

Original Article

Photorefractoriness and energy availability interact to permit facultative timing of spring breeding

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In seasonally breeding mammals, vernal reproductive development is not directly triggered by increases in day length, rather, an endogenous program of photorefractoriness to short winter days initiates spontaneous development in advance of spring. The transition to the reproductive phenotype is energetically demanding. How food availability in late winter and early spring impacts the onset and expression of photorefractoriness is not known. In this study, male Siberian hamsters were born into a simulated natural photoperiod, and at the winter solstice, they were subjected to a restricted feeding protocol in which a daily food ration was provided in an amount equal to ad libitum (AL) intake during the weeks preceding the solstice. Over the next several months, AL-fed control hamsters exhibited spontaneous recrudescence or spontaneous development. In contrast, vernal reproductive development was abolished in most food-rationed hamsters. In food-rationed hamsters that did exhibit recrudescence, conspicuous delays in the onset of gonadal development and decreases in the magnitude of growth were evident. In all hamsters, the termination of food rationing triggered rapid gonadal development. The data indicate that late winter/early spring increases in environmental food availability are required for the normal manifestation of photorefractoriness-induced reproductive development and suggest that a function of photorefractoriness may be merely to disinhibit the reproductive axis from photoperiodic suppression. Vernal gonadal development or recrudescence appears to be strongly affected by proximate energy availability. *Key words:* energy balance, food availability, photoperiodism, seasonality, Siberian hamster. [*Behav Ecol*]

INTRODUCTION

Seasonal cycles in the timing of the reproductive effort have evolved to permit synchrony of energetically costly phases of the reproductive cycle (e.g., growth, lactation, reproductive behaviors) with energetically favorable environmental conditions (Prendergast et al. 2009). To achieve this, reproductive responses to proximate cues such as day length must permit anticipation of biotic (food) and abiotic (temperature) environmental factors critical to survival and reproduction (Bronson 1989).

In Siberian hamsters, intermediate-duration photoperiods of late summer inhibit reproductive physiology in advance of the appearance of energetically challenging winter conditions (Stetson et al. 1986; Gorman and Zucker 1995a; Paul et al. 2009). The vast majority of animals born after the summer solstice thus delay puberty until the following spring. In overwintering animals, vernal (spring) reproductive development is not directly triggered by longer or increasing day lengths (Gorman 1995; Gorman and Zucker 1995b; Butler, Trumbull, et al. 2007); rather, an endogenous program of photorefractoriness (i.e., unresponsiveness) to short days is initiated, and after many weeks of exposure to short days, an interval timer triggers gonadal growth in mid-to-late winter

(Gorman 1995; Prendergast et al. 2000; Butler et al. 2010). In males, photorefractoriness begins the lengthy process of gonadal growth (recrudescence) and restoration of spermatogenesis while day lengths are still relatively short (Lerchl and Schlatt 1993; Gorman 1995; Schlatt et al. 1995). This mechanism presumably allows hamsters to produce litters coincident with the appearance of moderate temperatures and abundant food in spring rather than initiating gonadal activation in response to the appearance of such favorable conditions (Weiner 1987).

The transition to the spring phenotype is energetically demanding. Hamsters typically increase body mass by 30–50% (Wade and Bartness 1984), initiate growth of the testes (Hoffmann 1978), molt to a less insulative fur (Heldmaier and Steinlechner 1981; Kauffman et al. 2001), and undergo substantial increases in gonadal steroidogenesis (Tsutsui et al. 1989; Prendergast et al. 2006). Not surprisingly, in laboratory conditions of ad libitum (AL) food availability, hamsters undergoing gonadal recrudescence exhibit marked increases in voluntary food intake (Masuda and Oishi 1995). Unknown, however, is how the persistent scarcity of food during mid-to-late winter impacts the development of photorefractoriness and gonadal recrudescence in hamsters' natural habitat (Weiner 1987). The overwhelming majority of investigations of reproductive photorefractoriness in rodents have been conducted under conditions of AL food availability. In the single exception, hamsters housed for an extended interval in a fixed short photoperiod exhibited high rates of mortality following restricted feeding, prompting the conjecture that increased energy expenditure associated with the development of photorefractoriness may have exceeded a fixed energy budget

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(Masuda and Oishi 1995). This remarkable outcome suggested that refractoriness-induced development of the reproductive system may have an absolute requirement for increases in energy availability. Ambiguities associated with reproductive measurements, supplemental feeding during the putative restriction period, and stress responses to extended intervals of food deprivation preclude unambiguous conclusions from this study (Masuda and Oishi 1995); however, if an increase in energy availability is mandatory for photorefractoriness-induced vernal reproductive development to occur, then this would pose a direct challenge to current notions of the ecological significance of photorefractoriness.

In the following study, hamsters were born into a simulated natural photoperiod (SNP), and a restricted feeding protocol was instantiated at the winter solstice. The timing of reproductive recrudescence or spontaneous development was assessed over the next several months. If increases in energy availability are required for refractoriness-induced gonadal development, then food rationing should inhibit vernal reproductive development. In contrast, if spontaneous gonadal development occurs independent of the appearance of additional energy in the environment, then food-rationed hamsters should initiate gonadal development in a manner indistinguishable from that of ad libitum-fed controls.

MATERIALS AND METHODS

Animals and breeding

A dedicated breeding colony generated male offspring for this experiment. Adult (60–120 days of age; $n = 20$) female Siberian hamsters (*Phodopus sungorus*) from a breeding colony maintained on 15 h light/day (15L) were singly housed and introduced into a SNP controlled by an electronic timer (Paragon EC71ST). The midpoint of the SNP light phase was matched to the photophase midpoint of the laboratory breeding colony. The SNP generated a sinusoidally changing pattern of day length which mimicked that occurring at 53°N latitude (zenith = 16.9L; nadir = 7.6L); this latitude approximates the northern extent of this species' range and is near the collection site of the founder animals of our colony (Figure 1; Ross 1998; Wynne-Edwards et al. 1999). Breeder females were moved into the SNP 8 weeks prior to the summer solstice photoperiod (i.e., on week -8; all week designations are relative to the solstice). During week -6, week +2, and week +9, stud males from the lab breeding colony were placed in females' cages and were removed 7 days later; 18–25 days later, females' cages were inspected for litters. Litters were born during week -3 (Cohort 1, late spring; $n = 12$ litters, 36 male offspring), week +5 (Cohort 2, midsummer; $n = 16$ litters, 61 male offspring), and week +12 (Cohort 3, late summer; $n = 11$ litters, 35 male offspring); the last births correspond to mid-September, the end of the breeding season in nature (Weiner 1987). Pups were weaned at 18–25 days of age and were singly housed in the SNP.

All animals were housed in polypropylene cages (28 × 17 × 12 cm) with wood shaving beddings (Harlan Sani-Chips; Harlan Inc., Indianapolis, IN). Ambient temperature of the room was 20 ± 0.5 °C, and relative humidity was maintained at $53 \pm 2\%$. Filtered tap water was available ad libitum. Food (Teklad Rodent Diet 8604; Harlan Inc.) was available ad libitum except during periods of food restriction (see below). All procedures conformed to the USDA Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Somatic and reproductive measures

Offspring were weighed (to 0.1 g) at 4 weeks of age and weekly thereafter.

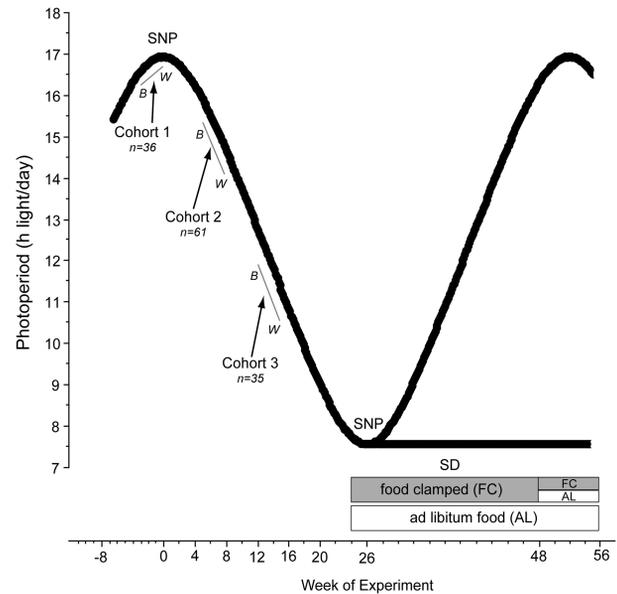


Figure 1

The SNP (53°N latitude) is shown schematically, along with the birth (B) and weaning (W) times for each of 3 birth cohorts. Food measurements began on week 18, and food clamping (FC) manipulations began on week 24. A subset of hamsters from each cohort was transferred to the winter solstice photoperiod (7.6L; "SD") on week 26; all others remained in the SNP. On week 48, a subset of FC hamsters was returned to AL feeding.

For each hamster, an estimated testis volume (ETV) was determined on week +3 (Cohort 1) and again on weeks +10 (Cohorts 1 and 2), +17, and +24 (Cohorts 1–3). Beginning on week +24, ETV was determined at 2-week intervals. ETVs were obtained under light isoflurane anesthesia (3% in medical O₂). The fur ventral to the left testicle was shaved, the testicle was palpated externally with forceps, and the length (L) and width (W) were then measured (to 0.1 mm) using analog calipers. ETV is calculated as the product of testis ($L^2 \times W$); this measure correlates ($R^2 > 0.9$) with testis mass and is not significantly affected by repeated measurements (Gorman and Zucker 1995b).

ETVs obtained through week +24 were used to identify hamsters as reproductively responsive to short days and to identify whether puberty was delayed in response to the SNP. Hamsters with ETVs ≥ 400 on week +24 were considered to be short-day nonresponders (Prendergast et al. 2001) and were excluded from all subsequent analyses (Butler, Turner, et al. 2007).

Also on week +24, the reproductive developmental phenotype of photoresponsive hamsters was established (Hoffmann 1978). ETVs ≥ 400 on either week +5 or week +12 weeks of age indicated rapid gonadal development (RGD) followed by inhibition in response to the decreasing SNP. ETVs < 400 on both week +5 and week +12 indicated delayed puberty (DP).

Food manipulations

In order to identify basal (winter nadir) food intake and tailor food restriction to each individual animal, weekly food consumption in short days was measured for each hamster between weeks +19 and +24. Food in the cage hopper was weighed weekly (to 0.01 g), and uneaten food in the cage bottom was subtracted. Weekly food intake was divided by 7 to generate mean daily food intake. Intake was examined over 5 weeks, and it was determined that groupwise intake reached a plateau between weeks +22 and +24. Therefore, for each food-restricted hamster, mean daily food intake during these 2 weeks identified winter food intake.

On week 24, hamsters were randomly assigned to 1 of 2 feeding treatments. In one group, each hamster was provisioned daily with an amount of food equal to its mean daily food intake as measured between weeks +22 and +24 (food clamped [FC] group, $n = 58$). Food was preweighed and delivered daily within 20 min of lights-off. Food aliquots ranged from 1.6 to 4.3 g (mean \pm standard deviation = 3.0 ± 0.6 g). Hamsters were not permitted to cache food. Each day, on food delivery, the cage floor was inspected for unconsumed pieces of the previous food aliquot, and hamsters were observed for distended cheek pouches. Any leftover food was removed and weighed; in the course of the experiment, no hamsters were observed to have food in the cheek pouches at the time of daily food delivery. The remaining hamsters were allowed free access to food at all times (ad libitum, "AL" group, $n = 49$). Weekly food intake was measured, as described above, in AL hamsters.

Hamsters were inspected daily for signs of severe adverse consequences due to food restriction. Evidence of death, or hunched or recumbent posture, combined with unresponsiveness to experimenter handling; evidence of absence of grooming behavior and general inactivity in the absence of torpor (Elliott et al. 1987; Kirsch et al. 1991) were considered symptoms of severe intolerance of the FC procedure. Hamsters that exhibited such symptoms on 3 successive days with no signs of recovery were determined to be intolerant of the food restriction protocol and in unnecessary distress and were removed from the study. A total of 8 hamsters were intolerant of the FC protocol and were removed from the study.

Photoperiod manipulations

On week +26 (winter solstice), all AL and FC hamsters were randomly assigned to 1 of 2 photoperiod treatments. One group of hamsters was transferred into a photoperiod that remained fixed at the solstice photoperiod of 7.6L for the remainder of the study ("SD"; FC: $n = 29$; AL: $n = 24$). The other group remained in the increasing SNP ("SNP"; FC: $n = 29$; AL: $n = 25$). The time of food delivery remained locked to within 20 min of lights-off within each photocycle.

Removal of food clamp

Photoperiod and food manipulations remained as above until week 48, at which time FC hamsters in both photoperiods were randomly assigned into 1 of 2 groups. In one group, FC hamsters was returned to AL food (FC \rightarrow AL; SNP: $n = 13$; SD: $n = 11$). In the other group, FC hamsters continued to receive the FC treatment (FC \rightarrow FC; SNP: $n = 12$; SD: $n = 12$).

Statistical analyses

Changes in body mass, ETV, and food intake were analyzed using a between groups, repeated measures analysis of variance (ANOVA) with photoperiod (SD and SNP) and feeding condition (AL and FC) as between group variables. Pairwise comparisons were conducted using Fisher's protected least-significant difference tests, where permitted by a significant F -statistic. Within-group longitudinal changes in ETV and body mass were analyzed using repeated measures ANOVA; increases in these measures were also examined using paired t -tests. Frequencies of categorical events were compared using χ^2 tests. Differences were considered significant if $P < 0.05$.

RESULTS

Reproduction in the SNP

A total of 39 litters yielding 132 male offspring were born across the 3 cohorts (Figure 1). The incidence of reproductive non-

responsiveness to the SNP ranged from 18% to 20% across cohorts, and these animals were omitted from all subsequent analyses. Reproductive developmental trajectories differed markedly across cohorts: 100% ($n = 29$) of photoresponsive males in Cohort 1 exhibited RGD; in contrast, 20% ($n = 10$) and 14% ($n = 4$) of males in Cohorts 2 and 3 exhibited RGD, whereas the remainder exhibited DP (Figure 1).

Reproductive and somatic responses to the SNP

Between birth and week 24, hamsters of both developmental trajectories exhibited reproductive inhibition in the SNP (Figure 2A). RGD hamsters underwent gonadal regression (Figure 2B), and DP hamsters maintained undeveloped testes (Figure 2C). In all cohorts, progressive postnatal increases in body mass reached a plateau at 25–30 g by week 24 (Figure 2D–F). On week 24, neither ETV ($F_{1,105} = 0.28$; $P > 0.50$) nor body mass ($F_{1,105} = 0.07$; $P > 0.70$) differed between groups that had previously undergone different developmental trajectories.

Somatic and reproductive responses to food manipulations

Post-solstice food intake

AL food intake changed significantly over time following the solstice ($P < 0.001$, all comparisons). In the SNP, pre-solstice reproductive developmental phenotype (RGD vs. DP) modestly affected the pattern of change in food intake ($F_{21,483} = 1.6$, $P < 0.05$): Sustained increases in food intake (relative to nadir food intake levels) were evident beginning at week 36 in DP hamsters and at week 38 in RGD hamsters (Figure 3A). In SD, increases in food intake were less robust relative to those evident in the SNP, but their patterns differed between reproductive phenotype ($F_{21,441} = 2.9$, $P < 0.001$): Elevations in food intake were first evident on at week 27 in DP hamsters and at week 45 in RGD hamsters (Figure 3B).

Hamsters exhibiting DP

Food manipulations began on week 24, and photoperiod manipulations were implemented on week 26. In the SNP, FC treatments significantly inhibited testis size ($F_{1,24} = 6.2$, $P < 0.05$). DP hamsters that remained in the SNP and were fed AL likewise maintained small testes. The first significant increase in ETV relative to winter solstice (week 26) values occurred on week 30 in AL hamsters ($t_{11} = 4.28$, $P < 0.001$) and on week 38 in FC hamsters ($t_{14} = 2.64$, $P < 0.05$). Further increases in ETV occurred between weeks 36 and 38 ($t_{11} = 2.53$, $P < 0.05$), followed by a marked increase between weeks 38 and 40 ($t_{11} = 9.16$, $P < 0.001$; Figure 4A). ETVs were comparable between AL and FC hamsters from weeks 26 to 38 ($P > 0.20$, all comparisons), but FC significantly inhibited testis size thereafter (Figure 4A; $P < 0.005$, all comparisons). In the SNP, AL hamsters exhibited mean ETV > 400 on week 40, whereas ETVs of FC hamsters remained below this value until week 48. At week 48, ETV > 400 in 12 of 17 FC hamsters and in 12 of 12 AL hamsters ($\chi^2 = 2.5$, $P > 0.10$).

DP hamsters that were kept in SD after the winter solstice also maintained gonadal involution, followed by an interval of gonadal growth ($F_{1,29} = 36.8$, $P < 0.001$). AL hamsters in SD had small testis sizes between weeks 26 and 38, after which ETV increased significantly over the next 10 weeks ($F_{10,130} = 14.5$, $P < 0.001$), indicative of refractoriness to the SD photoperiod. The first significant increase in ETV relative to winter solstice values occurred on week 40 in SD-AL hamsters ($t_{13} = 2.44$, $P < 0.05$) and on week 48 in FC hamsters ($t_{16} = 2.63$, $P < 0.05$). Significant increases in ETV occurred in AL hamsters between weeks 38 and 40 ($t_{13} = 5.36$, $P < 0.001$;

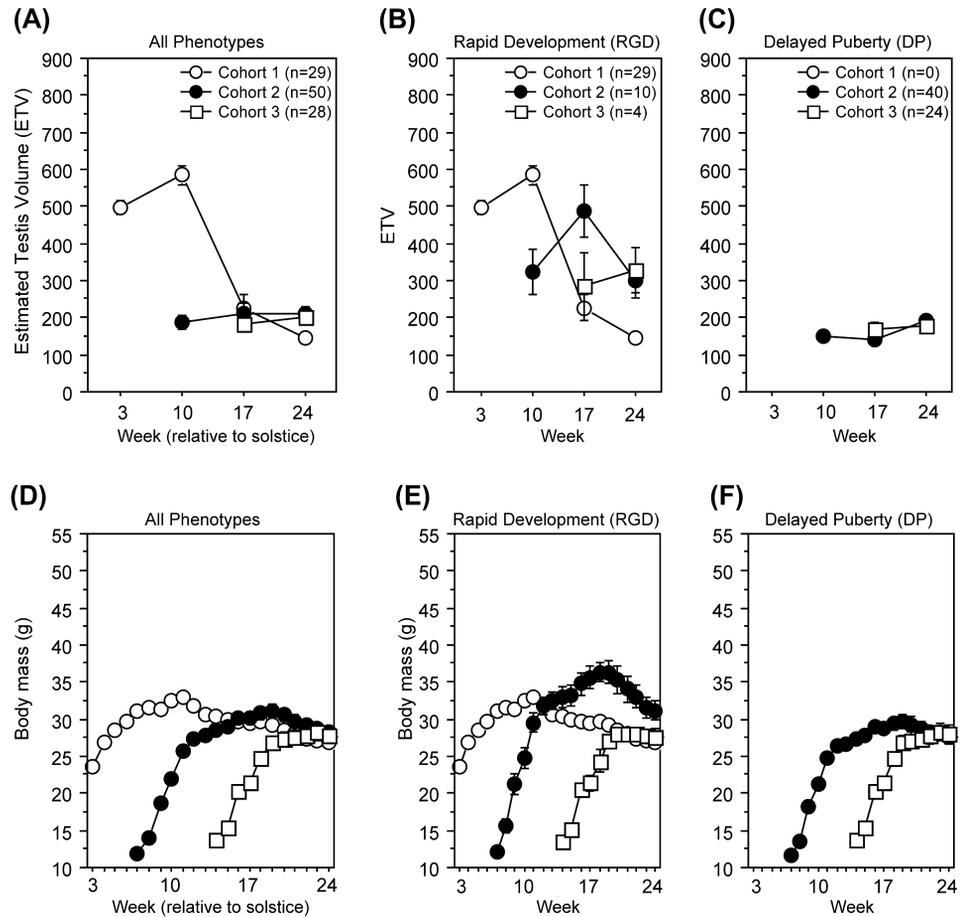


Figure 2

Mean \pm standard error of the mean ETV (mm^3) (A–C) and body mass (D–F) of photoreponsive male Siberian hamsters born into an SNP -3 weeks (Cohort 1), $+5$ weeks (Cohort 2), and $+12$ weeks (Cohort 3) relative to the summer solstice (week 0). Hamsters exhibited either rapid gonadal development (RGD) (B and E) followed by gonadal regression or delayed puberty (DP) (C and F) in the SNP.

Figure 4B). FC hamsters in SD exhibited modest, gradual increases in testis size between weeks 26 and 48 ($F_{10,160} = 6.0$, $P < 0.001$); testes of FC hamsters were significantly smaller than those of AL hamsters on weeks 30 through 48 (Figure 4B; $P < 0.05$, all comparisons). In SD, AL hamsters exceeded mean ETV >400 on week 40, whereas mean ETV of FC hamsters remained <400 through week 48. On week 48, ETV >400 in 13 of 14 FC hamsters but in only 4 of 21 AL hamsters ($\chi^2 = 15.5$, $P < 0.001$).

For both RGD and DP hamsters receiving AL treatments, body masses of SNP hamsters were significantly lower than those of SD hamsters on week 26. This initial difference is not explicable by prior photoperiod treatments, which were identical in the 2 groups prior to week 26, and is likely due to chance. Because of these initial differences, comparisons of absolute body mass values of SD and SNP hamsters were not warranted. Body mass was likewise inhibited by FC treatments in both photoperiods (SNP: $F_{1,24} = 13.2$, $P < 0.005$; SD: $F_{1,29} = 20.7$, $P < 0.001$). In the SNP, body mass of AL hamsters increased by 80% between weeks 26 and 48 ($F_{22,242} = 123.2$, $P < 0.001$), with the first significant 2-week increase evident between weeks 34 and 36 ($P < 0.05$); body mass of FC hamsters increased by 15% during this interval ($F_{22,286} = 9.1$, $P < 0.001$; Figure 4C). In SD, body mass of AL hamsters increased by 38% between weeks 26 and 48 ($F_{22,286} = 48.7$, $P < 0.001$), whereas that of FC hamsters did not change significantly (3% decrease; $F_{22,352} = 1.4$, $P > 0.10$). Body mass of AL hamsters exceeded that of FC hamsters as early as week 26, and successive 2-week increases in body mass were first evident in SD-AL hamsters beginning on week 33 (Figure 4D).

Hamsters exhibiting RGD

Food manipulations significantly altered patterns of testis growth in hamsters that had exhibited autumnal RGD (SNP: $F_{1,22} = 75.4$, $P < 0.001$; SD: $F_{1,14} = 5.7$, $P < 0.05$; Figure 5A). In the SNP, both AL ($F_{10,120} = 28.9$, $P < 0.001$) and FC ($F_{10,100} = 20.9$, $P < 0.001$) hamsters exhibited gonadal regression followed by regrowth, but ETVs of AL hamsters significantly exceeded those of FC hamsters from weeks 34 through 48 ($P < 0.005$, all comparisons; Figure 5A). The first significant increase in ETV relative to winter solstice (week 26) values occurred on week 28 in RGD-AL hamsters ($t_{12} = 2.30$, $P < 0.05$) and on week 40 in FC hamsters ($t_{10} = 3.23$, $P < 0.01$). In the SNP, AL hamsters achieved mean ETV >400 on week 40, whereas FC hamsters first exceeded this criterion on week 48. In SD, neither AL nor FC hamsters exceeded mean ETV of 400.

In SD, ETVs were smaller in FC hamsters on several measurement weeks between weeks 26 and 48 ($P < 0.05$, all comparisons; Figure 5B). Both AL and FC hamsters maintained gonadal involution, and neither group exhibited significant testicular recrudescence by week 48 (AL: $F_{10,90} = 0.2$, $P > 0.9$; FC: $F_{10,50} = 1.1$, $P > 0.3$).

Body mass increases were inhibited by FC in both photoperiods (SNP: $F_{1,22} = 41.7$, $P < 0.001$; SD: $F_{1,14} = 10.6$, $P < 0.01$; Figure 5C,D). In the SNP, body mass of AL hamsters increased by 71% between weeks 26 and 48 ($F_{22,264} = 83.6$, $P < 0.001$), with the first significant 2-week increase evident between weeks 33 and 35 ($P < 0.05$). Body mass of FC hamsters increased by 8% during this interval ($F_{22,220} = 17.9$, $P < 0.001$; Figure 5C), with significant increases beginning on week 39. Mean body mass of SNP-AL hamsters exceeded that of SNP-FC hamsters beginning on week 28 ($P < 0.05$, all

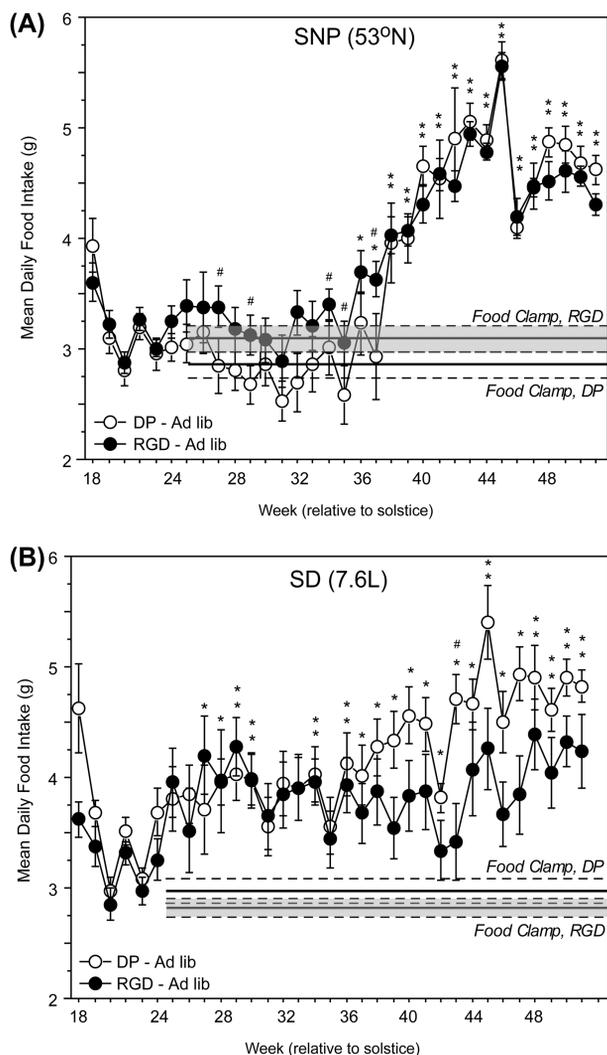


Figure 3
Mean \pm standard error of the mean daily food intake between week 18 and week 52 of hamsters exhibiting rapid gonadal development (RGD) or delayed puberty (DP) in the SNP. Food was weighed weekly, and a daily average was calculated. Food clamp manipulations began on week 24, and on week 26, hamsters either (A) remained in the SNP or (B) were maintained at the solstice photoperiod of 7.6L (SD) for the next 26 weeks. AL-fed values are depicted with symbols, and food clamp (FC) values are depicted with a horizontal line. * $P < 0.05$ versus FC value, within developmental phenotype group. # $P < 0.05$ versus RGD-AL.

comparisons). In SD, body mass of AL hamsters increased by 10% between weeks 26 and 48 ($F_{22,198} = 0.7$, $P > 0.80$), whereas that of FC hamsters decreased by 8% over this interval ($F_{22,110} = 2.5$, $P < 0.005$). FC hamsters weighed less than AL hamsters from weeks 26 to 48 (Figure 5D).

Early removal/mortality

A total of 8 hamsters were removed from the study early or did not survive the FC treatment. The mean (\pm standard deviation) time of removal/death was week 35.5 (± 4), with a range from week 29 to week 41. All cases of early removal/death were in FC hamsters. Neither developmental trajectory nor photoperiod significantly affected the probability of mortality/removal ($\chi^2 < 1.0$, $P > 0.10$, all comparisons).

Responses to discontinuation of food clamp

DP hamsters

In the SNP, termination of FC treatment on week 48 significantly altered testis size ($F_{2,23} = 16.6$, $P < 0.001$) and body mass ($F_{8,92} = 27.5$, $P < 0.001$). Gonadal growth was evident in FC \rightarrow AL hamsters within 4 weeks (Figure 6A), and increases in body mass were evident within 1 week (Figure 6B). A similar pattern of outcomes obtained in SD hamsters (ETV: $F_{2,28} = 7.2$, $P < 0.005$; body mass: $F_{8,112} = 16.4$, $P < 0.001$; Figure 6C,D).

RGD hamsters

In the SNP, testis size ($F_{2,21} = 20.3$, $P > 0.0001$) and body mass ($F_{8,84} = 41.8$, $P < 0.001$) of RGD hamsters likewise increased following discontinuation of the FC treatment. Testis sizes of FC \rightarrow AL hamsters were comparable to those of AL hamsters within 4 weeks (Figure 7A), and sustained body mass increases were evident within 1 week of ad lib feeding (Figure 7B). In SD, termination of FC significantly affected the pattern of change in testis size ($F_{2,13} = 5.1$, $P < 0.05$) and body mass ($F_{8,52} = 41.7$, $P < 0.0001$) over the next 4 weeks. Testis responses were highly variable across groups, but ETV of FC \rightarrow AL hamsters increased significantly from weeks 48 to 52 ($P < 0.05$; Figure 7C), and sustained increases in body mass were also evident in this group (Figure 7D).

DISCUSSION

In the present study, hamsters born into an SNP exhibited gonadal regression, and extended maintenance in inhibitory photoperiods (winter SNP or static SD) yielded refractoriness-induced gonadal growth. The experimental goal was to determine if preventing increases in energy intake would affect the development of photorefractoriness. Food rationing that mimicked winter solstice energy availability conspicuously altered the timing and/or magnitude of refractoriness-induced vernal reproductive development, and these effects varied as a function of autumnal reproductive trajectory (DP vs. RGD) and experimental photoperiod (SNP vs. SD). In the most extreme case, DP hamsters, food restriction delayed the onset of gonadal growth in SD and inhibited the magnitude of growth in both SD and SNP. In RGD hamsters, the timing of recrudescence was delayed, and gonadal growth was inhibited by food restriction in the SNP. Together, these data suggest that refractoriness-induced gonadal growth requires increases in environmental food availability and food intake.

Limits to food availability inhibited vernal reproductive development, and these effects were exaggerated in the static SD photoperiod. In SD, DP hamsters fed AL exhibited spontaneous puberty by week 40; in contrast, FC hamsters exhibited sizeable decrements in vernal reproductive development (Figure 4). At week 48, both groupwise (mean ETV < 400) and idiographic (only 19% of individuals exhibited ETV > 400) analyses indicated that DP-FC hamsters had still not accomplished substantial testicular growth. Reproductive effects of FC were largely absent among RGD hamsters in SD: Neither FC nor AL groups had exhibited recrudescence by week 48 in SD (Figure 5). The majority (67%) of these RGD hamsters were born prior to the summer solstice; delayed recrudescence in hamsters born prior to the solstice has been reported previously in this species (Butler, Trumbull, et al. 2007; Butler, Turner, et al. 2007).

Reproductive responses of AL hamsters in SD were consistent with earlier work in this species, indicating that sinusoidally changing photoperiod information acquired prior to the winter solstice is sufficient to set the interval timer that

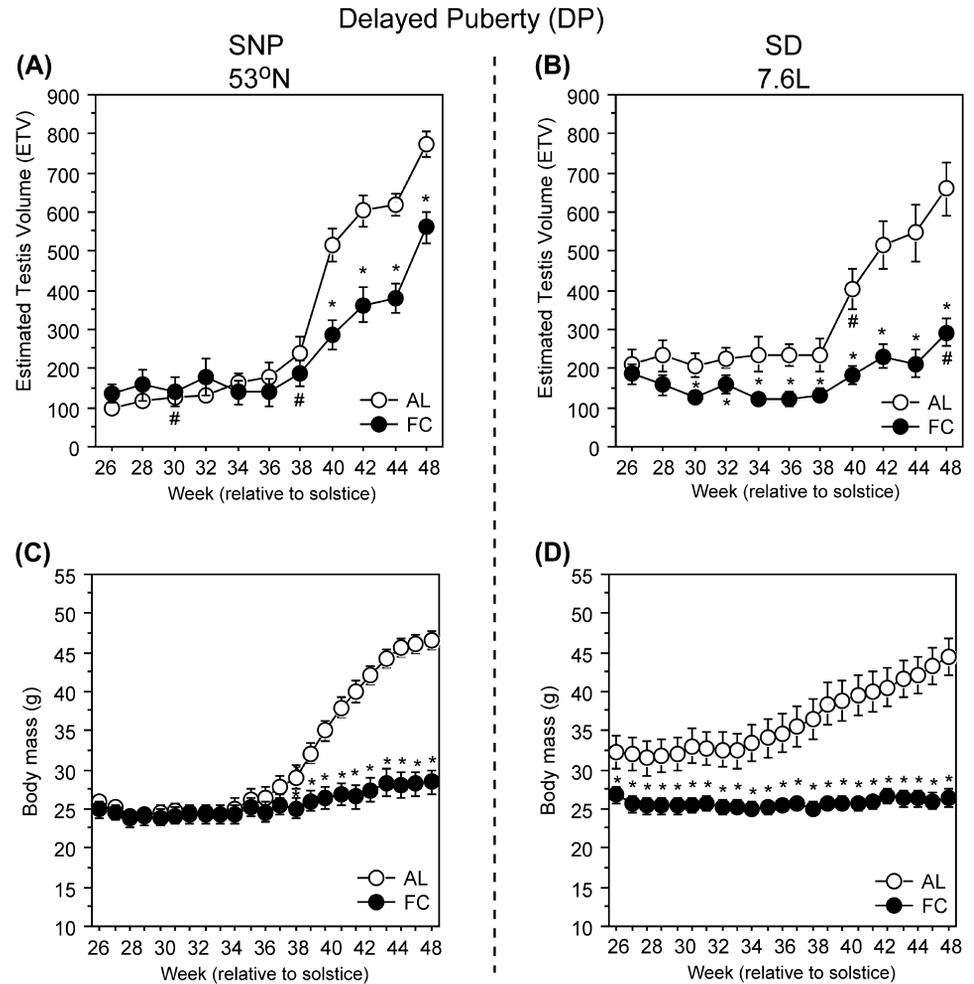


Figure 4

Mean \pm standard error of the mean testis volume (ETV [mm³]; A and B) and body mass (C and D) of male Siberian hamsters exhibiting delayed puberty (DP) following birth into the SNP. On week 24, hamsters were provided with a daily ration of food equal to their nadir short-day food intake (food clamp, FC) or were fed AL. On week 26, hamsters either (A and C) remained in the SNP or (B and D) were maintained at the solstice photoperiod of 7.6L (SD) for the next 26 weeks. * $P < 0.05$ versus AL value. # (below symbol) $P < 0.05$ versus solstice value, within group.

phases vernal gonadal regrowth (Gorman 2003; Butler, Turner, et al. 2007) and that post-solstice increases in day length are not required for growth to occur (Gorman and Zucker 1995a). Among AL hamsters, increases in food intake were also occurring during this interval (weeks 35–40) of gonadal growth. The interval timer evidently triggers parallel increases in food intake and reproductive development, but absent this ability to acquire additional energy, substantial delays in gonadal growth are evident.

In the SNP condition, both RGD-AL and DP-AL hamsters exhibited post-solstice gonadal growth. But in contrast to the SD condition, gonadal development was not abolished altogether by the FC treatment in the SNP condition. Rather, growth was delayed and decreased in magnitude by FC (Figures 4A and 5A). Whereas AL-fed hamsters in the SNP initiated gonadal growth in advance of the appearance of long (14L) day lengths (DP-AL: week 30; RGD-AL: week 28), FC hamsters initiated gonadal growth on week 38, only after day lengths exceeded 14L. Thus, in the SNP condition, FC treatments inhibited gonadal development, but this effect was diminished relative to the SD condition. Moreover, the timing of recrudescence among FC hamsters in the SNP was delayed in a noteworthy fashion: the coincidence of photostimulatory long days with gonadal growth, circa week 38, in SNP-FC hamsters suggests that such growth may have been a result of direct photostimulation by categorically long days rather than driven solely by refractoriness to categorically short-day lengths.

Qualitatively, similar effects of FC were evident on the accretion of body mass (Figures 4C,D and 5C,D). In DP and RGD hamsters, FC completely inhibited body mass gain between weeks 26 and 48 in SD hamsters and markedly suppressed body mass gain in SNP hamsters. Even in DP-FC hamsters exhibiting gonadal recrudescence, body mass did not increase coincidentally. These data are reminiscent of effects described in male mice and rats, which when maintained at a fraction (45% or 60%) of adult body mass via restricted feeding, nevertheless undergo qualitatively normal reproductive development (Hamilton and Bronson 1986). Relative to mice and rats, Syrian hamsters are more reproductively responsive to energy restriction—maintenance of Syrian hamsters at 75% of normal body mass causes a substantial, albeit incomplete, inhibition of testis size (Eskes 1983). Siberian hamsters appear to be capable of initiating gonadal recrudescence absent any appreciable increases in body mass; indeed, in the face of a finite energy budget, testicular recrudescence appears to take precedence over somatic recrudescence, and pubertal development can be initiated absent increases in body size.

In all conditions, the discontinuation of food rationing on week 48 triggered gonadal growth (Figures 6 and 7). Increases in ETV during the first 4 weeks after restoration of AL feeding were greatest in DP hamsters: ETV increased by 75% in SNP hamsters and by >90% in SD hamsters. Simultaneous increases in ETV were not evident in hamsters that remained on the FC treatment from weeks 48 to 52. In SD hamsters, we interpret this rapid growth to indicate that the return to AL feeding disinhibited an already refractory hypothalamo-

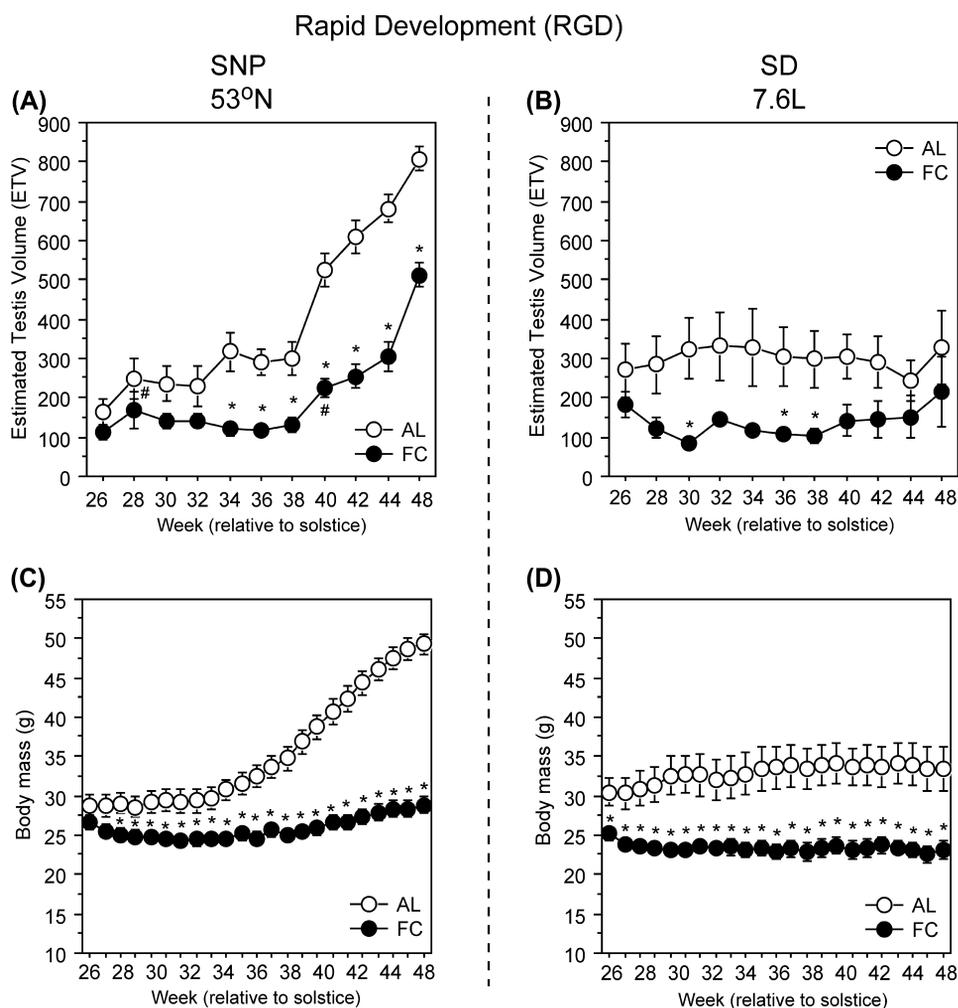


Figure 5
Mean \pm standard error of the mean testis volume (ETV [mm³]; A and B) and body mass (C and D) of male Siberian hamsters exhibiting rapid gonadal development (RGD) following birth into the SNP. On week 24, hamsters were provided with a daily ration of food equal to their nadir short-day food intake (food clamp, FC) or were fed AL. On week 26, hamsters either (A and C) remained in the SNP or (B and D) were maintained at the solstice photoperiod of 7.6L (SD) for the next 26 weeks. * $P < 0.05$ versus AL value. # (below symbol) $P < 0.05$ versus solstice value, within group.

pituitary-gonadal (HPG) axis. In SNP hamsters, rapid growth following restoration of ad libitum feeding was presumably driven by a combination of refractoriness and a direct stimulatory effect of photoperiod (which had exceeded 15L by week 48).

The present report is one of few studies examining properties of the photorefractoriness interval timer under conditions that approximate limited food availability in nature. In a related report, hamsters housed for an extended interval in a fixed short photoperiod were subjected to restricted feeding; this was accomplished via an episodic starvation protocol consisting of bolus food delivery every 2 days beginning shortly before the onset of SD photorefractoriness or coincident with transfer from long to short days (Masuda and Oishi 1995). Approximately 70% of these hamsters died during the first 10 weeks of food restriction, leading the authors to conclude that increased energy expenditure associated with gonadal growth exceeded a finite energy budget (Masuda and Oishi 1995). Mortality was comparatively lower in the present study: 8 of 107 hamsters died or were determined to be intolerant of the FC treatment. When mortality was evident, it typically occurred during the interval immediately preceding the onset of gonadal growth, a coincidence which suggests the conjecture that individuals that do attempt to initiate gonadal development in the face of limited food availability rapidly enter a state of negative energy balance which may be so severe that it leads to mortality. The lower incidence of mortality in the present study may have been a result of the

daily availability of small amounts of food. In nature, Siberian hamsters forage nightly throughout the winter months (Weiner 1987); daily food intake may be essential to over-winter survival and anticipatory breeding in late winter.

Prolonged exposure to short days is a common laboratory model for mechanistic studies of photorefractoriness (e.g., Hoffmann 1982; Gorman and Zucker 1995b; Prendergast et al. 2002; Johnston et al. 2003; Watanabe et al. 2007). In a fixed SD paradigm, FC treatments block the onset of gonadal growth, and restoration of AL food unmasks a refractory HPG axis, triggering rapid development. The data suggest that, under this paradigm, the development of photorefractoriness arises from a disinhibition of the reproductive axis from photoperiodic control (i.e., from inhibition by short days). In the majority of laboratory studies, food is available ad libitum and vernal gonadal development, or spontaneous recrudescence, reliably occurs following prolonged exposure to short days. In nature, when food is scarce and unreliable in the timing of its spring appearance (Paul et al. 2009), refractoriness may occur in late winter, leaving the HPG axis primed for rapid responses to the appearance of food in the environment. Vernal recrudescence may be strongly dependent on proximate control by energy availability, with full gonadal development only occurring under permissive conditions of abundant food.

Gonadal regrowth occurred substantially later in DP hamsters subjected to the food clamping manipulation, but the present data do not permit inferences regarding whether

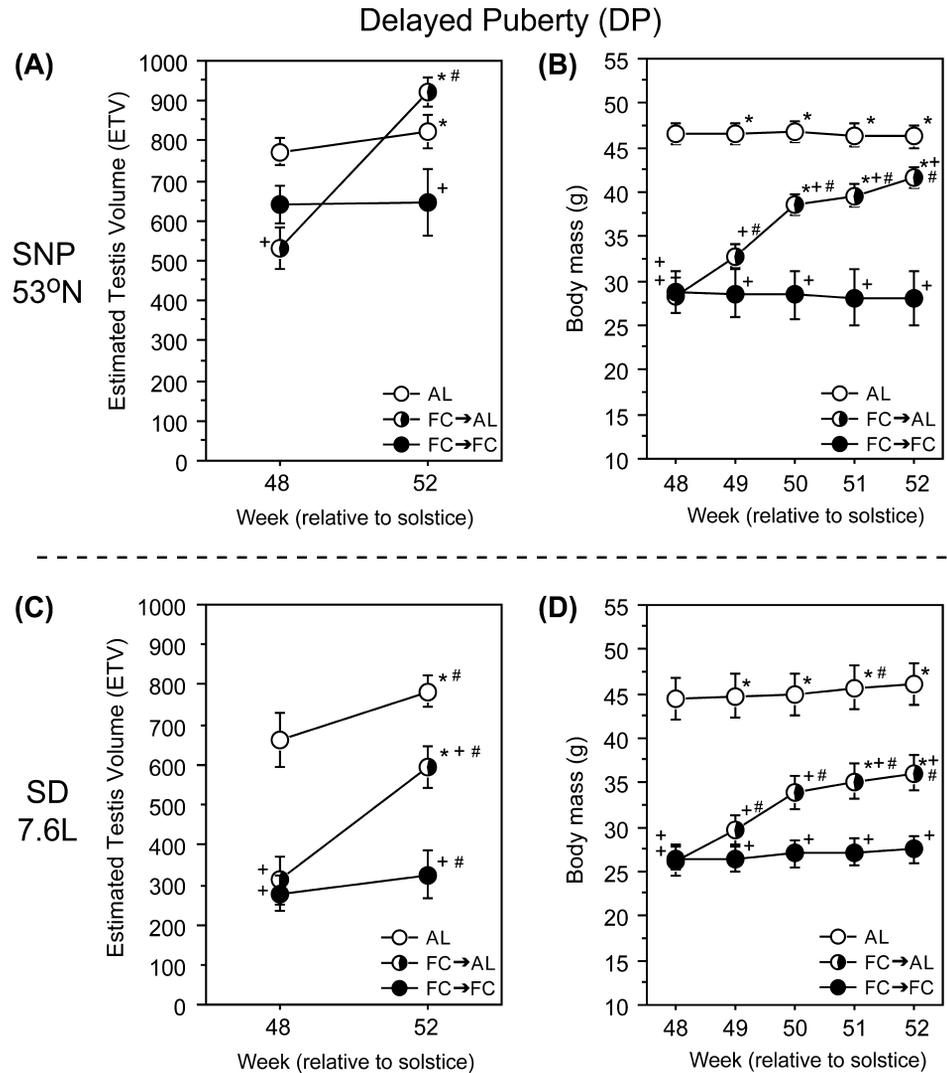


Figure 6

Mean \pm standard error of the mean testis volume (ETV [mm^3]; A and C) and body mass (B and D) of male Siberian hamsters exhibiting delayed puberty (DP). From weeks 24 to 48, hamsters were provided with daily food rations (FC) or AL access to food. On week 48, a subset of previously FC hamsters was allowed AL access to food (FC \rightarrow AL), whereas the remainder of the FC hamsters remained on food rationing (FC \rightarrow FC). * $P < 0.05$ versus FC \rightarrow FC; + $P < 0.05$ versus AL; # $P < 0.05$ versus week 48 value, within group.

the timing of neuroendocrine photorefractoriness was delayed in these hamsters. It is possible that neuroendocrine refractoriness occurred along a normal timecourse in DP-FC hamsters, but gonadal recrudescence per se was masked by the effects of FC on gonadotrophin secretion (Sisk and Bronson 1986). Such a hypothesis could be critically evaluated via measures of gonadotrophin secretion. Photorefractoriness to short days is accompanied by enhanced gonadal hormone-independent stimulation of luteinising hormone (LH) secretion and diminished sensitivity to inhibitory negative feedback effects of testosterone (T) on LH secretion (Prendergast et al. 2006). If SD-FC hamsters on week 42 (after recrudescence had occurred in AL-fed hamsters, but before FC hamsters had exhibited significant gonadal growth) were to exhibit diminished sensitivity to negative feedback effects of T on LH secretion, then this would suggest that neuroendocrine events associated with refractoriness to short days were occurring but were evidently being masked by caloric restriction. Alternatively, if changes gonadotrophin sensitivity did not occur until after week 48 (when recrudescence was finally evident in DP-FC hamsters in SD hamsters), then such an outcome would suggest that FC had indeed inhibited the development of refractoriness to short days. Evaluation of changes in LH sensitivity to steroid-dependent and -independent restraint awaits further study.

In summary, the present data provide additional insights into the ecological significance of the refractoriness interval timer. The timing of vernal reproductive development is markedly influenced by environmental food availability. Under photoperiod conditions where refractoriness to short days is providing the sole stimulatory drive on the reproductive axis, food restriction largely blocked gonadal recrudescence. Under photoperiod conditions where both refractoriness to short-day and long-day photostimulation are collectively driving the HPG axis, gonadal growth was both delayed in onset and reduced in magnitude by food restriction. The data are compatible with a model in which the development of photorefractoriness incurs a disinhibition, rather than an obligate stimulation, of the HPG axis. Under the highly permissive and ecologically unlikely conditions of ad libitum food availability in late winter, gonadal growth proceeds unabated. But under conditions in which abundant food does not appear coincident with the development of photorefractoriness, full reproductive development does not occur until photoperiods become categorically long, and even then, gonadal growth is significantly attenuated in magnitude.

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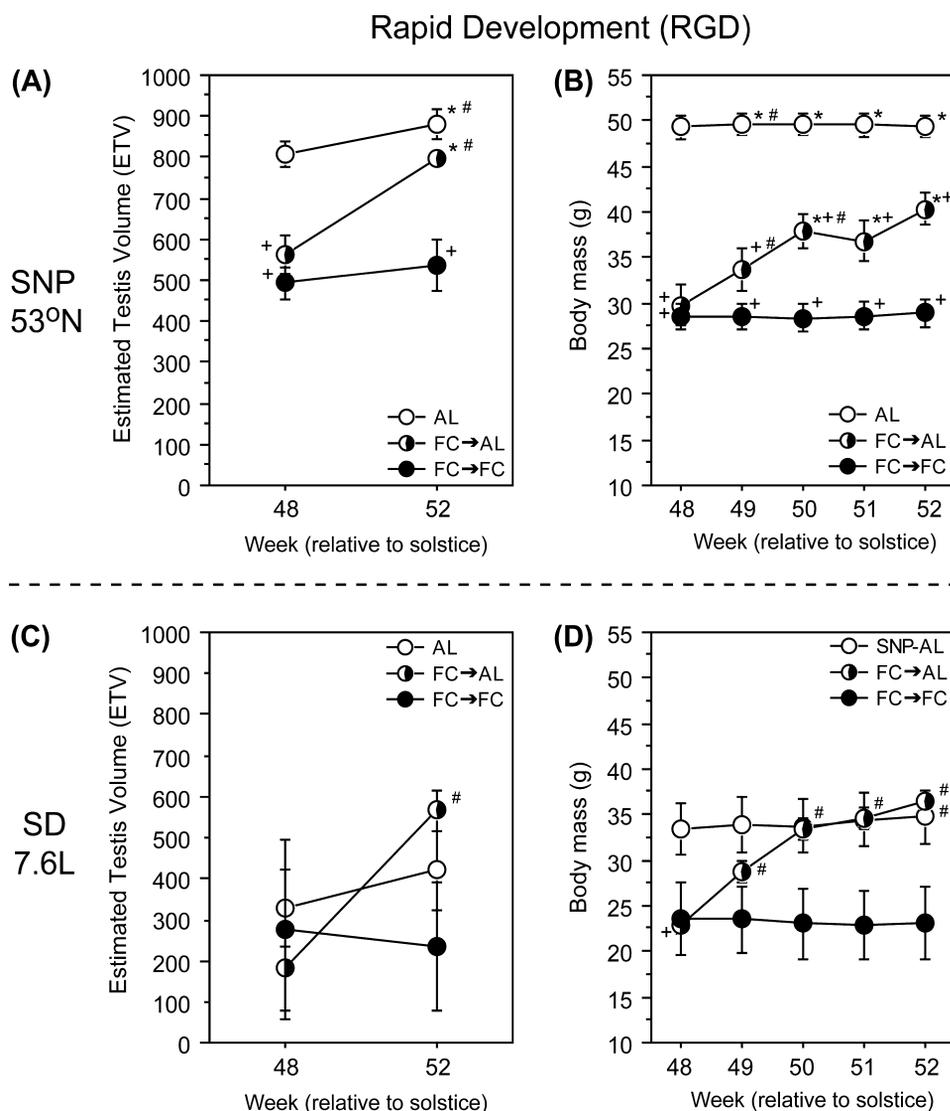


Figure 7
Mean \pm standard error of the mean testis volume (ETV [mm³]; A and C) and body mass (B and D) of male Siberian hamsters exhibiting rapid gonadal development (RGD). From weeks 24 to 48, hamsters were provided with daily food rations (FC) or AL access to food. On week 48, a subset of previously FC hamsters was allowed AL access to food (FC→AL), whereas the remainder of the FC hamsters remained on food rationing (FC→FC). * $P < 0.05$ versus FC→FC; + $P < 0.05$ versus AL; # $P < 0.05$ versus week 48 value, within group.

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REFERENCES

- Bronson FH. 1989. Mammalian reproductive biology. Chicago (IL): University of Chicago Press.
- Butler MP, Trumbull JJ, Turner KW, Zucker I. 2007. Timing of puberty and synchronization of seasonal rhythms by simulated natural photoperiods in female Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol*. 293:R413–R420.
- Butler MP, Turner KW, Park JH, Butler JP, Trumbull JJ, Dunn SP, Villa P, Zucker I. 2007. Simulated natural day lengths synchronize seasonal rhythms of asynchronously born male Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol*. 293:R402–R412.
- Butler MP, Turner KW, Park JH, Schoomer EE, Zucker I, Gorman MR. 2010. Seasonal regulation of reproduction: altered role of melatonin under naturalistic conditions in hamsters. *Proc R Soc B Biol Sci*. 277:2867–2874.
- Elliott JA, Bartness TJ, Goldman BD. 1987. Role of short photoperiod and cold exposure in regulating daily torpor in Djungarian hamsters. *J Comp Physiol A*. 161:245–253.

- Eskes GA. 1983. Gonadal responses to food restriction in intact and pinealectomized male golden hamsters. *J Reprod Fertil*. 68:85–90.
- Gorman MR. 1995. Seasonal adaptations of Siberian hamsters. I. Accelerated gonadal and somatic development in increasing versus static long day lengths. *Biol Reprod*. 53:110–115.
- Gorman MR. 2003. Melatonin implants disrupt developmental synchrony regulated by flexible interval timers. *J Neuroendocrinol*. 15:1084–1094.
- Gorman MR, Zucker I. 1995a. Seasonal adaptations of Siberian hamsters. II. Pattern of change in daylength controls annual testicular and body weight rhythms. *Biol Reprod*. 53:116–125.
- Gorman MR, Zucker I. 1995b. Testicular regression and recrudescence without subsequent photorefractoriness in Siberian hamsters. *Am J Physiol*. 269:R800–R806.
- Hamilton GD, Bronson FH. 1986. Food restriction and reproductive development: male and female mice and male rats. *Am J Physiol*. 250:R370–R376.
- Heldmaier G, Steinlechner S. 1981. Seasonal control of energy requirements for thermoregulation in the Djungarian hamster (*Phodopus sungorus*) living in natural photoperiod. *J Comp Physiol [B]*. 142:429–437.
- Hoffmann K. 1978. Effects of short photoperiods on puberty, growth and moult in the Djungarian hamster (*Phodopus sungorus*). *Reproduction*. 54:29–35.
- Hoffmann K. 1982. The critical photoperiod in the Djungarian hamster, *Phodopus sungorus*. In: Aschoff J, Daan S, Gross G, editors.

- Vertebrate circadian systems: structure and physiology. New York: Springer. p. 297–304.
- Johnston JD, Cagampang FRA, Stirland JA, Carr A-JF, White MRH, Davis JRE, Loudon ASI. 2003. Evidence for an endogenous per1- and ICER-independent seasonal timer in the hamster pituitary gland. *FASEB J.* 17:810–815.
- Kauffman AS, Cabrera A, Zucker I. 2001. Energy intake and fur in summer- and winter-acclimated Siberian hamsters (*Phodopus sungorus*). *Am J Physiol Regul Integr Comp Physiol.* 281:R519–R527.
- Kirsch R, Ouarour A, Pévet P. 1991. Daily torpor in the Djungarian hamster (*Phodopus sungorus*): photoperiodic regulation, characteristics and circadian organization. *J Comp Physiol A.* 168:121–128.
- Lerchl A, Schlatt S. 1993. Influence of photoperiod on pineal melatonin synthesis, fur color, body weight, and reproductive function in the female Djungarian hamster, *Phodopus sungorus*. *Neuroendocrinology.* 57:359–364.
- Masuda A, Oishi T. 1995. Effects of restricted feeding on the light-induced body weight change and locomotor activity in the Djungarian hamster. *Physiol Behav.* 58:153–159.
- Paul MJ, Galang J, Schwartz WJ, Prendergast BJ. 2009. Intermediate-duration day lengths unmask reproductive responses to nonphotic environmental cues. *Am J Physiol Regul Integr Comp Physiol.* 296:R1613–R1619.
- Prendergast BJ, Flynn AK, Zucker I. 2000. Triggering of neuroendocrine refractoriness to short-day patterns of melatonin in Siberian hamsters. *J Neuroendocrinol.* 12:303–310.
- Prendergast BJ, Hotchkiss AK, Wen J, Horton TH, Nelson RJ. 2006. Refractoriness to short day lengths augments tonic and gonadotrophin-releasing hormone-stimulated lutenising hormone secretion. *J Neuroendocrinol.* 18:339–348.
- Prendergast BJ, Kriegsfeld LJ, Nelson RJ. 2001. Photoperiodic polyphenisms in rodents: neuroendocrine mechanisms, costs, and functions. *Q Rev Biol.* 76:293–325.
- Prendergast BJ, Nelson RJ, Zucker I. 2009. Seasonal rhythms of mammalian behavioral neuroendocrinology. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, Rubin R, editors. *Hormones, brain, and behavior.* 2nd ed. San Diego (CA): Academic Press. p. 93–157.
- Prendergast BJ, Wynne-Edwards KE, Yellon SM, Nelson RJ. 2002. Photorefractoriness of immune function in male Siberian hamsters (*Phodopus sungorus*). *J Neuroendocrinol.* 14:318–329.
- Ross PD. 1998. *Phodopus sungorus*. *Mamm Species.* 595:1–9.
- Schlatt S, De Geyter M, Kliesch S, Nieschlag E, Bergmann M. 1995. Spontaneous recrudescence of spermatogenesis in the photoinhibited male Djungarian hamster, *Phodopus sungorus*. *Biol Reprod.* 53:1169–1177.
- Sisk CL, Bronson FH. 1986. Effects of food restriction and restoration on gonadotrophin and growth hormone secretion in immature male rats. *Biol Reprod.* 35:554–561.
- Stetson MH, Elliott JA, Goldman BD. 1986. Maternal transfer of photoperiodic information influences the photoperiodic response of prepubertal Djungarian hamsters (*Phodopus sungorus sungorus*). *Biol Reprod.* 34:664–669.
- Tsutsui K, Kawashima S, Masuda A, Oishi T. 1989. Changes in the testicular binding of luteinizing hormone and plasma testosterone concentrations in the Djungarian hamster subjected to different photoperiods and temperatures and effects of long-term testosterone treatment on the binding. *J Exp Zool.* 251:91–100.
- Wade GN, Bartness TJ. 1984. Effects of photoperiod and gonadectomy on food intake, body weight, and body composition in Siberian hamsters. *Am J Physiol.* 246:R26–R30.
- Watanabe T, Yamamura T, Watanabe M, Yasuo S, Nakao N, Dawson A, Ebihara S, Yoshimura T. 2007. Hypothalamic expression of thyroid hormone-activating and -inactivating enzyme genes in relation to photorefractoriness in birds and mammals. *Am J Physiol Regul Integr Comp Physiol.* 292:R568–R572.
- Weiner J. 1987. Limits to energy budget and tactics in energy investments during reproduction in the Djungarian hamster (*Phodopus sungorus sungorus*). *Symp Zool Soc Lond.* 57:167–187.
- Wynne-Edwards KE, Surov AV, Telitzina AY. 1999. Differences in endogenous activity within the genus *Phodopus*. *J Mammal.* 80:855–865.