Role of Gonadal Steroids and Inhibitory Photoperiod in Regulating Body Weight and Food Intake in Deer Mice (*Peromyscus maniculatus*) (43777)

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Abstract. **We investigated the role of declining daylength and gonadal steroids on body weight and food intake in male deer mice (*Peromyscus maniculatus*). This species was chosen for study because individual males display different reproductive responses to inhibitory daylength. About one-third of all mice exposed to short days undergo testicular regression and exhibit reduced circulating levels of luteinizing hormone and testosterone (reproductive responsive males). In contrast, testicular function and circulating levels of both these hormones remain unaffected in an equal number of mice (reproductive nonresponsive males). Previous studies have shown that each phenotype exhibits a distinct set of metabolic responses to short days, including adjustments in body weight. These characteristics make deer mice a useful animal model in which to study the interactive effects of gonadal steroids and photoperiod on neural substrates regulating body weight. A gonadectomy/steroid replacement experimental model was used to test the role of testosterone in regulating body weight and food intake in short day-housed male deer mice. Among gonad-intact males, short daylength caused a decline in body weight in both reproductive responsive and nonresponsive individuals. However, reproductive responsive mice lost significantly more body weight than did nonresponsive mice. Furthermore, while the weight loss was accompanied by a significant reduction in food intake in responsive mice, the relatively minor weight loss in nonresponsive mice was not accompanied by a change in food intake. Because changes in body weight and food intake (data not shown) occurred nearly simultaneously during the 8-week exposure to short daylength, results suggest that modifications in body weight are not responsible for the decline in food consumed, and vice versa. Gonadectomized reproductive responsive mice lost the same amount of weight as intact responsive mice but ate significantly more food. Among nonresponsive males, gonadectomy led to significantly greater weight loss, relative to intact mice, but caused an increase in food intake per gram body weight. Steroid replacement prevented weight loss and increased food intake in both gonadal phenotypes. Despite the observations that food intake was steroid dependent and the magnitude of the effect differed between reproductive phenotypes, changes in food consumption do not fully explain the inhibitory effects of short days on body weight in either phenotype. Taken together with previous studies, these results suggest that reproductive quiescence confers significant metabolic benefits to individual deer mice by reducing the amount of daily energy requirements via a reduction in body weight. Conversely, maintenance of reproductive function during the nonbreeding winter season carries greater metabolic costs; these costs lead to increased amounts of food required to maintain body weight.** [P.S.E.B.M. 1994, Vol 206]

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Many seasonally breeding mammals exhibit annual fluctuations in body weight during the transition from breeding to nonbreeding condition. For members of the Muridae (mice, voles, and hamsters) these fluctuations are apparently not caused by an underlying circannual rhythm in weight gain or loss, but rather occur in response to seasonal variation in some physical aspect of the environment

(1, 2). Declining daylength is the principle proximate environmental factor triggering seasonal changes in body weight, although other factors such as temperature, food type or availability, and gonadal steroids may influence the magnitude and/or direction of the change (3–6). Seasonal adjustments in body weight among north temperate zone species are generally thought to provide energy savings to individuals that are exposed to an annual decline in ambient temperature and food resources (7, 8).

Body weight is potentially modified by any physiological factor that affects energy assimilation or expenditure. Among these, modifying food intake has been frequently cited as the primary way in which declining photoperiod evokes seasonal changes in body weight. However, the association between food intake and body weight is not always so direct. For example, in the meadow vole (*Microtus pennsylvanicus*), short day-housed voles lose body weight because of a reduction in caloric intake (9). By comparison, short days cause an increase in body weight in the Syrian hamster (*Mesocricetus auratus*), despite the absence of an increase in food intake (2, 10). Siberian hamsters (*Phodopus sungorus*) lose weight and eat less, but the decline in body weight precedes the decline in food intake by 2–3 weeks (2, 11).

We undertook an investigation of the role of both declining daylength and gonadal steroids on body weight and food intake in male deer mice (*Peromyscus maniculatus*). This animal model was selected for study because individual deer mice differ in their reproductive and metabolic responses to declining photoperiod (12, 13). About one-third of all males undergo gonadal regression when exposed to short daylength (reproductively responsive males), while the reproductive axis is unaffected by short days in an equal number of mice (reproductive nonresponsive males); intermediate spermatogenic responses are exhibited by the remaining mice (13). These disparate gonadal responses are paralleled by endocrine measures: responsive males exhibit reduced circulating levels of testosterone and luteinizing hormone, while nonresponsive males exhibit long day levels of both hormones (13). This variation has a genetic basis (14) and both breeding and nonbreeding individuals have been found in winter populations of this species (15). A variety of evidence indicates that all mice respond to the short photoperiod signal at a postpineal site-of-action, albeit with different hypothalamic-pituitary responses (16). Thus, different testicular responses observed within populations of deer mice have been hypothesized to reflect individual differences in hypothalamic responsiveness to declining daylength (16, 17).

Responsive and nonresponsive deer mice have been shown to differ in their body weight response to short days in several studies. Responsive males lose about 10% of their long-day weight during short-day exposure, while nonresponsive males either maintain body weight or show only minor reductions (17). Because these two phenotypes differ in circulating levels of testosterone (13), and because this steroid has been widely shown to influence body weight, deer mice provide a unique animal model in which to investigate the role of both photoperiod and gonadal steroid in regulating seasonal changes in body weight.

We employed a gonadectomy/testosterone replacement model to evaluate the role of testosterone in regulating the effects of inhibitory photoperiod on body weight and food intake in responsive and nonresponsive deer mice. Data presented herein demonstrate that short photoperiod evokes changes in body weight and food intake among deer mice of both reproductive phenotypes. Both groups retain sensitivity to steroid following short day treatment, although data suggest differing effects of steroid on body weight. Intact responsive and nonresponsive mice also differ substantially in body weight loss and food intake, indicating differing metabolic responses to the short-day signal. Taken together with previous findings, these data support the view that short-day adjustments made by each phenotype are regulated, in part, by differential responses to the internal steroid milieu.

Materials and Methods

Animals and Breeding Conditions. Deer mice used in this experiment were obtained from the F_2 generation of an outbred F_1 breeding colony maintained at Kent State University, Kent, OH. Parental breeders were captured in Wind Cave National Park, Hot Springs, SD (43° 30′ W Lat; 103° 34′ N Long). In the laboratory, breeding pairs and their offspring were maintained under long photoperiod (16:8-hr light:dark cycle) from birth and provided with food (Purina Lab Chow; Ralston, St. Louis, MO) and water *ad libitum*. Reproductive maturity was assessed at 90 days of age by external measurement of the right testis, as described elsewhere (13). Males with a testis size of 54 mm^2 (length × width) or larger are capable of inseminating females (18) and were selected for further study.

General Methodology. Body weight and food intake was measured at 48-hr intervals, between 1 and 2 hr after lights-on, for all treatment groups. During the experiment, animals were fed ground diet (Formulab) that had been placed in a porcelain food cup beneath a 2-mm wire mesh. This presentation allowed mice free access to food, reduced spillage and contamination, and allowed collection of uneaten food scattered from the cup (19). Deer mice were exposed to the caging conditions (16:8-hr light:dark cycle, 23°C) and ground diet for 10 days. At the end of this period, no deviation from original body weight was observed. During each

experiment, a decline in mass of food in the container over a 48-hr period was used to calculate the amount of food consumed per day for each animal. At autopsy, testes and epididymides were removed, weighed, and homogenized. The numbers of elongated spermatids and/or spermatozoa shaped like them were assessed by hemocytometric counting (13).

Determination of Reproductive Phenotype. Previous work (16) demonstrated that male deer mice exhibit the same testicular response to a second short photoperiod exposure as to an initial exposure following a 20-week refractory period. To produce a cohort of males of known phenotype, deer mice were maintained on short daylength (8:16-hr light:dark cycle) for 8 weeks after which external testis size was measured. Males with a testis size of 54 mm^2 and larger, or 24 mm^2 and smaller, were classified as reproductively nonresponsive (n = 12) or reproductively response (n = 12) to short photoperiod, respectively. Males were returned to long daylength for 24 weeks; a period sufficient to stimulate sensitivity to a second short-day exposure, and used in experiment 2.

Experiment 1. Photoperiod Effects on Gonad-Intact Males. Intact adult males of unknown reproductive phenotype and uniform body weight were housed as described above, provided with a ground diet, and exposed to long photoperiod for 2 weeks. Following this period, body weight and food intake were measured every 2 days for an additional 10-day period. Thereafter, mice were placed on short daylength for 8 weeks, and body weight and food intake were measured at 2-day intervals. After the 8 week period, mice were categorized into reproductive phenotypes based on testis measurements (see above), and data were analyzed, as described below for six reproductive response and six reproductive nonresponsive males.

Experiment 2. Photoperiod Effects on Gonadectomized Males. To evaluate the effects of steroid and short photoperiod on body weight and food consumption, we adopted a steroid removal and replacement experimental model. Long day-housed males of known reproductive phenotype of uniform body weight (see Methods) were gonadectomized under Metophane (Pitman-Moore, Mundelein, IL) anesthesia. After a two week recovery period, food consumption was measured for a 10-day period as described above. Thereafter, responsive (n = 12) and nonresponsive (n = 12) deer mice were implanted subcutaneously with a silastic capsule (Silastic Medical Grade Tubing, O.D. 0.125 in, I.D. 0.078 in; Dow Corning, Inc., Midland, MI) containing either 5 mm packed testosterone or no testosterone and placed on short photoperiod (8:16-hr light:dark cycle) for 8 weeks; treatment groups did not significantly (F = 2.25, P = not significant) differ in initial body weight. Preliminary work indicated a testosterone capsule of this size produced mean blood concentrations of testosterone about twice that of long day-housed intact deer mice, but within maximum levels previously measured in this species (13). We chose to deliver a higher amount of testosterone, but at a concentration known to be within physiological levels, in order to maximize any effect testosterone might have on body weight and food intake. Additionally, this level of circulating testosterone, coupled with plasma concentrations in intact mice or those implanted with empty capsules, provided for analysis of dose-response effects of testosterone on body weight and food consumption. At the end of the 8-week period, food intake and body weight were measured, as described above, for 10 days.

Serum Collection and Testosterone Assay. All mice were sacrificed after short-day treatment by sodium pentobarbital overdose. Arteriovenous blood was collected in polyethylene tubes and placed on ice until centrifuged; plasma was stored at −70°C until hormone concentrations were measured by radioimmunoassay (RIA). Testosterone was also measured in blood collected from long day and reproductively mature mice (n = 10) to provide a comparison with animals maintained under each experimental treatment.

Plasma concentrations of testosterone were determined in 20 μl of serum using a procedure modified after Lee *et al.*, 1986. The antiserum (kindly provided by Dr. B. Bruot, Kent State University) cross-reacts less than 10% with dihydrotestosterone, 1% with androsterone, and less than 0.1% with pregnenolone, progesterone, 20a-dihydroprogesterone, corticosterone, androsterone, estradiol, and estrone (20). Extracted hormone and standards were dried under nitrogen and resuspended in 0.1 ml 0.01 M phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (Sigma Chemical Co., St. Louis, MO). To this sample was added 0.1 ml PBS containing 15,000 dpm (^{125}I-testosterone, Hazelton) and 0.1 ml of a 1:35,000 dilution of antiserum. Following a 24-hr incubation at 4°C, free and bound steroid were separated by incubation with 0.5% charcoal (Norit A)/0.2% dextran solution for 20 min. Samples were centrifuged at 3000g for 20 min and supernatant counted. The minimum amount of testosterone detectable was 0.7 pg/ml. Samples were run in two assays; the within-assay coefficient of variation for five replicates made on a pool of blood plasma from intact male deer mice were 4.34% and 7.80%, respectively, and recovery was 94%.

Testosterone Levels. Mean plasma concentrations of testosterone were significantly (F = 65.10, P < 0.0001) lower among reproductive responsive males and were unaffected by short-day treatment among nonresponsive mice (Table I). Mean testosterone con-

centrations among mice implanted with testosterone capsules were about two times that of long day controls (Table I). Plasma concentrations were positively correlated with the change in body weight (r^2 = 0.3158, $P < 0.0001$).

Statistics. Effects of short days on food intake and body weight are plotted as a deviation from long day levels for each treatment group. Each individual's long-day value for body weight and food intake served as its own control to reduce within-group variability. Short-day effects were assessed with a paired Student's t test using the difference between an individual's long-day and short-day values for body weight and food intake. Differences between groups were evaluated with a one-way ANOVA; post-hoc comparisons were made using an SNK test. The 0.05 level of acceptance was equated with significance.

Results

Experiment 1. After 8 weeks exposure to short days, males were categorized into either reproductive responsive or nonresponsive phenotypes, depending upon the absence or presence of epididymal sperm. Epididymal sperm represent the numbers of mature spermatozoa available for ejaculation and are, therefore, a conservative measure of reproductive capability. On this basis, epididymides and testes of reproductive responsive males (n = 12) were found to contain significantly (F = 25.15, $P < 0.0001$; F = 74.59, $P < 0.0001$, respectively) fewer sperm compared with long-day controls (Table II). In contrast, spermatogenesis was unaffected in nonresponsive males exposed to short daylength, as confirmed by sperm numbers equal to or greater than those of long-day controls (Table II).

The effect of short days on body weight and food consumption was subsequently examined among reproductively responsive (no sperm) and reproductively nonresponsive (long-day sperm numbers). Short days caused a significant (t = 10.46, $P < 0.0001$) decline in body weight in gonad-intact responsive males (Fig. 1). Mean body weight was 7.14% lower by Week 3 of short-day exposure, relative to initial long-day levels, and continued to decline through the 8-week exposure period. At autopsy, mean body weight had declined by 2.68 ± 0.26 g, a 12.27% reduction relative to the control period (Fig. 1). These changes in body weight were paralleled by reductions in food consumed per gram body weight. By Week 8 of short-day exposure, reproductive responsive deer mice ate significantly (t = 2.62, $P < 0.0178$) less food per gram body weight than during the long-day exposure period (Fig. 2).

Table I. Testosterone Concentrations in Silastic Implanted or Gonad-Intact Deer Mice[a]

Group	Testosterone (ng/ml serum)
Intact long-day	1.66 ± 0.22[b]
T implanted	3.14 ± 0.20[c]
Intact nonresponsive	1.71 ± 0.15[b]
Intact responsive	0.10 ± 0.07[d]
Gonadectomized	0.17 ± 0.03[d]

[a] All animals other than intact long-day mice were housed under short photoperiod for 8 weeks. Means with different letter superscripts are significantly different.

Short days also caused a significant (t = 3.94, $P < 0.001$) reduction in body weight of intact reproductive nonresponsive deer mice (Fig. 1). However, the decline reached a plateau by Week 3 of short-day exposure and remained constant until autopsy (Fig. 1). At autopsy, nonresponsive mice weighed 0.88 ± 0.22 g less than during the control period (Fig. 1). This decline was about one-third that observed among responsive males; the difference between response groups was significant (t = 5.29, $P < 0.0001$). Surprisingly, even though body weight declined, food consumed per gram body weight was unchanged (t = 0.48, not significant) from long-day levels (Fig. 2).

Experiment 2. Gonadectomized responsive mice exhibited a significant (t = 5.5, $P < 0.0001$) reduction in body weight (Fig. 3). This decline closely paralleled that of intact mice: the rate of decline in body weight (Fig. 3) and the total amount of weight lost at the end of 8 weeks of short-day exposure did not differ from intact responsive males (r^2 = 0.7213, P = not significant and t = 1.24, P = not significant, respectively). These similarities were noted despite observing that gonadectomized mice significantly (t = 2.79, $P < 0.0125$) increased food consumed per gram body weight (Fig. 4), whereas intact mice significantly (t = 2.62, $P < 0.0178$) reduced food intake (see above).

Gonadectomy dramatically increased the suppressive effect of short days on body weight among reproductive nonresponsive deer mice (Fig. 3). The rate of weight loss among these mice closely paralleled that of responsive males (Fig. 3). By the end of short-day exposure, the amount of weight lost was nearly three times (2.66 g ± 0.71) that of intact nonresponsive males (Fig. 3), but was not significantly (t = 0.68, P = not significant) different than that of reproductive responsive mice. Again, despite the nearly 3-fold reduction in body weight among gonadectomized nonresponsive mice, short days caused a significant (t = 3.37, $P < 0.0037$) increase in food consumed per gram body weight (Fig. 4).

Steroid replacement prevented weight loss and increased food consumption among gonadectomized males of both phenotypes. At the end of the 8 weeks short-day treatment, body weights of testosterone-implanted gonadectomized deer mice were either not

Table II. Paired Testes and Epididymides Weight and Sperm Numbers in Long Photoperiod or Short Photoperiod (Responsive and Nonresponsive) Exposed Deer Mice[a]

Group	Testis wt (mg)	Sperm ($\times 10^{-6}$)	Epididymides wt (mg)	Sperm ($\times 10^{-6}$)
Long-day	338.70 ± 24.7[b]	72.38 ± 3.14[b]	103.10 ± 10.37[b]	159.67 ± 18.64[b]
Nonresponsive	382.87 ± 22.8[b]	87.50 ± 9.31[b]	106.25 ± 7.71[b]	160.20 ± 19.07[b]
Responsive	63.80 ± 14.2[c]	0.00 ± 0.00[c]	19.72 ± 3.70[c]	0.00 ± 0.00[c]

[a] Means with different letter superscripts are significantly different.

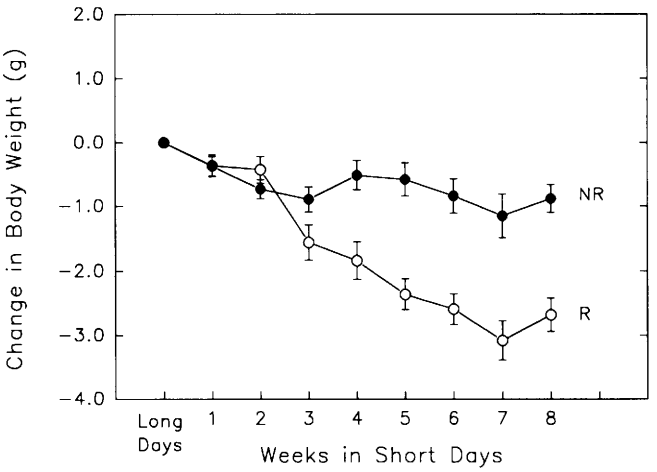

Figure 1. Effect of short photoperiod on body weight in either reproductively responsive (regressed testes) or nonresponsive (nonregressed testes) deer mice. Body weights are expressed as a change from long-day levels. Body weight declined in both phenotypes, although responsive males lost significantly more weight than nonresponsive males over the course of the treatment period.

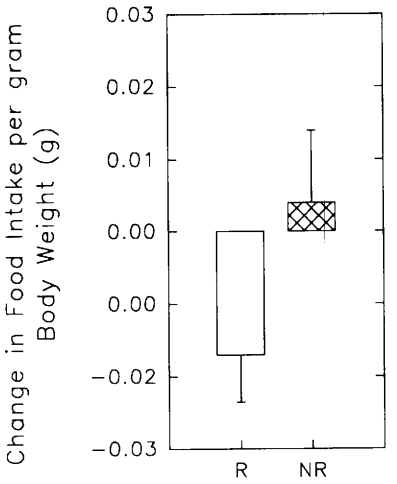

Figure 2. Change in food consumed per gram body weight in responsive and nonresponsive males after 8-week exposure to short photoperiod. Data are expressed as a change from long-day levels. Short days suppressed food consumption only among reproductively responsive deer mice; short days did not affect food consumed/g body weight among nonresponsive males. Differences between the phenotypes were significant. Alterations in the absolute amounts of food eaten during short-day treatment are noted in the text.

significantly different from (responsive males, t = 0.78, not significant) or greater than (nonresponsive males, t = 2.17, $P < 0.04$) long-day levels (Fig. 3). Body weight of responsive mice did not differ from long-day levels at any point during short-day exposure (Fig. 3). In contrast, body weight of nonresponsive males increased from long-day levels during Week 1 and remain elevated throughout the short-day period (Fig. 3). However, the effects of steroid replacement on food intake differed between the two phenotypes. Responsive mice exhibited no change in food intake per gram body weight (t = 0.45, P = not significant, Fig. 4). In contrast, short day-housed reproductive nonresponsive males consumed significantly more (t = 3.06, $P < 0.007$) food per gram body weight (Fig. 4).

Discussion

Body weight is influenced by physiological pathways that regulate energy intake and expenditure. The pathway by which short photoperiod modifies body weight in the deer mouse is not completely described. However, the effector systems that detect and respond to changes in photoperiod are widely thought to reside in central nervous system (CNS) structures (21). Endogenous gonadal steroids may influence body weight via central nervous system effector systems. Steroid concentrating neurons reside within the hypothalamus and testosterone has been shown to modify the reproductive response to short daylength by modifying hypothalamic-pituitary function (22, 23). With respect to body weight, a putative neural path by which steroid exerts an influence may be mitigated by the well-demonstrated anabolic effects of steroid at peripheral tissues. Therefore, a complete evaluation of effector systems which govern body weight requires assessment of the interactive effects of photoperiod and steroid both within the central nervous system and at the site of peripheral tissues. Our experiment dealt solely with the former site of action.

Results obtained from Experiment 1 demonstrate that short daylength alters body weight of deer mice differentially depending upon the reproductive response to an inhibitory photoperiod. Reproductive responsive mice (regressed testes) exhibited a 12.3% reduction in body weight during short-day treatment, relative to long-day levels. This weight loss was associated with an 11.5% reduction in food intake per gram

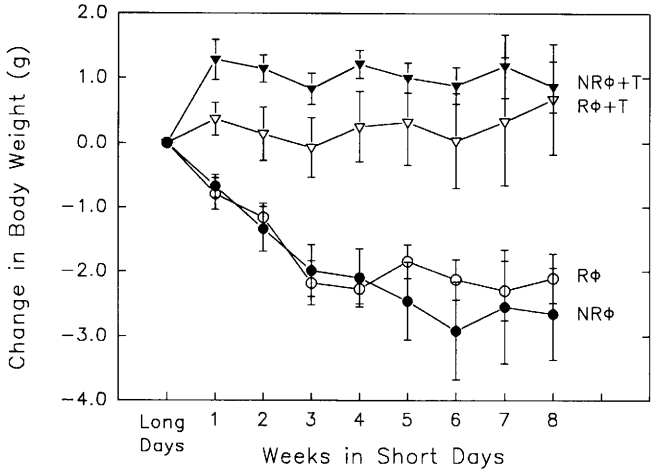

Figure 3. Effect of short photoperiod on body weight of gonadectomized responsive and nonresponsive deer mice implanted with either empty (ϕ) or testosterone-filled (ϕ + T) silastic capsules. Reproductive responses of all males were known from prior exposure to short days (see Methods). Data are expressed as a deviation from long-day levels. Nonresponsive males implanted with empty capsules (NRϕ) lost significantly more weight during short-day exposure than intact nonresponsive males (cf. Fig. 1). In contrast, gonadectomy did not alter the effect of short days on body weight of responsive (Rϕ) deer mice (cf. Fig. 1). Steroid replacement in gonadectomized mice of both phenotypes (Rϕ + T and NRϕ + T) prevented these responses to short daylength.

body weight. By comparison, reproductive nonresponsive males (functional testes) also exhibited weight loss (3.8%), although significantly less than responsive males. Further, this weight loss occurred in the absence of any change in food intake from long-day levels. Presumably, the weight loss occurred in nonresponsive males because energy expenditure increased or energy assimilation declined; we have no data that address either possibility.

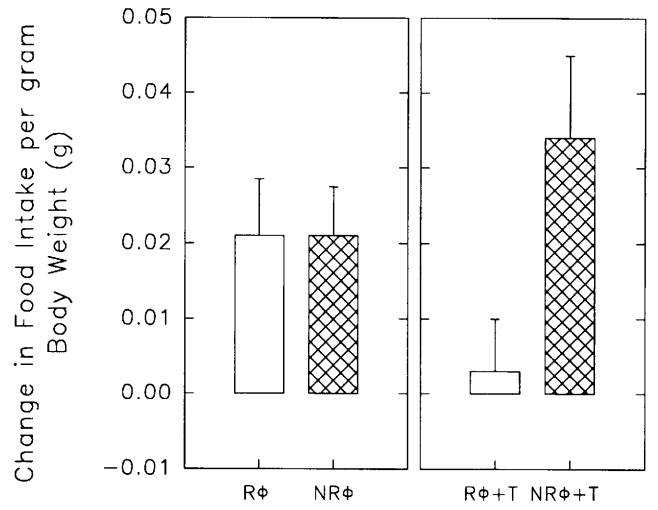

Figure 4. Change in food consumed per gram body weight in gonadectomized responsive and nonresponsive males implanted with empty (Rϕ and NRϕ) or testosterone-filled (Rϕ + T and NRϕ + T) silastic capsules after 8-week exposure to short photoperiod. Data are expressed as a deviation from long-day levels. Short days caused an increase in food consumed per gram body weight among all gonadectomized mice implanted with empty silastic capsules (left panel). Steroid replacement (right panel) significantly increased food consumption of short day-exposed mice, relative to short day-exposed intact males (cf. Fig. 2). However, steroid replacement caused a significant increase in food consumed, relative to long-day levels, only among nonresponsive mice.

Experiment 2 was designed to evaluate the effect of steroid on the photoperiod-induced changes in body weight and food intake. We performed this experiment because short photoperiod has been argued to cause testicular regression by increasing the negative feedback sensitivity to testosterone of hypothalamic effector systems which regulate hypothalamic-pituitary-gonadal function (e.g., 23). A key component of this hypothesis is that the reduced amounts of testosterone produced by the regressed gonad are sufficient to inhibit release of gonadotropins supporting testicular function. Studies conducted on the Syrian hamster support both the concept of hypothalamic supersensitivity and the effectiveness of reduced levels of testosterone in inhibiting gonadotropin release (22). As noted in the introduction, the deer mouse reproductive phenotypes serve as a useful animal model to study steroid feedback effects. In this species, differential gonadal responses have been shown to result from differences in hypothalamic response to the short photoperiod signal and not due to a deficiency in the ability of the central nervous system to detect changes in daylength (17). One possible mechanism for the cause of different reproductive responses in deer mice would be the lack of photic-induced supersensitivity to negative steroid feedback in the nonresponsive phenotype.

Among responsive males, gonadectomy did not alter weight loss, but eliminated the suppressive effect of short days on food intake. This suggests that the inhibitory effect of short days on body weight in responsive males is independent of lower plasma testosterone in this phenotype. Further, the absence of short-day inhibition of food intake among gonadectomized responsive mice suggests that short days suppress food intake in this phenotype by a steroid dependent pathway. The observation that steroid replacement also prevents a short-day suppression of food intake would seem to contradict this hypothesis. However, although exogenous testosterone was delivered at a dose twice that of long-day mice, this dose is more than an order of magnitude greater than that of short day-exposed intact responsive mice (Table I). We suggest at this higher level, testosterone may be exerting anabolic effects on metabolism, one outcome of which is increased food intake. This hypothesis is consistent with the observation that short days also do not suppress body weight in steroid-treated responsive mice.

Among nonresponsive deer mice, gonadectomy

resulted in weight loss about three times that of intact males, while food intake remained unchanged. These results suggest that short days suppresses body weight in this phenotype, and this effect is significantly magnified in the complete absence of circulating steroid. It appears that long-day levels of testosterone in nonresponsive mice attenuates the suppressive effect of short days on body weight. The absence of weight loss among nonresponsive males provided with twice long-day levels of testosterone is consistent with this argument. Again, we suggest that with increasing amounts of steroid, testosterone may be exerting anabolic effects on metabolism, one effect of which is increased food consumption.

Testosterone was delivered exogenously to gonadectomized mice to evaluate the effect of steroid on body weight and food intake. We delivered exogenous steroid at a blood concentration twice that of long-day levels to maximize any effect of steroid. In both deer mouse phenotypes, this amount of testosterone overrode the inhibitory affects of short days on body weight. Further, food intake either remained unchanged (responsive group) or increased (nonresponsive group) relative to long-day levels. Because testosterone was not replaced at doses equivalent to that of long-day mice, these data only indicate that steroid at the dose provided (two times long-day levels) exerts a stimulatory effect on central and/or peripheral pathways governing body weight and food intake. These data do not unequivocally address the question of whether mice of each phenotype differ in steroid feedback sensitivity, either with respect to body weight or food intake. Although the testes of reproductive responsive mice are devoid of sperm, testosterone is detectable in the plasma, indicating that steroidogenesis is not inhibited completely by short days. Short days may induce supersensitivity of CNS loci governing body weight and/or food intake to steroid feedback. If so, then it is possible that the reduced amount of circulating testosterone in reproductive responsive deer mice could exert regulation over these traits.

These results indicate that all deer mice, regardless of reproductive phenotype, undergo a short day-induced decline in body weight. However, reproductive responsive mice lose significantly more body weight than nonresponsive mice, and this weight loss is accompanied by a significant reduction in food intake per gram body weight. Time course changes in body weight and food intake (data not shown) indicate that both occur nearly simultaneously. This suggests that the decline in body weight is not responsible for the decline in food consumed and vice versa. This suggestion is supported by the observation that all gonadectomized mice lose body weight, albeit without a reduction in food intake.

In summary, results of the present experiment show that weight loss depends on circulating levels of testosterone; the lower the amount of testosterone, the greater the amount of weight lost. Further, although the amount of food eaten per gram is steroid-dependent, changes in food consumption do not fully explain the inhibitory effects of short days on body weight in either phenotype.

While the gonadectomy/steroid replacement model is suitable to evaluate the steroid dependence of physiological adjustments, it has little relevance to the issue of the cost and benefits of weight loss to animals in their natural habitat. Deer mice used in this study were obtained from latitudes characterized by severe winters. The adaptive significance of the observed differences between the deer mouse phenotypes may be related to differences in energy metabolism following short-day exposure. Our data show that reproductive responsive mice eat an average of 0.72 g (22%) less food per day than do nonresponsive mice. This large difference indicates that the daily energy requirements of responsive mice are substantially less than that of nonresponsive mice. This possibility dovetails with recent findings that responsive deer mice have a significantly greater tolerance of cold temperatures than do nonresponsive mice and that this difference is due, in large part, to differences in body weight (24).

The hypothesis that individuals cease breeding during the winter in order to conserve energy at a time when both food resources and the probability of offspring survival are low has gained nearly universal adherance among physiologists. This hypothesis assumes that reproductive processes comprise a major proportion of an individual's daily energy budget. While data are widely supportive of this assumption (25), a significant problem with the above hypothesis is that winter breeding is a widely observed and even common event among individuals of many different species living in north temperate or even arctic habitats (15, 24). This single observation indicates that many individuals are far more flexible in their physiological responses to the environment than has been previously appreciated.

If the metabolic advantages of ceasing reproduction are significant, why do individuals of many species breed during the short and cold days of winter? The answer to this question may reside in two observations. First, previous studies have shown that while short photoperiod does not inhibit reproductive function in nonresponsive deer mice, it does evoke a number of metabolic adjustments that moderately increase the ability of nonresponsive males to tolerate thermoregulatory challenges. While these thermogenic responses are not as completely developed as those of responsive males, they may be adequate to meet environmental challenges during mild winters (24). A second observation consistent with this possibility is

that winter conditions over much of the range of this species, including the habitat from which deer mice used in this study were obtained, are quite variable (17). Thus, during mild winters deer mice capable of producing offspring and also meeting metabolic challenges would be at a selective advantage relative to those individuals in which reproduction is obligately inhibited.

The present and previous findings support the contention that reproductive quiescence confers significant metabolic benefits to an individual; these benefits presumably increase survivorship. Conversely, maintenance of reproductive function during the nonbreeding season carries greater metabolic costs, the cost translating into greater amounts of food required to fuel both breeding and thermoregulatory demands. Within natural populations of deer mice, one could envision maintenance of both phenotypes, with their relative frequency varying from year-to-year depending upon the severity of local winter conditions and the ability of either phenotype to contend with these conditions. This suggestion points to the importance of evaluating the functional connections between short photoperiod-induced adjustments in body weight and reproductive function in the context of an individual's natural life history.

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1. Hoffman J. The influence of photoperiod and melatonin on testis size, body weight and pelage colour in the Djungarian hamster (Phodopus sungorus). J Comp Physiol **85**:267–282, 1973.
2. Bartness TJ, Wade GN. Photoperiodic control of seasonal body weight cycles in hamsters. Neurosci Biobehav Rev **9**:599–612, 1985.
3. Bartness TJ, Wade GN. Seasonal obesity in Syrian hamsters: Effects of age, diet, photoperiod, and melatonin. Am J Physiol **247**:R328–R334, 1984.
4. Steinlechner S, Heldmaier G, Bockler H. Influence of photoperiod on body temperature rhythms in the Djungarian hamster. J Interdiscipl Cycle Res **16**(4):329–330, 1983.
5. Slusser WN, Wade GN. Testicular effects of food intake, body weight, and body composition in male hamsters. Physiol Behav **27**:637–640, 1981.
6. Heldmaier G. Seasonal acclimation of energy requirements in mammals: Functional significance of body weight control, hypothermia, torpor and hibernation. In: Wieser W, Knaiger E, Ed. Energy Transformations in Cells and Organisms. Stuttgart: Thieme, 1989.
7. Heldmaier G, Steinlechner S. Seasonal control of energy requirements for thermoregulation in the Djungarian hamster (*Phodopus sungorus*), living in natural photoperiod. J Comp Physiol **142**:429–437, 1981.
8. Lovegrove BG, Heldmaier G, Ruf T. Perspectives of endothermy revisited: The endothermic temperature range. J Therm Biol **16**(4):185–209, 1991.
9. Dark J, Zucker I. Seasonal cycles in energy balance: Regulation by light. Ann NY Acad Sci **453**:170–181, 1985.
10. Hoffmann J, Davidson K, Steinberg K. Influence of photoperiod and temperature on weight gain, food consumption, fat pads and thyroxine in male golden hamsters. Growth **46**:150–162, 1982.
11. Wade GN, Bartness TJ. Effects of photoperiod and gonadectomy on food intake, body weight, and body composition in Siberian hamsters. Am J Physiol **246**:R26–R30, 1984.
12. Desjardins C, Lopez MJ. Environmental cues evoke differential responses in pituitary-testicular function in deer mice. Endocrinology **112**:1398–1406, 1983.
13. Blank JL, Desjardins C. Photic cues induce multiple neuroendocrine adjustments in testicular function. Am J Physiol **250**:R199–R206, 1986.
14. Desjardins C, Bronson FH, Blank JL. Genetic selection for photoperiodic responsiveness in deer mice. Nature **322**:172–173, 1986.
15. Millar JS. Reproduction and survival of *Peromyscus* in seasonal environments. In: Merritt JF, Ed. Winter Ecology of Small Mammals. Pittsburgh, PA: Carnegie Museum of Natural History Publication No. 10, pp253–266, p352, 1984.
16. Blank JL, Freeman DA. Differential reproductive response to short photoperiod in deer mice: Role of melatonin. J Comp Physiol **169**:501–506, 1991.
17. Blank JL. Phenotypic variation in physiological response to seasonal environments. In: Mammalian Energetics: Interdisciplinary Views of Metabolism and Reproduction. Ithaca, NY: Cornell University Press, pp186–212, 1992.
18. Lopez MJ. Reproductive and temporal adaptation to seasonal change in the deer mouse, *Peromyscus maniculatus*. PhD Diss, University of Texas, Austin, 1981.
19. Blank JL, Desjardins C. Spermatogenesis is modified by food intake in mice. Biol Reprod **30**:410–415, 1983.
20. Lee M, Bruot BC, Adams WC. Hormonal changes during the early development of ovarian cysts in the rat. Biol Reprod **35**:542–548, 1986.
21. Moore RY. Organization and function of a central nervous system oscillator: The suprachiasmatic hypothalamic nucleus. Fed Proc **42**:2783–2789, 1983.
22. Turek FW. The interaction of the photoperiod and testosterone in regulating serum gonadotropin levels in castrated male hamsters. Endocrinology **101**:1210–1215, 1977.
23. Bartke A, Steger RW. Seasonal change in the function of the hypothalamic-pituitary-testicular axis in the Syrian hamster. Proc Soc Exp Biol Med **199**:139–148, 1992.
24. Blank JL, Ruf T. Effects of reproductive function and cold tolerance in deer mice. Am J Physiol Reg Comp Integ Phys **32**:R820–R826, 1993.
25. Thompson SD. Gestation and lactation in small mammals: Basal metabolic rate and the limits of energy use. In: Mammalian Energetics: Interdisciplinary Views of Metabolism and Reproduction. Ithaca, NY: Cornell University Press, pp213–259, 1992.