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The Effect of Diet on Reproductive Inhibition in Photoresponsive White-Footed Mice (Peromyscus leucopus)

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The Effect of Diet on Reproductive Inhibition in Photoresponsive White-Footed Mice (*Peromyscus leucopus*)

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by

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Abstract

Variation in reproduction is a characteristic of many different species. Reproductive variation can be caused by genetic variation, phenotypic plasticity, or a combination of both factors. The genes of an animal set up reaction norms, which include phenotypically plastic responses to different environmental cues. One environmental cue that has a powerful effect on reproductive variation in many mammalian species is photoperiod. Animals that are reproductively responsive to photoperiod demonstrate suppressed reproductive capacity in short, winter-like photoperiods. A second environmental cue that has a powerful effect on reproduction is food availability. The importance of food availability in reproductive variability is unsurprising, because increased energy is required for reproductive activity. Previous studies have shown that reduced food availability can result in suppressed reproduction. This study tests whether an abundance of high quality food can also effect reproductive variation. In this study, we tested reproductively photoresponsive male white-footed mice, *Peromyscus leucopus*, to determine if a high fat diet could overcome the reproductive suppression usually seen in short day photoperiods. This study also tested whether *P. leucopus* provided with a high fat diet consumed more kilocalories than mice provided with a defined control diet. Our results indicated increased caloric intake and gonad mass in mice provided with a high fat diet, but not to statistically significant levels.

Chapter 1: The Effects of Photoresponsiveness and Energy on Reproduction

One of the defining features of living organisms is reproduction, the ability of an individual organism to pass on its genes to a new generation. Reproduction is strongly linked to the fitness of animal, because fitness is often maximized when an animal is able to produce a

large number of offspring. The creation and maintenance of a large number of offspring involves a wide variety of costs. The costs associated with reproduction include opportunity costs, risk costs, and direct energetic costs. Many of the costs of reproduction occur when reproduction diverts energy and resources away from other important processes of the body (Harshman and Zera 2006). These other body processes must then receive less energy, or the organism must take in more energy in order to reproduce. Alternatively, animals may reduce reproduction in order to offset some of the energetic and risk costs of reproduction. Alternative reproductive strategies may result in variability in reproductive capacity. Variability in reproductive capacity may be due to variability in the costs of reproduction. When costs of reproduction are at their greatest, an animal may increase its fitness by suppressing reproduction until energy resources are more readily available (Heideman et al. 2005). Because food intake allows an animal to obtain energy, variation in quantity and quality of food may cause variation in reproduction.

Because of its relation to energetic costs, food availability may have a major effect on reproduction. However, measuring and predicting food availability is a complex process, making it a complicated cue for animals to use to regulate reproductive capacity. In addition, reproductive variability is related to factors other than food. One of these other factors is photoperiodism, which plays a role in the reproduction of a number of mammalian species. Photoperiodism is the ability of organisms to use day length to regulate the changes in behavior and physiology that occur from season to season. An organism that responds to photoperiodism uses the proximate factor of day length, which has no direct effect on fitness, because of the correlations of day length with ultimate factors. These ultimate factors, including seasonal changes in temperature, food availability, and predation, are difficult for organisms to predict

directly. However, organisms can predict these future changes using a correlated change, such as day length, that is regular and easy to predict (Goldman 2001). Low temperatures, low food availability, and high predation risks that are correlated with shortened day lengths favor seasonal reduced reproduction in many mammals.

In mammals, seasonal changes are detected by the neuroendocrine system, specifically through a retinal-hypothalamic-pineal pathway (Prendergast et al. 2002). Light signals enter the pathway through retinal photoreceptors, and the signal travels into the brain to the hypothalamus. In the hypothalamus, the signal passes through the suprachiasmatic nucleus (SCN), which plays a key role in the body's circadian rhythms. From the SCN, neuronal signals travel to the paraventricular nuclei of the hypothalamus, and via the sympathetic nervous system to the pineal gland, causing the release of the hormone melatonin (Gorman and Lee 2002). Melatonin secretion occurs at night, and is secreted for a longer duration if night length is longer, such as in the winter. The melatonin signal allows a signal from photoperiod to be transferred to multiple tissues of the body (Goldman 2001).

Both melatonin and light act as signals that affect two distinct processes, photoperiodism and circadian rhythms. Circadian rhythms are based on 24 hour periods of light and dark and thus control daily patterns of behavior. In contrast, photoperiodism is based on annual changes in day length and so controls seasonal changes in behavior and physiology (Goldman 2001). For these seasonal changes, it is the duration of melatonin secretion that is important (Prendergast et al. 2002). The duration of nightly melatonin signals gradually increases as winter approaches and nights lengthen, while the opposite occurs when nights shorten in spring. The direction of change in melatonin secretion, either increased or decreased secretion, is able to convey information about seasonal change (Prendergast et al. 2002).

Melatonin causes seasonal changes in reproduction through a specific neuroendocrine pathway. In the white-footed mouse (*Peromyscus leucopus*), studies have indicated that melatonin alters neurons in the hypothalamus that are responsible for secreting gonadotrophin-releasing hormone (GnRH) (Avigdor et al. 2005). GnRH is a major regulator of reproduction in mammals, controlling the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH regulate many components of reproduction, including the release and production of sex steroids and gonad development (Baum 2002). This pathway allows photoperiod to alter reproduction in an animal, with either increased or reduced secretions of GnRH.

The culminating effect of photoperiod on these neuroendocrine pathways is seasonal changes in reproduction. Animals in which photoperiod signals result in suppressed reproduction in the winter are defined as being reproductively photoresponsive. However, many photoresponsive mammals use internal timers as well as exogenous cues such as day length. The combination of exogenous and endogenous cues control seasonal rhythms in most short-lived, small mammals in temperate zones (Prendergast et al. 2002). In a typical small mammal, the exogenous signal of short day length will cause a reproductively photoresponsive male to undergo testes regression, and therefore a period of reproductive inactivity. However, many small mammals kept indefinitely in a short, winter-like photoperiod will eventually fail to be reproductively suppressed and undergo spontaneous gonadal regrowth (Prendergast et al. 2002). Full reproductive activity is eventually restored, even in short photoperiod. Because the animal is no longer responsive to the cue of short day length, it is defined as photorefractory. The onset of the photorefractory period is controlled by an endogenous internal timer, not requiring any exogenous cues. Only a switch to a long, summer-like photoperiod will break the

photorefractory period, allowing a new period of reproductive suppression in short days (Fig. 1) (Prendergast et al. 2002). The combined role of both exogenous and endogenous cues in seasonal reproductive variation allows animals to adapt to situations in which reliance on only one set of cues might lead to a reduction in fitness. For example, an animal would have zero fitness in constant short day conditions if reproduction was suppressed indefinitely. However, reproductive suppression is eventually removed due to the action of the internal timer.

The physiological processes allowing reproductive photoresponsiveness are very complex. The evolution of such complex processes for this function indicates that it is potentially beneficial for some animals to reduce reproductive capacity in the winter. Winter reduction of reproductive capacity can be beneficial for multiple reasons. Reproduction requires large quantities of energy to dedicate to the various aspects of reproduction, including gamete production and searching for a mate. To fulfill the energetic costs of reproduction an animal needs to consume greater quantities of food. However, food availability is often reduced in the winter, making it more difficult for animals to procure the extra energy needed for reproduction during the winter months. If this is the case, it would be plausible that reduction or elimination of reproductive activity in the winter could actually increase fitness.

Increased fitness may not always result from reproductive suppression in the winter. If winter conditions are mild and food is abundant, fitness will often be greater for an animal that continues to reproduce during the winter. However, if conditions are severe and food availability is low, fitness will often be greater for an animal that suppresses reproduction during the winter. Because of the variability of fitness outcomes in different situations, no one reproductive strategy during the winter will maximize fitness in a population. In fixed reproductive strategies, all members of a population will always respond to short day cues in the same way. Therefore, in

each individual, short days cues will either always cause reproductive suppression or will always cause maintenance of reproductive activity during the winter. In contrast, members of a population that exhibits a variable strategy will sometimes respond differently to short, winter-like photoperiods. Therefore, individuals may sometimes have reproductive suppression, may remain reproductively active, or show intermediate levels of reproductive activity.

Variation in reproductive response to photoperiod can be caused by genetic variation and by phenotypic plasticity. Fixed reproductive strategies are determined by genetics. Because the genes of an individual do not change over time, individuals of a fixed reproductive strategy always have the same reproductive response. However, genetic variation can allow the reproductive response of a population to evolve over time. The existence of genetic variation in photoresponsiveness would allow for rapid selection of responses to photoperiod that lead to the highest fitness in different environmental situations. Over time, selection would favor animals that had the more adaptive response to photoperiod (Prendergast et al. 2002). In contrast, variable reproductive strategies involve phenotypic plasticity. Phenotypic plasticity is included in the potential reaction norms set up by genes. Genes are acted on by natural selection, while phenotypic plasticity is affected by environmental cues. The combination of these two factors in a variable reproductive strategy provides an animal with a greater potential to have an adaptive reproductive response.

Seasonal variation in reproduction occurs in many populations of rodents found in temperate areas. This includes the species used in this study, *Peromyscus leucopus*, the white-footed mouse (Heideman et al. 1999). In *P. leucopus*, maintaining reproductive activity in the winter requires more time spent foraging for food resources that are less abundant than they would be during the summer. Increased foraging time means increased risks of predation, which

can reduce the fitness of these mice. Unlike larger mammals, small mammals such as *P. leucopus* usually cannot depend on large stores of fat and nutrients to dedicate to winter reproduction (Bronson 1987; Bronson et al. 1991). Therefore, food intake is needed to support the high energy requirements of reproduction, making increased foraging time during times of low food availability unavoidable. However, foraging time could potentially be reduced if reproductive capacity is reduced during periods when there is likely to be low food availability, such as the winter season.

Reproductive photoresponsiveness in *P. leucopus* can be demonstrated in laboratory conditions (Heideman et al. 1999; Heideman et al. 2005). In the laboratory, winter-like conditions can be mimicked by placing mice in short day photoperiods (SD), in which they are exposed to more dark hours than light hours during a 24 hour period. In turn, long day photoperiods (LD), in which the mice are exposed to more light hours than dark hours, mimic summer conditions. Placing photoresponsive white-footed mice in SD conditions results in mice that are reproductively suppressed. This makes *P. leucopus* a useful model to study a variety of aspects of reproduction and reproductive control.

Another characteristic of *P. leucopus* is that individuals show variation in their reproductive response to photoperiod (Heideman et al. 1999; Goldman 2001). When mice are placed in summer-like LD conditions, mice are reproductively active. In male mice, relatively large testes masses and high sperm counts are indications of reproductive capacity. In contrast, much greater reproductive variation occurs when mice are placed in SD conditions. Some of these mice have testes sizes and sperm counts comparable to the mice placed in LD conditions. These mice that are not reproductively suppressed in SD conditions are referred to as being

nonresponsive. However, some mice have much smaller testes sizes and very low or zero sperm counts when placed in SD conditions. These mice are termed responsive.

In *P.* leucopus, variation in the reproductive response to photoperiod has a genetic basis (Heideman et al. 1999; Goldman 2001; Prendergast et al. 2002). Evidence for a genetic basis has included the ability to artificially select for either responsiveness or nonresponsiveness to photoperiod (Heideman et al. 1999). Heideman and coworkers produced a selection line of *P. leucopus* that suppresses reproduction in short winter-like photoperiods, and a line that shows little reproductive suppression in these SD conditions. Further evidence that this variability has a genetic basis is that photoresponsiveness seems to be significantly heritable (Heideman et al. 1999). Existence of this genetic variation in photoresponsiveness indicates that different individuals of *P. leucopus* may have different fitness in different conditions. For example, if a winter was mild, and food was more abundant than usual, it would be beneficial for these short-lived mice to use this time to reproduce. In this case, the mice that were unresponsive to photoperiod and did not suppress reproduction would have higher fitness than the responsive mice that were unable to reproduce during the winter. However, in another year, winter might be particularly harsh with very scarce food availability. These conditions would select for mice that did not need to spend extra, dangerous time foraging to obtain the energy needed to remain reproductively active.

Another important characteristic of *P. leucopus* is that of phenotypic plasticity, the ability to change phenotype in response to the environment. As described above, responsive mice show plasticity in reproductive ability depending on the environmental cue of photoperiod. In LD conditions these mice will be reproductively active, while in SD conditions these mice will be reproductively suppressed. A number of other environmental factors have been shown to

interact with photoperiod to affect the phenotypically plastic reproductive response of several rodent species. Some of these factors include ambient temperature, social factors, and food availability (Prendergast et al. 2002). For *P. leucopus* specifically, food availability, in conjunction with photoperiod, has been shown to play a role in reproductive variability. Unresponsive individuals, which normally remain reproductively active in SD conditions, can be reproductively suppressed when food access is restricted in SD conditions (Reilly et al. 2006). This indicates that genes are not the only factor in determining the reproductive status of an individual mouse. The combination of genetic variability and phenotypic plasticity in determining overall reproductive response indicates that *P. leucopus* may use a variable reproductive strategy.

Food intake and availability appear to have an effect on reproductive variation seen in *P. leucopus* (Heideman et al. 2005). As might be expected, differences in food intake seem to be related to the reproductive response of *P. leucopus*. Unresponsive mice that are reproductively active in short, winter-like photoperiods eat approximately 50% more than mice in a responsive line that are reproductively suppressed in the winter-like conditions (Heideman et al. 2005). This provides more evidence that winter reproduction is costly, thus requiring this increase in food intake (Heideman et al. 2005). Furthermore, mice placed in short day conditions also tend to undergo decreases in body mass. Smaller body mass reduces somatic energy requirements (Prendergast et al. 2002). If a mouse does not need to dedicate as much energy resources to basic needs, it can theoretically afford to dedicate energy to reproduction.

The availability of energy resources is a very important component of reproductive capacity. Since increased energy intake is important for reproductive activity, it follows that available food and nutrients should also play a role in reproduction. It has already been shown

that restricting food access can result in reproductive suppression in *P. leucopus* (Reilly et al. 2006). Therefore, we might predict that providing mice with an abundance of food rich in energy content would be able to overcome the reproductive suppression normally seen in SD conditions. In the same way that restricted food acts as a signal to repress reproductive capacity, perhaps a high-energy food source can signal the availability of resources that can aid in reproductive activity.

Reproductive capacity is directly related to the fitness of an animal. Because of the importance of reproduction, it is understandable why organisms have evolved intricate mechanisms to regulate reproductive activity. Much still remains to be learned about genetic and phenotypic variation in the multitude of signals and physiological processes that contribute to the regulation of reproduction. This includes the roles that food, energy, and nutrients play as potential regulatory signals for reproduction. Gaining a better understanding of the effect of food on reproductive processes opens up a wealth of possibilities for further research on nutritional regulation of reproduction in both animals and humans.

Chapter II: The Effect of Diet on Reproductive Inhibition in Photoresponsive White-Footed Mice (*Peromyscus Leucopus*)

Introduction

Variability is present in a wide number of physical and physiological traits, including reproductive strategy. Variability in reproductive strategy is affected by both genetic variation and phenotypic plasticity. Genes interacting with the environment create the reaction norms that define the possible reproductive responses of an animal. An animal's reaction norm includes the

effects of phenotypic plasticity, which is defined as the ability of an organism with a given genotype to change its phenotype in response to changes in the environment. An assortment of environmental cues affects reproductive variability in mammalian species. For many mammals, the most powerful of these cues are food availability and day length (Goldman 2001; Prendergast et al. 2002). Studies have shown that both short, winter-like photoperiods (Goldman 2001; Prendergast et al. 2002) and reduced food availability (Schneider 2004) can both lead to reproductive suppression.

An animal that is reproductively photoresponsive uses the cue of shortened day length, or photoperiod, to suppress reproductive fertility in the winter (Prendergast et al. 2002; Goldman 2001). Photoperiod, which does not have a direct effect on fitness, is a reliable cue that can predict factors that do have an effect on fitness such as food availability and temperature (Goldman 2001). Low food availability and low temperature, features that often characterize winter months, can be predicted by shortened photoperiod. Limited food availability is detrimental to the maintenance of reproductive processes because reproductive processes require large quantities of energy. In order to continue reproduction during times of low food availability, an animal would have to increase foraging time, potentially increasing the risk of predation and lowering fitness (Heideman et al. 2005). In this case, fitness might be increased by suppressing reproduction during times of low food availability. Because low food availability regularly occurs during the winter months, the use of short photoperiods as a cue to suppress reproduction can be beneficial.

The response to photoperiod shows natural genetic variation in the animal model used in this study, the white-footed mouse, *Peromyscus leucopus* (Heideman et al. 1999). Some mice show reproductive suppression in short winter-like photoperiods and are termed reproductively

photoresponsive (referred to as 'responsive' hereafter). In contrast, mice that retain reproductive capacity in short winter-like photoperiods are termed reproductively nonphotoresponsive (referred to as 'nonresponsive' hereafter). The genetic basis behind this variation in photoperiod response has allowed the artificial selection of lines of mice with specific responsive or nonresponsive photoperiod responses. Photoperiod is a factor which can easily be manipulated in laboratory conditions. Placing mice selected to be responsive in either short day photoperiods (SD) or long day photoperiods (LD) allows experimental control of their reproductive capacity. This allows *P. leucopus* to act as a useful model to test a variety of factors that can have an effect on reproductive variation.

In addition to having genetic variation in photoresponsiveness, *P. leucopus* show variability in reproductive response through phenotypic plasticity. Phenotypic plasticity is defined as the ability of an individual to change phenotype in response to environmental cues. Photoperiod is an environmental cue that can result in phenotypic plasticity of the reproductive response. A responsive mouse that is placed in LD conditions will be reproductively active. However, if the same mouse is placed in SD conditions, it will exhibit reproductive suppression. Other studies have shown that food availability can also result in phenotypic plasticity of the reproductive response. Nonresponsive mice that are placed in SD conditions will usually remain reproductively active. However, if restricted food access is combined with these SD conditions, mice show significant reproductive suppression (Reilly et al. 2006). This demonstrates the important role that food availability can have in the reproductive response.

It has been shown that limited food availability can contribute to reproductive suppression (Reilly et al. 2006; Schneider 2004). It is not known if abundant or high quality food can overcome reproductive suppression in SD. The purpose of this study is to test the role

that an abundance of food rich in nutrients such as fat plays in reproductive ability of *P. leucopus*. There are two questions. First, can a high fat diet overcome reproductive suppression observed in short days? The ultimate cause of some mice suppressing reproduction in the winter may be to avoid increased predation risk due to increased foraging time needed to support reproduction. It would be potentially adaptive if, when there is an abundance of nutrients available, the reproductive suppression induced by photoperiod could be overcome. Second, if mice are provided with a high fat diet, will they consume more calories than when provided with a lower fat diet? Previous studies have shown that providing rodents with more food, or a richer diet, does not necessarily mean that they will consume more calories. Instead, these animals will often compensate their food intake in order to maintain a particular caloric intake (Meyer and Elashoff 2001; Treit and Spetch 1985). If *P. leucopus* do not actually consume more calories when provided with a high fat diet, then it is less likely that this kind of diet will be able to overcome reproductive suppression.

We tested whether a high fat diet can overcome reproductive suppression in responsive male mice. When placed in SD conditions, responsive mice normally suppress reproduction, as indicated by reduced testes mass, reduced seminal vesicle mass, and reduced sperm counts. Experimental mice were fed a defined high fat diet and high nutrient supplement to test whether these diets could overcome the normal reproductive suppression seen in SD conditions. We hypothesized that if mice fed the high fat diet consumed more calories than mice fed a control diet, then the extra energy provided by the high fat diet would cause these mice to overcome reproductive suppression in SD conditions. In this case, we predicted that mice provided with a high fat diet in SD conditions would have larger testis and seminal vesicle masses than mice given a control diet in SD conditions. In contrast, if mice given a high fat diet calorie adjusted

and consumed a similar number of calories as mice given the control diet, we hypothesized that the high fat diet would not cause mice to overcome reproductive suppression in SD conditions. In this case, we predicted that responsive mice placed in SD conditions would have small, suppressed testis and seminal vesicle masses regardless of whether they were given the high fat or control diets.

Methods

All animals used in this study were males from one of two different lines of white-footed mice. The first was a selected line of mice that had been bred to be responsive. Most of the mice in this line respond to short photoperiods with repressed reproduction (Heideman et al. 1999). The second was a control line of mice not selected for photoresponsiveness. The control line more closely mimics the natural variation in photoresponsiveness found in white-footed mice (Heideman et al. 1999). The reason for using mice from both of these lines was to test for differences in responses between the two lines.

For the purposes of this experiment, only mice with a responsive phenotype from each line were used. Mice were defined as having a responsive phenotype according to measurements of the length and width of a single testis. These measurements were used to calculate an estimated testis volume (ETV = width^2 × length × 0.523). Measurements were taken when the mice were 63 ± 4 days old. The mice were considered photoresponsive if the ETV was less than 50 mm^3.

Mice were born into a LD photoperiod (L16:D8; lights on at 0400 EST) and transferred within three days to a SD photoperiod (L8:D16; lights on at 0800 EST). At age 21-23 days, mice were weaned and moved to individual housing in 27×13×16 cm cages with wire tops and

provided with wood pulp bedding (7099 Harlan Teklad Tek-Fresh laboratory animal bedding, Madison, WI, USA). After a problem early in the study from an escaped mouse affecting food intake measurements of the experimental mice, all cages were also covered with micro-filter tops. Food (7012 Harlan Teklad LM-485 mouse/rat sterilizable diet, Madison, WI, USA) and water were provided ad libitum. Light was provided by overhead fluorescent lights with an intensity of 100-1,000 lux at the level of the cages. Room temperatures were 22°C ± 4°C during the experiments.

Palatability Pilot Experiment

Because this experiment used two new defined diets heretofore unused by our lab, a palatability study tested whether changes in food intake by the mice given the defined diets might be caused by aversion to the defined diets. Two defined diets were tested: a defined control diet with 10% kcal from fat (Open Source Diets D12450B, New Brunswick, NJ, USA) and a defined high fat diet with 45% kcal from fat (Open Source Diets D12451, New Brunswick, NJ, USA).

The protocol for this initial palatability experiment was adapted from Hambly et al. (2005). Two groups of mice were used to test each defined diet. Each group contained 12 male mice chosen at random from mice in the responsive and control lines. Mice were kept in SD conditions throughout the course of the pilot study.

Mice were first acclimated to the defined diets during a seven day period when no food intake measurements were taken. All mice were given the defined diet assigned for their group (either the defined control diet, or the defined high fat diet). The defined diet was alternated with the standard chow every day for the seven day period. After this initial seven day introduction

period, the mice were provided with both the defined diet and the standard chow in a divided hopper. Every day, for 10 days, foods were alternated between sides of the hopper to control for directional bias, and measurements of food intake for each type of food were taken.

Additional studies were done to test the use of possible high fat supplemental food that could be provided for mice. Foods tested included walnuts, Brazil nuts, and sweetened condensed milk. The studies observed whether the mice ate these supplemental foods and also tested the ease of measuring intake of these supplements. Sweetened condensed milk was chosen as a suitable high fat supplemental food because of its palatability to the mice and the ease of intake measurement.

Experiment 1: effect of artificial diet on reproduction

The purpose of experiment 1 was to test whether the defined diets might affect reproductive variability. Experimental set-up for Experiment 1 is outlined in Fig. 2. For this experiment, at 49 ± 4 days of age mice from the reproductive and control lines were either kept on the standard chow or switched to the defined control diet. Initial body mass and food intake measurements were taken for two weeks. After two weeks, when mice were 63 ± 4 days old, measurements of testis length and width were taken to calculate the ETV. Mice that were defined as responsive were then divided into experimental groups. Groups were matched by weight as closely as possible, and no siblings were placed in the same experimental groups. Each group contained 11-13 mice and a nearly equal number of control line and responsive line mice. Mice that had been switched to the defined control diet were place in either a LD group or a SD group. Mice that were maintained on the standard chow were also placed in either a LD control group or a SD control group. It was found that mice kept on the standard chow were

much more likely to grind and discard their food. In an attempt to reduce food grinding, mice in all four groups were provided with wooden chewing blocks throughout the length of the study. Since the decision to use the wooden blocks was made after the study had already begun, some mice received wooden blocks at later ages than others. However, analysis showed that the time the wooden block was received by mice had no significant effect on caloric intake, body mass, testis mass, or seminal vesicle mass (data not shown).

Measurements of body mass and food intake were taken weekly for 14 weeks. In order to adjust for food intake to remove ground food that was not consumed, estimates of the amount ground food were made for all 16 of the 24 mice on the standard diet that ground more than 1 gram of food per week. Estimates of ground food were made for 2-4 weeks for each of these 16 mice, and for each mouse these estimates were averaged to produce a correction to subtract from the weekly food intake values. The other 8 mice given standard chow did not grind more than 1 gram of food per week. Therefore, these 8 mice did not have estimated ground food measurements taken and did not have their food intake values adjusted.

At week 9, measurements of the testis length and width were taken again. At week 14, mice were euthanized using CO_2 gas. Wet masses of a single testis and a single seminal vesicle (not stripped of fluid) were taken for each mouse. Caloric intake for each mouse was calculated on a weekly basis using food intake measurements. For mice on the standard diet that ground more than 1 gram of food per week, caloric intake calculations were adjusted using the estimated ground food measurements. These adjusted weekly caloric intake values were used to calculate the total caloric intake for the duration of the experiment.

Experiment 2: effect of a high fat diet on reproduction

Experiment 2 was designed to test whether a diet rich in fat can overcome reproductive suppression of responsive mice in short days. Experimental set-up for Experiment 2 is outlined in Fig. 3. Mice from the responsive and control lines were placed on the defined control diet at 49 ± 4 days of age for two weeks, during which initial body mass and food intake measurements were taken. After two weeks, when mice were 63 ± 4 days old, measurements of testis length and width were taken to calculate the ETV. Mice that were defined as responsive were then divided into four experimental groups. Mice in the groups were matched by body mass as closely as possible, and it was ensured that siblings were not placed in the same experimental groups. 11-12 animals were placed in each group, with approximately equal numbers of mice from the control line and the responsive line placed in each group. The groups included two experimental groups, maintained in short day conditions, and fed a diet high in fat. One of these groups was fed only the high fat defined diet. The other group was fed the high fat defined diet along with a sweetened condensed milk supplement, administered through a bottle. Both the high fat defined diet and the condensed milk supplement were provided ad libitum. The other two groups in this study were control groups, which were fed the control defined diet. One of the control groups was kept in SD conditions, while the other group was moved to LD conditions.

Measurements of body mass and food intake were taken weekly for 14 weeks. At week 9, measurements of testis length and width were taken again. At week 14, mice were euthanized via CO_2 gas. Wet masses of a single testis and a single seminal vesicle (not stripped of fluid) were taken for each mouse. Caloric intake for each mouse was calculated on a weekly basis using food intake measurements. Weekly caloric intake values were used to calculate the total caloric intake for the duration of the experiment.

Statistical Analysis

Data were analyzed by Analysis of Variance (ANOVA) using Statview 4.5 or SuperANOVA (Abacus Concepts, Inc.) on a Macintosh. Statistical Significance was defined as $P < 0.05$. In the text and in figures, means are presented with their standard errors unless indicated otherwise.

Results

Palatability Pilot Experiment

Mice showed no preference for the standard chow over either of the defined diets. For mice given a choice between the control defined diet and the standard chow, 53% of the food mass they consumed on average was the control defined diet (Fig. 4). For mice given a choice between the high fat defined diet and the standard chow, 79% of the food mass consumed on average was the high fat defined diet (Fig. 5). We concluded that the defined diets were sufficiently palatable to the mice.

Experiment 1: effect of artificial diet on reproduction

The first goal of Experiment 1 was to test for a difference in total caloric intake between experimental groups (Table 1; Table 2; Fig. 6). Groups that were fed the defined diet consumed fewer total calories than groups that were fed the standard chow, but although these results indicated a trend, the difference was not significant (F=3.90, P=0.06, df=1). Mice from the control line consumed significantly fewer calories than mice from the responsive line (F=14.26, P<0.001, df=1). When comparing mice in different photoperiods, mice from the SD groups consumed approximately 5% fewer calories than mice in the LD groups. However, difference in

total calorie consumption between the LD and SD groups was not significant (F=1.85, P=0.18, df=1).

There was concern about the effect of the grinding and discarding of food by mice on our food and caloric intake measurements for the mice on the standard diet. A series of three analyses were performed to test whether our estimated ground food measurements were adequately accounting for the effects of ground food on the caloric intake data. First, mice that on average ground more than five grams of food per week were excluded from the caloric intake analysis (four mice excluded in total). Second, mice that on average ground more than three grams of food per week were excluded (eight mice excluded in total), and then, third, all mice that exhibited measurable levels of grinding were excluded (sixteen mice excluded in total, 2/3 of mice on the standard diet). The analyses from which individuals that ground and discarded some food had been excluded produced results similar to those including all animals, except that in some of these analyses mice in SD consumed significantly fewer kilocalories than mice in LD (results not shown).

Average body mass of the mice was also analyzed (Table 3). Average body mass of mice in the SD groups was approximately 6% less than mice in the LD groups. However, the difference in body mass between the SD and LD groups was not significant (F=2.20, P=0.15, df=1). There were also no significant difference between mice on the defined or standard diets (F=1.38, P=0.25, df=1).

The second goal of Experiment 1 was to test the reproductive response of mice in the different experimental groups. There was a significant difference in both final testis mass (Table 4; Fig. 7; F=11.32, P=0.002, df=1) and final seminal vesicle mass (Table 5; Fig. 8; F=9.95, P=0.003, df=1) between the LD and the SD experimental groups. The different foods, however,

did not affect reproductive capacity. There was no significant difference in either final testis mass (Table 4; Fig. 7; $F<0.001$, $P=1.00$, df=1) or final seminal vesicle mass (Table 5; Fig. 8; $F=0.61$, $P=0.44$, df=1) between groups fed the standard and defined diets. ETV values from the 9 week testes checks followed the same statistical trends observed in the testis and seminal vesicle masses (Fig. 9). ETV was significantly lower for mice in the SD experimental groups than mice in the LD experimental groups ($F=8.69$, $P=0.005$, df=1). There was no significant difference in ETV between mice fed the standard and defined diets ($F=0.70$, $P=0.41$, df=1).

Experiment 2: effect of a high fat diet on reproduction

One goal of Experiment 2 was to test the caloric intake of mice provided with one of two diets, the control diet or a high fat diet (Table 6; Table 7; Fig. 10). Total caloric intake showed no significant difference between all of the four experimental groups (Table 7; Figure 10; $F=1.46$, $P=0.24$, df=3). Total caloric intake also did not vary significantly between the LD and SD control groups fed the lower fat defined diet ($F=0.36$, $P=0.56$, df=1), or between the two high fat diet groups ($F=.51$, $P=0.49$, df=1). While no differences were significant, a higher total caloric intake was seen in the experimental groups provided with high fat diets. Highest caloric intake occurred in the group fed both the defined high fat diet and condensed milk.

Like in Experiment 1, mice from the control line consumed significantly fewer total calories than mice from the responsive line ($F=8.42$, $P=0.006$, df=1). When comparing only the two control groups, there was also a significant difference in total caloric intake between control and responsive line mice ($F=4.79$, $P=0.04$, df=1). When comparing only the two high fat food experimental groups, there was a similar statistical trend between the control and responsive line mice ($F=3.97$, $P=0.06$, df=1).

Average body mass of the mice was also analyzed (Table 8). Overall, there was no significant difference in average body mass among all four experimental groups (F=0.32, P=0.81, df=3). When comparing only the two control groups, there was also no significant difference in average body mass between mice in the LD and SD groups (F=0.26, P=0.62, df=1).

The second goal of Experiment 2 was to test the reproductive effect of the different diets. No significant difference existed between the final testis masses of the two high fat diet groups and the LD control group (F=0.42, P=0.66, df=2). The mean values of the final testis masses of the high fat diet groups and the LD control group were all larger than the mean final testis mass for the SD control group (Table 9; Fig. 11). There was also no significant difference between the final seminal vesicle masses of the two high fat diet groups and the LD control group (F=1.30, P=0.29, df=2). However, when comparing the LD and SD control groups, there was also no significant difference in both final testis mass values (F=2.35, P=0.14, df=1) and the seminal vesicle mass values (F=2.36, P=0.14, df=1). Overall, no significant difference existed in final single testis mass (Table 9; Figure 11; F=0.75, P=0.53, df=3) or seminal vesicle mass (Table 10; Figure 12; F=1.23, P=0.31, df=3) among the four experimental groups.

ETV values from the 9 week testes checks followed the same statistical trends observed in the testis and seminal vesicle masses (Fig. 13). There was no significant difference in ETV among the four experimental groups (F=0.34, P=0.80, df=3).

Discussion

In Experiment 1, there was a significant difference in both testis and seminal vesicle masses between the groups exposed to different photoperiods. These results match the expected

effect of short photoperiod on reproductive capacity, with mice in SD conditions having smaller testis and seminal vesicle masses.

The results of Experiment 1 do not indicate that the defined diets had any effect on reproductive capacity. There was no significant difference in testis or seminal vesicle masses between groups fed the defined or standard diet. We concluded that the defined diet did not affect testis or seminal vesicle mass.

Line, photoperiod, or food type did not result in any significant differences in body mass in either Experiment 1 or Experiment 2. These results suggest that body mass is unrelated to the factors being tested in this study. The effect of body mass was not further considered in any of the results reported here. However, an analysis of covariance with body mass as a covariate will be done in the future to test this further.

In other studies, mice in SD conditions have had significantly lower body mass than mice in LD conditions (Heideman et al. 1999; Heideman et al. 2005). While SD control mice in both Experiment 1 and Experiment 2 of this study had smaller body masses, they did not differ significantly from LD control mice. The previous studies reporting this difference had larger sample sizes than this study. Our sample sizes may have been too small to have enough statistical power to detect these differences if they were present in the mice in this study.

The series of analyses performed for Experiment 1 that excluded from the caloric intake analysis the mice that ground and discarded food allowed us to conclude that our method of accounting for ground food was sufficiently accurate for our caloric intake measurements. Because the magnitude of caloric intake between the defined and standard diets, control and responsive lines, and LD and SD photoperiods were almost unchanged by the exclusion of

grinders from the analysis, we concluded that our data and calculations for caloric intake were accurate.

In both Experiment 1 and Experiment 2, photoperiod did not have a significant effect on caloric intake. However, although differences were not significant, higher caloric intake occurred in LD groups in both experiments. Based on previous studies (Heideman et al. 2005), higher caloric intake might be predicted in LD conditions.

The caloric intake data for Experiment 2 suggest that mice compensated food intake in order to calorie adjust, although perhaps not completely. Mice that were provided with a high fat diet had as much as 20% higher total caloric intake, but the difference was not significant. Because the mice provided with a high fat diet did not consume significantly more energy to dedicate to reproductive processes, it is less plausible that access to a high fat diet might have any effect on reproduction. This is consistent with our results of no significant difference in either testis mass or seminal vesicle mass between high fat and control diet groups in Experiment 2.

We can predict from previous studies that an increase in caloric intake might cause testes growth. Previous studies have shown that nonresponsive mice consume approximately 50% more calories than responsive mice (Heideman et al. 2005). Therefore, in order for a responsive mouse to become nonresponsive, we would predict a 50% increase in caloric intake of that mouse. However, our results show that mice provided with a high fat diet were not consuming significantly more calories than mice provided with a control diet. Although there was a 20% increase in caloric intake in the high fat groups, this increase was not statistically significant. In any case, the 20% increase in caloric intake is not close to the 50% we predict for a mouse to overcome reproductive suppression in SD. Therefore, our results provide no evidence that

genetically and/or phenotypically responsive *P. leucopus* are phenotypically plastic to food quality.

Studies performed on a *Peromyscus* species closely related to the *P. leucopus* used in this study indicate that reproductive differences between responsive and nonresponsive mice may be closely linked to differences in general metabolism (Cronin and Bradley 1988; Staubs and Bradley 1998). Responsive animals consumed significantly less food than nonresponsive mice. Responsive animals also had significantly lower levels of body fat than nonresponsive mice (Cronin and Bradley 1988). Finally, responsive animals also had significantly lower levels of oxygen consumption than nonresponsive animals (Staubs and Bradley 1998). These results suggest that fundamental differences in metabolism may be responsible for the reproductive differences between responsive and nonresponsive mice.

The testis mass and seminal vesicle mass data from Experiment 2 do not allow us to conclude that a high fat diet overcomes reproductive suppression in SD. Because there was no significant difference between the final testis and seminal vesicle masses of the two high fat experimental groups and the LD control group, it may initially appear that the high fat diet allowed the experimental mice to reach a reproductive capacity similar to mice placed in LD conditions. However, there was also no statistically significant difference between the testis and seminal vesicle masses of the LD and SD control groups. Testis and seminal vesicle masses of the SD control group were smaller than those of the LD control group, as would be expected based on previous studies (Heideman et al. 1999; Prendergast et al. 2002), but the difference did not reach statistical significance. Because we did not see a significant difference between our control groups in SD and LD, our test for the effect of a high fat diet on reproduction is inconclusive.

If a high fat diet is able to overcome reproductive suppression in SD conditions, we would predict mice provided with a high fat diet in SD to have similar testes and seminal vesicle masses to nonresponsive mice in SD. Data from Reilly et al. (2006) indicate that nonresponsive mice in SD conditions had an average paired testes mass of approximately 400 mg and an average seminal vesicle mass of approximately 90 mg. In Experiment 2, mice provided with a high fat diet in SD did have larger testes and seminal vesicle masses than mice in the SD control group. However, their testes and seminal vesicle masses did not have the same magnitude as those of nonresponsive mice from previous studies. Mice provided with the high fat defined diet only had an average paired testes mass of 246 mg and an average seminal vesicle mass of 61 mg. Mice provided with the high fat diet and condensed milk supplement had an average paired testes mass of 264 mg and an average seminal vesicle mass 77 mg. Because the magnitudes of the testes and seminal vesicle masses are not as great as those seen in nonresponsive mice, we are less confident in the ability of a high fat diet to overcome reproductive suppression in SD.

Despite testes and seminal vesicle masses not reaching the same magnitude as those seen in nonresponsive mice, our results from Experiment 2 still indicated larger than expected testes and seminal vesicle masses in the SD groups. From the results of Experiment 1, we concluded that the defined diets did not play any role in the larger than expected testis and seminal vesicle masses. Therefore, we cannot cite the use of the defined diets as a potential explanation for the lack of significant difference between the testis and seminal vesicle masses of the LD and SD control groups in Experiment 2. However, there are several other potential explanations for the lack of significant differences between the testis and seminal vesicle masses of the control groups. The first potential explanation is that there may have been an error in our experimental set-up which prevented mice in the SD control group from undergoing the normal level of

reproductive suppression. Perhaps the experimental set-up inadvertently allowed currently unknown factors other than food and photoperiod to have an effect on reproductive capacity.

A second potential explanation for the lack of significant difference in testis and seminal vesicle masses between the control groups in Experiment 2 may be statistical sampling error. The direction of the results seen in our control groups was as predicted, with smaller testis and seminal vesicle masses in the SD control group. However, the amplitude of the difference was less than typical, preventing the difference from being statistically significant. As is the case with any experiment that tests a sample of a population, the sample does not always accurately represent the entire population. It is possible that the sample of mice in our SD control group developed larger testis and seminal vesicle masses in SD conditions than would the majority of mice in the population.

A third potential explanation for the larger than expected final testis masses in the SD control group would be that the mice had started to become photorefractory. At the conclusion of the study, mice in SD groups had been exposed to SD conditions for approximately 23 weeks. In Siberian hamsters, photorefractoriness can occur after 20 weeks of exposure to SD conditions (Prendergast et al. 2006). Therefore, it is plausible that the mice in this experiment had become photorefractory and were undergoing spontaneous gonadal regrowth. This may also potentially explain the lack of significant difference in testis and seminal vesicle masses between the high fat groups and LD control group in Experiment 2. Instead of the high fat diet causing the experimental groups to have larger testis and seminal vesicle masses, onset of the photorefractory period may have been the cause. The results of Experiment 2 support photorefractoriness as a potential explanation for the larger than predicted testis and seminal vesicle masses in the SD groups. However, the results of Experiment 1 suggest that

photorefractoriness is not a potential explanation. Mice in Experiment 1 were tested for the same number of weeks as mice in Experiment 2, but mice in SD groups in Experiment 1 did not show larger than predicted testis and seminal vesicle masses. If photorefractoriness had been the cause of the larger testes and seminal vesicle masses in Experiment 2 mice, we would have predicted similar results in Experiment 1 mice. Although Experiment 1 results do not support photorefractoriness as a potential explanation, it is still possible that a statistical sampling error resulted in unusually rapid photorefractory response in the SD groups in Experiment 2.

An unexpected result in both Experiment 1 and Experiment 2 was that mice from the control line consumed significantly fewer calories than mice from the responsive line. In contrast, line did not have any significant effect on other factors such as testis mass, seminal vesicle mass, or body mass. Previous studies have shown that when the food intake of a random sample of mice from the control line is compared to the food intake of mice from the responsive line, control line mice have significantly higher food intake (Heideman et al. 2005). However, in Heideman et al. (2005), the control line sample was drawn at random and thus included both responsive and nonresponsive mice. In contrast, in our study only a small subset of mice from the control line, approximately 30%, had a responsive phenotype. Thus, the control line as a whole and mice from the control line with a responsive phenotype used in this study are not equivalent and comparable.

The finding that responsive mice from the control line consumed significantly fewer calories than mice from the responsive line warrants further study. These data suggest that, in terms of food intake, the control line should not be viewed as merely an intermediate between the responsive and nonresponsive lines. If the control line was an intermediate between the responsive and nonresponsive lines, we would have expected responsive phenotype mice from

the control line to be very similar to mice from the responsive line. However, mice in the responsive line were selected for based on testes size, while mice in the control line did not undergo any deliberate selection. The differences in the development of the two lines suggest that the differences in caloric intake between the two lines may be genetic. The responsive line was developed by artificial selection on gonadal development in SD. Selection on gonadal development had the unexpected result of correlated differences in food intake (Heideman et al. 2005). In addition to alleles for food intake, there are potentially many other alleles that are correlated with testes size. Development of the responsive line, for which mice were selected for based on testes size, was more likely to favor alleles that are specifically related to small testes size, including alleles that might decrease food intake. However, if non-food related alleles can also cause small testes size, then some mice in the responsive line may demonstrate reduced food intake, and others may not. In contrast, the responsive phenotype mice in the control line may require low food intake in a mixture of variable alleles for testes size in SD. Therefore, it is more likely that a single allele correlated to small testes size may be seen in the responsive phenotype in the control line. If this is true, it may explain our results of responsive phenotype control line mice having significantly lower food intake than the responsive line mice.

Chapter III: Future Research and Prospects

This study left us with many questions about the relationship between diet and reproduction. The results of this study did not allow us to come to a clear conclusion about the effects of a high fat diet on reproductive capacity. We can make no final conclusion because there was no significant difference in testis mass and seminal vesicle mass between our control groups in Experiment 2. These results indicate that testis and seminal vesicle mass may have

been affected by other factors in the experimental set-up besides diet and photoperiod. This prevents us from concluding that the high diets had an effect in the larger testis and seminal vesicle masses seen in the two experimental groups in Experiment 2. Therefore, it could be beneficial to perform a careful repetition of Experiment 2, trying to eliminate any experimental errors that may have occurred in the previous study. If a repetition of this experiment yielded results that made us confident in our experimental setup, then we could better conclude whether or not a high fat diet has an effect on reproductive capacity.

Although we were not able to make clear conclusions about the effect of a high fat diet on reproductive capacity, many of our results indicated that the high fat diet did not result in the changes thought necessary to allow mice to overcome reproductive suppression in SD. For example, mice provided with a high fat diet did not consume significantly greater kilocalories than mice on a control diet. In addition, mice provided with a high fat diet did not have testes and seminal vesicle masses that were as great as those of nonresponsive mice in SD conditions. One possible reason that a high fat diet does not appear to overcome reproductive suppression in SD may be the age that mice in this study were started on the high fat diet. Mice were placed into SD conditions shortly after birth, but mice were not started on the high fat diet until they were approximately 63 days old. Most mice that are kept in LD conditions will have already reached maturity by the age of 63 days. It is plausible that starting mice on the high fat diet at 63 days of age is too late a point in the development of the mouse for the high fat diet to significantly effect reproduction. A potential future experiment might begin mice on the high fat diet at weaning and then test the effect on reproductive capacity. This would allow us to test whether the effect of a high fat diet on reproduction may be dependent upon the stage of development that the diet is provided.

When looking at the larger question of the link between food and reproduction, there is much that remains to be discovered. Studies have shown that food availability and intake do have an effect on reproduction (Schneider 2004; Prendergast et al. 2002; Reilly et al. 2006). Much research has also been done to study the specific physiological mechanisms that allow food availability and intake to have an effect on reproduction, such as sensory stimuli and hormones. However, several processes are not yet understood. For example, the hormone leptin appears to act as a signal to provide the body with information about energy resources. It still remains inconclusive whether or not leptin plays any role in the regulation of reproduction (Schneider 2004). Future studies will undoubtedly bring more details to light about the physiological mechanisms linking food and reproduction.

There are multiple reasons why further studies about the connection between food and reproduction may prove beneficial to society. Several human clinical issues can be linked to food and reproduction, including nutritional infertility and reproductive problems that coincide with eating disorders (Schneider 2004). The role of nutrition in all aspects of health is gaining more scientific and popular attention in recent years. By gaining a clearer, more detailed understanding of food intake and its effect on all aspects of physiology, including reproduction, we can begin to gain insight into some of the issues facing human health today. Some of these issues include obesity, eating disorders, and infertility. More indirectly, a thorough understanding of the link between food and reproduction effects human health through agricultural practices. Diet and reproduction play a central role in the improvement of the care and breeding of dairy and meat animals (Schneider 2004). Improvement of agricultural practices can help farmers economically and result in improved food sources for all of human society.

Because reproduction has a direct effect on fitness, gaining a more thorough understanding of reproduction can have ecological applications as well. There is much concern today about the maintenance of endangered species and ecosystems. A straightforward method to ensure that species and ecosystems remain viable is to ensure that conditions for reproduction are sufficient. The availability of energy to support reproduction is an important factor than can affect the continued endurance of a species. The better we understand the ways in which animals use energy resources to fuel reproduction, the better we can understand how to make conditions optimal for species survival.

Advances in human health, agricultural practices, and ecological understanding are exciting examples of applications for research exploring the relationship between food and reproduction. However, before these applications can come to a full effect, more basic research needs to be done. Basic research, such as that done in this study, will allow us to continue to expand our knowledge about the physiological mechanisms that link food and reproduction. As our knowledge expands, we will be able to confidently approach applied research that will concentrate on issues that can directly benefit both humans and the natural world. The future for this area of research is full possibilities even beyond addressing questions of scientific curiosity.

Figures and Tables

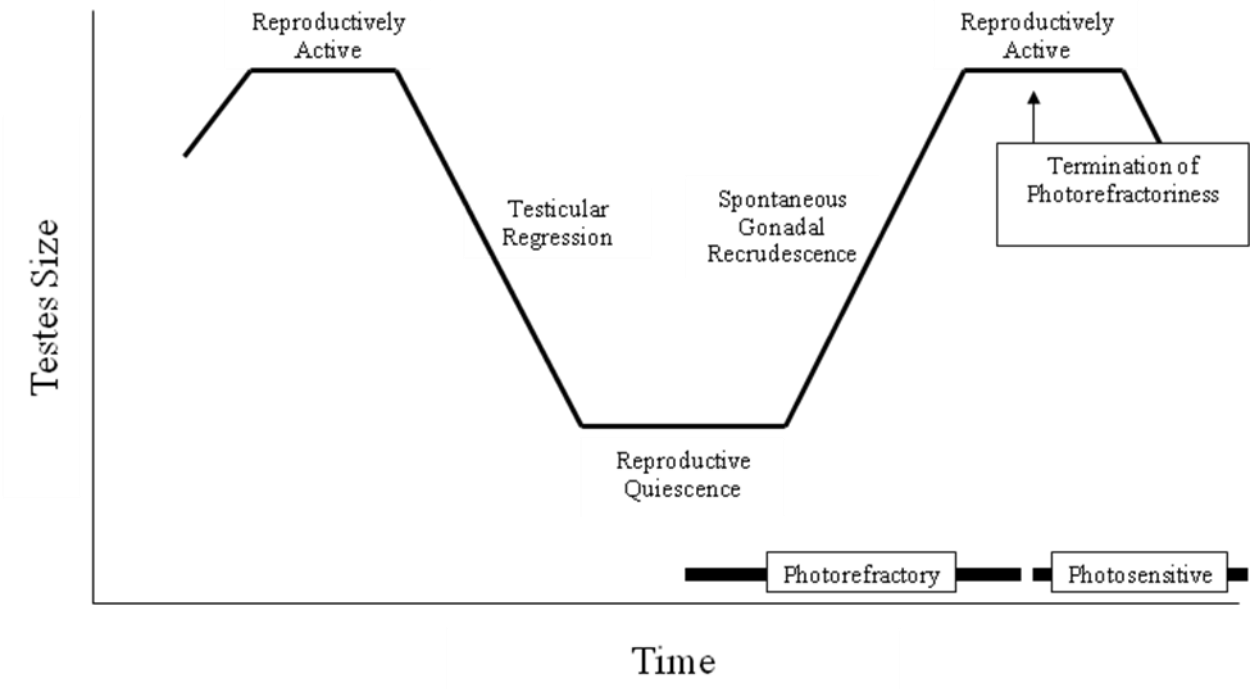

Fig. 1 Adapted from Prendergast et al. (2002). Schematic representation of seasonal rhythms, showing the onset of the photorefractory period. A reproductively photoresponsive male animal subjected to the exogenous cue of decreasing day length will undergo testicular regression. This triggers an endogenous interval timer, which after a period of time renders the animal refractory to short day lengths. Photorefractoriness causes the animal to undergo spontaneous gonadal recrudescence. Only exposure to long day lengths breaks the photorefractory period, causing the animal to become photosensitive once again.

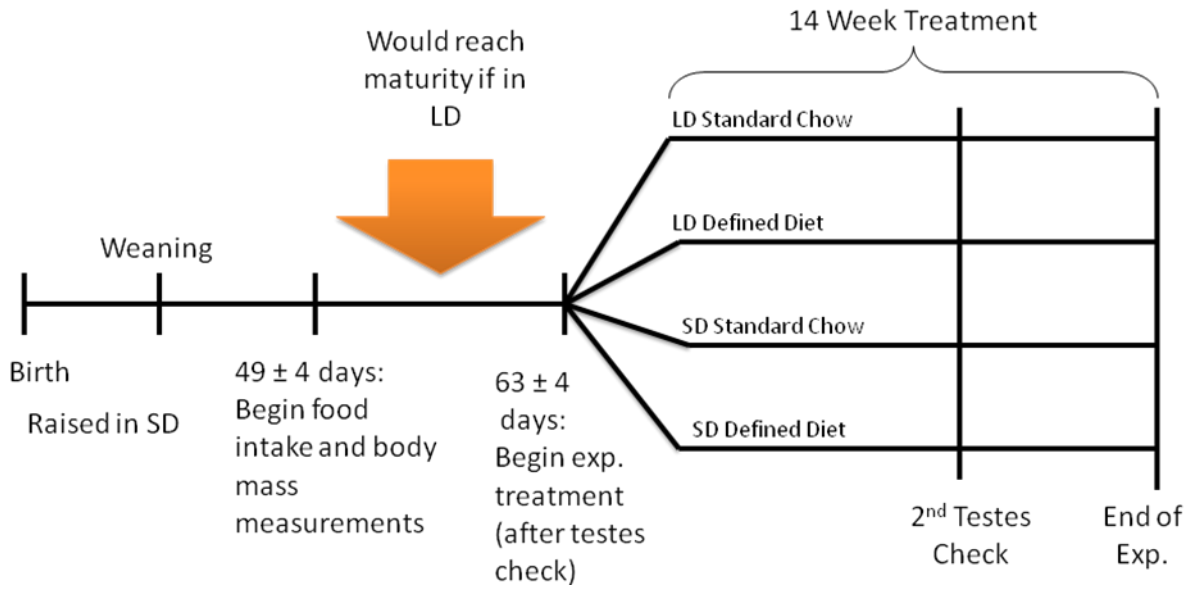

Fig. 2 Experiment 1 set-up. At approximately 63 days of age, mice were divided into four experimental groups with different diet and photoperiod treatments. At the end of the experiment mice were euthanized and testis and seminal vesicle masses were taken.

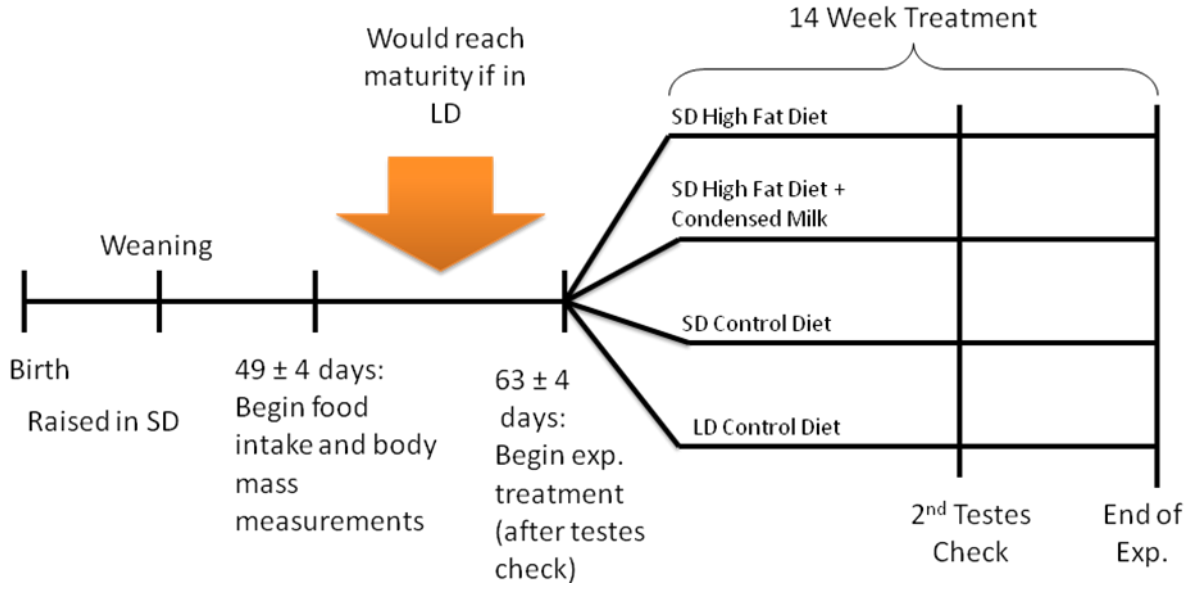

Fig. 3 Experiment 2 set-up. At approximately 63 days of age, mice were divided into four experimental groups: two groups given high fat diets, one SD control group, and one LD control group. At the end of the experiment mice were euthanized and testis and seminal vesicle masses were taken.

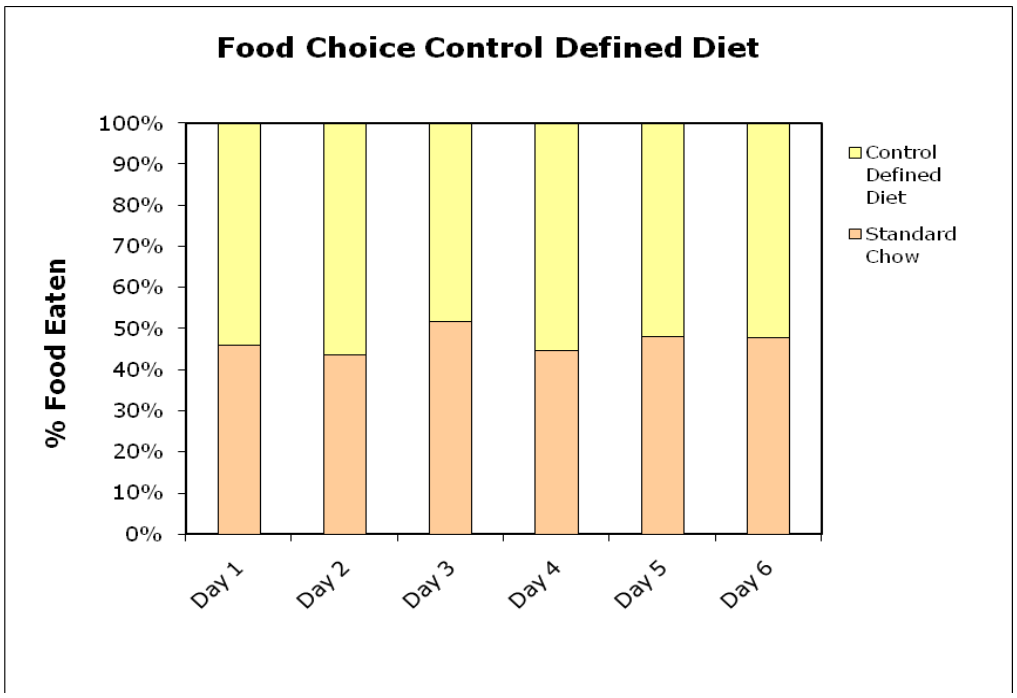

Fig. 4 Control defined diet results from the Palatability Pilot Experiment. Data are presented as percentage of total food eaten for each of the two diets provided to mice.

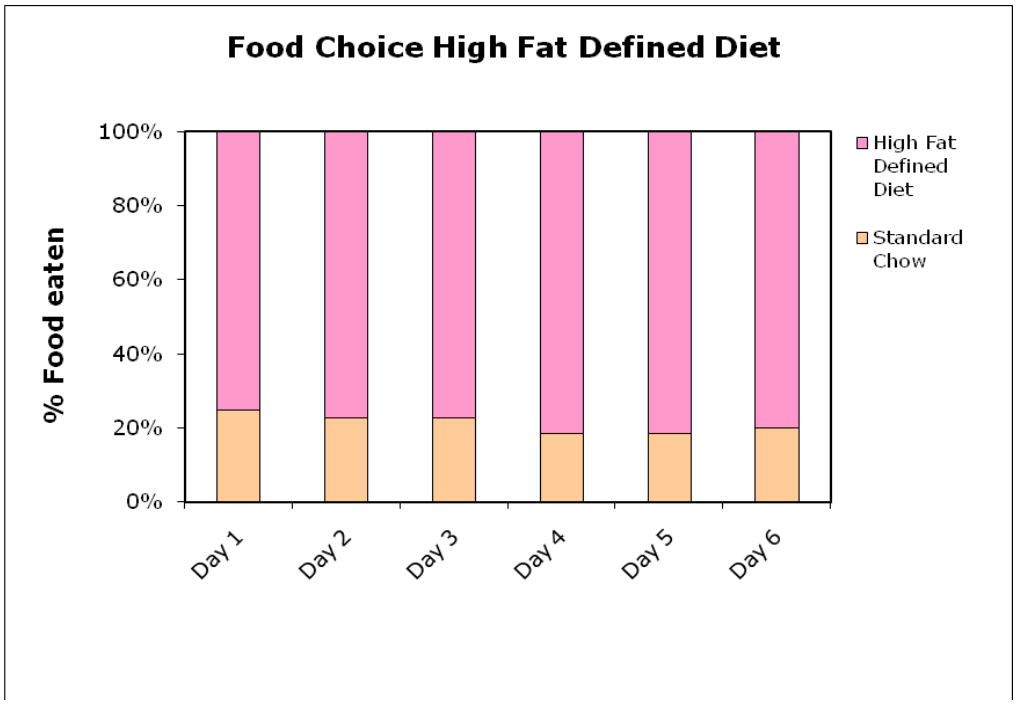

Fig. 5 High fat defined diet results from the Palatability Pilot Experiment. Data are presented as percentage of total food eaten for each of the two diets provided to mice.

Table 1 – Experiment 1 food intake for all experimental groups.

	Food Intake (g/day)
Standard Chow, SD	3.29†
Standard Chow, LD	3.23†
Control Defined Diet, SD	2.50
Control Defined Diet, LD	2.81

†Adjusted to remove effect of ground and discarded food

Table 2 – Experiment 1 mean total caloric intake and standard error for all experimental groups.

	Mean Total Caloric Intake (kcals)	Standard Error
Standard Chow, SD	1076.41†	58.24
Standard Chow, LD	1077.41†	46.69
Control Defined Diet, SD	924.05	54.96
Control Defined Diet, LD	1057.81	53.48

†Adjusted to remove effect of ground and discarded food

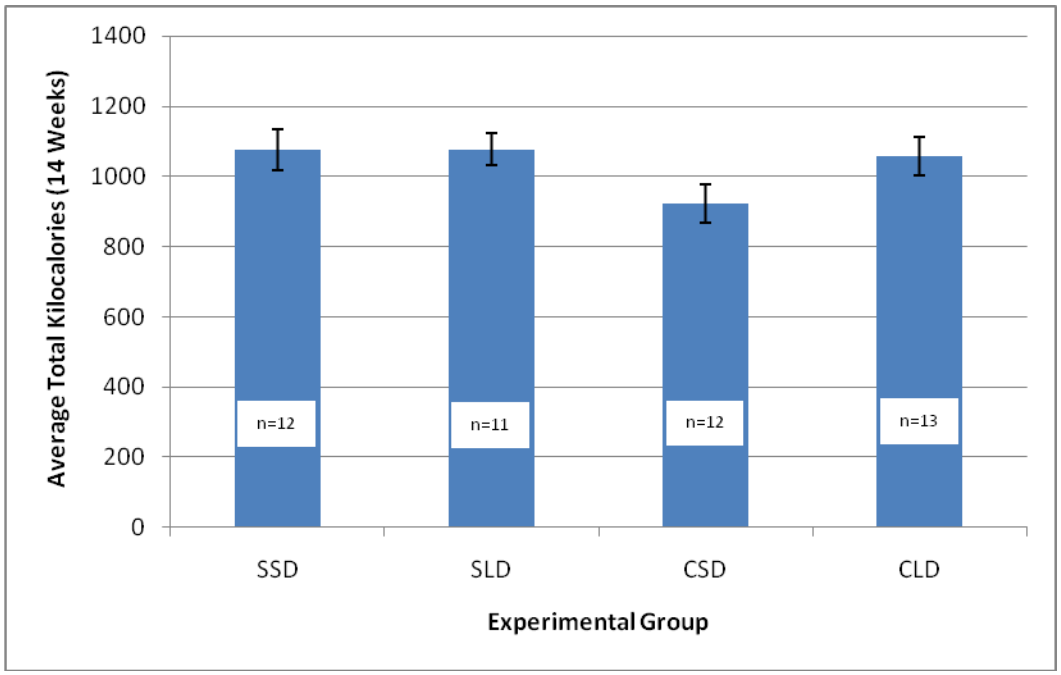

Fig. 6 Experiment 1 total caloric intake for all for standard chow long day (SLD), standard chow short day (SSD), control defined diet long day (CLD), and control defined diet short day (CSD) groups. Data are presented as mean ± standard error.

Table 3 – Experiment 1 average body mass and standard error for all experimental groups.

	Average Body Mass (g)	Standard Error
Standard Chow, SD	19.79	0.76
Standard Chow, LD	20.89	0.60
Control Defined Diet, SD	18.49	0.89
Control Defined Diet, LD	20.07	1.16

Table 4 – Experiment 1 average single testis mass and standard error for all experimental groups.

	Average Single Testis Mass (mg)	Standard Error
Standard Chow, SD	83	16
Standard Chow, LD	135	14
Control Defined Diet, SD	87	11
Control Defined Diet, LD	131	15

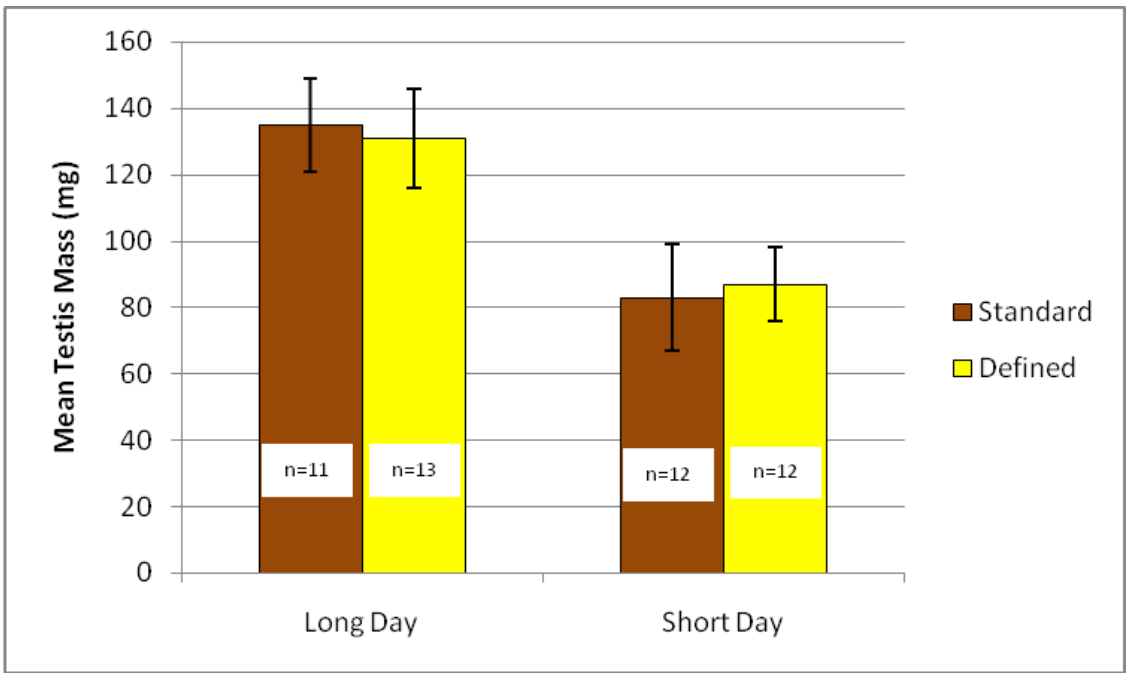

Fig. 7 Experiment 1 single testis mass for experimental groups placed in long days or short days and provided with either standard chow or the control defined diet. Data are presented as mean ± standard error.

Table 5 – Experiment 1 average seminal vesicle mass and standard error for all experimental groups.

	Average Seminal Vesicle Mass (mg)	Standard Error
Standard Chow, SD	31	11
Standard Chow, LD	66	11
Control Defined Diet, SD	36	12
Control Defined Diet, LD	81	16

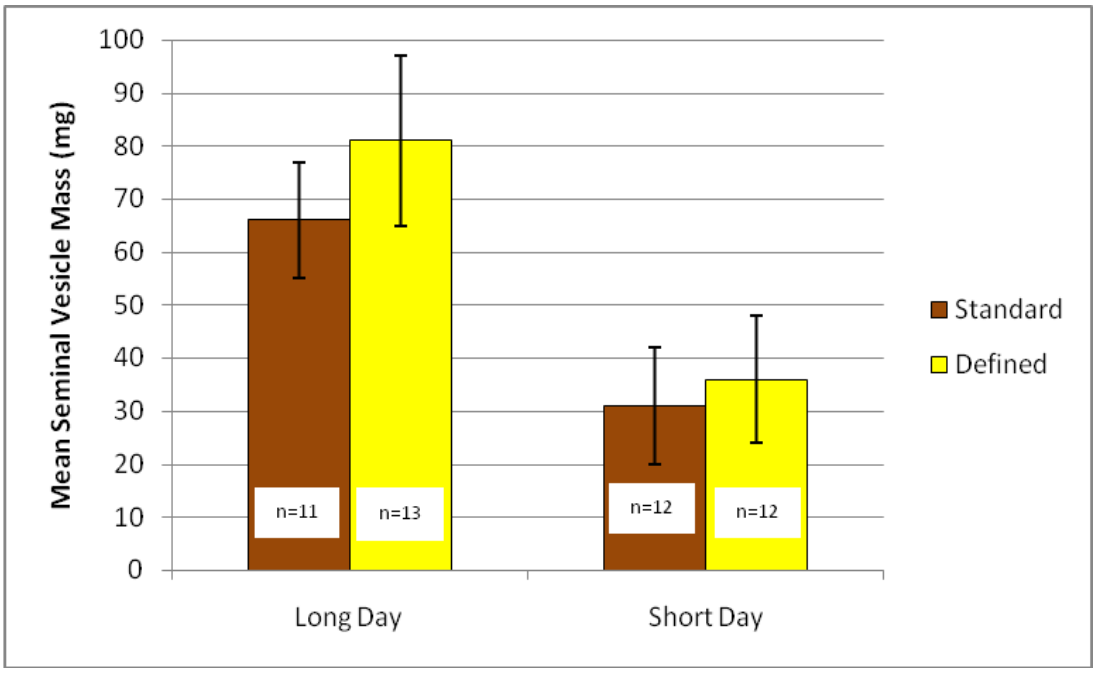

Fig. 8 Experiment 1 seminal vesicle mass for experimental groups placed in long days or short days and provided with either standard chow or the control defined diet. Data are presented as mean ± standard error.

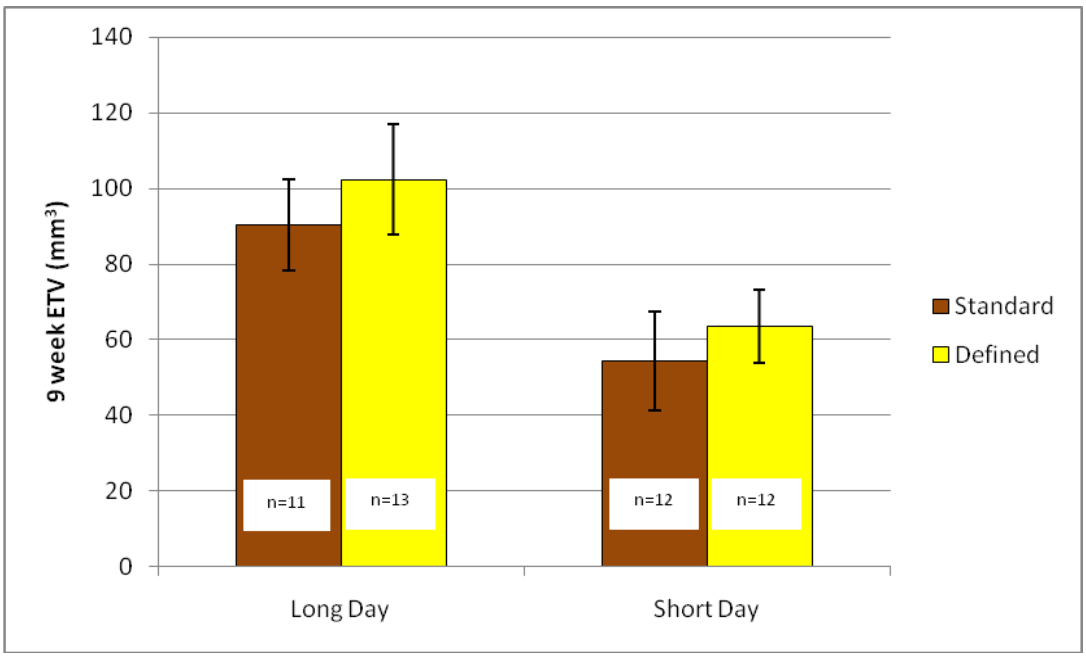

Fig. 9 Experiment 1 Estimated Testis Volume (ETV) calculated from external testis measurements taken during week 9 of the experiment. ETV is shown for experimental groups placed in long days or short days and provided with either standard chow or the control defined diet. Data are presented as mean ± standard error.

Table 6 – Experiment 2 food intake for all experimental groups. The group that was provided with the high fat defined diet and condensed milk includes separate daily intake values for each.

	Intake of Chow (g/day)	Intake of Condensed Milk (g/day)
Control Defined Diet, LD	2.94	-
Control Defined Diet, SD	2.73	-
HF defined diet, SD	2.42	-
HF defined diet + CM, SD	1.71	1.29

Table 7 – Experiment 2 mean total caloric intake and standard error for all experimental groups.

	Mean Total Caloric Intake (kcals)	Standard Error
Control Defined Diet, LD	1106.51	77.97
Control Defined Diet, SD	1023.75	89.91
HF defined diet, SD	1172.62	96.11
HF defined diet + CM, SD	1271.67	115.41

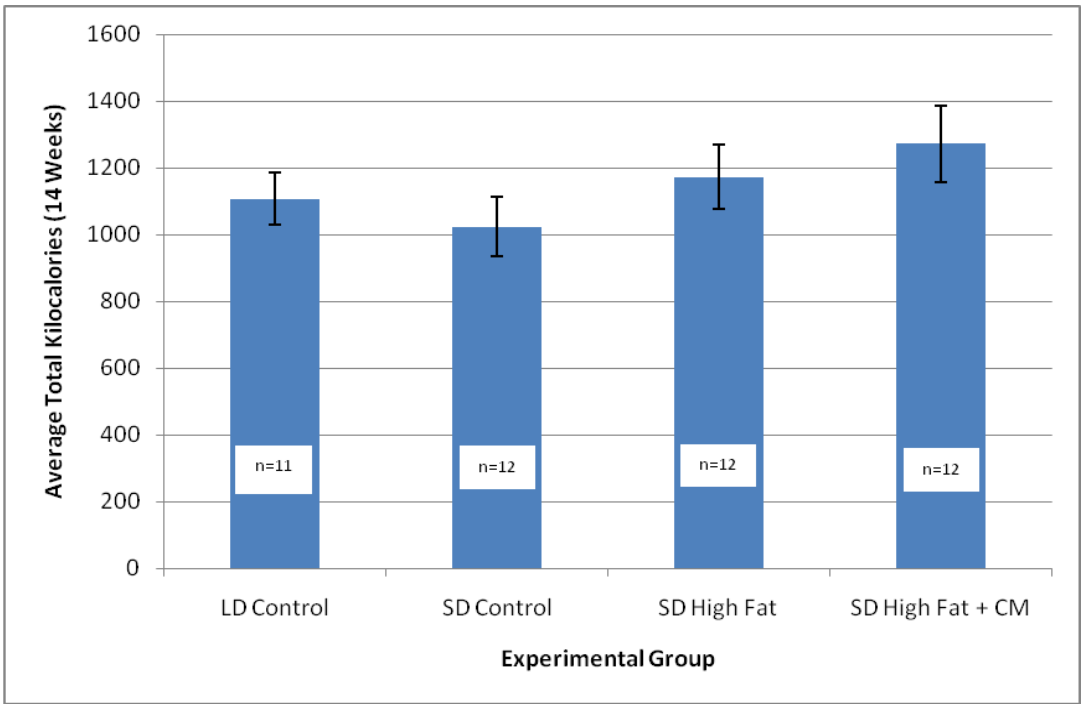

Fig. 10 Experiment 2 total caloric intake for the long day control group, short day control group, high fat defined diet experimental group, and high fat defined diet + condensed milk experimental group. Data are presented as mean ± standard error.

Table 8 – Experiment 2 average body mass and standard error for all experimental groups.

	Average Body Mass (g)	Standard Error
Control Defined Diet, LD	21.43	1.24
Control Defined Diet, SD	20.30	1.40
HF defined diet, SD	21.84	1.67
HF defined diet + CM, SD	20.24	1.35

Table 9 – Experiment 2 average single testis mass and standard error for all experimental groups.

	Average Single Testis Mass (mg)	Standard Error
Control Defined Diet, LD	147	12
Control Defined Diet, SD	111	18
HF defined diet, SD	123	19
HF defined diet + CM, SD	133	19

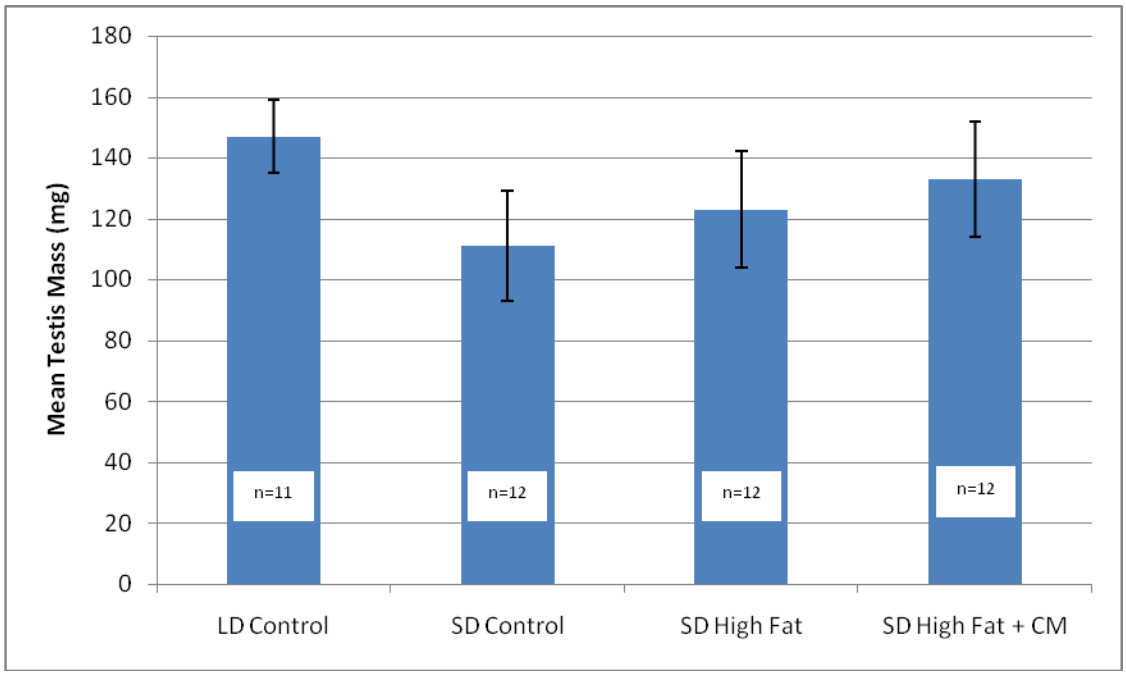

Fig. 11 Experiment 2 single testis mass for the long day control group, short day control group, high fat defined diet experimental group, and high fat defined diet + condensed milk experimental group. Data are presented as mean ± standard error.

Table 10 – Experiment 2 average seminal vesicle mass and standard error for all experimental groups.

	Average Seminal Vesicle Mass (mg)	Standard Error
Control Defined Diet, LD	107	20
Control Defined Diet, SD	64	17
HF defined diet, SD	61	20
HF defined diet + CM, SD	77	17

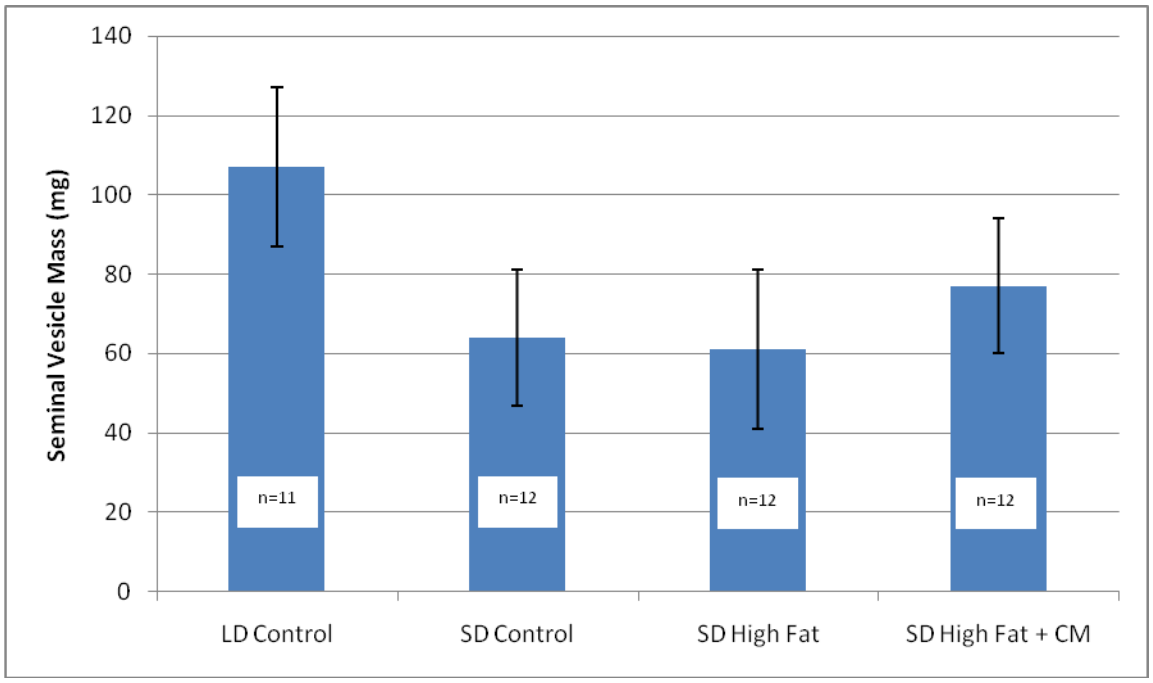

Fig. 12 Experiment 2 seminal vesicle mass for the long day control group, short day control group, high fat defined diet experimental group, and high fat defined diet + condensed milk experimental group. Data are presented as mean ± standard error.

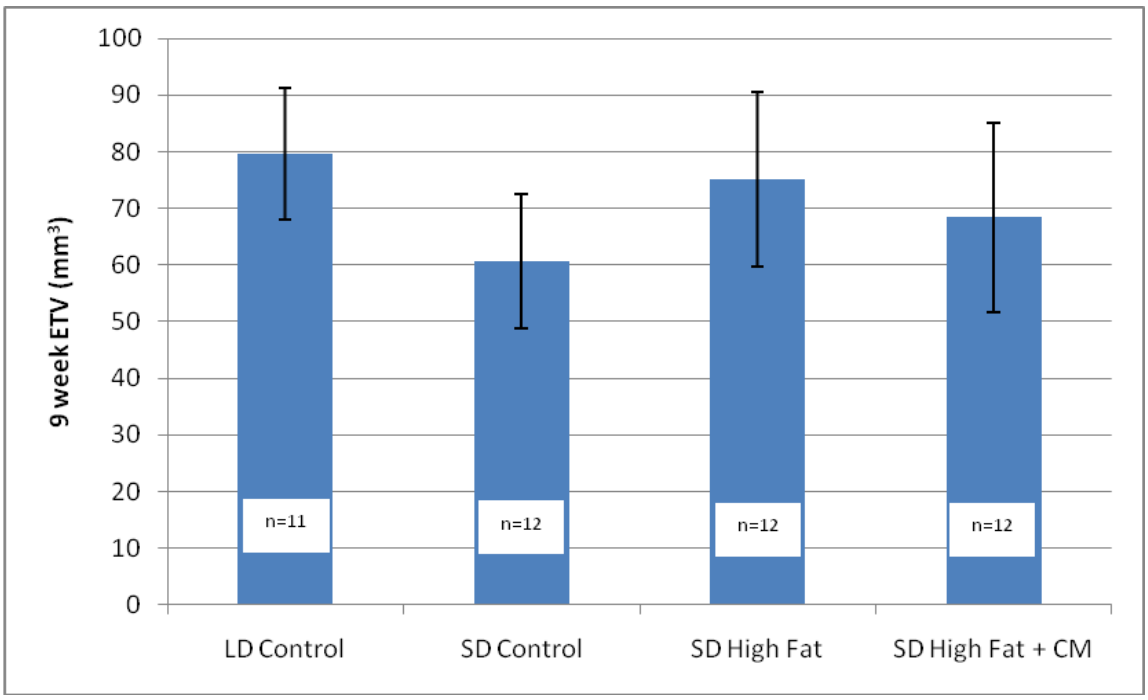

Fig. 13 Experiment 2 Estimated Testis Volume (ETV) calculated from external testis measurements taken during week 9 of the experiment for the long day control group, short day control group, high fat defined diet experimental group, and high fat defined diet + condensed milk experimental group. Data are presented as mean ± standard error.

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