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Food restriction during development delays puberty but does not affect adult seasonal reproductive responses to food availability in Siberian hamsters (*Phodopus sungorus*)

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Abstract

Seasonally breeding animals respond to environmental cues to determine optimal conditions for reproduction. Siberian hamsters (*Phodopus sungorus*) primarily rely on photoperiod as a predictive cue of future energy availability. When raised in long-day photoperiods (>14 h light), supplemental cues such as food availability typically do not trigger the seasonal reproductive response of gonadal regression, which curtails reproduction in unsuitable environments. We investigated whether recognition of food availability as a cue could be altered by a nutritional challenge during development. Specifically, we predicted that hamsters receiving restricted food during development would be sensitized to food restriction (FR) as adults and undergo gonadal regression in response. Male and female hamsters were given either ad libitum (AL) food or FR from weaning until d60. The FR treatment predictably limited growth and delayed puberty in both sexes. For 5 weeks after d60, all hamsters received an AL diet to allow FR hamsters to gain mass equal to AL hamsters. Then, adult hamsters of both juvenile groups received either AL or FR for 6 weeks. Juvenile FR had lasting impacts on adult male body mass and food intake. Adult FR females exhibited decreased estrous cycling and uterine horn mass indiscriminately of juvenile food treatment, but there was little effect on male reproductive measurements. Overall, we observed a delay in puberty in response to postweaning FR, but this delay appeared not to affect seasonal reproductive responses in the long term. These findings increase our understanding of seasonal reproductive responses in a relevant environmental context.

KEYWORDS

development, food restriction, gonadal regression, puberty, reproduction, seasonality

1 | INTRODUCTION

Animals inhabiting temperate climates experience extreme fluctuations in environmental conditions on a seasonal basis. To maximize survival and reproductive success, many such animals have evolved to breed seasonally, limiting energetic investment in reproduction to only times of year in which resources are abundant enough to support survival of self and offspring (Bronson, 1985; Bronson & Heideman, 1994; Goldman, 2001). Many seasonally breeding animals rely on photoperiod as their primary cue signaling future changes in resource availability, but breeding activity can also be regulated by environmental conditions such as temperature, precipitation, presence of conspecifics, and food availability. Photoperiod acts as a salient "proximate" cue that serves as a reliable, easily detectable

signal of environmental change that functions to prepare animals for changes in the latter "ultimate" cues, those which directly influence survival and reproductive success (Baker, 1938; Wingfield & Farner, 1980).

It is critical for seasonally breeding animals to accurately interpret these environmental cues signaling resource availability, as insufficient energy can have dramatic consequences for reproductive function, particularly during reproductive development (Schneider & Wade, 2000; Schneider, 2004). The phenomenon of delayed puberty, most notably in females, as a result of undernutrition is well-documented in mammals (Bronson, 1986; Bronson & Rissman, 1986; Castellano et al., 2005; Hamilton & Bronson, 1985; Parent et al., 2003; Schillo et al., 1992; Wayne et al., 1991). Puberty is initiated when energy stores are sufficient to support reproduction, and is characterized by the increased pulsatile activity of gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus. This triggers increased production and secretion of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone from the anterior pituitary and subsequently the steroids testosterone and estrogen from the gonads (Bronson, 2000; Bronson & Rissman, 1986; Ebling, 2005). Interestingly, the mechanisms of puberty initiation in all mammals, that is, the stimulation of pulsatile GnRH secretion, significantly overlap with those involved in the coordination of seasonal reproductive condition in seasonally breeding species (e.g., activation of the neuropeptide kisspeptin) (Clarke & Caraty, 2013; Greives et al., 2008; Revel et al., 2007; Smith & Clarke, 2010). Thus, common endocrine mechanisms exist to regulate reproductive function in response to the environment throughout a seasonally breeding animal's lifetime.

Siberian hamsters (*Phodopus sungorus*) are seasonally breeding rodents that respond to multiple, interacting environmental cues to coordinate seasonal changes in physiology and behavior (Bailey et al., 2016). They are reproductively active in long-day (LD), summer-like (>14 h of light per day) photoperiods and undergo robust gonadal regression in short-day (SD), winter-like (<12 h of light per day) photoperiods (Bartness & Wade, 1985; Hoffmann, 1982). When housed in either of these "extremes" of natural photoperiod variation in the laboratory, reproductive status is heavily reliant on photoperiod compared to other cues. Recent work has shown that minor decreases in food availability do not influence reproductive physiology in LD-housed Siberian hamsters, but when hamsters experience mild food restriction (FR) in an intermediate, fall-like (13.5 h of light per day) photoperiod, gonadal regression is triggered (Bailey et al., 2017; Paul, Galang, et al., 2009; Paul, Pyter et al., 2009). Thus, whereas food availability has little influence on reproduction when photoperiod is an accurate predictor of conditions in the near future, it can act as a "supplementary" cue in times when photoperiod cues, and thus future resource availability are uncertain (i.e., intermediate photoperiods can occur in fall, when resources will decline, or in spring, when resources will increase) (Wingfield & Farner, 1980). The relevance of food availability as an accurate cue, therefore, appears to be dependent on other environmental signals, specifically photoperiod.

The goal of the present study was to investigate whether the interpretation of food availability as a supplementary cue could be altered depending on developmental nutritional experience. Specifically, we asked whether experiencing delayed puberty as a consequence of undernutrition during development would affect Siberian hamsters' reproductive responses to FR as adults. We hypothesized that moderate FR during development would result in delayed puberty and would subsequently affect perception of food availability as a relevant cue in adulthood. We predicted that animals receiving restricted food availability as juveniles would be more sensitive to FR as adults; that is, that animals that developed in food-restricted conditions would show greater alteration of reproductive physiology in response to FR as adults compared to animals that received unlimited food until adulthood. In Experiment 1, we provided male and female Siberian hamsters with either AL access to food or a moderate restriction of food (FR, 70% of AL intake) in the period between weaning and 60 days of age (typical early adulthood) and monitored reproductive development. In Experiment 2, adult hamsters from both juvenile treatment groups in Experiment 1 were provided AL or slightly milder FR (80% of AL intake) and reproductive responses were determined. Between Experiments 1 and 2, all animals received an AL diet for a time sufficient to allow juvenile FR hamsters to regain body mass to equal juvenile AL hamsters.

2 | MATERIALS AND METHODS

2.1 | Experiment 1: Effects of postweaning FR on reproductive development

2.1.1 | Animals and housing

Male and female Siberian hamsters ($n = 116$, 62 females and 54 males) were procured from a breeding colony (one litter each from 20 breeding pairs) housed in a long-day (LD, 16:8 h light:dark cycle, lights on at 0400 Eastern Standard Time, EST) photoperiod at Indiana University. Animals were weaned at 18 days of age (d18) and individually housed under the same photoperiod in polypropylene cages (27.5 × 17.5 × 13.0 cm) with Sani-chip bedding material. Animals received AL access to food (Lab Diet 5001; PMI Nutrition) until the start of food treatments on d20, and AL access to tap water at all times. Temperature and humidity were maintained at 20 ± 2°C and 50 ± 10%, respectively. All animal procedures were reviewed and approved by the Indiana University Bloomington Institutional Animal Care and Use Committee.

2.1.2 | Post-weaning food treatments

Hamsters of each sex were randomized by litter into food treatment groups (AL or FR). Restricted food rations were calculated dynamically, based on AL intake. On d18 and d19, baseline AL intake was monitored in all hamsters to establish the first FR ration, which was

calculated as 70% of the average daily intake of each individual and provided just before lights out on d20. From then on, FR rations were calculated based on AL animals' increasing intake, to ensure that the developing hamsters would receive appropriate rations to avoid excessive nutritional stress. AL animals' intake was assessed every other day, and after each AL intake measurement, a new FR ration would be calculated as 70% of the average daily intake of all AL hamsters. Each day, upon providing FR rations, any remaining ration in the cage food hopper from the previous day was collected and weighed (±0.1 g) to gain a better approximation of actual intake.

2.1.3 | Assessment of puberty onset

Every 5 days, body mass was recorded and hamsters were evaluated for puberty onset. Females were checked for vaginal patency by applying a sterile pipet tip to the genital area and gently probing to investigate the existence of an opening. Male estimated testis volume (ETV) was measured under light anesthesia with isoflurane vapors. Fur covering the scrotum was moistened with 70% ethanol to facilitate visualization of testes, and calipers were used to measure the length and width of the right testis (±0.01 mm). ETV was calculated as the length × width^2, which is directly correlated with testis mass and spermatogenesis (Gorman & Zucker, 1995). An ETV of 400 mm^3 indicates a mass of approximately 200 mg, at which stage viable spermatids begin to emerge in increasing numbers (Gorman & Zucker, 1995; Schlatt et al., 1995). ETV assessment for each individual continued until reaching this threshold of 400 mm^3.

2.1.4 | Blood sampling and tissue collection

On d60, a blood sample was collected at 1030 h EST to determine serum LH concentrations as an indicator of basal hypothalamo-pituitary-gonadal (HPG) axis activity. The time of sampling avoided that of the preovulatory LH surge, which occurs several hours before lights off (Dodge et al., 2002). Hamsters were lightly anesthetized with isoflurane vapors and blood (~3.5% of the animal's total blood volume) was drawn from the retro-orbital sinus and collected into microcentrifuge tubes. Samples were allowed to clot at room temperature for 1 h, clots were removed, and samples were centrifuged at 4°C for 30 min at 2500 rpm. Serum was collected from the tubes and transferred to a −20°C freezer for storage until performing hormone assays. Immediately after blood collection, a subset of hamsters (n = 24 females and 20 males, randomized by litter and food treatment) was deeply anesthetized in isoflurane vapors and necropsies were performed to collect ovaries, uterine horns, and parametrial white adipose tissue (PWAT) from females and testes and epididymal white adipose tissue (EWAT) from males. PWAT is a fat pad that surrounds the ovary and likely provides metabolic support to reproductive functions (Gui et al., 2006; Jaubert et al., 1995; McInroy et al., 2000). Similarly, EWAT is a fat pad that surrounds the testis and promotes spermatogenesis (Chu et al., 2010). Tissues were weighed to determine morphological development as a result of food treatment.

2.2 | Experiment 2: Effects of subsequent FR in adulthood on reproductive responses to environmental cues

2.2.1 | Adult food treatments

For 5 weeks after d60 ("adult AL period"), all remaining hamsters (38 females and 34 males) were provided with AL access to food, to allow juvenile FR hamsters to gain mass to equal juvenile AL hamsters. During this time, body mass and AL intake were assessed weekly. At the end of 5 weeks, adult hamsters of each sex were randomized by juvenile food treatment and litter into four new experimental groups: juvenile AL-adult AL (AL-AL), juvenile AL-adult FR (AL-FR), juvenile FR-adult AL (FR-AL), and juvenile FR-adult FR (FR-FR). For the last 5 days of the adult AL period, AL intake was assessed daily in each animal to calculate individual adult FR rations (80% of individual average daily intake). Adult FR animals received their rations daily just before lights out for 6 weeks ("adult food treatment period"), and adult AL animals' intake continued to be assessed weekly. Remaining FR rations were collected and weighed daily as previously.

2.2.2 | Assessment of adult reproductive responses

During the 6-week adult food treatment period, body mass was measured weekly and reproductive measures were taken regularly. In males, ETV was measured weekly to track the occurrence of gonadal regression over time. In females, estrous cycling was determined through cytological examination of vaginal epithelial cells for three 5-day periods in the experimental timeline (one set of 5 days before the start of food treatment [preadult food treatments], one set 3 weeks after the beginning of food treatment [mid-adult food treatments], and one during the last 5 days of the experiment [end-adult food treatments]). A sample of vaginal epithelial cells was collected from each female daily at 1200 h EST by lavaging 30 µl of sterile 0.9% saline through the vaginal canal 3–5 times. The sample was placed on a glass microscope slide, allowed to dry, fixed in methanol, and stained with a 10% Giemsa solution (Sigma-Aldrich® Procedure No. GS-10). Cells were visualized using a light microscope and estrous cycle stages were identified according to the presence and proportions of nucleated and cornified epithelial cells, as well as leukocytes (Caligioni, 2009). Vaginal cytology is not as precise a method in this species as it is for other rodents (Dodge et al., 2002); thus, rather than analyzing an individual female's specific cycle progression, we instead determined whether individual females were cycling (the population of cells progressed through clear estrous cycle stages during the 5-day evaluation period) or noncycling (the population of cells was unchanging, usually in a late diestrous/early proestrous-like state).

2.2.3 | Blood sampling and tissue collection

Two blood samples, one just before the adult food treatment period, and one on the final day of the experiment, were collected and processed as described above to monitor changes in basal HPG axis activity as determined by serum LH concentrations. On the final day of the experiment, hamsters were euthanized and necropsies were conducted as described above. Reproductive tissues (ovaries, uterine horns, and PWAT in females, testes, and EWAT in males) were weighed to assess the occurrence of gonadal regression in response to adult food treatments.

2.2.4 | Hormone measurement

Serum LH concentrations were determined via a radioimmunoassay (RIA) described previously (Legan et al., 2009) with slight modifications. The standard (rat LH, RP-3) and purified LH for iodination were obtained from Dr. A.F. Parlow at the National Hormone and Peptide Program, Torrance, CA, USA. Single 200 µl aliquots of each sample were diluted in 0.05 M phosphate-buffered saline (PBS) containing 0.1% gelatin (gel-PBS). The primary antibody was CSU 120 (provided by Dr. Terry Nett, Colorado State University), diluted 1:10,000 in 1:100 normal rabbit serum (Millipore). The tubes were incubated for 24 h at 22°C after addition of 100 µl primary antibody, and after adding radiolabeled LH (~60,000 counts per minute/100 µl gel-PBS, iodinated by the iodogen method), and again following addition of the secondary antibody (anti-rabbit gamma globulin, diluted 1:50 in gel-PBS; Millipore). The LH results reported herein were obtained from four assays for which the mean sensitivity was 0.05 ng/ml, determined as two standard deviations below the maximum binding. Two replicates each of two standard serum pools from male hamsters that inhibited binding on average to 71.4% and 83.7% were analyzed at the beginning, middle, and end of each assay for determination of inter- and intra-assay coefficients of variation (CVs). The inter-assay CV was 13.4% and the mean intra-assay CV was 8.6%.

2.2.5 | Statistical analysis

Statistical tests were performed using JMP versions 12.1.0 and 15 (SAS Institute, Inc.). A value of $p < 0.05$ was considered to be statistically significant for all tests. Sample sizes for each group in Experiment 1 were: juvenile AL: 31 females and 27 males; juvenile FR: 31 females and 27 males. Sample sizes for tissues collected at d60 were: juvenile AL: 12 females and 10 males; juvenile FR: 12 females and 10 males. Sample sizes in Experiment 2 were: AL-AL: 10 females and 8 males; AL-FR: 9 females and 7 males; FR-AL: 9 females and 8 males; FR-FR: 10 females and 9 males. One male in the AL-AL group was excluded from LH analysis in Experiment 2, as his final blood sample did not include enough serum for assay.

Analysis of variance (ANOVA) models including food treatments and sex in a full factorial design were used to assess all measurements. Before analysis, data distributions were checked for homogeneity of variance and for normality of model residuals. Those distributions with unequal variances or producing nonnormal residuals were transformed to best meet these assumptions for parametric tests (Experiment 1: serum LH was log-transformed and uterine horn mass was square-root transformed. Experiment 2: serum LH, uterine horns, body mass, and food intake during the adult AL period were log-transformed). In Experiment 1 and in the adult AL period of Experiment 2, some groups contained more than one individual littermate. Litter was therefore included as a covariate in all analyses in these two study phases. In addition, body mass was included as a covariate in all ETV and reproductive tissue mass analyses.

Food intake analysis in Experiment 1 focused on comparing AL intake between the sexes, as FR rations were calculated based on AL intake. Proportions of males and females achieving benchmarks of puberty were compared between food treatment groups via nominal logistic regression. For analysis of estrous cycling, treatment effects on proportions of cycling females in each group at each time point were assessed via nominal logistic regression. For measurements of body mass and food intake in both Experiment 1 and 2, repeated measures ANOVAs were used to detect treatment effects over time within and between subjects, with time as a within-subjects variable. Within-subjects comparisons violated assumptions of sphericity and were Greenhouse-Geiser (G-G) corrected. Finally, direct comparisons of group means were conducted using one-way ANOVAs with Tukey's HSD post-hoc tests.

3 | RESULTS

3.1 | Experiment 1: Effects of postweaning FR on reproductive development

3.1.1 | Body mass and food intake during development

At weaning (d18), males were significantly larger than females (sex effect: $F_{1,93} = 20.41$, $p < 0.001$). Body mass did not differ significantly among prospective food treatment groups at this baseline time point ($F_{1,93} = 1.26$, $p = 0.26$). Starting on d25 (the first time point at which body mass was measured after beginning the FR treatment), and continuing for each measurement day through d60, each of the four sex/food treatment groups was significantly different from one another in body mass (AL males > AL females > FR males > FR females) (e.g., Day 25: $F_{22,93} = 11.05$, $p < 0.001$) (Figure 1a). At d60, the total percent change in mass showed effects of sex ($F_{1,93} = 28.16$, $p < 0.001$), food ($F_{1,93} = 502.00$, $p < 0.001$), and a sex × food interaction ($F_{1,93} = 17.09$, $p < 0.001$), with AL hamsters of both sexes gaining more body mass than FR hamsters, and AL males gaining more mass than AL females (Figure 1b).

There were no significant treatment effects on baseline AL intake (sex: $F_{1,93} = 2.95$, $p = 0.09$; food: $F_{1,93} = 1.69$, $p = 0.20$; sex × food: $F_{1,93} = 0.35$, $p = 0.56$). On most days throughout the experiment, there was no significant difference in AL intake between the sexes. However, among days when there was an effect of sex, there was a temporal shift between which sex was removing more food: AL females removed more food than males early in the experiment (Days 24 and 26; e.g., Day 24 sex

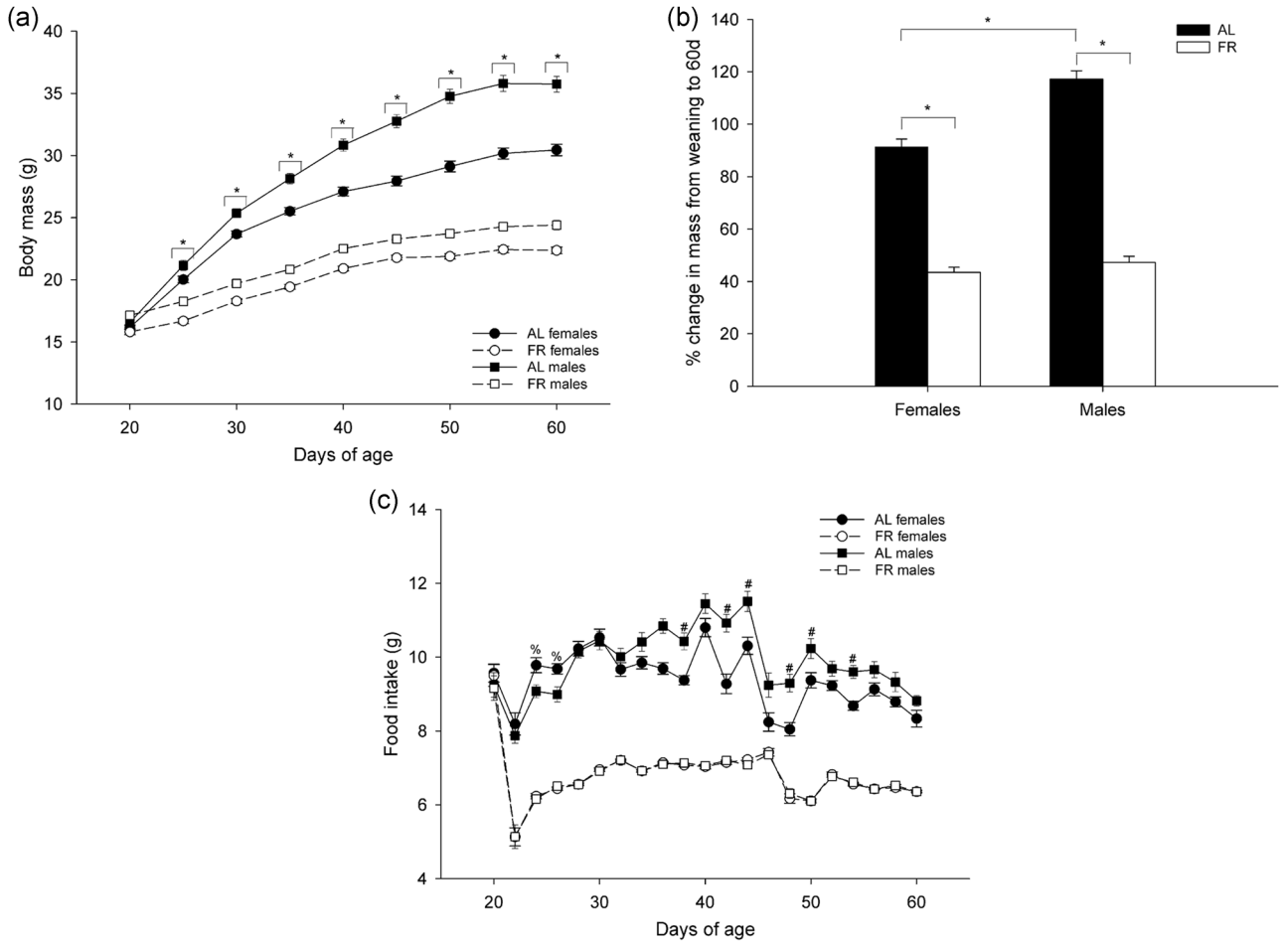

FIGURE 1 Mean (±SEM) (a) body mass over time, (b) percent change in body mass from Day 20 to Day 60, and (c) food intake over time in male and female juvenile Siberian hamsters. (a) *Over bracket indicates that all groups are significantly different from one another ($p < 0.05$). (b) * Ad libitum-fed (AL) hamsters gained significantly more mass than food-restricted (FR) hamsters and that AL males gained significantly more mass than AL females ($p < 0.05$). (c) % AL females removed significantly more food than AL males; #AL males removed significantly more food than AL females ($p < 0.05$)

effect: $F_{1,38} = 8.83$, $p = 0.005$), whereas AL males removed more food than females later in the experiment (Days 38, 42, 44, 48, 50, and 54; e.g., Day 38 sex effect: $F_{1,38} = 17.82$, $p < 0.001$) (Figure 1c).

3.1.2 | Onset of puberty, HPG axis function, and reproductive tissue mass at d60

There was a significant effect of food treatment on the proportions of females achieving vaginal patency at d40 ($X^2(1) = 8.32$, $p = 0.004$), which resulted in a lower proportion of FR females having achieved patency at this time point compared to AL females (Figure 2a). All females exhibited vaginal patency by d45. Similarly, there were significant effects of food treatment on the proportions of males achieving the ETV threshold of 400 mm^3 at d30 and d35 (d30: $X^2(1) = 4.71$, $p = 0.03$; d35: $X^2(1) = 5.00$, $p = 0.03$), which resulted in lower proportions of FR males achieving the threshold at these time points compared to AL males. All males reached the ETV threshold by d40 (Figure 2b).

Serum LH at d60 was significantly different between AL and FR males, with FR males having lower levels of circulating LH, but did not differ significantly between food treatments in females ($F_{3,93} = 6.00$, $p < 0.001$; post-hoc tests significant between male, but not female, groups) (Figure 2c).

There was no significant effect of food treatment on reproductive tissue mass in females (ovaries: $F_{1,8} = 0.10$, $p = 0.76$; uterine horns: $F_{1,8} = 0.46$, $p = 0.52$; PWAT: $F_{1,8} = 0.30$, $p = 0.60$); however, uterine horns in FR females were half the size of those in AL females (16.2 mg vs. 32.6 mg average mass, respectively). A large standard error in the AL females' data (11.7 mg), perhaps due to differences in estrous cycle stage at the time of tissue collection, contributed to the lack of statistical significance. In males, despite the dramatic decrease in LH seen in FR animals, food treatment did not have a significant effect on paired testes mass (testes: $F_{1,3} = 2.07$, $p = 0.25$). EWAT mass, however, was significantly lower in FR males ($F_{1,3} = 17.92$, $p = 0.02$) (Table 1).

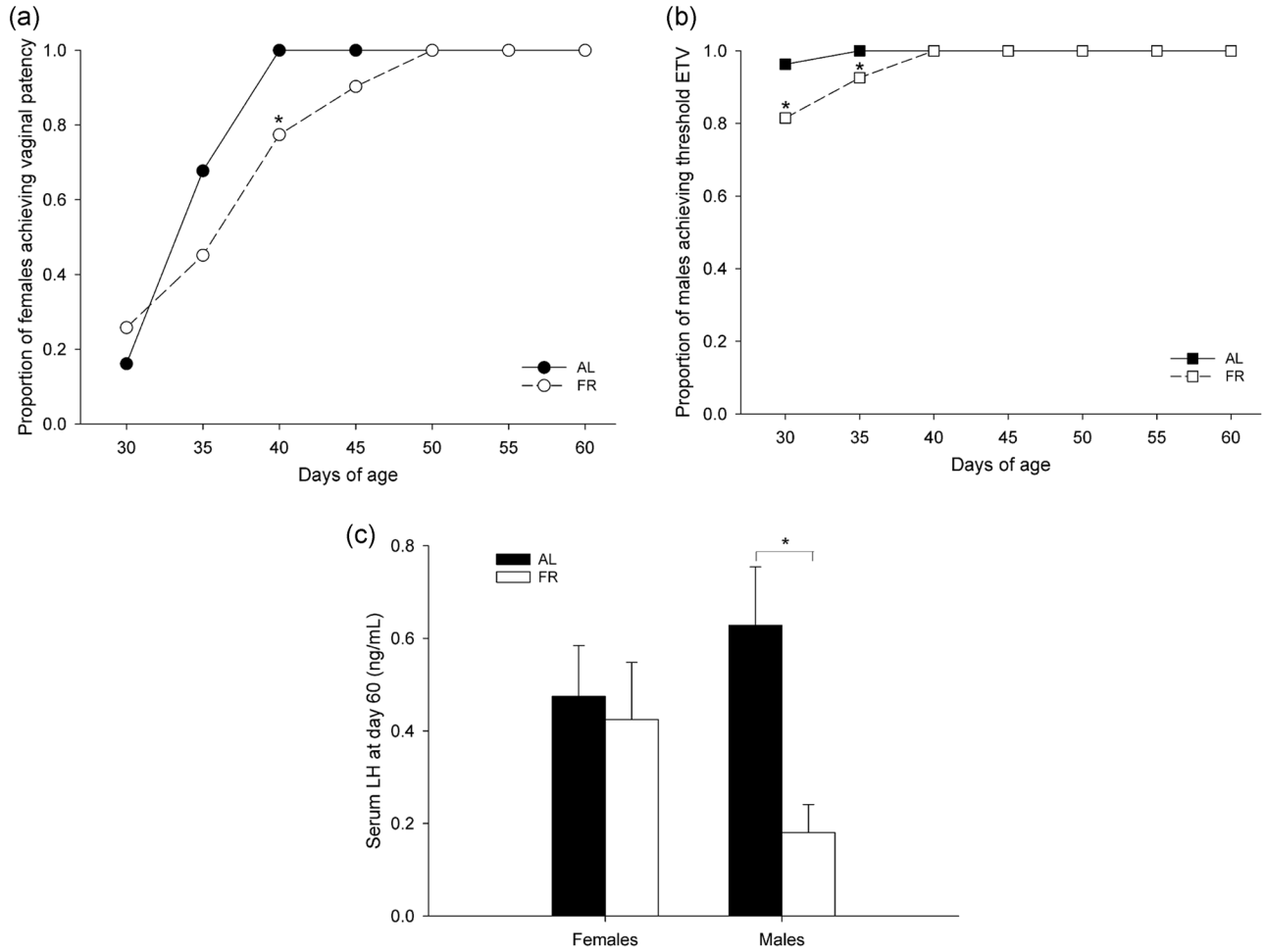

FIGURE 2 (a) Proportion of female hamsters achieving vaginal patency, (b) proportion of male hamsters achieving estimated testis volume (ETV) puberty threshold, and (c) serum luteinizing hormone (LH) at Day 60. (a) *A significantly smaller proportion of food-restricted (FR) females had achieved vaginal patency at Day 40 than ad libitum-fed (AL) females ($p < 0.05$). (b) *A significantly smaller proportion of FR males had reached the ETV threshold of 400 mm^3 than AL males. (c) *Serum LH concentration at d60 differed significantly between AL and FR males, but not females ($p < 0.05$)

3.2 | Experiment 2: Effects of subsequent FR in adulthood on reproductive responses to environmental cues

3.2.1 | Body mass and food intake during adult AL period

Throughout the 5-week AL period post-d60, body mass showed effects of juvenile food treatment and sex in weeks 1–4, with juvenile FR hamsters and females being smaller than juvenile AL hamsters and males, respectively (week 1: juvenile food: $F_{1,47} = 45.30$, $p < 0.001$; sex: $F_{1,47} = 60.44$, $p < 0.001$. Similar results were observed in weeks 2–4) (Figure 3a). By Week 5, the juvenile food treatment effect dissipated ($F_{1,47} = 3.28$, $p = 0.08$), but the sex difference in mass persisted ($F_{1,47} = 50.47$, $p < 0.001$), with females maintaining significantly lower body mass than males. In accordance with these results, repeated measures analysis of body mass during this period revealed between-subjects effects of sex ($F_{1,47} = 54.05$, $p < 0.001$) and juvenile food treatment ($F_{1,47} = 13.80$, $p < 0.001$), with a within-subjects effect of time × juvenile food treatment (G-G corrected: $F_{1.88,88.32} = 43.36$, $p < 0.001$).

AL food intake during the AL period was affected by juvenile food treatment in Weeks 1 and 2 (e.g., Week 1: $F_{1,47} = 38.19$, $p < 0.001$), with juvenile FR hamsters eating more than juvenile AL hamsters (Figure 3b). This effect was lost by Week 3 ($F_{1,47} = 4.25$, $p = 0.35$). AL intake was also significantly affected by sex (significant in every week, e.g., Week 1: $F_{1,47} = 14.33$, $p < 0.001$), with males showing greater intake than females (Figure 3b). Repeated measures analysis similarly revealed between-subjects effects of juvenile food treatment ($F_{1,47} = 11.20$, $p = 0.002$) and sex ($F_{1,47} = 17.00$, $p < 0.001$), with a within-subjects effect of time × juvenile food treatment (G-G corrected: $F_{2.66,125.13} = 20.22$, $p < 0.001$) (Figure 3b).

TABLE 1 Reproductive tissue mass from female and male juvenile (60 days of age) Siberian hamsters following postweaning food treatments

	AL	FR
Female tissues		
Ovaries (mean mg ± SEM)	12.3 ± 0.8	10.3 ± 0.8
Uterine horns (mean mg ± SEM)	32.6 ± 11.7	16.2 ± 2.8
PWAT (mean g ± SEM)	0.21 ± 0.02	0.08 ± 0.01
Male tissues		
Testes (mean g ± SEM)	0.71 ± 0.02	0.50 ± 0.06
EWAT (mean g ± SEM)	0.89 ± 0.05	0.38 ± 0.05*

Abbreviations: AL, ad libitum (hamsters received ad libitum food access from weaning to Day 60); EWAT, epididymal white adipose tissue; FR, food restricted (hamsters received restricted food access from weaning to Day 60); PWAT, parametrial white adipose tissue.

*EWAT was significantly smaller in FR males compared to AL males ($p < 0.05$).

3.2.2 | Body mass and food intake during adult food treatment period

Body mass did not differ significantly among groups at the end of the 5-week AL period ($F_{1,61} = 0.40$, $p = 0.53$). Throughout the 6-week adult food treatment period, effects of sex, adult food treatment, and an interaction of juvenile food treatment × adult food treatment were present each week (e.g., Week 1 [Week 6 in Figure 3a]: sex: $F_{1,61} = 31.47$, $p < 0.001$; adult food: $F_{1,61} = 5.10$, $p = 0.027$; juvenile food × adult food: $F_{1,61} = 4.77$, $p = 0.033$), with the exception of Week 5 (Week 10 in Figure 3a), in which the interaction effect just missed significance ($F_{1,61} = 3.96$, $p = 0.051$) (Figure 3a). This was reflected in repeated measures analysis, which revealed between-subjects effects of sex ($F_{1,61} = 29.20$, $p < 0.001$), adult food treatment ($F_{1,61} = 17.84$, $p < 0.001$), and juvenile food treatment × adult food treatment ($F_{1,61} = 4.79$, $p = 0.033$), with within-subjects effects of time × sex × juvenile food treatment (G-G corrected: $F_{1.50,91.40} = 5.42$, $p = 0.011$) and time × adult food treatment (G-G corrected: $F_{1.50,91.40} = 59.37$, $p < 0.001$). These effects can be more clearly summarized by visualizing the percent change in body mass over the 6 weeks (Figure 3c). Hamsters of both sexes in both juvenile food treatment groups lost mass in response to adult FR (adult food treatment effect: $F_{1,61} = 105.29$, $p < 0.001$) and those in the FR-AL treatment group continued to gain mass throughout the adult food treatment period. Females, but not males, in the AL-AL group also continued to gain mass (sex × juvenile food treatment effect: $F_{1,61} = 7.04$, $p = 0.010$) (Figure 3c).

Adult food treatment and an interaction of sex × juvenile food treatment affected food intake for all 6 weeks of the adult food treatment period, with adult AL hamsters eating more than adult FR hamsters and FR-AL males eating more than their other adult AL counterparts (e.g., Week 1 [Week 6 in Figure 3b]: adult food: $F_{1,61} = 155.82$, $p < 0.001$; sex × juvenile food: $F_{1,61} = 33.10$, $p = 0.024$) (Figure 3b). Thus, juvenile FR led to an increase in AL intake in adulthood in males, but not in females. An effect of sex persisted for Weeks 1–4 (Figure 3b: Weeks 6–9), but not in Weeks 5 or 6 (Figure 3b: Weeks 10–11), as male AL intake declined to meet that of females (e.g., Week 1 [Figure 3b Week 6]: $F_{1,61} = 6.01$, $p = 0.017$. Similar results were observed in Weeks 2–3 [Figure 3b Weeks 7–8], with a weakening effect in Week 4 [Figure 3b Week 9] [$F_{1,61} = 4.10$, $p = 0.047$]. Week 5 [Figure 3b Week 10]: $F_{1,61} = 1.02$, $p = 0.32$. Week 6 [Figure 3b Week 11]: $F_{1,61} = 0.002$, $p = 0.96$). Repeated measures analysis similarly showed between-subjects effects of sex ($F_{1,61} = 5.00$, $p = 0.029$), sex × juvenile food ($F_{1,61} = 8.11$, $p = 0.006$), adult food ($F_{1,61} = 125.06$, $p < 0.001$), and sex × juvenile food × adult food ($F_{1,61} = 4.94$, $p = 0.030$), with within-subjects effects (G-G corrected) of time × sex ($F_{2.77,168.95} = 2.95$, $p = 0.038$), and time × adult food ($F_{2.77,168.95} = 50.24$, $p < 0.001$).

3.2.3 | Reproductive responses to adult food treatments

Proportions of females exhibiting estrous cycling before the adult food treatment period did not differ significantly among groups ($\chi^2(3) = 1.00$, $p = 0.80$). After 3 weeks of adult food treatments, estrous cycling showed a significant effect of adult food treatment ($\chi^2(1) = 7.38$, $p = 0.007$), as well as a trend toward an effect of juvenile food treatment ($\chi^2(1) = 3.69$, $p = 0.055$). By the end of the adult food treatment period, the effect of adult food treatment persisted ($\chi^2(1) = 8.65$, $p = 0.003$), with FR-treated females being significantly less likely to exhibit cycles (Figure 4a). In males, ETV did not differ among groups before adult food treatments ($F_{4,27} = 1.20$, $p = 0.33$) and was subsequently unaffected by adult food treatments (e.g., Week 1: $F_{4,27} = 0.87$, $p = 0.50$) (Figure 4b).

Serum LH levels were very low at all experimental time points, with group means ranging between 0.1 and 1.1 ng/ml, representing basal levels (Figure 4c). Repeated measures analysis of LH levels from d60 to the end of the experiment showed a between-subjects effect of juvenile food treatment × adult food treatment ($F_{1,60} = 9.98$, $p = 0.003$), but functional significance of this effect is difficult to determine. The same interaction effect of juvenile food treatment × adult food treatment was revealed in the preadult food treatment time point, before adult food treatments had begun ($F_{1,60} = 12.35$, $p < 0.001$), which was likely due to LH concentrations being highly variable within some experimental groups. In the final blood sample obtained at the end of adult food treatments, serum LH concentrations did not differ among the groups ($F_{7,60} = 0.42$, $p = 0.88$). Female paired ovary mass and PWAT mass did not differ significantly among treatment groups (ovaries: $F_{3,32} = 0.39$, $p = 0.76$; PWAT: $F_{3,32} = 0.62$, $p = 0.61$) (Table 2). Uterine horn mass was affected by adult food treatment, with adult FR females having smaller uterine horns than adult AL females, regardless of juvenile food treatment ($F_{1,32} = 5.34$, $p = 0.027$), and without a significant change in circulating LH levels. Male paired testes showed a significant interaction effect of juvenile food treatment × adult food treatment ($F_{1,27} = 4.31$, $p = 0.048$), but this effect was not significant enough to reveal any post-hoc

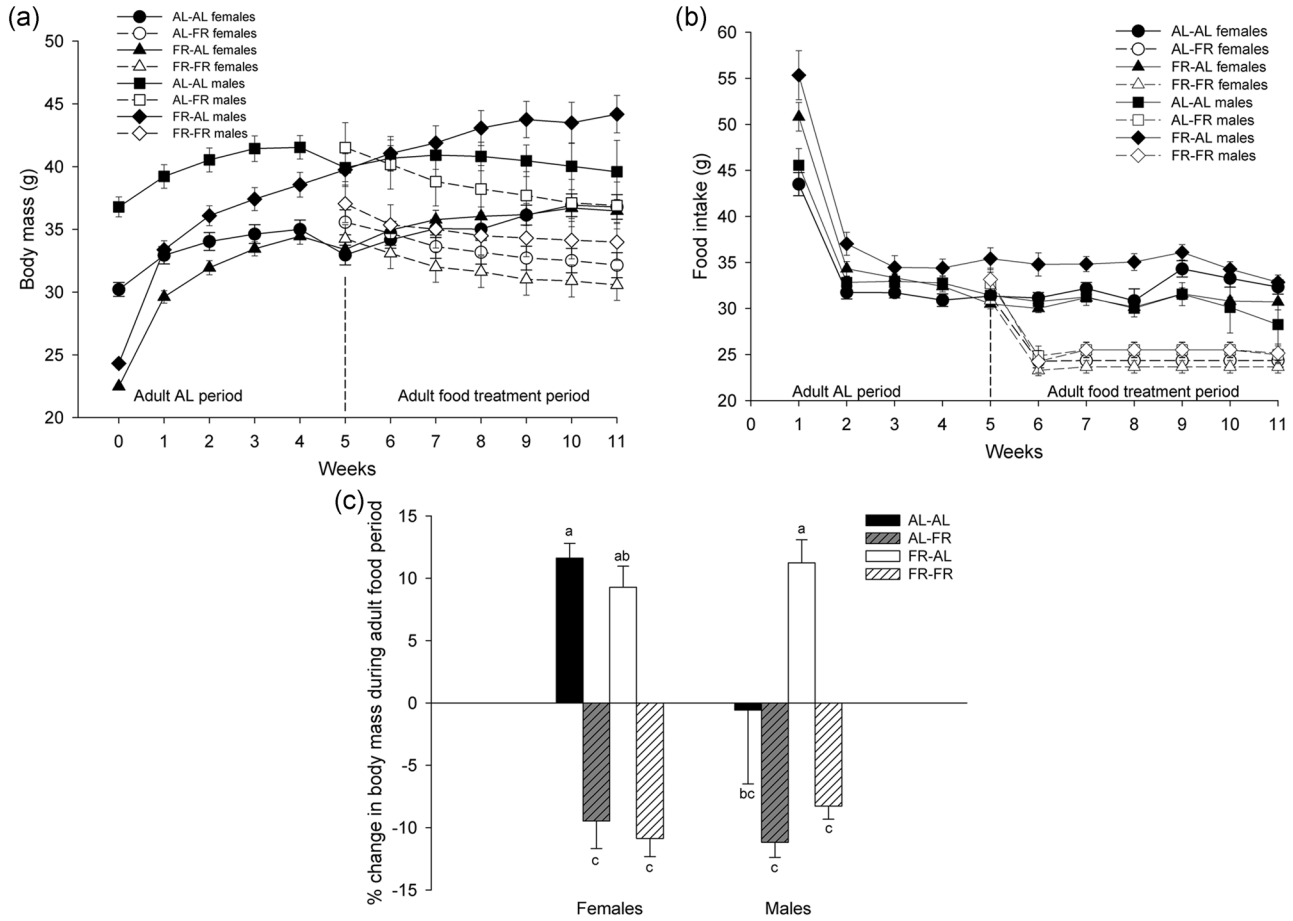

FIGURE 3 Mean (±SEM) (a) body mass over time and (b) food intake over time in male and female Siberian hamsters during 5 weeks of all ad libitum (AL) access to food after having received AL access or food restriction (FR) as juveniles (left of dotted vertical line; "Adult AL period") and a subsequent 6 weeks of AL access or FR (right of dotted vertical line "Adult food treatment period"). Juvenile and adult food treatments are notated in sequence; that is, "FR-AL" indicates hamsters that were food restricted as juveniles and received AL food as adults. (a) Males maintained significantly higher body mass than females during the adult AL period. Juvenile FR hamsters regained mass to equal that of AL hamsters by Week 5. During the adult food treatment period, sex, adult food treatment, and an interaction of juvenile food treatment × adult food treatment significantly affected body mass. (b) Sex and juvenile food treatment influenced food intake during the adult AL period, with males removing significantly more food than females throughout, and juvenile FR hamsters removing more food than juvenile AL hamsters for the first 2 weeks. During the adult food treatment period, adult food treatment and an interaction of sex × juvenile food treatment affected food intake for all 6 weeks, with AL hamsters and FR-AL males removing significantly more food. (c) Percent change in body mass from the beginning to the end of the 6-week adult food treatment period. Groups with different letters indicate statistically significant differences between group means ($p < 0.05$); groups sharing the same letter are not significantly different

differences among experimental groups ($F_{3,27} = 1.59$, $p = 0.21$). EWAT was unaffected by treatments ($F_{3,27} = 0.06$, $p = 0.98$) (Table 2).

4 | DISCUSSION

We tested the hypothesis that FR during development would result in delayed puberty and have lasting effects on adult reproductive function in Siberian hamsters. We specifically predicted that postweaning FR would sensitize adult hamsters to recognize food availability as a relevant cue for seasonal reproduction in a photoperiod environment in which they otherwise would not. We provided male and female Siberian hamsters housed in a LD photoperiod with either AL food or a moderate (70% of AL) reduction in food availability during postweaning development, allowed FR hamsters to catch up to the body mass of AL hamsters in early adulthood, and subsequently provided either AL food or a milder (80% of AL) reduction in food availability to both juvenile treatment groups to assess the responses of adult hamsters. We found that moderate postweaning FR delayed puberty in male and female Siberian hamsters and had lasting effects on adult body mass and food intake, but had little impact on the perception of decreased food availability as a relevant cue for triggering gonadal regression in adulthood.

Both sexes exhibited restricted body mass growth and delays in puberty (as measured by achievement of vaginal patency in females

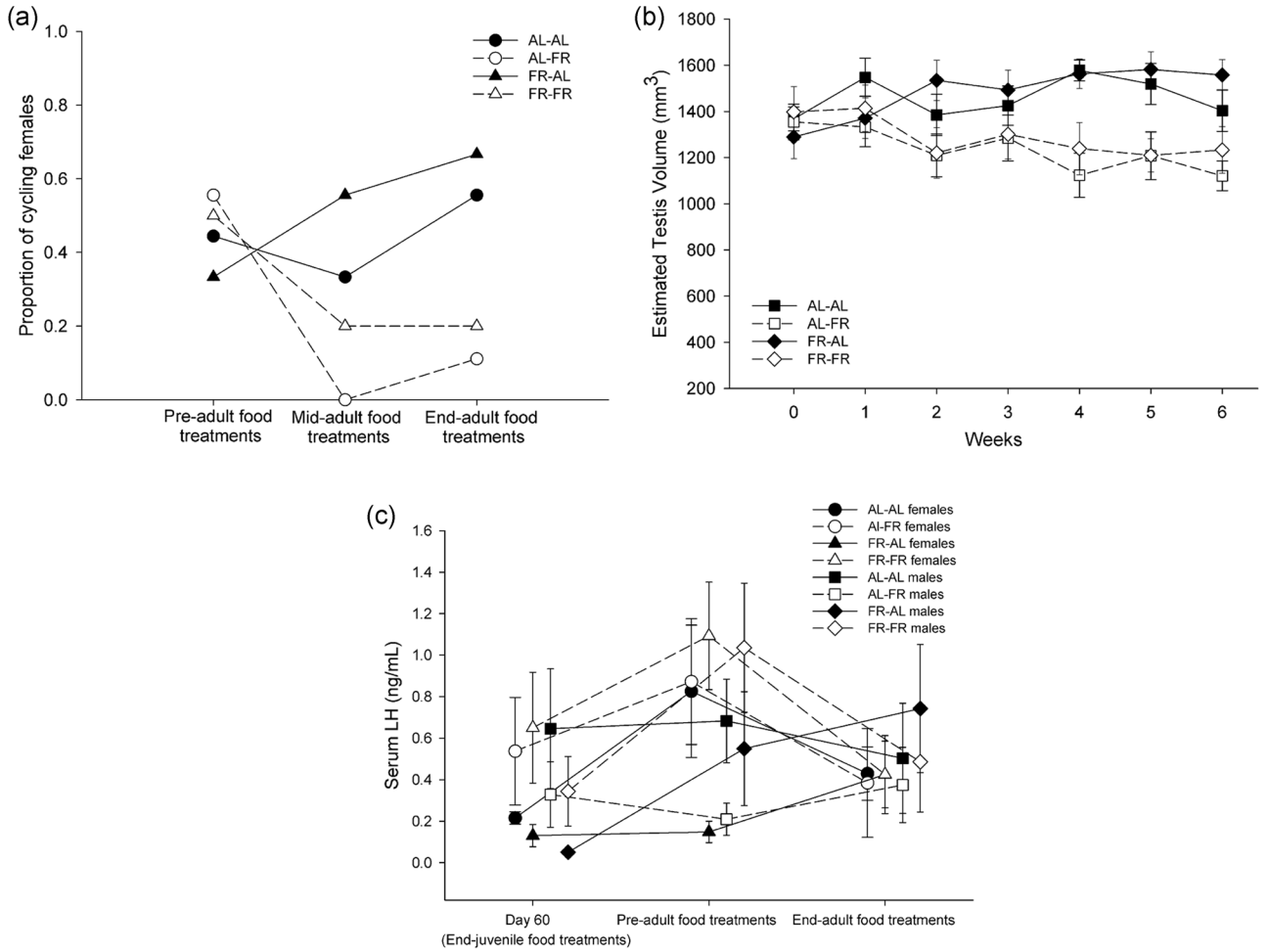

FIGURE 4 (a) Proportion of females exhibiting normal estrous cycles throughout 6 weeks of receiving either ad libitum (AL) access to food or food restriction (FR) in adulthood after previously receiving similar or opposite treatments as juveniles, (b) mean (±SEM) male estimated testis volume (ETV) throughout adult food treatments, and (c) mean (±SEM) serum luteinizing hormone (LH) before and after adult food treatments. Juvenile and adult food treatments are notated in sequence; that is, "FR-AL" indicates hamsters that were food restricted as juveniles and received AL food as adults. (a) Adult food treatment significantly affected the proportions of cycling females at the mid- and end-adult food treatments time points, with a trend ($p = 0.055$) toward juvenile food treatment affecting proportions at the mid-adult food treatments time point. (b) There were no treatment effects on ETV (this analysis included body mass as a covariate). (c) Serum LH showed an interaction effect of juvenile food treatment × adult food treatment between subjects over time, but LH concentrations did not differ significantly among groups by the end-experiment serum sample. Individual points have been formatted along the *x*-axis to facilitate interpretation; LH concentrations were assessed at fixed time points

and an ETV threshold of 400 mm^3 in males) in response to FR. Although we did not assess LH concentrations during the pubertal transition, it is interesting that d60 basal circulating LH levels in FR males were decreased to about one-third of those in AL males, but there was no change in d60 LH levels in FR versus AL females. A similar sexually dimorphic decrease in circulating LH after FR has been shown in adult male but not female rats, in response to the nutritional stress of being raised in a large litter (Sanchez-Garrido et al., 2013), and similarly in 8-week-old male but not female hamsters following 5–6 days of starvation (Howland & Skinner, 1973). While the present study focused on gaining a specific understanding of basal HPG axis activity as a result of experimental treatments, an expanded investigation into other endocrine and metabolic effects of FR during development in this species would greatly contribute to a broader comprehension. The combined results of Experiment 1 confirm that postweaning moderate FR effectively delays puberty in both sexes in this species, which seems to be associated with decreased LH secretion only in males.

Although we predicted disruption of the neuroendocrine regulators of reproduction in response to our juvenile FR treatment, our results did not support our hypothesis that the seasonal reproductive response in developmentally food-restricted hamsters would be more sensitive to FR in adulthood. In females, adult FR decreased the proportions of individuals exhibiting normal estrous cycles and uterine horn mass regardless of juvenile food availability. The difference in estrous cyclicity was observed in the absence of changes

TABLE 2 Reproductive tissue mass from female and male adult Siberian hamsters following 6 weeks of ad libitum or restricted food treatments

Female tissues	AL-AL	AL-FR	FR-AL	FR-FR
Female tissues				
Ovaries (mean mg ± SEM)	15.8 ± 0.7	14.4 ± 0.8	15.2 ± 0.2	13.6 ± 0.7
Uterine horns (mean mg ± SEM)	65.2 ± 9.5	43.1 ± 10.1*	84.0 ± 13.7	40.9 ± 9.2*
PWAT (mean g ± SEM)	0.25 ± 0.04	0.14 ± 0.03	0.24 ± 0.03	0.15 ± 0.024
Male tissues				
Testes (mean g ± SEM)	0.71 ± 0.05	0.59 ± 0.06	0.68 ± 0.02	0.61 ± 0.03
EWAT (mean g ± SEM)	0.91 ± ± 0.14	0.75 ± 0.08	1.08 ± 0.11	0.63 ± 0.07

Note: Hamsters had previously received similar or opposite food treatments as juveniles.

Abbreviations: AL-AL, Ad libitum-Ad libitum (hamsters received ad libitum food access as juveniles and adults); AL-FR, Ad libitum-Food Restricted (hamsters received ad libitum food access as juveniles and restricted food access as adults); EWAT, epididymal white adipose tissue; FR-AL, Food Restricted-Ad libitum (hamsters received restricted food access as juveniles and ad libitum food access as adults); FR-FR, Food Restricted-Food Restricted (hamsters received restricted food access as juveniles and adults); PWAT, parametrial white adipose tissue.

*Uterine horns were significantly smaller in adult FR females compared to adult AL females ($p < .05$).

in basal serum LH concentrations, which is not surprising because morning, or basal, LH levels are very low (<1 ng/ml), near the level of assay detectability, and are not different between noncycling and cycling female Syrian hamsters (Jorgenson & Schwartz, 1987). It is important to note that in view of the pulsatile nature of LH secretion, our sampling regimen, three single blood samples in a 6–7 week period, was too infrequent to detect any changes in LH pulse frequency or amplitude that may have occurred during development and adult FR, and that may be physiologically relevant to HPG axis function. However, the absence of estrous cycles would be accompanied by a lack of follicular development, resulting in decreased circulating estradiol levels, thereby causing atrophy of the uterine horns, as we observed, and which has also occurred after starvation of female hamsters (Howland & Skinner, 1973). In males, there was a small but significant interaction between juvenile food treatment and adult food treatment on testes mass, which suggests that male, but not female hamsters differed in their reproductive response to FR as adults depending on juvenile food treatment; however, the interaction effect on testes mass was not strong enough to elicit post-hoc differences among experimental groups. This could be related to the lack of a major effect of adult FR on basal serum LH levels; however, LH is not strongly correlated with the process of seasonal gonadal regression in this species (Bailey et al., 2017; Greives et al., 2007).

It is possible that FR to 80% of normal intake is not a severe enough signal in adult hamsters to result in more dramatic physiological or morphological effects than we observed. However, it is also possible that underlying regulatory mechanisms were altered. In adult male prairie voles (another seasonally breeding rodent), FR (70% of normal intake) did not alter tissue masses, but did increase GnRH immunoreactive neuron soma size and number (Kriegsfeld et al., 2001). In contrast, a study investigating a similar question in rats, in which prenatally undernourished rats were presented with FR as adults, found very little effect of a 48-h adult food deprivation on expression of GnRH-regulatory neuropeptides and their receptors, but found that serum LH and testosterone levels declined, indicating that the adult HPG axis of prenatally undernourished rats remained sensitive to FR (Iwasa et al., 2015). To gain a complete understanding of how a nutritional challenge early in life affects adult reproductive responses, further investigation of how the underlying neuroendocrine mechanisms of reproduction are affected will be essential.

In contrast to our observed lack of alteration of reproductive responses, juvenile FR to 70% of normal intake had lasting effects on adult changes in body mass and food intake, with interesting sex differences in these effects. At the end of the adult AL period, hamsters that had received juvenile FR had gained mass to equal their juvenile AL counterparts. During the adult food treatment period, male FR-AL hamsters continued to gain mass and remove more food. By comparison, AL females of both juvenile treatment groups continued to gain mass throughout the adult food treatment period, which most likely indicates a sex difference in growth curves at the age we investigated. These effects in males may illustrate the process of "developmental programming" of metabolism that occurs during a critical window within which perturbations to metabolic systems can have long-lasting effects on adult physiology (reviewed in: Remmers & Delemarre-van de Waal, 2011). Long-term effects of early-life nutritional stress have been studied in rodent models as well as humans; many investigations have discovered a tendency for animals that experience a nutritional challenge early in life to be more likely to exhibit dietary-induced obesity later in life, and some of these effects may be more common in males than females (Jones & Friedman, 1982; Gluckman & Hanson, 2004; Remmers & Delemarre-van de Waal, 2001). A related explanation of this phenomenon is the "thrifty phenotype" hypothesis, which describes the tendency of individuals that develop in a resource-scarce environment to be better prepared as adults to survive limited resources (Hales & Barker, 2001). This occurs through a developmental programming of metabolism to utilize scarce resources more efficiently. In the present study, juvenile FR males continued to

remove more food and gain mass in adulthood, which could be the result of differential developmental programming. We did not perform body composition analysis in this study; it would indeed be useful to employ this type of physiological analysis in the future to investigate these effects in seasonally breeding species.

Effects indicating the development of a "thrifty phenotype" are most often seen following prenatal, rather than postnatal/postweaning, undernutrition in rodent species (Remmers & Delemarre-van de Waal, 2011), although this may be different in seasonally breeding species that are more sensitive to environmental changes. Interestingly, there is at least one indication within our results that juvenile FR hamsters may have been more resistant to FR in adulthood, in opposite alignment with our predictions. Adult female hamsters were sensitive to FR in terms of estrous cycling, but it may be notable that no females in the AL-FR group showed signs of estrous cycling after 3 weeks of FR at the mid-experiment time point, while some females in the FR-FR group maintained cycling throughout the experiment. Further investigation of seasonally breeding rodents, particularly with larger sample sizes, may better elucidate the broad effects of developmental programming in animals that have evolved to cope with environmental change.

We have demonstrated that postweaning FR reliably restricts growth and delays puberty in male and female Siberian hamsters, a seasonally breeding species of rodent that is reproductively responsive to environmental cues indicating changes in resource availability. We observed lasting effects of juvenile FR on adult changes in body mass and food intake, particularly in males, but we did not find evidence to support our hypothesis that juvenile FR would sensitize adult hamsters to decreased food availability later in life. It is likely that neuroendocrine mechanisms involved in energy homeostasis, as well as regulation of reproduction, were affected by food treatments in both juvenile and adult hamsters; further investigation of these mechanisms will be essential to understanding how animals coordinate reproductive development as well as adult reproductive responses in fluctuating, unpredictable environments.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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