had unlimited access to water prior to metabolic measurements. Since mice are nocturnal, we measured the RMR of individuals during the day as that is the normal resting period for wild mice. We weighed each mouse just prior to and after taking metabolic rate measurements. We placed each mouse in a 280ml respirometry chamber inside an incubator illuminated by a 15W white fluorescent light and maintained at approximately 30ºC, within the thermal neutral zone for *Mus musculus* (Speakman and McQueenie 1996, Wone et al 2009). We allowed mice to acclimate to the respirometry chamber for 15 minutes and then an additional five minutes once placed in the incubator prior metabolic rate measurements. We monitored mouse movement inside the incubator using a SONY DCR-SX85 video camera (Sony Corporation, Tokyo, Japan). Each individual was sampled for a minimum of 30 minutes and maximum of 60 minutes. I recorded baseline O_2 and CO_2 levels at the beginning and end of each measurement. I recorded and analyzed data using the Datacon V acquisition and analysis software (Sable Systems, Henderson, NV, USA). We recorded oxygen consumption from the lowest stable 10-minute segment or from 2-3 averaged 3 minute stable

segments was used to calculate resting metabolic rate. We then calculated the respiratory quotient (RQ) by dividing carbon dioxide produced (V_{CO_2}) by oxygen consumed (V_{O_2}), correcting for body mass. Sample sizes for the HH, HL, LH and LL treatment groups were n= 4, 7, 8, and 10 respectively.

Age at First Reproduction

The putative mother of each litter was identified based on the recorded locations of the animals each day when we first entered the enclosure for husbandry. In general, I designated the female found on the nest greatest number of days as the mother of the litter. When the likelihood being the mother was equivocal between two females, I considered both the number of days female was on the nest during the first few days post-partum and the time between reproductive bouts. I used the female's age on the birth date of her first litter as her age at first reproduction.

Statistical Analyses

Only those females that were fasted and non-reproductive at the time of metabolic rate measurements were included in the analyses. I analyzed the effects of diet on body mass, RMR and age at first reproduction using a linear mixed effects model (lme), with enclosure included as a random factor. I performed all analyses using R v.3.0.1 (nlme library, Pinheiro et al.2007) (http://www.r-project.org/) and difference between groups were based on the results of a linear mixed effect model. I used a chi-squared test to determine if the effect of diet differed between reproductive and non-reproductive animals during the study. Significance was set at an α of $P < 0.05$.

RESULTS

Day 30

I observed no significant difference in body mass between high (H) and low (L) protein diets in mice at age 30 days ($P = 0.65$; fig. 1). After correcting for body mass, I observed no difference in levels of oxygen consumption between high (H) and low (L) treatment groups in 30 day mice ($P = 0.83$; fig. 2). I observed no differences in respiratory quotient (RQ) between high or low protein treatment groups at age 30 days ($P = 0.93$; fig. 3).

Day 75 (adulthood)

I observed no differences between body mass and treatment groups at age 75 days (fig. 4). However, it appeared that mice at adulthood in the LL treatment group were slightly heavier than those in the HH treatment group ($P = 0.13$), although this was not significant. I found differences in oxygen consumption, after correcting for body mass, in 75 day mice (fig. 4). Mice allocated to HH and LL treatment groups showed no differences in oxygen consumption in adulthood ($P = 0.36$; fig. 5). However, oxygen consumption was 0.007 O_2 $min^{-1}g^{-1}$ (±0.006 [95% C.I]) lower in the HL group relative to the HH group ($P = 0.04$) and there is a trend suggesting higher oxygen consumption in HL individuals relative to HH individuals ($P = 0.22$). In addition, oxygen consumption was 0.011 O_2 $min^{-1}g^{-1}$ (±0.004 [95% C.I.]) lower in the LH group relative to HL the group ($P = 0.001$). The RQ increased slightly in the HL group relative to the HH treatment group ($P = 0.10$), however this was not significant (fig. 6).

I observed a significant difference in age at first reproduction between treatment groups (fig.7). Individuals in the HL group first reproduced 47.5 days (± 47.32 [95% C.I]) earlier than the HH group ($P = 0.05$). Similarly, individuals in the LH group also bred 49.5 days (± 41.4 [95% C.I]) earlier than the HH group ($P = 0.02$). Although not significant, a similar trend was

seen between HH and LL individuals, with LL individuals reproducing 30.1 days (± 43.8 [95% C.I.]) earlier (P = 0.18). In addition, there were several individuals within each enclosure that remained non-reproductive throughout the course of the study. While not significant, it suggests that there is an effect of treatment on whether or not individuals reproduced (X^2 = 5.82, N=54, P=0.12; fig. 8).

DISCUSSION

In this investigation we studied the effects of early nutrition on metabolic rate and age at first reproduction in the house mouse. The dietary manipulation had no effect on body mass at 30 days of age (fig. 1) or at 75 days (fig. 4). Maternal diet also had no effect on offspring metabolic rate at weaning (fig. 2), but the interaction between maternal diet and the offspring's diet at independence resulted in significant differences in RMR between groups just before the onset of reproduction at 75 days (fig. 4). Mice in the matched treatment groups (HH and LL) exhibited similar levels of oxygen consumption at day 75 (fig. 5). The RQ for these groups was also similar suggesting that there was no difference between groups in the relative oxidation of lipids, proteins and carbohydrates in the production of ATP (fig. 6). Mice in the HL treatment had significantly lower levels of oxygen consumption and higher RQ at 75 days compared to the HH treatment group (fig. 5, 6). This suggests that the dietary shift to a low protein diet not only affected the metabolic of these mice but also they fuel metabolism, specifically relying on fats and carbohydrate to a greater degree than mice that born to mothers that consume a similarly low protein diet. Mice in the LH treatment group did not differ from HH females, but a trend suggests that they may experience a drop in metabolism in response their dietary shift to a high protein diet at independence (fig. 5). Hales and Barker (1992) argued that the dietary environment of an individual during development sets its metabolic trajectory. If this is true for

the house mouse, we can assume that the target metabolic rate is similar for mice born to mothers consuming a high protein and a low protein diet, even if there are underlying differences in metabolism between HH and LL mice. Yet, when a mouse's diet at independence did not match the programmed effect on metabolism, metabolic rate either shifted above or below that target. Criscuolo et al (2008) found a similar response to HH, HL, LH, and LL diet quality treatments in zebra finches, suggesting that the metabolic response to shifts in diet could be highly conserved.

Individuals that mature earlier are likely to incur a fitness advantage over those that mature later (Lindström 1999). Therefore, following the environmental matching hypothesis and evidence that the metabolic trajectory is similar for HH and LL females, we predicted that mice consuming a diet that matched their mothers diet (HH or LL) would reproduce earlier than those that experienced a dietary mismatch (HL or LH). Interestingly, our results did not support this prediction. Individuals in the LH and HL reproduced earlier than the HH treatment group, and the LL group displayed a similar trend. In many rodents, including mice, mass often determines when an individual reproduces (Singleton and Krebs 2007, Lantham and Masson 2004), with heavier individuals reproducing earlier. This effect was not apparent in our populations, as there was no difference in body mass between groups just before the onset of breeding, although there was a trend suggesting the females in the LL groups may be heavier than the females in the HH group. There was also a trend suggestion that the LL group also had a greater proportion of reproductive individuals (fig. 8).

These findings differ from prior work, suggesting that the cumulative quality of an individual's diet may drive fitness (Auer 2010, Dmitriew an Rowe 2011) For example, bank voles that had access to high quality food only as adults to those who had access to high quality food throughout their lives display comparable age at first reproduction, but reduced litter size

and offspring mass (Helle et al. 2012). There was a tremendous amount of variation of age at first reproduction within treatment groups and this may be attributed to factors in conjunction with dietary treatment. For example, social dominance can have strong impacts on reproduction (Vandenberg et al 1972). Reproductive suppression is known to occur in house mice under direct competition or when investment is high (Wasser and Barash 1983). It is possible that individuals in the mismatched groups (HL or LH) experienced increased competition and increased investment in reproduction due to dietary changes.

To our knowledge, this is the first study to evaluate the impact of metabolic rate and age at first reproduction within the context of environmental matching. Our results indicate that the RMR of an individual may not be a critical determinant of age at first reproduction. Bateson and Gluckman (2011) argued that dietary information that an individual received from its mother during develop can allow it to program a physiology that maximizes within a similar dietary context. Although we found no support for this hypothesis, it is likely that the cumulative reproductive effort of a house mouse presents a more meaningful measure of its probable fitness, given that the probability of successfully weaning the first litter in the house mouse is low relative to many other mammalian species.

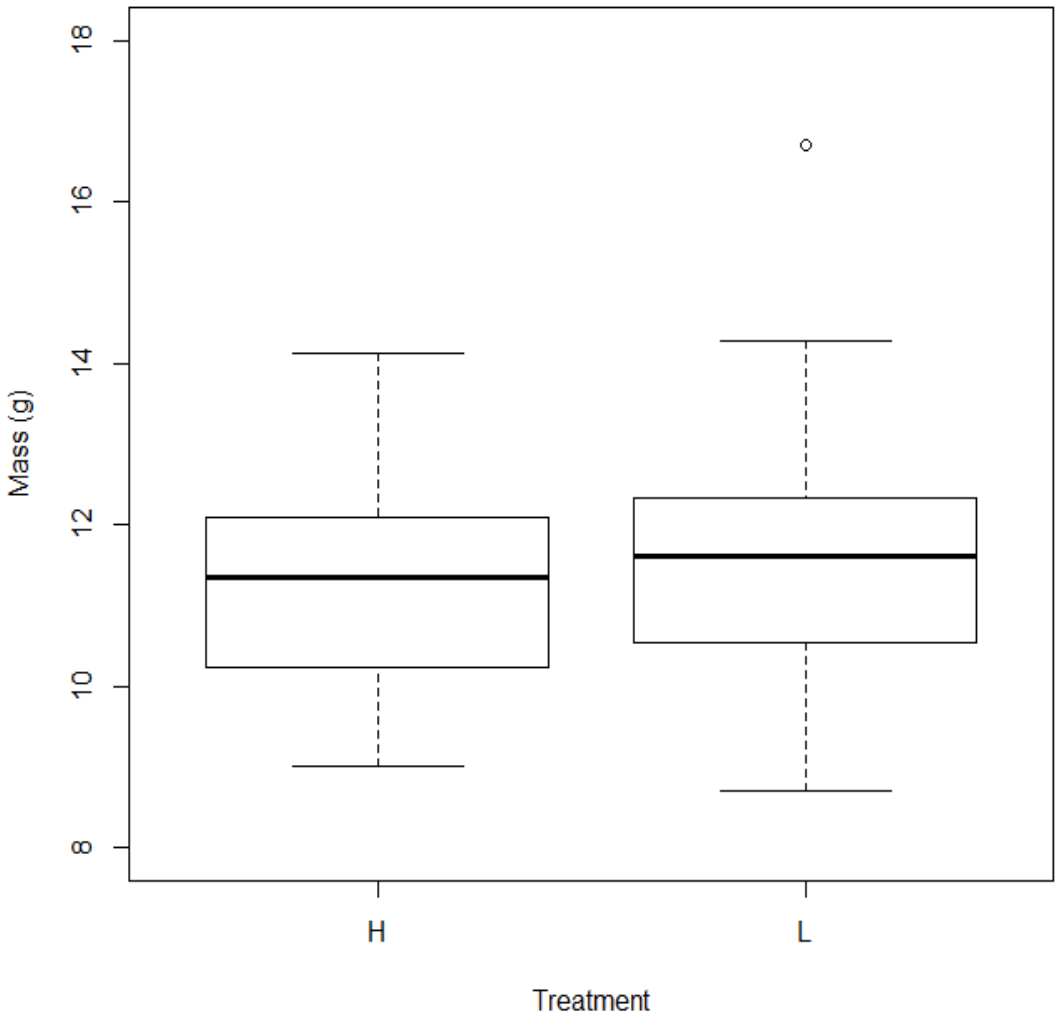

Figure1. Body mass (g) of F_1 mice at 30 days between high (H) and low (L) treatment groups

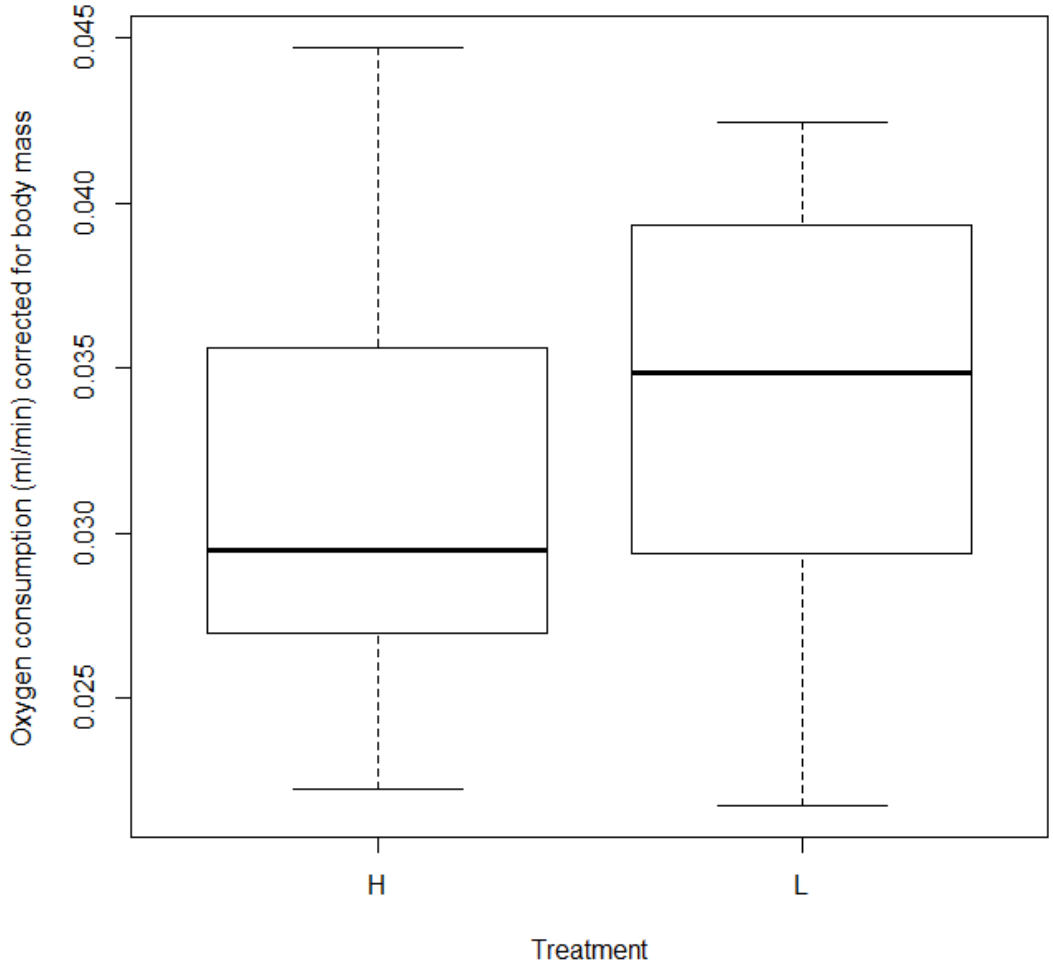

Figure2. Oxygen consumption (O_2 $min^{-1}g^{-1}$) corrected for body mass of F_1 mice in high (H) and low (L) treatment groups at 30 days

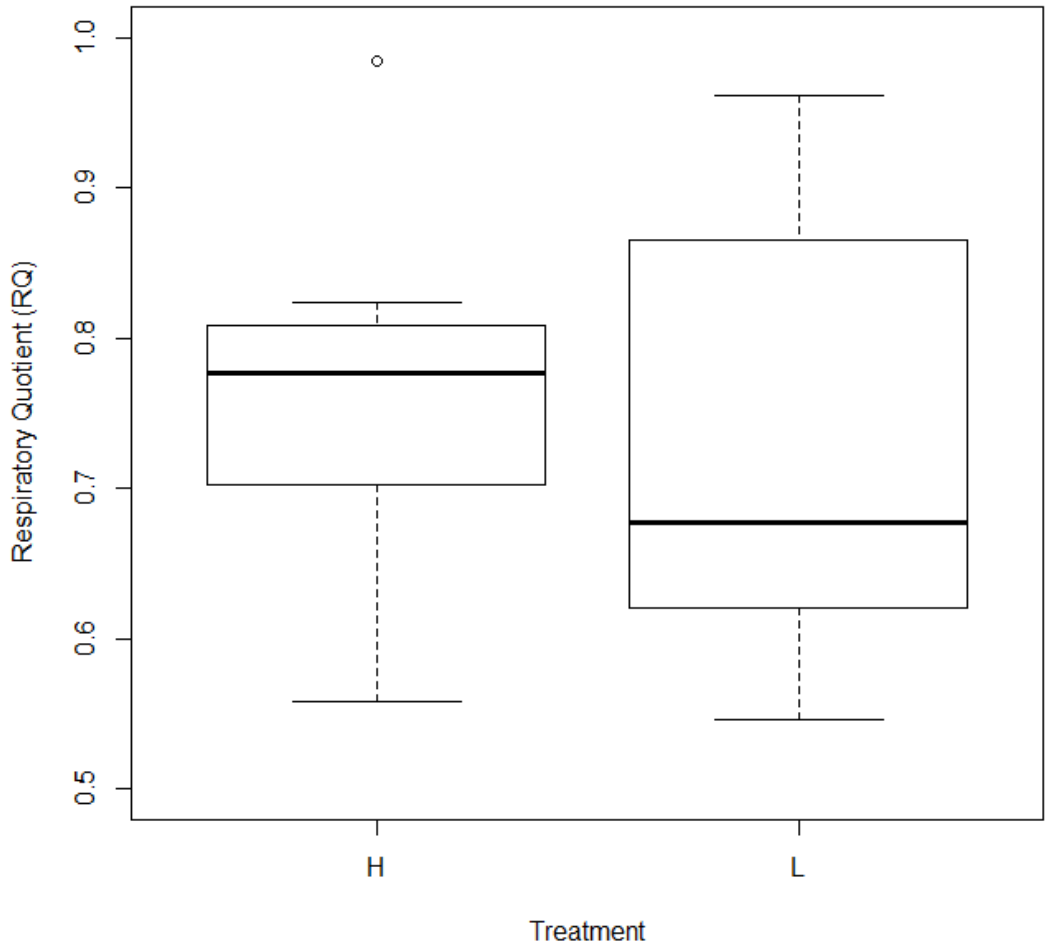

Figure3. Respiratory quotient (RQ) in F_1 mice at 30 days between high (H) and low (L) treatment groups

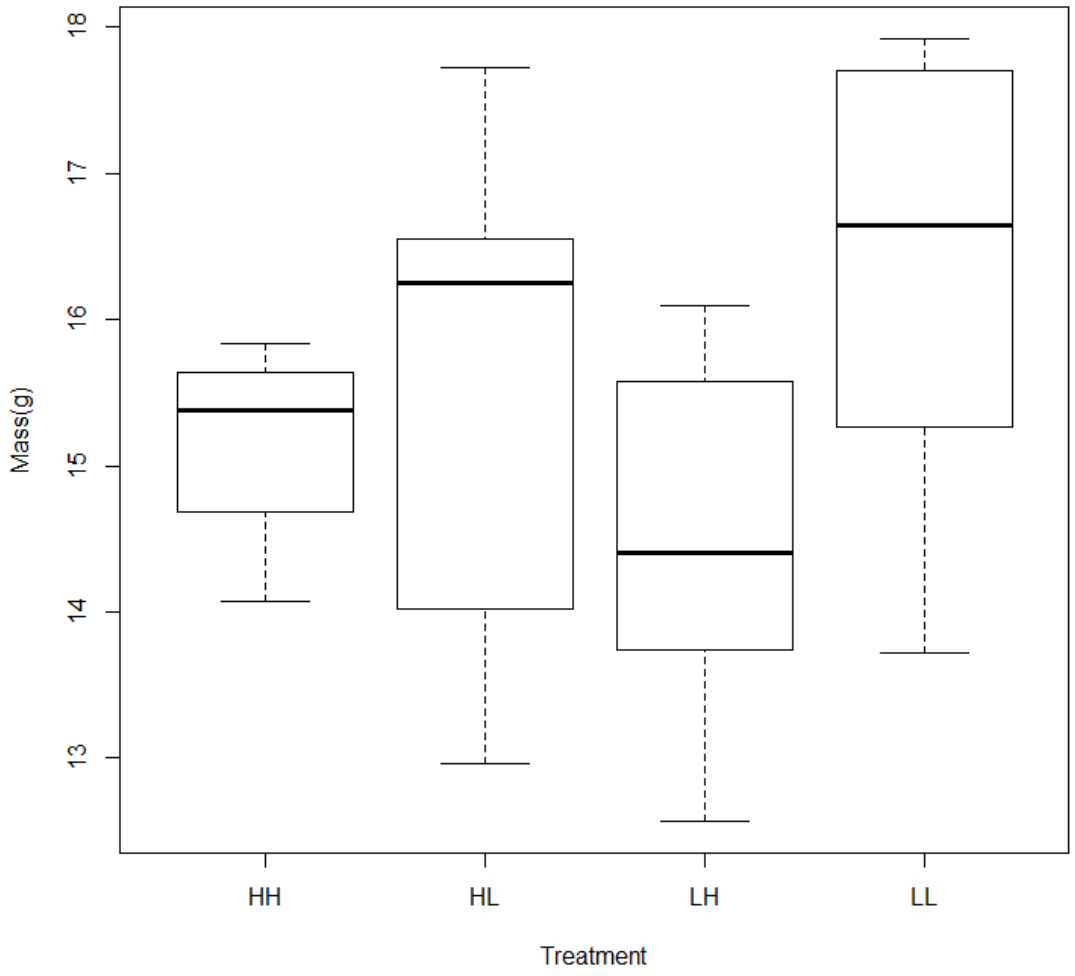

Figure4. Body mass (g) of F_1 mice at 75 days of age in HH, HL, LH, LL treatment groups

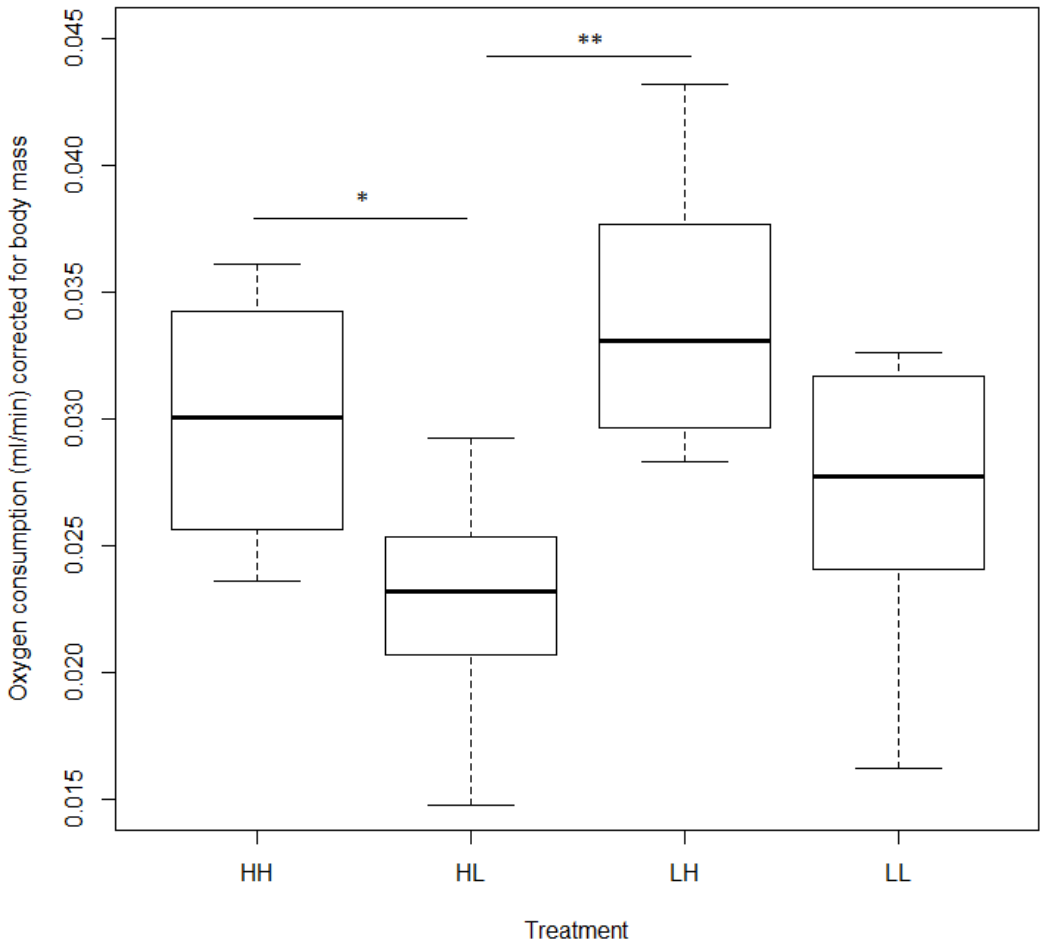

Figure5. Oxygen consumption of F_1 mice at 75 days of age in HH, HL, LH, and LL treatment groups. Asterisks indicate significant diet treatment differences (*p<.05, **p<.001)

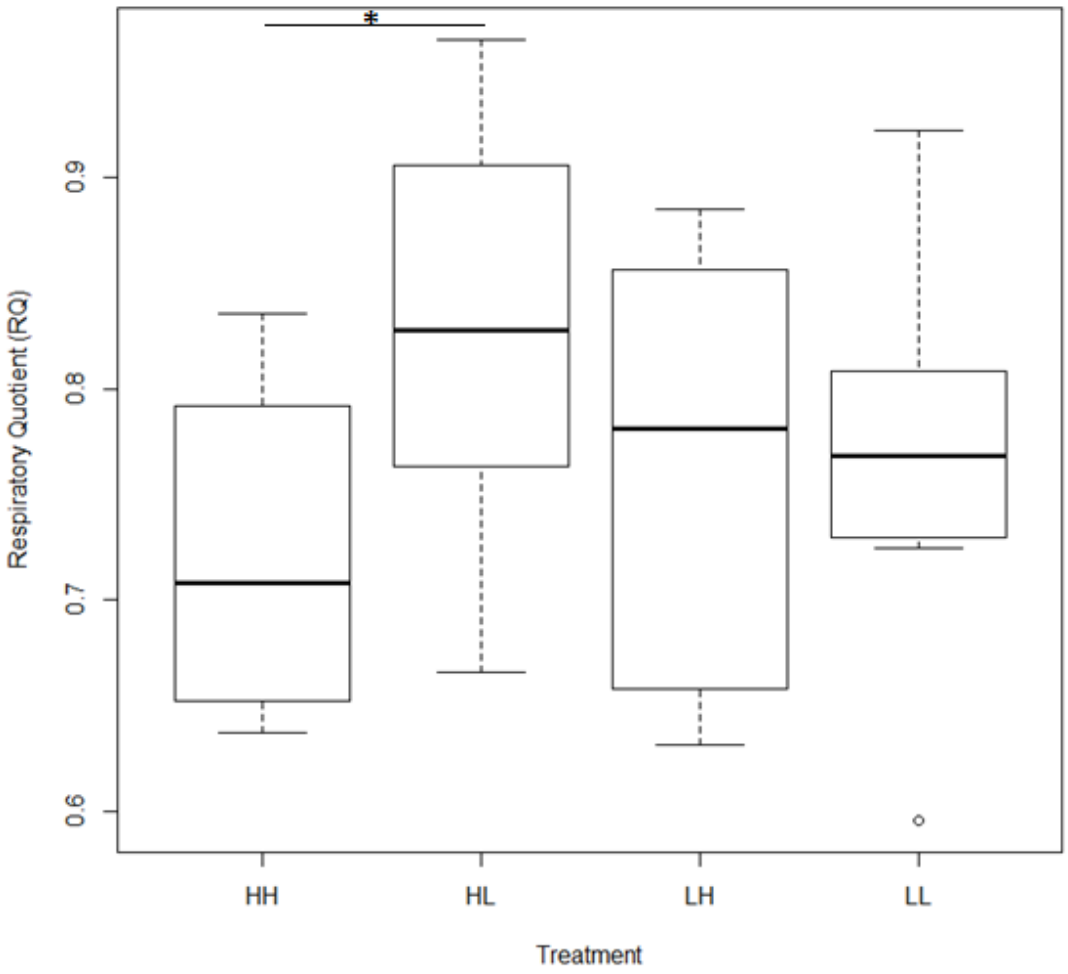

Figure6. Respiratory quotient (RQ) of F_1 individuals in HH, HL, LH, LL treatment groups. Asterisk indicates significant diet treatment differences (*p<.05).

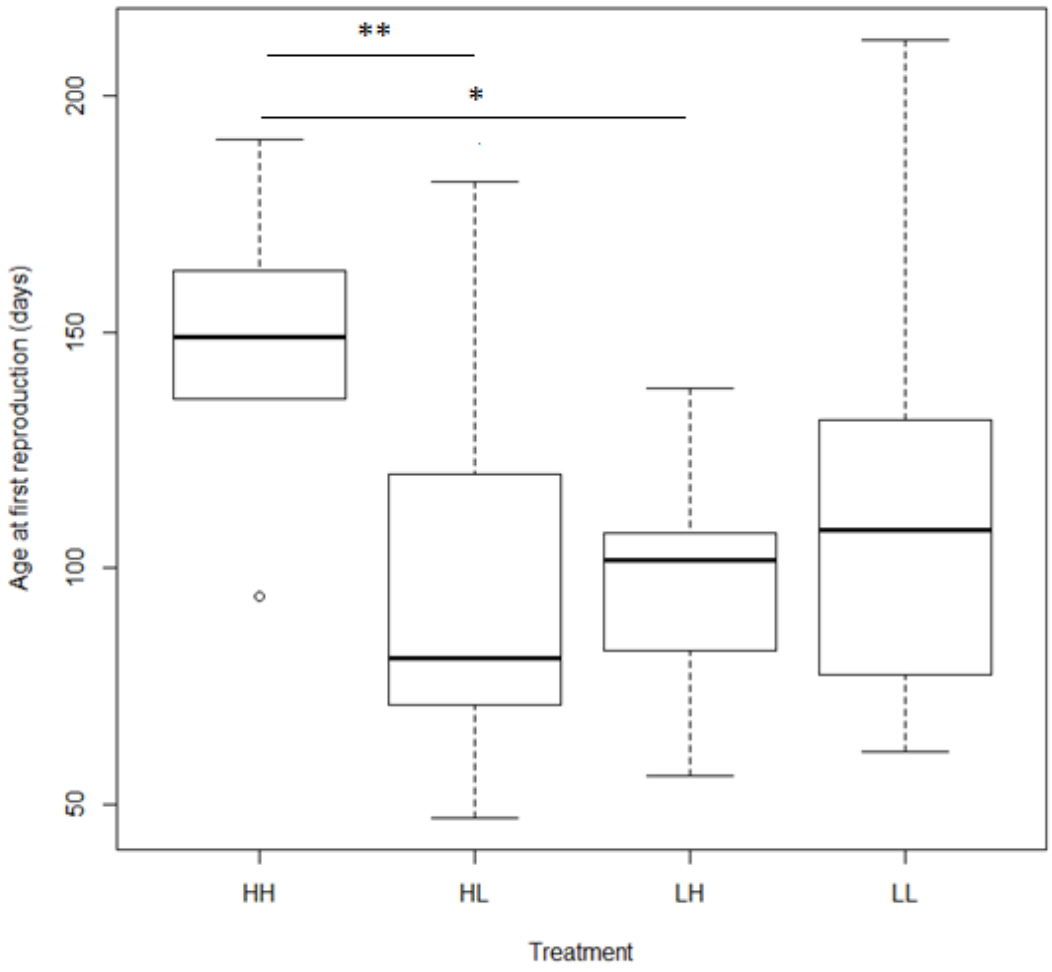

Figure7. Age at first reproduction for F_1 mice in HH, HL, LH, LL treatment groups. Asterisks indicate diet treatment difference (*p<.05, **p<.02).

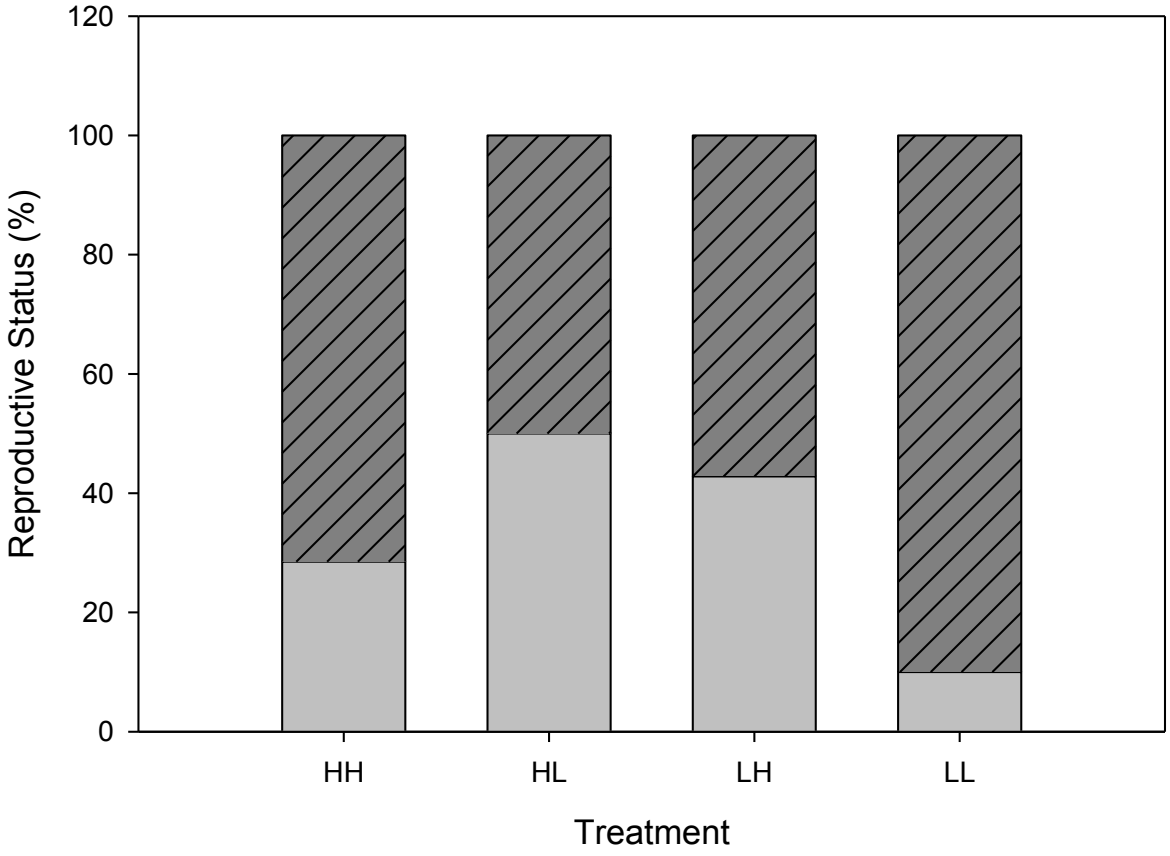

Figure8. Reproductive status of F_1 mice. Solid color refers to non-reproductive individuals and striped refers to reproductive individuals.

LITERATURE CITED

Auer, S. K.2010. Phenotypic plasticity in adult life-history strategies compensates for a poor start in life in Trinidadian guppies (Poecilia reticulata). The American naturalist, 176(6): 818–829.

Ayala-Moreno R., R. Racotta, B. Anguiano, C. Aceves, and L. Quevedo. 2013. Perinatal undernutrition programmes thyroid function in the adult rat offspring. The British journal of nutrition 110: 2207–2215.

Bateson, P., and P. Gluckman. 2011. Plasticity, robustness, development and evolution. Cambridge University Press.

Bomford M. 1987. Food and reproduction of wild house mice. Diet and breeding seasons in various habitats on irrigated cereal farms in New-South-Wales. Australian Wildlife Research 14: 183-196.

Boutin S.1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. Canadian Journal of Zoology 68*:* 203–220.

Burton T., S. S Killen, J. D. Armstrong, and N. B. Metcalfe.2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proceedings of the Royal Society B: Biological Sciences 278: 3465–3473.

Cameron G. N., and B. D. Eshelman .1996. Growth and Reproduction of Hispid Cotton Rats (Sigmodon hispidus) in response to naturally occurring levels of dietary protein. Journal of Mammalogy 77: 220–231.

Chastel O., A. Lacroix, and M. Kersten. 2003. Pre-breeding energy requirements: thyroid hormone, metabolism and the timing of reproduction in house sparrows Passer domesticus. Journal of Avian Biology 34: 298–306.

Criscuolo F., P. Monaghan, L. Nasir, and N. B. Metcalfe .2008. Early nutrition and phenotypic development: “catch-up” growth leads to elevated metabolic rate in adulthood. Proceedings of the Royal Society B: Biological Sciences 275: 1565–1570.

Desai M. and C. N. Hales.1997. Role of fetal and infant growth in programming metabolism in later life. Biological reviews of the Cambridge Philosophical Society 72: 329–348.

Dmitriew, C., and Rowe, L. 2011. The effects of larval nutrition on reproductive performance in a food-limited adult environment. PloS one, 6(3): e17399.

Dobson F. S., and J. D. Kjelgaard.1985. The influence of food resources on life history in Columbian ground squirrels. Canadian Journal of Zoology 63:2105–2109.

Duarte L. C., L. M. Vaanholt, R. E. Sinclair, Y. Gamo, and J. R. Speakman.2010. Limits to sustained energy intake XII: is the poor relation between resting metabolic rate and reproductive performance because resting metabolism is not a repeatable trait? The Journal of experimental biology 213: 278–287.

Faulks S. C., N.Turner, P. L. Else, and A J. Hulbert .2006. Calorie restriction in mice: effects on body composition, daily activity, metabolic rate, mitochondrial reactive oxygen species production, and membrane fatty acid composition. The journals of gerontology 61: 781–794.

Gittleman, J. L., and S. D. Thompson .1988. Energy Allocation in Mammalian Reproduction. American Zoologist 28: 863–875.

Guzmán C., R. Cabrera, M. Cárdenas, F. Larrea, P. W. Nathanielsz, and E. Zambrano .2006. Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. Journal of Physiology 572: 97–108.

Helle, H., Koskela, E., & Mappes, T. (2012). Life in varying environments: experimental evidence for delayed effects of juvenile environment on adult life history. The Journal of animal ecology, 81(3): 573–582.

Ilmonen P., D.J. Penn, K. Damjanovich, L. Morrison, L. Ghotbi and W.K. Potts. 2007. Major histocompatibility complex heterozygosity reduces fitness in experimentally infected mice. Genetics 176: 2501-2508.

Labocha M. K., E. T. Sadowska, K. Baliga, A. K. Semer, and P. Koteja.2004. Individual variation and repeatability of basal metabolism in the bank vole, Clethrionomys glareolus. Proceedings of the Royal Society B: Biological Sciences 271: 367–372.

Latham N., and G. Mason .2004. From house mouse to mouse house: the behavioural biology of free-living Mus musculus and its implications in the laboratory. Applied Animal Behaviour Science 86: 261–289.

Lindström, J.1999. Early development and fitness in birds and mammals. Trends in ecology & evolution 14: 343–348.

Mathot, K. J., S. Godde, V. Careau, D. W. Thomas, and L.A Giraldeau. 2009. Testing dynamic variance-sensitive foraging using individual differences in basal metabolic rates of zebra finches. Oikos 118: 545–552.

McAdam, A. G. and J. S Millar.1999. Dietary protein constraint on age at maturity: an experimental test with wild deer mice. Journal of Animal Ecology 68*:* 733–740.

McNab, B. K.2002. The physiological ecology of vertebrates: a view from energetics. Cornell University Press

Mcmillen, I., and J. Robinson.2005. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiological Reviews, 571–633.

Meagher S., and W. K. Potts, .1997. A microsatellite-based MHC genotyping system for house mice (Mus domesticus). Hereditas 127: 75–82.

Meagher S., Penn, D. J., and Potts, W. K.2000. Male-male competition magnifies inbreeding depression in wild house mice. Proceedings of the National Academy of Sciences of the United States of America 97: 3324–3329.

Metcalfe N. B., and P. Monaghan.2001. Compensation for a bad start: grow now, pay later? Trends in Ecology and Evolution 16: 254–260.

Miller, R. A., J. M. Harper, R. C. Dysko, S. J. Durkee, and S. N. Austad.2002. Experimental Biology and Medicine 227: 500-508.

Morgan I. J., and N. B. Metcalfe.2001. Deferred costs of compensatory growth after autumnal food shortage in juvenile salmon. Proceedings of the Royal Society B: Biological Sciences 268: 295–301.

Monaghan P. 2008. Early growth conditions, phenotypic development and environmental change. Philosophical transactions of the Royal Society of London. Series B, Biological Sciences 363: 1635–45.

Nilsson J., M. Akesson, , and J. F. Nilsson.2009. Heritability of resting metabolic rate in a wild population of blue tits. Journal of Evolutionary Biology 22: 1867–1874.

Oli M. K., G. R. Hepp, and R. A. Kennamer.2002. Fitness consequences of delayed maturity in female wood ducks, Evolutionary Ecology Research 4: 563–576.

Pinheiro J., D. Bates, S. DebRoy, D. Sarkar.2007. nlme: Linear and Nonlinear Mixed- Effects Models, R Package Version 3.1–86

Reeves P. G., F. H. Nielsen, and G. C. Fahey.1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of nutrition 123: 1939–1951.

Robbins C.T.1993. Wildlife Feeding and Nutrition. Academic Press, New York, New York.

Selman C., S. Lumsden, L. Bünger, W. G. Hill, and J. R. Speakman.2001. Resting metabolic rate and morphology in mice (*Mus musculus*) selected for high and low food intake. The Journal of Experimental Biology 204: 777–784.

Speakman J. R. 2008. The physiological costs of reproduction in small mammals. Philosophical transactions of the Royal Society of London. Series B, Biological Sciences 363: 375–398.

Speakman J. R., D. A Talbot, C. Selman, S. Snart, J. S. McLaren, P. Redman, E. Krol, et al. 2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. Aging cell 3: 87–95.

Speakman J.R.1997. Factors influencing the daily energy expenditure of small mammals. The Proceedings of the Nutrition Society 56: 1119–1136.

Speakman J. R., and J. McQueenie.1996. Limits to Sustained Metabolic Rate : The Link between Food Intake , Basal Metabolic Rate , and Morphology in Reproducing Mice , Mus musculus. Physiological Zoology 69: 746–769.

Singleton G. R., and C. J. Krebs.2007. The secret world of wild mice. The Mouse in Biomedical Research. I. History, Wild Mice, and Genetics, 25-51.

Stearns S. C.1992. The Evolution of Life Histories. Oxford University Press.

Tann C.R., G.R. Singleton, and B.J. Coman.1991. Diet of the house mouse, Mus domesticus, in the Mallee-Wheatlands of North-Western Victoria. Wildlife Research 18: 1-12.

Tobler, M., J.K. Nilsson, and J. F. Nilsson.2007. Costly steroids: egg testosterone modulates nestling metabolic rate in the zebra finch. Biology Letters 3:408–410.

Vandenbergh J.G., L. C. Drickamer, and D. R. Colby.1972. Social and dietary factors in the sexual maturation of female mice. J. Reprod.Fert 28: 397–405.

Verhulst S., M.J. Holveck and K. Riebel .2006. Long-term effects of manipulated natal brood size on metabolic rate in zebra finches. Biology Letters 2: 478–480.

Whittingham D. G., and M. J. Wood.1983. "Reproductive physiology." The mouse in biomedical research 3:137-164.

Wone, B., M. W. Sears, M. K. Labocha, E. R. Donovan, and J. P. Hayes.2009. Genetic variances and covariances of aerobic metabolic rates in laboratory mice. Proceedings of the Royal Society B: Biological Sciences 276: 3695–3704.

Zambrano E., P. M. Martínez-Samayoa, C. J. Bautista, M. Deás, L. Guillén, G. L. Rodríguez-González, C. Guzmán, et al.2005. 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. The Journal of Physiology 566: 225–236.

CHAPTER TWO

Short and long-term effects of the developmental environment on insulin-like growth factor 1 and reproductive effort in the house mouse (*Mus musculus*)

INTRODUCTION

The life history strategy that affords an individual the highest possible fitness typically varies with environmental conditions (Ricklefs and Wikelski 2002) and the environment that an individual experiences during early development can play an influential role in determining its physiological phenotype (Hales and Barker 2001, McMillen and Robinson 2005). Based on these observations, it has been suggested that these physiological changes can be maintained through adulthood and affect an individual's ability to function optimally in its adult environment (Monaghan 2008). In other words, the early developmental environment may be key in shaping an individual's life history strategy and therefore, determining how available resources are partitioned to growth, reproduction, and self-maintenance. Metabolic hormones play fundamental roles in coordinating energy allocation to such tasks and regulating physiological and behavioral activities. Therefore, hormonal cues may also underlie and mediate life history tradeoffs (Ketterson and Nolan 1992, Ricklefs and Wikelski 2002). Insulin-like growth factor 1 (IGF-1) is a metabolic hormone that has been shown to influence life history traits associated with growth, reproduction, and lifespan (Dantzer and Swanson 2012, Yakar et al. 2002, Sparkman et al. 2009). Given that IGF-1 is an effective stimulator of growth and is highly sensitive to nutritional status, IGF-1 may be one mechanism by which the early

developmental environment influences an individual's physiological phenotype and ultimately its life history strategy.

It is known that nutrition during early development can affect the physiology and metabolism of individuals (Desai and Hales 1997, McMillen and Robinson 2005), potentially altering their growth and developmental trajectories. Consistent with this idea, IGF-1 is highly plastic in response to fluctuations in nutrient availability (either food quality or quantity). For example, when Stellar sea lions undergo bouts of food restriction, levels of IGF-1 decrease (Jeannaird du Dot et al. 2010). Additionally, it is widely recognized that IGF-1 has positive effects on postnatal growth (Yakar et al. 2002, Stratikopoulos et al. 2008, Stuart and Page 2010). Furthermore, the nutrients available to young during early development, which are influenced by both the mother's phenotype and environmental conditions, can have long-lasting effects on IGF-1 production. In particular, restriction during prenatal and postnatal development has been shown to impact IGF-1 production later in life. For example, early restriction of the maternal diet results in reduced levels of IGF-1 in offspring up to 9 days following parturition (Woodall et al. 1996). Mice that experienced periods of food restriction after birth via an increase or decrease in litter size had significantly decreased plasma IGF-1 production compared to controls up to three months after restriction (Kappeler et al. 2009). The fetuses of female rats fed a low protein (8% protein) diet had lower levels of IGF-1 compared to the fetuses of control females (El Khattabi et al. 2003). Tissues exposed to such low levels of IGF-1 during development are proposed to suffer long-term consequences that alter metabolic regulation or growth trajectories (McMillen and Robinson 2005).

IGF-1 not only affects somatic growth but it is also necessary for proper function and maturation of the reproductive system. When levels of IGF-1 are low, sexual maturation is

delayed due to altered synthesis of reproductive hormones or cellular activity of reproductive organs (Chandrashekar et al. 2004, Woodall et al. 1996). For example, in female rats IGF-1 receptors (IGF-1R) are involved in stimulating estradiol and progesterone production (Demeestre et al. 2004). In addition to showing delayed postnatal growth, mice that lack the gene for IGF-1 are essentially infertile. Because of its essential role in the reproductive system, it has been suggested that variation in circulating levels of IGF-1 and in IGF-1 signaling may influence life history traits associated with reproduction (Dantzer and Swanson 2012). For instance, increased circulating levels of IGF-1 are generally associated with both reproductive success and onset of reproduction (Taylor et al. 2014, Velazquez et al. 2008). In male deer, IGF-1 is positively correlated with circulating testosterone and antler size during the breeding season (Ditchkoff et al. 2001). Circulating IGF-1 is also positively correlated with reproductive output in garter snakes (*Thamnophis elegans*) (Sparkman et al. 2009). Yet, the interactions among the environment, IGF-1, and reproductive variables in natural populations remain largely unexplored (but see Bronikowski and Arnold 2000, Sparkman et al. 2010).

The amount of energy a mother can allocate to reproduction is dependent on her own energy stores and resource availability. Reproduction is energetically expensive, particularly in small mammals. During this time, individuals can face significant protein and calcium demands and to a larger extent organ remodeling (Speakman 2008, Hammond 1997, Hood 2012). The plasticity of IGF-1 and IGF-1 signaling in response to nutrition may allow for individuals to cope with costs of reproduction, by delaying or altering high-energy activities such as reproduction. For example, during periods of negative energy balance, perhaps prompted by low resource availability, IGF-1 levels decrease (Du dot et al. 2009). This can result in a reduction in overall energy expenditure (Yambayamba et al. 1996, Ling et al. 1996). Therefore, a female's

reproductive effort, or the proportion of energy available to the female for reproduction, is likely to be mediated by changes in IGF-1 levels.

There is strong evidence suggesting that conditions an individual experiences during development can permanently alter individual metabolism (Lillycrop 2010, McMillen and Robinson 2005), and a mismatch between the developmental and adult environment can lead to metabolic dysfunction in adulthood (Hales and Barker 2001). The environmental matching hypothesis links these metabolic effects to reproductive fitness and predicts that fitness of an individual will be highest when individuals match their adult environment to conditions experienced during early development. Accordingly, when development and adult conditions are mismatched fitness is predicted to decrease. It is possible that the interaction between the development and adult diet programs differences in circulating levels of IGF-1 and expression the IGF-1 gene at its sites of production, such as the liver, and a result provides a mechanism that links metabolism, developmental conditions, and reproductive performance in adulthood.

The house mouse was an ideal candidate for this investigation due to their short generation time and their increased sensitivity to intrinsic and extrinsic stressors compared to their laboratory counterparts (Meagher et al. 2000, Ruff et al. 2013). I chose to manipulate dietary protein because 1) wild mice, as generalists, are likely limited by diet quality rather than quantity (Lochmiller et al. 2000) and 2) protein manipulation has been shown to impact physiology and reproduction in rodents (Guzman et al. 2006, Zambrano et al. 2005). Protein intake can be highly variable in wild mice varying from low protein for individuals consuming an entirely grain based diet (~10-13% for corn and wheat) or high protein (~20%) for individuals consuming an insectivorous diet (Bomford 1987, Tann et al. 1991, Robbins 1993). In addition, dietary protein has been shown to alter glucose, lipid, and protein metabolism (Zambrano et al.

2005, Gluckman 2007, Lillycrop et al. 2005). To investigate the impact of early nutrition during development on circulating IGF-1 levels, expression and reproductive effort in a population of wild-derived house mice, we manipulated dietary protein. The parental generation was given access to either a 20% high protein (H) or 10 % low protein (L) diet and allowed them to breed freely. After weaning, their offspring (F_1 generation) were either maintained on the maternal diet (HH, LL) or switched to the alternate diet (HL, LH). Therefore, we can examine the effect of the early developmental diet on reproductive success by switching from a low to high (LH) or high to low (HL) diet after weaning. Under the environmental matching model, we predict that mice in the HH and LL group will display greater reproductive effort than mice in the HL or LH groups. Given its association with greater reproduction, circulating IGF-1 and IGF-1 gene expression in the liver is predicted to be highest in the animals with highest reproductive performance. Therefore, if the predictions for reproductive effort are supported, mice in the HH and LL groups are predicted to have higher IGF-1 than mice in the HL and LH groups.

METHODS

Study Organism

The house mouse (*Mus musculus*) is an ideal model species to study the impacts of the early developmental environment on offspring physiology in a wild population. House mice are easy to maintain and a rapid generation time allows us to measure reproductive variables within a controlled environment. In addition, methods for quantifying physiological variables in mice were readily available and often standardized. The house mice that founded our populations

were obtained from established breeding populations maintained by W. Potts, University of Utah, Salt Lake City.

Diet Manipulation

To assess the impact of early environmental conditions on circulating and expression levels of IGF-1, I manipulated diet in F_0 parents and F_1 offspring after weaning. Mice were maintained on a custom 10% low protein diet (L) or a 20% high protein diet (H) prepared by TestDiet Inc. These diets were isocaloric with cornstarch making up the differences in energy content between the H and L diets as described in chapter 1.

Animal Husbandry

Mice were maintained in 10 semi-natural enclosures. In the wild, mice typically interact within breeding groups known as demes that include 7-12 adults and use a home-range size of just a few meters (Klein 1975). Each 1.4 m x 3.5 m enclosure was lined with 1 m aluminum flashing to prevent escapes. All enclosures were located in within 1 of 2 adjacent buildings. The windows in each building were covered with hardware cloth to expose the mice to ambient temperatures and keep predators out. When mice were first obtained, they were segregated by sex for approximately 8 weeks. Group assignments minimized relatedness among individuals and did not include female or male siblings from the same litter. Populations in each enclosure were founded with a maximum of 10 individuals, with up to 7 females and 3 males.

Females and males were allowed to breed and compete for mates as they would in a natural setting. I selected F_1 pups born between late December 2012 and March 2013 for this study. No more than two males and two females were taken from each litter. Littermates of opposite sex were placed on different diets to ensure that siblings were not mated. At the time of

collection all F_1 individuals were pit tagged subcutaneously (size: HPT8, Biomark, Inc) for future identification using a handheld reader. As a secondary form of identification, each F_1 individual were also ear punched (1-2 mm) in a unique pattern that referred a unique number.

The reproductive status of each female was monitored daily. On each occasion, we determined if any new females gave birth, the identity of all females on a nest of pups based on its pit tag number, monitored the condition of young and counted the number of young present in each litter from birth until weaning. I removed F_1 offspring at 4 weeks from each enclosure. These individuals were either euthanized or retained to examine the impact of the developmental diet on reproductive effort and circulating levels of IGF-1 and IGF-1 gene expression. Individuals that were retained were moved to a new enclosure and were either given a high or low protein that was either similar or dissimilar to their developmental diet, creating four distinct treatment groups (HH, LL, HL, and LH). After 8 months of breeding, the males were removed from each enclosure to stop breeding. When females were approximately 1 year (277-379 days), all remaining F1 adults were euthanized. Because breeding females often mated shortly after parturition, it was necessary prevent breeding so that the final IGF-1 values could be compared between females in a comparable non-reproductive state. Sample sizes for the HH, HL, LH, and LL group were 3, 7, 12, and 13 respectively.

Reproductive Effort

After the diet manipulation we allowed the retained F_1 individuals to breed. I followed each litter daily and recorded the number of offspring at birth through weaning. In general, I designated the female found on the nest the greatest number of days as the mother of the litter. When the likelihood of being the mother was equivocal between two females, I considered both

the identity of the female that was on the nest during the first few days post-partum and the amount of time between reproductive bouts. I removed the offspring of the F_1 females (F_2 estimated reproductive effort (RE) of each F_1 female by applying a modification of the lifetime reproductive effort equation (LRE) described by Charnov, Warne and Moses (2007). I applied the following equation to estimate reproductive effort per female:

$$\text{RE}= \sum \left(\frac{\#\ of\ offspring}{litter}\right) * (average\ total\ body\ mass\ per\ litter)$$

I included only those F_1 offspring that survived until independence (weaning) in these analyses.

Blood and liver collection

Following euthanasia, I collected blood from 30 day F_1 females following weaning and from 1 year old females following breeding. Approximately 1ml of blood was collected in EDTA tubes and centrifuged at 13,000 rpm for 5 minutes, and the plasma was removed from each sample. The intact liver was also removed from each female and placed in a tube with RNAlater (Life Technologies). Both the plasma and liver were then frozen at -80 C for future analysis.

Radioimmunoassay techniques

Plasma IGF-1 concentrations were measured using a radioimmunoassay (RIA) previously validated for mice, following the methods outlined in Richmond and Zinn (2009). The primary antibody was rabbit anti-human antisera (National Hormone & Peptide Program, AF Parlow). The secondary antiserum to γ-globulin was produced in goats (GARGG) (National Hormone & Peptide Program). The sensitivity of the validation assay was 20 ng/ml, the intraassay variation

was 16%, and percent recoveries were 53% for the juvenile pool and 80% for the adult pool. Linearity was determined using a linear regression analysis of the observed hormone concentrations vs. the expected hormone concentrations. A correlation coefficient (R^2) and slope of 1 were considered linear in our serially diluted adult and juvenile pools (fig.1). The sensitivity of the assay was 20 ng/ml , the interassay variations were 11.8 % and 20%, and the intraassay variations were 7% and 9% respectively. Internal controls were created by pooling samples from 3-4 juvenile and adult mice from the same cohort and sex. All samples were run in two assays in quadruplicate. Percent recoveries for the juvenile pool were 82% and 86%. Recoveries for the adult pool were 86% and 72% respectively.

Quantitative polymerase chain reaction (qPCR) for IGF-1 mRNA expression

I collected livers from F_1 juvenile females at 30 days after parturition and at adulthood. We extracted RNA using TRIzol (Invitrogen). I then treated the RNA with Turbo DNase (Ambion Inc.) for 30 minutes at 37ºC and further purified using a phenol-chloroform reaction. I converted 2µg of RNA to cDNA via reverse transcription using qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD). We used qPCR to determine levels of IGF-1 mRNA expression in F_1 females and used GADPH as a housekeeping control (as adapted from Yamamoto et al. 2013 and Sun et al. 2004) (see Table1 for primer sequences). We used 2X SYBR® green PCR Master Mix (Applied Biosystems) to complete the qPCR according to the manufacturer's instructions and 1ul of primers at 100 ng/µl concentration per reaction. Cycling conditions were: 10 minutes holding at 95ºC and 50 cycles of 95º C for 15 seconds followed by 60ºC for 1 minute (Eppendorf Mastercycler eprealplex^2).

Statistical Analyses

The effects of diet on levels of circulating IGF-1 and IGF-1 mRNA expression were analyzed using linear mixed effect models (lme), with mouse enclosure included as a random factor. Reproductive effort in relation to treatment group was also analyzed using a liner mixed effect model including enclosure as a random factor. We compared circulating levels of IGF-1 and reproductive effort using a standard linear model (lm). IGF-1 expression was analyzed by comparing the ratio of liver IGF-1 expression relative to expression of GADPH and then determining differences among groups using a linear model (lm). We performed all analyses using R v.3.0.1 (http://www.r-project.org/, nlme library from Pinheiro et al., 2007) and extracted all *p* values from the model summaries. Significance was set at α for $P \leq 0.05$.

RESULTS

Reproductive Effort

There was no observed difference between reproductive effort and treatment group in matched individuals (P = 0.4) (fig. 2). However, there is a trend suggesting that LH individuals average may have greater reproductive effort than individuals in the HH treatment group (P=0.12). Interestingly, this trend was not apparent in the HL group (P = 0.70). We also compared reproductive effort to concentrations of circulating IGF-1 in adult females (fig. 3). I found that circulating IGF-1 concentrations negatively correlated with reproductive effort (F = 6.004, df = 16, P = 0.02; fig.3).

Circulating IGF-1 concentrations

At 30 days of age we observed no significant difference in circulating levels of IGF-1 between high (H) and low (L) protein treatment groups (P = 0.35; fig. 4). However, diet had a significant effect on circulating levels of IGF-1 in adulthood (365 days; fig. 5). IGF-1 concentrations in the HL treatment group averaged 20.4 ng/ml (± 18.0 [95% C.I.]) greater compared with those in the LH treatment group (P = 0.04). Mice in the LH treatment group averaged 13.92 ng/ml (± 16.7 [95% C.I.]) higher IGF-1 concentrations compared to individuals in the LL treatment group, although this was not significant (P = 0.11).

IGF-1 Gene Expression in the liver

I observed no difference in the ratio of IGF-1 to GADPH expression between diets at 30 days (F = 0.109, P = 0.74) (fig. 6). This was also the case at adulthood as we observed no difference in the ratio of IGF-1to GADPH expression among diets (F = 0.123, P = 0.94 ; fig. 7). However, we observed a difference in the ratio of IGF-1 to GADPH expression when we compared juveniles to adults (fig. 8).Expression of IGF-1 to GADPH ratio was 0.05 lower (± .064 [95% C.I.]) in juvenile mice compared to adult mice (P = 0.09), although this was not significant.

DISCUSSION

At 30 days of age, IGF-1 hormone concentrations were similar among diet treatments suggesting that they were not impacted by the maternal diet during development (fig. 3). Derrickson and Lowas (2007) showed in laboratory mice that mothers consuming a 20% protein diet produced higher protein milk that mothers consuming a 10% protein diet. In addition, low protein intake has also been shown to reduce milk yield (Sampson 1984). However under the

conditions described herein, 30 day old mice born to mothers on the high and low protein diet did not differ in body mass (chapter 1). It is possible that nutrient intake did not differ between pups in each group or young compensated for low protein intake between initial consumption of solid foods (~14 days) and the body mass measurements.

At 1 year of age, circulating levels of circulating IGF-1 were similar among mice in the HH and LL groups but mice in the LH treatment group exhibited significantly lower concentrations of circulating IGF-1 compared to mice in the LL group. Mice in the HL treatment group had significantly higher circulating levels of IGF-1 compared to the LH treatment group (fig. 5). Similarities in reproductive effort and concentrations of IGF-1 between the HH and LL suggest that these two different environments produce similar metabolic phenotypes; but the results of for HL and LH high were unexpected. It's important to note that since mice were fed ad libitum animals in the low treatment groups may have adjusted protein deficiencies by increasing intake, however this does not explain differences between the mismatched groups (HL and LH). There is a trend that suggests reproductive effort in the LH animals could be higher than the other groups (fig. 2). Based on results of previous studies (Sparkman et al. 2009, Sparkman et al. 2010), IGF-1 was expected to be highest in the group with highest reproductive effort. However, this effect was not observed. Interestingly, when treatment group was ignored and IGF-1 was compared to reproductive effort using a simple linear model, we observed a negative relationship (fig. 3), suggesting that IGF-1 is lower in mice that have displayed greater reproductive effort than those that displayed lower effort. These IGF-1 values represent females that had an opportunity to mate for approximately 8 months and all mice were approximately 1 year old. Females with the highest reproductive effort would have continuously bred for the full 8 months that they were paired with a male, whereas a female with low reproductive effort

would have breed discontinuously and may not have been reproductively active at the time males were removed. If females with high reproductive effort could have readily bred again, we would have expected concentrations of IGF-1 to be higher than those that had put little effort into reproduction. In addition, IGF-1 has also been shown decline with age (Breese et al. 1991), it is possible that reduced IGF-1 among females with high reproductive effort reflects the costs of reproduction.

Given that most of circulating IGF-1 production originates in the liver, we predicted that expression levels could be altered by diet during early development. Treatment group had no effect on differences in IGF-1 expression at 30 days (fig.5). However, our data suggests that there is a difference in IGF-1 expression between juveniles (30days) and adults, with juveniles exhibiting lower IGF-1 expression than adults (fig.8). This response may be largely due to the fact that individuals were just weaned and IGF-1 production was only beginning to increase. We also observed no effect of diet on IGF-1 expression at adulthood (fig. 7). This is surprising given that there appeared to be changes in circulating levels at adulthood. While we found no differences among diets and levels of IGF-1 expression it is possible that local IGF-1 production and signaling such as IGF-1 receptors (IGF-1R) had an additional impact on offspring metabolism. Like IGF-1 gene expression, IGF-1R can also be altered during early development. For example, it is known that increased activity of IGF-1 receptors (IGF-1 R) caused by an increase in plasma IGF-1 can have effects postnatal growth (Baker et al. 1993), which can have potential fitness consequences. Reproduction is also influenced by the expression of specific IGF-1 binding proteins (IGF-1BP) (Gay et al. 1997, Fowler et al.2000). It is possible that the effect of treatment on components of the IGF-1 system may be altered at the level of IGF-1 receptor or binding protein expression and in turn influences reproduction.

Investment in reproduction and lifespan thought to be constrained by physiological tradeoffs making it impossible for both to increase simultaneously (Reznick 1985, Stearns 1992). Within the IGF-1 system, high levels of circulating IGF-1 and local IGF-1 signaling are generally associated with increased reproductive output and decreased longevity (Dantzer and Swanson 2012, Sparkman et al 2009, Bartke 2005). Mice with genetic mutations that result in permanent decreases in levels of plasma IGF-1 exhibit resistance to oxidative stress and greater lifespan than wild-type controls (Bartke 2005, Holzenberger et al. 2003). We also measured liver mitochondrial respiratory efficiency, oxidative damage, and antioxidant production the 1-year-old mice described in this study. Our results suggest that although ROS production and oxidative damage was higher in animals that had reproduced (4-HNE), females that bred displayed higher mitochondrial efficiency (respiratory control ratio, SOD2, catalase) and higher antioxidant production than those that did not breed (Mowry, Kavazis, and Hood unpublished data). This suggests that despite oxidative damage, reproductive females did not display a decline in metabolic function and repair that commonly observed during aging (Balaban et al. 2005; Speakman et al. 2004). Low circulating IGF-1 in females with high reproductive performance and high mitochondrial respiratory efficiency in females that bred, question whether enhanced reproductive effort would have been associated with reduced longevity in these animals.

The results of this study suggest that reproductive effort in house mice is not influenced by matching metabolic phenotype to maternal diet. Yet, similarities in patterns of reproductive effort, circulating IGF-1 levels, and a negative correlation between reproductive effort and circulating IGF-1 suggest that IGF-1 may still be important in matching reproductive effort to dietary environment. Interestingly, circulating IGF-1 levels countered the predicted relationship

between IGF-1 and reproduction and this may be due to the relative age of animals at the time of samples collection. Most of the prior work examining IGF-1 during reproduction and aging evaluated each of these life history variables independently (but see Sparkman et al. 2009). To further understand the role that IGF-1 plays in life-history variation, future work should consider the inevitable interaction between these variables.

Table1

qPCR Primer Sequences

Gene	Primer Orientation	Nucleotide Sequence (from 5' to 3')	Source
GADPH	Forward	GAAGACACCAGTAGACTCCAC	Sun et al. 2005
	Reverse	AACGACCCCTTCATTGAC	
IGF-1	Forward	GTCTGCTCACCTTCACCAGC	Yamamoto et al. 2013
	Reverse	AATGTACTTCCTTCTGAGTCT	

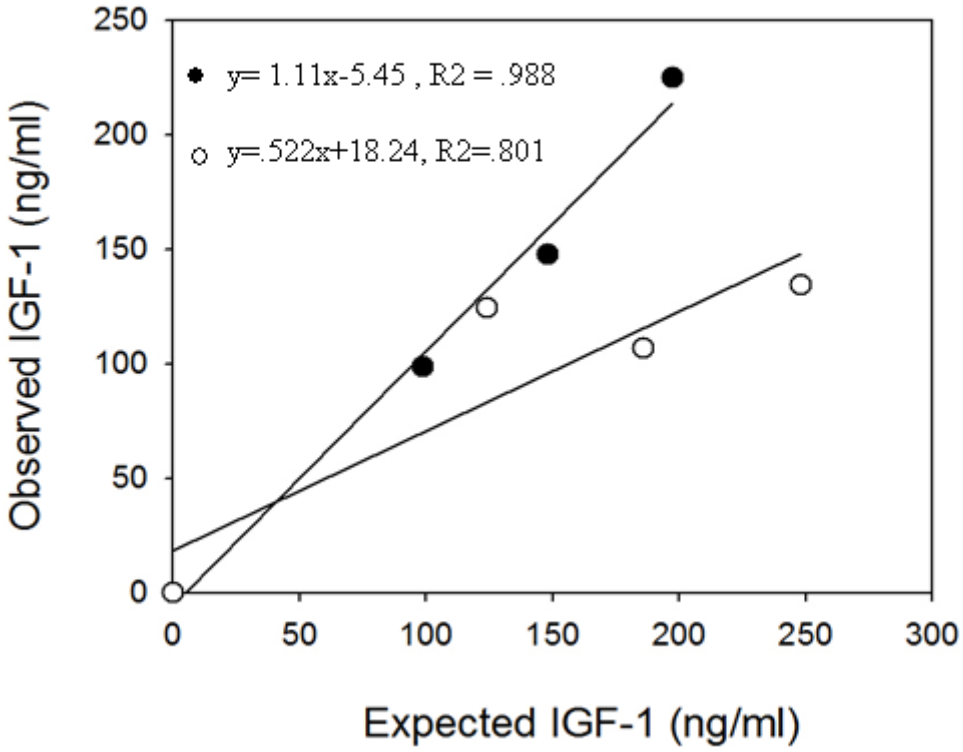

Figure 1. Insulin-like growth factor (IGF)-1 linearity; Adult and juvenile pool for Mus musculus. Adult pool exhibited a significant linear regression with a slope and correlation coefficient close to one. Linear regression equations and correlation coefficients for both the adult and juvenile pool are displayed on the graph. Closed circles represent the adult pool, open circles represent the juvenile pool.

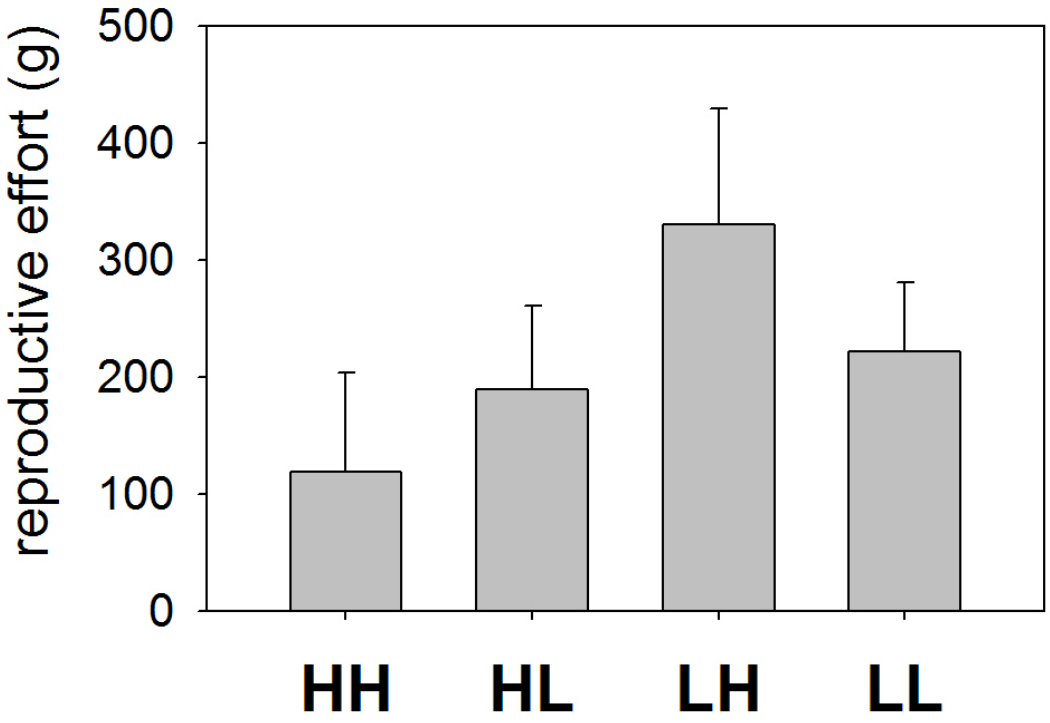

Figure2. Reproductive effort (grams) in adult females for HH, HL, LH, and LL protein treatment groups. P = 0.12

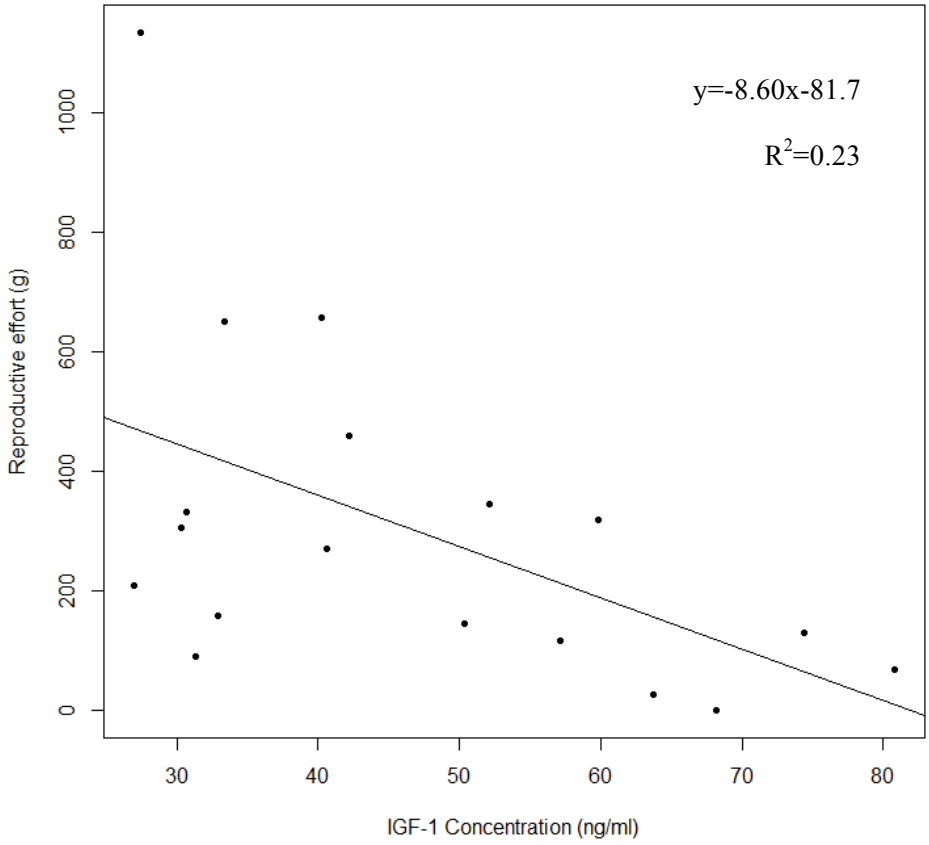

Figure3. Reproductive effort (grams) and circulating IGF-1 concentrations (ng/ml) in adult mice (P=.02)

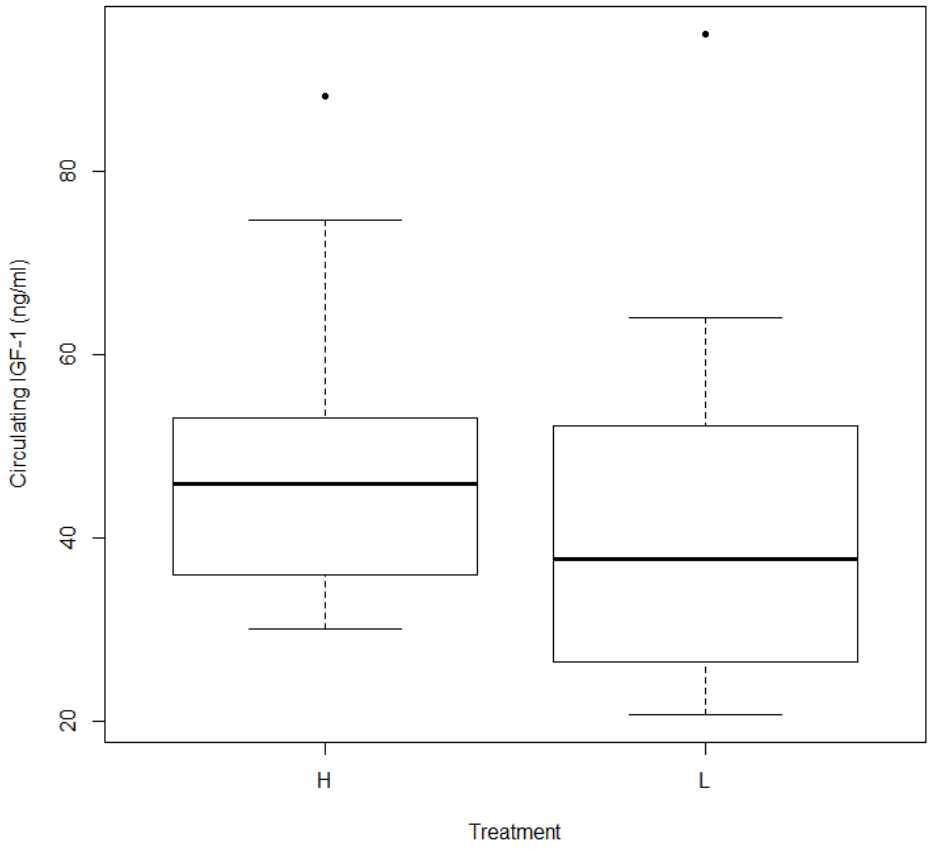

Figure4. Circulating insulin-like growth factor 1 (ng/ml) in mice at 30 days for high (H) and L (low) protein treatment groups. P=0.35

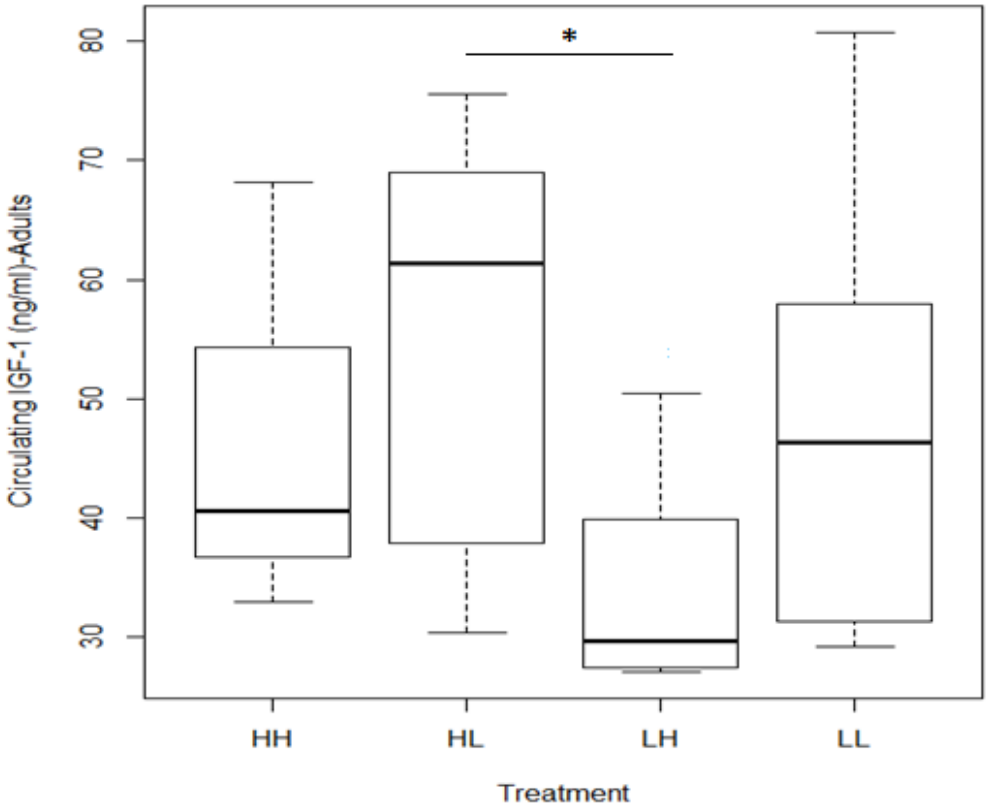

Figure5. Circulating insulin-like growth factor1 (ng/ml) in adult mice for HH, HL, LH, and LL treatment groups (*P<.05).

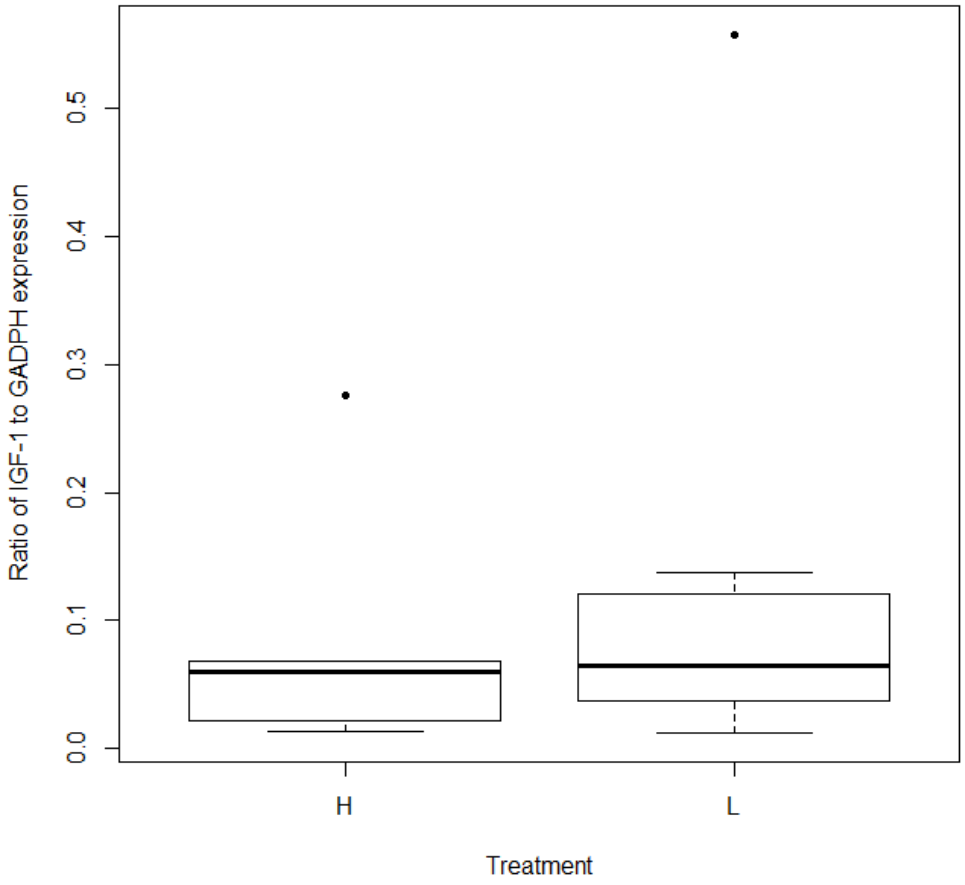

Figure6. Ratio of IGF-1 to GADPH expression between high (H) and low (L) treatment groups at 30 days. P = .74

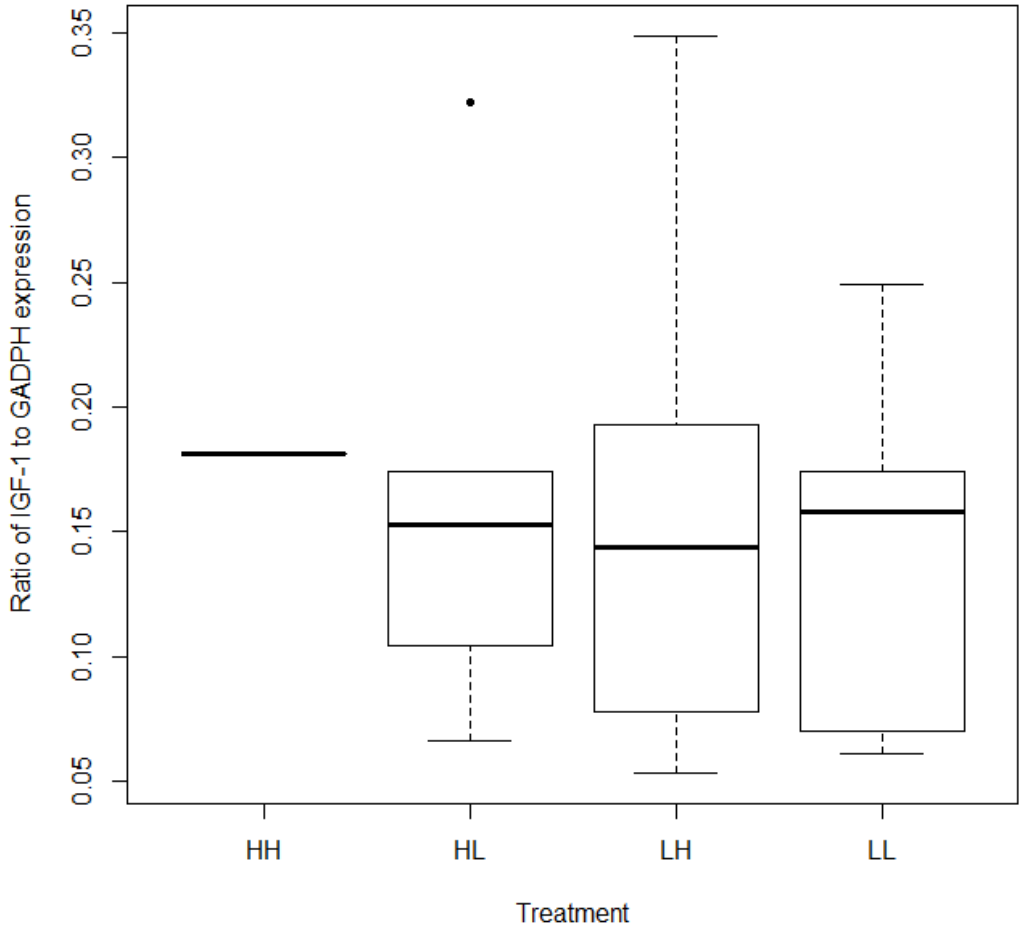

Figure7. Ratio of IGF-1 to GADPH expression between HH, HL, LH, and LL treatment groups at adulthood.

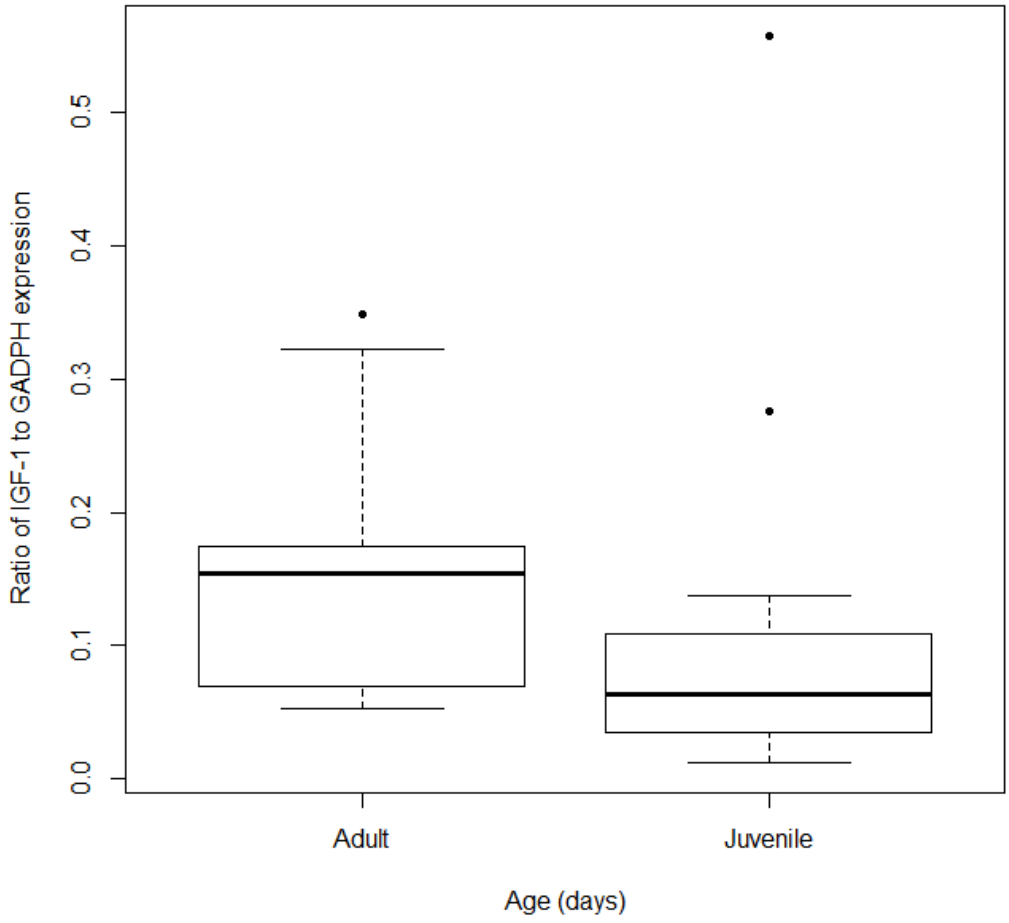

Figure8. Ratio of IGF-1 to GADPH expression between adults (A) and juveniles (J).

P= 0.06

LITERATURE CITED

Baker J., L. Jeh-Ping, E.J. Robertson, and A. Efstratiadis.1993.Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75 :73-82.

Balaban, R.S., S. Nemoto and T. Finkel. 2005. Mitochondria, oxidants, and aging. Cell 120: 483-495.

Bartke, A. 2005. Minireview: role of the growth hormone/insulin-like growth factor system in mammalian aging. Endocrinology 146: 3718–23.

Bomford, M. 1987. Food and reproduction of wild house mice. Diet and breeding seasons in various habitats on irrigated cereal farms in New-South-Wales. Australian Wildlife Research 14: 183-196.

Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. Canadian Journal of Zoology 68: 203–220.

Breese C. R., R.L. Ingram, and W. E. Sonntag.1991. Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. Journal of gerontology 46: B180-B187.

Bronikowski A.M. 2000. Experimental evidence for the adaptive evolution of growth rate in the garter snake Thamnophis elegans. *Evolution*; international journal of organic evolution, 54, 1760–1767.

Chandrashekar V., D. Zaczek, , and A. Bartke.2004.The consequences of altered somatotropic system on reproduction. Biology of reproduction 71: 17–27.

Charnov E. L.2007. Fish growth: Bertalanffy k is proportional to reproductive effort. Environmental Biology of Fishes 83: 185–187.

Dantzer B., and E. M. Swanson.2012. Mediation of vertebrate life histories via insulin-like growth factor-1. Biological reviews of the Cambridge Philosophical Society 87: 414–29.

Demeestere I., C. Gervy, J.Centner, F. Devreker, Y. Englert, and A. Delbaere.2004. Effect of insulin-like growth factor-I during preantral follicular culture on steroidogenesis, in vitro oocyte maturation, and embryo development in mice. Biology of reproduction 70: 1664–1669.

Derrickson E.M., and S.R. Lowas.2007.The effects of dietary protein levels on milk protein levels and postnatal growth in laboratory mice (*Mus musculus*). Journal of Mammology 88: 1475–1481.

Desai M., and C. N. Hales.1997. Role of fetal and infant growth in programming metabolism in later life. Biological reviews of the Cambridge Philosophical Society 72: 329–348.

Ditchkoff S. S., L. J. Spicer, R. E. Masters, and R. L. Lochmiller.2001.Concentrations of insulin-like growth factor-I in adult male white-tailed deer (Odocoileus virginianus): associations with serum testosterone, morphometrics and age during and after the breeding season. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 129: 887–95.

Dot T. J., D.A.S. Rosen, J. P. Richmond, A. S. Kitaysky, S. A. Zinn, and A. W. Trites.2009. Changes in glucocorticoids, IGF-I and thyroid hormones as indicators of nutritional stress and subsequent refeeding in Steller sea lions (Eumetopias jubatus). Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 152: 524–534.

Fowler D. J., K. H. Nicolaides, and J. P. Miell.2000.Insulin-like growth factor binding protein-1 (IGFBP-1): a multifunctional role in the human female reproductive tract. Human Reproduction Update 6: 0–1.

Gay E., D. Seurin, S. Babajko, S. Doublier, M. Cazillis, and M. Binoux.1997. Liver-specific expression of human insulin-like growth factor binding protein-1 in transgenic mice: repercussions on reproduction, ante- and perinatal mortality and postnatal growth. Endocrinology 138: 2937–47.

Guzmán C., R.Cabrera, M.Cárdenas, F. Larrea, P. W. Nathaniels, and E. Zambrano.2006. Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. The Journal of physiology 572: 97–108.

Hales C. N., and D. J. Barker.2001. The thrifty phenotype hypothesis. British medical bulletin, 60: 5–20.

Hammond K. A.1997. Adaptation of the maternal intestine during lactation. Journal of Mammary Gland Biology and Neoplasia 2: 243–252.

Holzenberger M., J. Dupont, B. Ducos, P. Leneuve, A. Géloën, P. C. Even, P. Cervera, et al. 2003. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature, 421: 182–187.

Kappeler L., C.D.M Filho, P. Leneuve, J. Xu, N. Brunel, C. Chatziantoniou, Y. Le Bouc, et al. 2009. Early postnatal nutrition determines somatotropic function in mice. Endocrinology, 150: 314–323.

Ketterson E. D., and V. Nolan.1992. Hormones and life histories: an integrative approach. The American Naturalist 140: S33–S62.

Klein J. 1975. Natural History of the Mouse Histocompatibility-2 complex. Springer-Verlag, New York.

Lillycrop K. A., E. S. Phillips, A. A. Jackson, M. A. Hanson, and G. C. Burdge.2005. Nutrient-Gene Interactions Dietary Protein Restriction of Pregnant Rats Induces and Folic Acid

Supplementation Prevents Epigenetic Modification of Hepatic Gene Expression in the Offspring. The Journal of nutrition: 1382–1386.

Ling P. R., C. Gollaher, E. Colon, N. Istfan and B. R. Bistrian.1995. IGF-I alters energy expenditure and protein metabolism during parenteral feeding in rats. The American journal of clinical nutrition 61: 116–20.

Lochmiller R. L., S.S. Ditchkoff, and J.A. Sinclair.2000. Developmental plasticity of postweanling cotton rats (Sigmodon hispidus) as an adaptation to nutritionally stochastic environments. Evolutionary Ecology 14: 127–142.

El-Khattabi I., F. Grégoire, C. Remacle, and B. Reusens.2003. Isocaloric maternal low-protein diet alters IGF-I, IGFBPs, and hepatocyte proliferation in the fetal rat. American journal of physiology. Endocrinology and metabolism 285: E991–E1000.

Mcmillen I.C., and J.S Robinson.2005. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiological Reviews: 571–633.

Monaghan P.2008. Early growth conditions, phenotypic development and environmental change. Philosophical transactions of the Royal Society of London. Series B, Biological sciences: 363, 1635–1645.

Meagher S., and W. K. Potts .1997. A microsatellite-based MHC genotyping system for house mice (Mus domesticus). Hereditas 127: 75–82.

Pinheiro J., D. Bates, S. DebRoy, D. Sarkar.2007.nlme: Linear and Nonlinear Mixed- Effects Models, R Package Version 3.1–86

Reznick D.1985. Costs of Reproduction : An Evaluation of the Empirical Evidence Costs of reproduction : an evaluation of the empirical evidence. Oikos, 44: 257–267.

Richmond J. P., and S. A. Zinn.2009. Validation of Heterologous Radioimmunoassays (RIA) for Growth Hormone (GH) and Insulin-Like Growth Factor (IGF)-I in Phocid, Otariid, and Cetacean Species. Aquatic Mammals 35:19–31.

Ricklefs, R.E., and M. Wikelski. 2002. The physiology/life-history nexus. Trends in Ecology & Evolution 17: 462-468.

Robbins, C.T. 1993. Wildlife Feeding and Nutrition. Academic Press, New York, New York.

Speakman, J.R., D.A. Talbot, C. Selman, S. Snart, J.S. McLaren, P. Redman, E. Krol, D.M. Jackson, M.S. Johnson and M.D. Brand. 2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. Aging Cell 3: 87-95.

Sparkman A. M., D. Byars, N. B. Ford, and A. M. Bronikowski.2010. The role of insulin-like growth factor-1 (IGF-1) in growth and reproduction in female brown house snakes (Lamprophis fuliginosus). General and comparative endocrinology 168: 408–414.

Sparkman A.M, C.M. Vleck, and A. M Bronikowski.2009. Evolutionary Ecology of Endocrine-Mediated Life-History Variation in the Garter Snake (*Thamnophis elegans*).Ecology, 90: 720–728.

Stearns S. C. 1992. The Evolution of Life Histories. Oxford University Press.

Stratikopoulos E., M. Szabolcs, I. Dragatsis, A. Klinakis, and A. Efstratiadis. 2008. The hormonal action of IGF1 in postnatal mouse growth. PNAS: 19378–19383.

Stuart J. A, and M. M. Page.2010. Plasma IGF-1 is negatively correlated with body mass in a comparison of 36 mammalian species. Mechanisms of ageing and development:131, 591–8.

Sun L. Y., K. Al-Regaiey, M. M. Masternak, J. Wang, and A. Bartke.2005. Local expression of GH and IGF-1 in the hippocampus of GH-deficient long-lived mice. Neurobiology of aging, 26: 929–937.

Tann C.R., G.R. Singleton and B.J. Coman. 1991. Diet of the house mouse, Mus domesticus, in the Mallee---Wheatlands of North---Western Victoria. Wildlife Research 18: 1-12.

Taylor V. J., Z.Cheng, P. G. A. Pushpakumara, D. E. Beever, and D. C. Wathes.2002. Papers & Articles Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. The veterinary record 155: 583-588

Velazquez M. A, L. J. Spicer, and D. C. Wathes.2008. The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. Domestic animal endocrinology 35: 325–342.

Woodall S. M., B. H. Breier, B. M. Johnston,and P. D. Gluckman.1996. A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. Journal of Endocrinology 150: 231–242.

Yamamoto M., G. Iguchi, H. Fukuoka, K. Suda, H. Bando, and M. Takahashi.2013. SIRT1 regulates adaptive response of the growth hormone – insulin-like growth factor-I axis under fasting conditions in liver. PNAS 110: 1–6.

Yambayamba E. S., M. A. Price, and G. R. Foxcroft.1996. Hormonal status, metabolic changes, and resting metabolic rate in beef heifers undergoing compensatory growth. Journal of animal science74: 57–69.

Zambrano, E., P. M. Martínez-Samayoa, C. J. Bautista, M. Deás, L. Guillén, G. L. Rodríguez-González, C. Guzmán, et al. 2005. 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. The Journal of physiology 566: 225–236.