Energetic challenges unmask the role of ovarian hormones in orchestrating ingestive and sex behaviors

Candice M. Klingerman, Kaila Krishnamoorthy, Kevin Patel, Andrew B. Spiro, Chris Struby, Anand Patel, Jill E. Schneider *

Department of Biological Sciences, Lehigh University, Bethlehem, PA 18015, USA

ABSTRACT

Effects of ovarian hormones on sex and ingestive behavior are well studied, and yet, their role in diverting attention from food to sex has not been examined directly, possibly because these functions are masked under conditions of excessive food abundance typical of the laboratory. Female Syrian hamsters were either fed *ad libitum* or food-restricted to 75% of their *ad libitum* intake for 8 days and then tested every day of the estrous cycle for their preference for males versus food, food hoarding and food intake in an apparatus designed to mimic aspects of their natural habitat. The food-restricted, but not the fed females, varied significantly over the estrous cycle in appetitive behaviors, which included their preference for males versus food and in the amount of food hoarded, with low food hoarding and high male preference on the night of ovulation. In contrast, there were no significant differences between restricted and *ad libitum*-fed females in the consummatory behaviors, namely, food intake or lordosis duration. In ovariectomized females, estradiol plus progesterone treatment delayed food restriction-stimulated hoarding and hastened feeding-inhibited hoarding without affecting food intake or lordosis duration. In summary, energy restriction and the presence of males unmasked an effect that was obscured in the normal laboratory conditions characterized by isolation and an over abundance of readily available food. These results are consistent with the idea that ovarian hormones orchestrate appetites for food and sex to optimize reproductive success under fluctuating energetic conditions.

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Estradiol and progesterone are well-known reproductive hormones that have garnered attention for their effects on energy balance. Female mice that lack receptors for estradiol are hyperphagic, obese, and display deficits in sex behavior (Heine et al., 2000; Rissman et al., 1997). In many different species with spontaneous ovulatory cycles, including women, natural increases in circulating estradiol concentrations are correlated with maximum fertility, sex behavior and high levels of sexual motivation (Blaustein and Erskine, 2002; Ciaccio et al., 1979; Lisk and Nachtigall, 1988; Steel, 1980) and decreases in food intake and body weight (Blaustein and Wade, 1976; Butera, 2010; Fessler, 2003; Wade and Gray, 1979; Wade et al., 1985). Food intake and body weight increase with ovariectomy and decrease with estradiol treatment in many species including women (Blaustein and Wade, 1976; Butera, 2010; Fessler, 2003; Wade and Gray, 1979; Wade et al., 1985) and the subjects of the present study, Syrian hamsters (Morin and Fleming, 1978; Schneider et al., 1986). There is a great deal of circumstantial evidence suggesting that ovarian hormones orchestrate reproduction and energy balance, but very

few experiments are designed explicitly to address the functional significance of the pleiotropic effects of estradiol and progesterone.

We hypothesize that the main function of 'satiety' hormones, including estradiol, is to set behavioral priorities for the purpose of optimizing reproductive success in natural habitats where food availability varies unpredictably (Schneider, 2006; Schneider et al., 2007b; Schneider and Watts, 2009). According to our hypothesis, the effects of estradiol on sexual motivation are expected to differ under different levels of energy availability. Under extreme shortages, when energy demands far outstrip energy availability, reproduction is expected to be inhibited in favor of intense interest in foraging, hoarding and eating. Evidence in support of this prediction has been documented in every species in which it has been studied (reviewed by Bronson, 1989; Schneider and Watts, 2009), including Syrian hamsters (Morin, 1986; Schneider and Wade, 1989). Under less extreme challenges, however, when energy demand is high but does not exceed availability, or when food availability is unpredictable, reproductive hormones are expected to increase sexual motivation while decreasing the hunger for food during the fertile period. Thus, even when females must fatten and hoard food in anticipation of future shortages, the temporary increase in ovarian steroids boosts sexual motivation in order to make sex a priority over eating and food hoarding during the time when mating is most likely to pay off

* Corresponding author. Department of Biological Sciences, 111 Research Drive, Bethlehem, PA 18015, USA. Fax: +1 610 758 4004.
E-mail address: js0v@lehigh.edu (J.E. Schneider).

in terms of reproductive success. In contrast, when energy availability is in excess of energy demand, the role of fluctuating ovarian hormones in overcoming hunger might be masked because hunger motivation is low relative to sexual motivation under these conditions.

This idea is supported by the following ideas from ecological neuroendocrinology. Food supplies in nature are rarely as predictable as in the laboratory and our western industrialized societies. Animals in the wild must expend considerable energy and time obtaining food, and in many cases, must anticipate future energy needs that are greater than the requirements for immediate survival (Bronson, 1989). Energy can be stored internally as adipose tissue or externally as food caches in anticipation of the increased energetic demands of pregnancy, lactation, seasonal changes in food availability or unpredictable natural disasters. Taking time and energy for reproductive activity in such energetically labile habitats can be risky because lack of vigilance to energetic demands could preclude any future genetic contribution to the next generation. Because evolutionary adaptation involves reproductive success in addition to survival, one role of ovarian steroids is to incite sexual motivation in the face of substantial risks (Wallen, 2000). Our aim, therefore, was to unmask the role of hormones in coordinating sex and eating by measuring several aspects of sex and ingestive behavior over the estrous cycles in Syrian hamsters housed under different energetic conditions in a setting that approximates the energetic conditions of their wild ancestors.

Syrian hamsters (*Mesocricetus auratus*) were used because they show consistent four-day estrous cycles that are easily monitored and behaviors that allow us to assess their motivations independent of their ability to perform behaviors. For example, appetitive aspects of ingestion, such as food hoarding, are highly sensitive to energetic challenges and can increase independent of the "consummatory" act of eating in Syrian hamsters (Buckley and Schneider, 2003; Schneider et al., 2007b) and other hamster species (Bartness, 1990; Bartness and Clein, 1994). Appetitive aspects of sex behavior include "male preference" measured in our experiments as the time spent with males minus the time spent with food divided by the total testing time (Schneider et al., 2007b). In contrast, the consummatory aspect of sex behavior, lordosis, is routinely studied in the laboratory by injections of estradiol followed by progesterone (Feder et al., 1974; Jones et al., 2002).

We compared appetitive and consummatory sex and ingestive behaviors in female hamsters with high, medium and low food availability and with and without mating partners. In Experiment 1, we measured these behaviors every six hours over the four-day estrous cycle in solitary female hamsters that were either fed *ad libitum* or food-restricted. The results guided our experimental design in other experiments. In Experiment 2, we measured the choice between hoarding and sex behavior at the onset of the dark phase of the photoperiod every night of the estrous cycle in females with *ad libitum* access to food and made comparisons to those with either a) restricted food availability or b) unlimited food and limited time for hoarding and mating. According to our hypothesis, females living in conditions of energy scarcity were expected to show high levels of food hoarding and low interest in males on all nights of the estrous cycle except the night of proestrous when they were expected to show the opposite pattern. Our hypothesis predicted that hamsters living in energy abundance would have little variation in food hoarding over the estrous cycle and prefer to spend their time assessing the whereabouts of opposite-sex conspecifics every night. Conversely, our hypothesis would be refuted if food availability had no effect on estrous cycle fluctuations in hoarding and male preference. In Experiment 3, females were ovariectomized and treated with estrous-inducing doses of estradiol and progesterone or vehicle to confirm that the results of Experiment 2 were due to these hormones.

Materials and methods

Animals and housing

Experiments were conducted according to the American Physiological Society guiding principles for research, the National Institutes of Health, the Lehigh University Institutional Animal Care and Use Committee, and the United States Department of Agriculture. Female Syrian hamsters (*M. auratus*) weighing between 100 g and 160 g were obtained from a colony bred at the Lehigh University animal facility or purchased from Charles River Breeding Laboratory (previous generations of animals were also obtained from Charles River Breeding Laboratories; Wilmington, MA). Animals were singly housed in opaque, Nalgene cages (31 × 19 × 18 cm) in a room maintained at 22 ± 1 °C with a 14:10 light–dark cycle (lights on at 2100 h). Animals were fed Harlan Rodent Chow 2016 and water was available at all times.

Hamster estrous cycles

All hamsters showed two consecutive 4-day estrous cycles as determined by a positive test for sex behavior (the lordosis posture) with a sexually experienced male hamster one hour before the onset of the dark period on day 4, termed 'proestrous' in rats, and in this experiment, the 'periovulatory day.' The next day, estrous cycle day 1, is characterized by a copious vaginal discharge, high levels of agonistic behavior and is termed the 'postovulatory day.' The next day is termed 'follicular 1.' The next day is 'follicular 2,' the day of peak vaginal marking.

Experiment 1: Characterization of food hoarding throughout the light: dark cycle and over the estrous cycle

Our aim was to document food hoarding and food intake every 6 h over the estrous cycle in order to determine whether our female laboratory hamsters, like wild hamsters, increase hoarding near the onset of the dark period, and to determine whether food hoarding varies over the estrous cycle in the absence of males under food restriction and *ad libitum* feeding.

Eleven adult, estrous-cycling female hamsters, 110–160 g in body weight, were acclimated to their home cage and trained to recognize food in the food boxes attached by plastic Habitrail tubing to their home cages. No males were present in the room during this experiment. A pre-weighed amount of food was placed in the food box (600–800 g) on the first day, and no food was placed in the home. Thereafter, the females' body weight and the amount of food remaining in the food box and the amount of food in the home were measured every 6 h for 6 days. At each time point, after food intake and body weight were recorded, all food was removed from the home, and pre-weighed food was placed in the food boxes. "Food hoarded" was the weight of food found in the home, or, if any of the food was soiled, "food hoarded" was calculated as the amount of food in the food box subtracted from the amount in the food box 6 h earlier. "Food intake" was the amount of food remaining in the food cage and in the home subtracted from the amount of food in the food box 6 h earlier. Half of the hamsters were restricted to 75% of their *ad libitum* food intake and the other half remained on *ad libitum* food rations for one week. Two hamsters were removed from the study because they began to show irregular estrous cycles. The experiment was repeated, measuring body weight, food intake and food hoarding every 6 h for 6 days. After the animals were placed on *ad libitum* feeding for 2 months, all of the previously food-restricted females were fed *ad libitum*, while the previously *ad libitum*-fed females were food-restricted for one week. After two replicates, there were 9 females in the food-restricted and 9 females in the *ad libitum*-fed groups.

Estrous cyclicity was determined by a 5-min lordosis test that occurred during the dark period, and females that showed lordosis

were designated as periovulatory females (day 4 of the estrous cycle). Testing began for all females on the same date, and the females were not synchronized with regard to the day of estrous. Thus, about one fourth of the females started the experiment on day 1 of the estrous cycle whereas others started on day 2, 3 or 4.

Experiment 2: Sex and ingestive behaviors over the estrous cycle during food scarcity and abundance

Our aim was to see whether energy restriction unmasks the effects of periovulatory hormones on hunger motivation. If the role of ovarian hormones is to divert attention toward sexual stimuli and away from food stimuli, females living in energy abundance might be expected to show little or no change in food hoarding over the estrous cycle, whereas females living in energy deficit would be expected to show more dramatic fluctuations in hoarding over the estrous cycle. More precisely, we predicted that 1) food hoarding would be lower at the time of ovulation when circulating estradiol levels are highest, 2) food hoarding would be maximal when circulating estradiol levels are low, and 3) food hoarding would be high in food-restricted females except on the periovulatory day when females would show a preference for males over food, producing a marked fluctuation in these behaviors over the estrous cycle. We also tested whether fluctuations in hoarding over the estrous cycle would be exaggerated by restricting the time available for hoarding.

The preference apparatus

The female hamsters' motivation to engage in either ingestive or sex behaviors was assessed using a specially-designed preference apparatus described previously (Schneider et al., 2007a) that consisted of a home cage, a food source box, and a male box (a box that contained an adult, sexually experienced male hamster). All three boxes were connected by plastic tubes. When females were not being trained or tested, they were housed in the home cage made from opaque, Nalgene cages ($31 \times 19 \times 18$-cm) lined with fine ground wood shavings with a specialized door at one end that was kept closed. During training and testing, the door was opened and led via the plastic tubes to the male box and the food box. The tube from the home cage was approximately 134 cm in length and led vertically to two horizontal tubes 40–50 cm in length, connected in a T configuration. One horizontal tube was connected to the food box, a disc-shaped, clear plastic chamber that contained a weighed amount of hoardable pellets. Hoardable pellets were made from standard laboratory chow pellets cut into 2 cm pieces, a size that readily fits into hamster cheek pouches and allows the females to travel through the tubes with their cheek pouches full. The second horizontal tube was connected to the male cage, a clear, Plexiglas cage ($27 \times 20 \times 15$-cm) that contained an adult, sexually experienced male and no food or water. Within this male cage the male was restrained within a wire box so that he could not leave the male cage.

Acclimation to the home cage

Prior to training, female subjects were placed in the home cage with fine wood chip bedding, food, and water, and allowed to live there for four to seven days with the door closed and no access to the male or to the food boxes. This allowed them to be acclimated to their home cage and reduced any tendency to sleep in or carry bedding or food to the other chambers.

Training in the preference apparatus

Once acclimated to the home cages, hamsters were trained to expect food in the food boxes and males in the male boxes. Training sessions (like testing sessions) began within one hour of the onset of the dark phase of the photoperiod and extended 1 to 2 h into the dark period, because this is near a time when hamsters are active in nature (Gattermann et al., 2008) and overlaps with the nocturnal hoarding period in our laboratory (result of Experiment 1). Training sessions with access to the food box lasted 90 min, and were given over 4 sequential days, encompassing all days of the estrous cycle. Training with the food box occurred every day of the cycle, whereas training with the male box was on days 3 and 4 of the estrous cycle. Females were allowed to discover the food box and to keep all of the food that they hoarded from the food box in their home cage. On days 3 and 4 of the estrous cycle, the females were allowed access to the male box, which contained an unrestrained adult sexually experienced male. The male and female were allowed to interact under close supervision by the experimenter for 2–5 min so that the females received anogenital sniffs, licks and ectopic mounts but no intromissions or ejaculations.

Testing in the preference apparatus

Preference testing began within 1 h of the onset of the dark phase of the photoperiod. To start the test, the door to the vertical tube was opened and the female subjects were allowed access to both the food and male boxes for a total of 90 min. During the first 15 min, the females' behaviors and locations were recorded every 5 s by an experimenter. The experimenter recorded vaginal marking, flank marking, hoarding, and eating. At the end of 15 min, the experimenter stopped recording, but females still had access to both the food box and the male box for 75 min. At the end of the final 75 min (90 min total), access to the food and male boxes ended, and the weight of the food in the apparatus (the home cage and the food box) was measured and recorded. This gave a measure of food hoarding and eating within the context of an available mating partner.

Procedures

Twenty-four female hamsters 100–150 g in body weight were acclimated and trained in the preference apparatus as described above. Baseline food intake was measured for 4 days by giving a weighed amount of food in the home cage and weighing the food remaining (minus pouched and spilled food). The hamsters were divided into 3 groups that did not differ in body weight or food intake prior to treatments: Hamsters in Group 1 had unlimited access (24 h per day) to the food box and were given as much food as they cared to eat each day ("*Ad libitum*-time and food"). Hamsters in Group 2 were limited in the amount of food they could eat each day but were unlimited in the time access to the food box ("Food-limited"). Hamsters in Group 3 were limited in time, i.e., they were limited to only 90 min access to the food box, but had more than enough food to eat each day ("Time-limited"). More detail for each treatment group is given below.

Ad libitum-time and food group

At the start of the 8-day treatment period, 8 control hamsters were allowed access 24 h a day to the food box containing at least 200–250 g of food for 24 h a day. They were allowed to hoard as much as they wanted, and their access remained unlimited for 8 days. The food in the food box was replenished as necessary.

Food-limited group

Each hamster in the Food-limited group was given 75% of its own average daily baseline food intake (4.5–8.25 g). This food was placed in the food box, and the females had unlimited access to the food box for 8 days. In the Food-limited group, the pre-weighed food was provided in two daily rations placed in the food box at random times throughout the day so as not to entrain activity rhythms (Rusak et al., 1988). Each ration was provided at least 6 h later than the last ration.

Hamsters in this group tended to visit the food box many times a day, but only received 75% of their daily baseline intake every 24 h. After each test period, food intake was measured and the females were provided only with enough food to remain restricted at the same level as before the start of testing.

Time-limited group

The hamsters in the Time-limited group were given 200–250 g of food in their food box each day, but were only allowed to visit the food box for 90 min each day, beginning at the onset of the dark phase of the photoperiod, for 8 days. They were allowed to keep all the food that they hoarded from the food box to the home cage each day. If a hamster failed to hoard any food, 20 g of food was placed in its home cage that day.

After the 8 days of the above treatments, each female was tested once per day on 4 consecutive days of the estrous cycle in the preference apparatus as described previously. The 90-min tests were scheduled to begin around the start of the dark phase of the photoperiod because it has been reported that wild hamsters hoard daily at this time (Gattermann et al., 2008), and a nocturnal hoarding rhythm in our laboratory hamsters was confirmed in Experiment 1 when food hoarding was measured around the clock every 6 h over the estrous cycle.

Estrous cycles were not synchronized, so testing began on each of the 4 estrous cycle days for roughly one quarter of the hamsters in each group. Hamsters were tested in counterbalanced order with regard to their experimental group so that no one group would be tested at a particular time of day relative to the other groups. The preference test occurred as described in Methods for Experiment 2.

Repeated measures analysis of variance (ANOVA) was used to analyze changes over the estrous cycle in the amount of food hoarded and eaten, male preference ((time with male − time with food) / total time), and the number of vaginal and flank marks. Differences among the groups on each day of the estrous cycle were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test when main effects were significant. Differences were considered statistically significant if P was less than 0.05.

Experiment 3: Effects of exogenous estradiol and progesterone in food scarcity and abundance

Our aim was to determine whether the hormone treatment known to bring ovariectomized (OVX) hamsters into estrous also decreases food hoarding and/or food intake. We hypothesized that one role of ovarian hormones is to attenuate the urge to hoard food and increase the urge to mate. If so, removal of ovarian hormones by OVX would increase food hoarding, and treatment of OVX females with the hormones characteristic of estrus would do the opposite, and furthermore, the conditions of energy scarcity would exaggerate the difference in hoarding between OVX-vehicle and OVX + hormone groups. Thus, we suggested that food restriction would unmask the effects of exogenous hormones on appetitive aspects of ingestive and sex behavior. This time, instead of comparing females at different stages of the estrous cycle, we compared OVX females treated with estrous-inducing doses of estradiol benzoate (E) and progesterone (P) or the oil vehicle. If our hypothesis were supported, we would expect to see larger differences in food hoarding between OVX and OVX-E + P groups when the females were food-restricted than when they were allowed to eat *ad libitum*. In addition, we asked whether the effects of E are dose-dependent or threshold effects by varying the dose of E and keeping the dose of P constant.

Doses of E and P

As a first step toward understanding the role of ovarian steroids in the appetite for food and sex, we used a hormonal treatment regimen known to induce estrous behavior in OVX Syrian hamsters. Hamsters received a subcutaneous (S.C.) injection of E 48 h before testing followed by an injection of P 6 h before testing) (Feder et al., 1974; Jones et al., 2002). If our hypothesis about the role of ovarian steroids in decreasing the urge to forage while at the same time increasing the urge for mating were supported, it would be expected that experimental regimens that are most effective in inducing sex behavior would also reduce food hoarding.

In addition, we varied the dose of E to determine whether this hormone produced a linear dose–response or threshold effect on food hoarding and preference for males. The different doses of E produce circulating levels within the range of hamsters on follicular day 1 (the low dose 1.5 μg), follicular day 2 (the medium dose, 2.5 μg) or at a superphysiological level (the high dose, 20 μg) (Baranczuk and Greenwald, 1973; Jones et al., 2002).

Forty-four female Syrian hamsters 105–145 g in body weight were deeply anesthetized using sodium pentobarbital (80 mg/kg; Sigma Aldrich, St. Louis, MO) and ovariectomized through bilateral flank incisions closed with suture (muscle incision) and wound clips (skin incision). An analgesic (Metacam, Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO, 0.06 ml) was given at the time of anesthesia to minimize pain and discomfort after surgery. After recovery, hamsters were randomly placed into treatment groups that did not differ significantly in body weight and would receive different doses of E (Sigma Aldrich, St. Louis, MO) dissolved in the canola oil vehicle.

This experiment employed a 2 by 4 factorial design, with hamsters receiving one of 2 energy treatments (*ad libitum*-fed or food-restricted) and one of 4 hormone treatments. One of the 4 hormone groups was vehicle-treated (canola oil), the other 3 groups were all P-treated, but each of the three groups received one of 3 doses of E. E was always given S.C. at 1.5, 2.5 or 25 μg per female. In total, there were 8 groups of 5–6 hamsters per group. Each hormone-treated female received their respective dose of E 48 h prior to testing, and 500 mg P 6 h before testing.

Prior to the start of the experiment, daily food intake was measured for 4 days to find each hamster's individual average daily food intake. Then, at the start of the experiment, half were fed either *ad libitum* or restricted to 75% of their *ad libitum* daily food intake, separated into two portions, one of which was provided every 12 h. These feeding regimens were scheduled to continue for 12 days, with hormone treatment and testing on days 4, 8 and 12, after which time, the previously food-restricted hamsters were returned to *ad libitum* feeding to be treated with hormones and tested at 4 and 8 days after re-feeding. The only exception was that if any particular food-restricted female that lost body weight failed to show lordosis in response to an unrestrained male, she was immediately returned to *ad libitum* feeding and tested 4 and 8 days later.

Testing procedures were the same as in Experiment 2, except that in the first ten minutes of testing, the male was restrained in a wire box and the female received gentle flank stimulation by the experimenter, and in the next ten minutes of testing the male was unrestrained and the female did not receive flank stimulation by the experimenter.

Differences among the groups on days 4 and 8 after the start of food restriction and on day 4 after re-feeding were analyzed using two-way ANOVA followed by planned comparisons. Differences were considered statistically significant if P was less than 0.05.

Results

Experiment 1: Daily rhythms in hoarding, body weight and food intake

Food hoarding

Food hoarding progressively increased over the initial time points, and then began to settle into a nocturnal pattern (Fig. 1, top). Only data from the last 4 of the 6 days were used for analysis. Both fed and food-restricted females that had no access to males hoarded more in

the dark than in the light period ($P<0.001$, Fig. 1, top). All daily peaks in food hoarding occurred during the dark, and all nadirs appeared in the light period, although small amounts of food were hoarded in the light period. Neither the highest hoarding score for each animal nor the lowest hoarding score for each animal differed significantly over days of the estrous cycle. The group that was fed *ad libitum* decreased nocturnal food hoarding over time, whereas the food-restricted females' nocturnal hoarding scores remained elevated and were significantly higher than those of the *ad libitum*-fed females on at least one time point every night ($P<0.05$, Fig. 1, top).

Food intake

Food intake showed a different pattern (Fig. 1, bottom). There was no significant difference between the amount of food eaten during the dark period and the amount of food eaten during the light period on 3 days of the estrous cycle, whereas very little food was eaten during the dark period of proestrous, the periovulatory period in the females fed *ad libitum* (Fig. 1, bottom). Food-restricted females had elevated food intake compared to *ad libitum*-fed females on only two of the 24 time points measured ($P<0.05$).

Body weight

Body weight gain and loss showed cyclic fluctuations with regard to the light and dark phases of the photoperiod, with weight gain at night and weight loss during the light period, and no significant differences over days of the estrous cycle (data not shown).

Experiment 2: Sex and ingestive behaviors over the estrous cycle during food scarcity and abundance

For all variables that were measured, the *ad libitum*-fed females did not differ significantly from the females that were time-restricted but given *ad libitum* access to food (Table 1), and thus, these two groups are combined into one group and named "Energy-abundant" in all graphs. The food-restricted females are referred to as "Energy-limited."
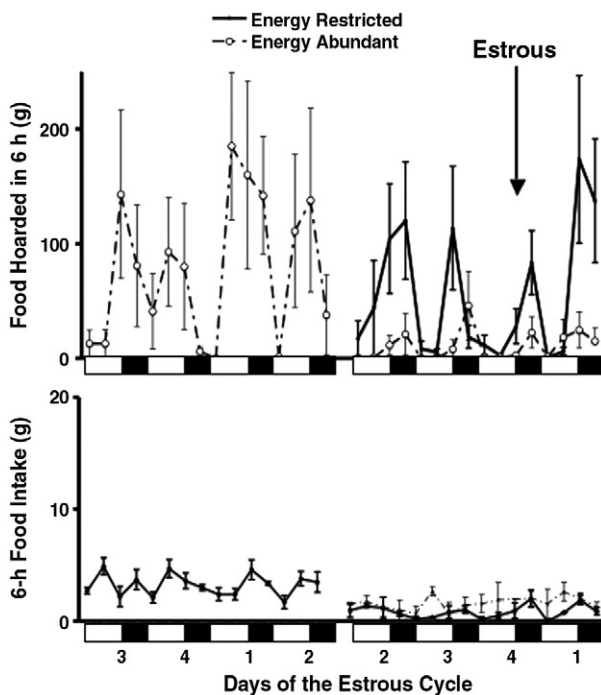
Table 1
Preference Behavior in Experiment 2.

	Follicular day 1	Follicular day 2	Periovulatory day	Postovulatory day
Number of vaginal marks (mean ± s.e.m.)				
Time-restricted	4.0 ± 1.2	4.4 ± 2.3	1.0 ± 1.0	3.0 ± 1.1
Ad libitum-time	3.6 ± 2.5	10.6 ± 3.1	0 ± 0	2.6 ± 1.6
Number of flank marks				
Time-restricted	2.8 ± 1.9	4.4 ± 2.9	0 ± 0	0.2 ± 0.2
Ad libitum-time	5.1 ± 2.8	11.1 ± 4.4	0 ± 0	3.3 ± 1.4
Food hoarded (g)				
Time-restricted	48.7 ± 40.7	12.4 ± 11.0	0.71 ± 0.31	44.7 ± 40.6
Ad libitum-time	4.0 ± 1.8	21.0 ± 18.7	0.29 ± 0.18	22.4 ± 21.4
Food eaten (g)				
Time-restricted	1.3 ± 0.39	1.20 ± 0.62	0.71 ± 0.31	1.2 ± 0.34
Ad libitum-time	1.2 ± 0.15	1.20 ± 0.71	0.29 ± 0.18	1.1 ± 0.66
Male preference ((time with male − time with food) / total time)				
Time-restricted	0.67 ± 0.15	0.53 ± 0.12	0.98 ± 0.01	0.60 ± 0.06
Ad libitum-time	0.38 ± 0.13	0.54 ± 0.12	0.95 ± 0.02	0.49 ± 0.10

Food hoarding

Food hoarding was significantly greater in Energy-limited compared to Energy-abundant females on the postovulatory day and on follicular day 1 ($P<0.009$ and 0.003 respectively, Fig. 2, top). Energy-limited hamsters showed a marked decrease in food hoarding on the night of follicular day 2 (the night of vaginal scent marking) to a level not significantly different from Energy-abundant hamsters (Fig. 2, top). On the night of proestrous, the periovulatory period, food hoarding was at
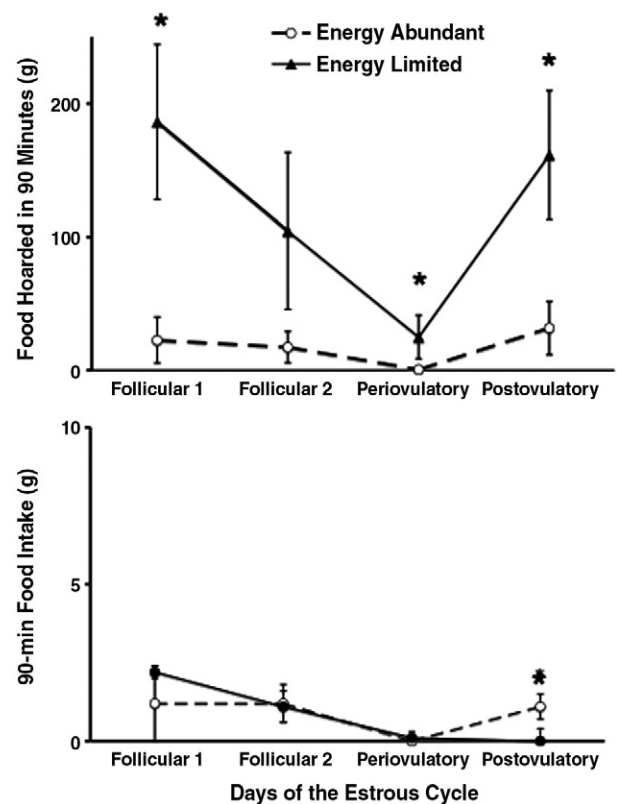

Fig. 1. Mean and standard error of the mean for amount of food hoarded (top) and food intake (bottom) in hamsters tested every 6 h of the 4-day estrous cycle. All females were singly housed. All were fed *ad libitum* prior to and during testing for the first 6 days (hatched lines, open circles), and half food-restricted (solid lines, filled circles), half fed *ad libitum* for the last 6 days of testing.

Fig. 2. Mean and standard error of the mean for amount of food hoarded (top) and food intake (bottom) in hamsters tested during a 90 min period that spanned the onset of the dark phase of the photoperiod each day of the 4-day estrous cycle. Female hamsters were either fed *ad libitum* ("Energy-abundant") or food-restricted to 75% of their baseline *ad libitum* daily intake ("Energy-limited") for 8 days before testing. *Significantly different from *ad libitum* at $P<0.05$.

its nadir but was still significantly higher in the Energy-restricted group compared to the Energy-abundant group because the latter group hoarded no food and was in persistent lordosis on the periovulatory day ($P<0.04$, Fig. 2, top). In the Energy-limited group, repeated measures ANOVA revealed significant changes over the estrous cycle in food hoarding ($F(3,15)=3.7$, $P<0.04$, Fig. 2, top), whereas fluctuations over the estrous cycle in hoarding were not statistically significant in the Energy-abundant group (Fig. 2, top).

Food intake

In contrast to food hoarding, there were no significant changes over days of the estrous cycle in 24-h food intake in either group or in the groups combined, and females that were Energy-limited did not differ from Energy-abundant in food intake during testing (Fig. 2, bottom).

Male preference

In the Energy-limited, but not Energy-abundant females, there were significant changes over the estrous cycle in the preference for males ((time with males − time with food) / total time) (Fig. 3), and the Energy-restricted hamsters showed significantly lower preference for males than Energy-abundant on all days of the estrous cycle ($P<0.004$, 0.001, 0.01 and 0.003 for the four days shown in Fig. 3).

Lordosis and scent marking

There were no significant differences between Energy-restricted and Energy-abundant females in lordosis duration (measured on the periovulatory day), vaginal marking, or flank marking (measured on Follicular 2, Fig. 4). In Energy-abundant but not restricted females, vaginal scent marking changed significantly over the estrous cycle with the highest level of vaginal scent marking on follicular day 2 of the estrous cycle, the day before mating and ovulation ($F(3,15)=4.3$, $P<0.01$, Table 1). There were significant changes over the estrous cycle in flank marking in the Energy-abundant females ($F(3,12)=4.4$, $P<0.01$, Table 1).

Experiment 3: Effects of exogenous progesterone and estradiol in food scarcity and abundance

Food hoarding

First, the data were analyzed with the hoarding scores of hamsters receiving different doses of estradiol divided into separate groups (Fig. 5, top). With regard to food hoarding on day 4 of food restriction, two-way ANOVA showed a significant main effect of hormone
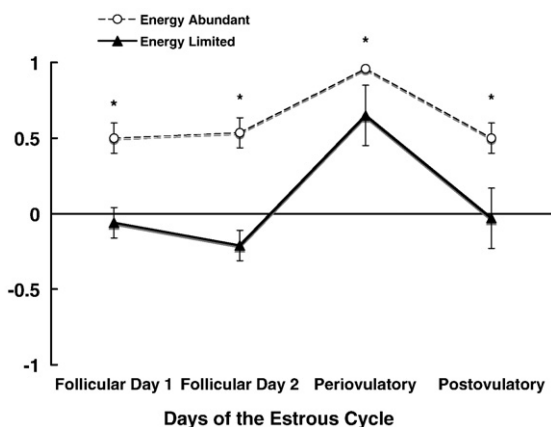
Fig. 3. Mean and standard error of the mean for male preference, calculated as the (time spent with males − the time spent with food) / the total time. Preference was measured during a 15 min test each day of the 4-day estrous cycle. Female hamsters were either fed *ad libitum* ("Energy-abundant") or food-restricted to 75% of their baseline *ad libitum* daily intake ("Energy-limited") for 8 days before testing. *Significantly different from *ad libitum* at $P<0.05$.
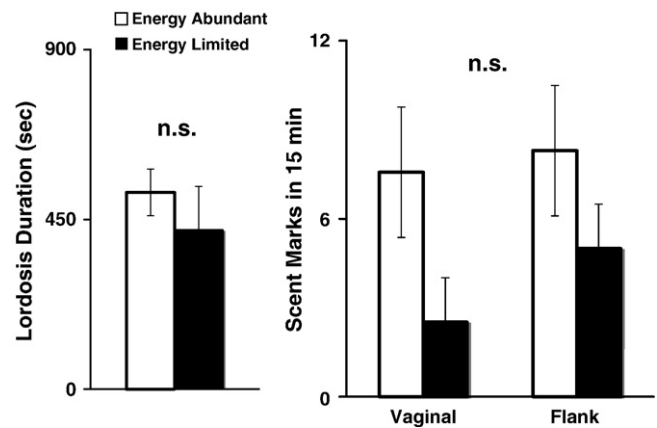
Fig. 4. Mean and standard error of the mean for lordosis duration (left), and scent marking, both vaginal and flank (right) in female Syrian hamsters. Female hamsters were either fed *ad libitum* ("Energy-abundant") or food-restricted to 75% of their baseline *ad libitum* daily intake ("Energy-limited") for 8 days before testing. n.s. = not significantly different.

treatment ($F(3,38)=3.0$, $P<0.04$). Planned comparisons showed that the hoarding scores of food-restricted, vehicle-treated hamsters were significantly higher than those of each of the food-restricted, E + P-treated groups ($P<0.01$, 0.05, and 0.03 for 1.5, 2.5 and $20\,\mu g$ respectively, Fig. 5, top).

There were no significant differences in hoarding among the three E + P-treated groups (Fig. 5 and Table 2), and food hoarding was not significantly correlated with estradiol doses between 1.5 and $20\,\mu g$, and so these groups were collapsed into one hormone-treated group. When hoarding on day 4 of restriction was analyzed this way, there was a significant main effect of diet ($F(1,40)=7.7$, $P<0.01$), hormone treatment ($F(1,40)=5.4$, $P<0.02$) and a significant interaction ($F(1,40)=4.3$, $P<0.04$), reflecting the fact that restriction-induced hoarding significantly increased in the OVX-vehicle-treated, but not in the OVX-E + P hamsters ($P<0.01$) (Fig. 5, bottom). On day 8, the main effect of restriction was significant ($F(1,40)=11.54$, $P<0.002$) but the main effect of hormone treatment was not, and both the vehicle and E + P-treated hamsters showed significant restriction-induced food hoarding ($P<0.04$ and 0.003 respectively). In particular, the food-restricted, E + P-treated hamsters had high levels of food hoarding on day 8 compared to *ad libitum*-fed, E + P-treated hamsters ($P<0.05$), while they had low levels of food hoarding on day 4 of restriction and on day 4 after re-feeding (Fig. 5, bottom right). On days 4 and 8 after re-feeding, there were no significant main effects or interactions, although inspection of the rank order of the means shows that the vehicle-treated, food-restricted group was the last to return to baseline hoarding levels (Fig. 5, bottom left).

Male preference

When we calculated the preference for males vs. food ((time with male − time with food) / total time), the different estradiol dose groups did not differ from each other and estradiol dose was not significantly correlated with male preference at 4 and 8 days after restriction (Table 3). The 3 groups treated with different doses of estradiol were collapsed into one E + P-treated group for days 4 and 8 after restriction. On day 4 of restriction, there was no significant main effect of energy availability and a significant main effect of hormone treatment on male preference ($F(1,42)=8.86$, $P<0.05$). Planned comparisons showed that food-restricted, vehicle-treated females had a significantly lower male preference than did the food-restricted, E + P-treated females ($P<0.008$). There were no other significant differences (Fig. 6). On day 8 of restriction, the main effect of diet ($F(1,42)=14.71$, $P<0.001$) and hormone treatment ($F(1,42)=11.06$, $P<0.002$) were significant (Fig. 6). Both the vehicle-treated
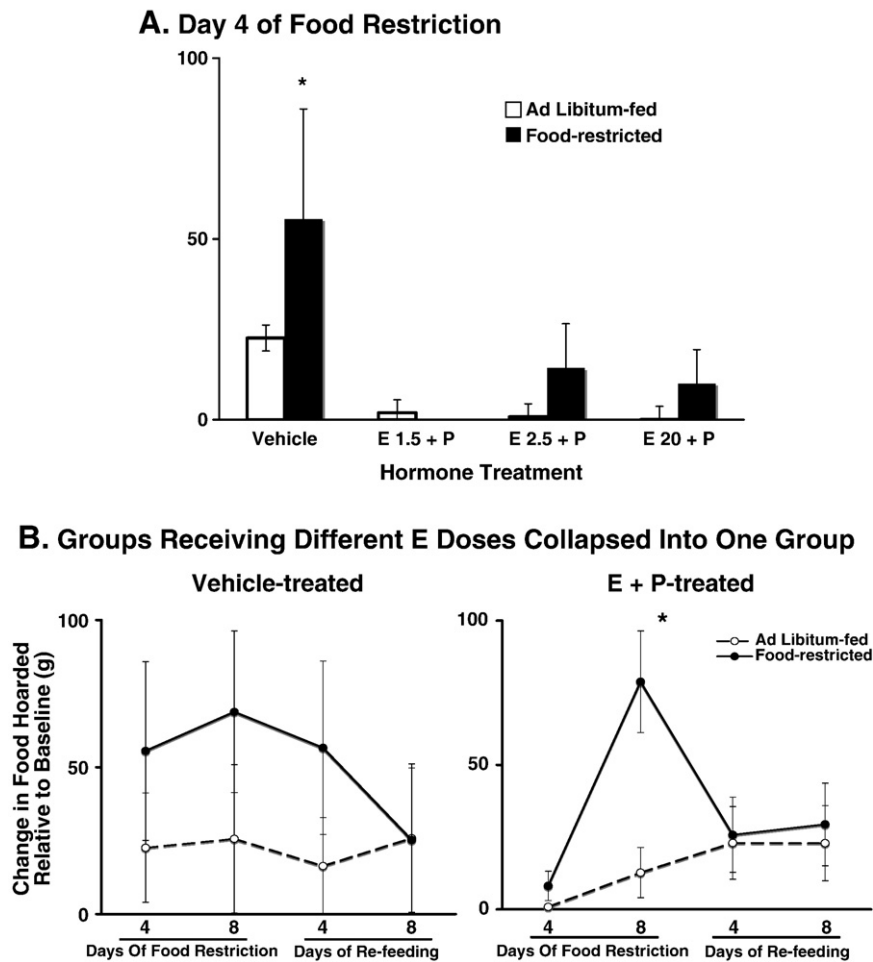
Fig. 5. Top: Mean and standard error of the mean for amount of food hoarded in hamsters tested during a 90 min period near the onset of the dark phase of the photoperiod. Hamsters were treated by s.c. injection with 500 μg progesterone or the oil vehicle 6 h before testing and with different doses of estradiol (1.5, 2.5 or 20 μg) or the oil vehicle 48 h before testing. Half of each group was either fed *ad libitum* or food-restricted to 75% of their baseline *ad libitum* daily intake. Bottom: Mean and standard error of the mean for amount of food hoarded in hamsters treated with vehicle (left) or estradiol plus progesterone (right). Females from three estradiol dose groups collapsed into one group. Data are shown for days 4 and 8 of restriction and days 4 and 8 after return to *ad libitum* access to food. *Significantly different from *ad libitum* at $P<0.01$.

and E + P-treated hamsters showed significant restriction-induced decreases in male preference (both $P<0.04$).

To analyze male preference on days 4 and 8 after re-feeding, estradiol dose groups were not combined because there were significant differences between the 20 μg group and the other groups, and male preference was significantly correlated with estradiol dose ($P<0.05$). On day 4 after re-feeding, the main effect of hormone treatment and the interaction were significant for male preference ($F(1,42)=12.9$, 3.32, $P<0.0001$, 0.03 respectively), whereas the effect of diet was not significant. In the previously food-restricted, vehicle-treated females, preference for males after re-feeding was significantly increased relative to *ad libitum*-fed females ($P<0.001$). In the food-restricted, E + P-treated females, male preference increased toward the levels of the *ad libitum*-fed females after 4 days of re-feeding (Fig. 6).

On day 8 after re-feeding, the main effects of diet and hormone treatment were significant for male preference ($F(1,42)=4.86$, 13.66, $P<0.03$, 0.0001 respectively). In the previously food-restricted, vehicle-treated females, preference for males was significantly increased relative to *ad libitum*-fed females ($P<0.002$), and in the food-restricted, E + P-treated females, male preference increased toward the levels of the *ad libitum*-fed females after 4 days of re-feeding (Fig. 6).

Hormone treatment significantly decreased time spent in the home cage. There was a significant main effect of hormone treatment

on the preference for spending time in the home on all four days ($F(1,40)=10.73$, 5.99, 3.9, and 5.7 and $P<0.002$, 0.02, 0.05 and 0.02 for each time point respectively), with no significant dose–response relation between 1.5 and 20 μg E + P (data not shown). The *ad libitum*-fed, E + P-treated females showed a significantly lower preference for the home compared to *ad libitum*-fed, vehicle-treated females at all time points ($P<0.03$). The food-restricted, E + P and the food-restricted, vehicle-treated hamsters all showed similarly low preference for the home cage (data not shown), and these groups did not differ significantly.

Lordosis duration

All E + P-treated groups decreased lordosis duration at 8 days of restriction and increased lordosis duration at 4 days of re-feeding, and repeated measures ANOVA showed a significant change over time ($F(3,45)=4.134$, $P<0.01$) in the food-restricted but not in the *ad libitum*-fed group. After the restriction period extended to 8 days, even those females treated with E + P decreased lordosis duration (Fig. 7). For hamsters fed *ad libitum* or food-restricted, there were no significant differences among the hamsters receiving different doses of E in lordosis duration on day 4 or 8 after re-feeding.

Only those hamsters treated with E + P showed lordosis. The frequency of *ad libitum*-fed females that showed lordosis in response to the restrained male and in response to the unrestrained male was not significantly different among the groups treated with different

Table 2
Lordosis duration and food hoarded and eaten during the preference test in Experiment 3.

	E dose	Length of restriction		Length of re-feeding	
		4 days	8 days	4 days	8 days
Duration of lordosis (s) (mean ± s.e.m.)					
Food-restricted	1.5 μg	310 ± 128	156 ± 108	158 ± 102	339 ± 109
	2.5 μg	301 ± 107	0 ± 0	126 ± 90	151 ± 89
	20.0 μg	364 ± 88	154 ± 85	388 ± 96	382 ± 96
	Veh	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ad libitum-fed	1.5 μg	548 ± 5	345 ± 105	423 ± 46	411 ± 73
	2.5 μg	367 ± 60	340 ± 60	242 ± 60	401 ± 43
	20.0 μg	470 ± 59	428 ± 63	422 ± 49	511 ± 27
	Veh	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Food hoarded (g) (mean ± s.e.m.)					
Food-restricted	1.5 μg	0 ± 0	47 ± 29	52 ± 27	8 ± 5
	2.5 μg	14 ± 12	100 ± 26	25 ± 26	72 ± 32
	20.0 μg	10 ± 9	64 ± 32	1 ± 1	0 ± 0
	Veh	56 ± 30	69 ± 28	57 ± 30	25 ± 24
Ad libitum-fed	1.5 μg	2 ± 1	28 ± 28	30 ± 30	31 ± 31
	2.5 μg	1 ± 1	4 ± 3	6 ± 3	2 ± 1
	20.0 μg	0 ± 0	7 ± 4	32 ± 24	33 ± 25
	Veh	23 ± 19	26 ± 25	16 ± 16	26 ± 25
Food eaten (g) (mean ± s.e.m.)					
Food-restricted	1.5 μg	1.6 ± 0.4	1.3 ± 0.5	0.7 ± 0.3	0.6 ± 0.2
	2.5 μg	0.5 ± 0.2	0.9 ± 0.6	1.0 ± 0.6	0.8 ± 0.5
	20.0 μg	1.0 ± 0.2	1.4 ± 0.4	0.7 ± 0.2	0.2 ± 0.1
	Veh	1.7 ± 0.5	1.5 ± 0.7	0.8 ± 0.5	1.7 ± 0.5
Ad libitum-fed	1.5 μg	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.4 ± 0.1
	2.5 μg	0.8 ± 0.4	0.8 ± 0.3	0.6 ± 0.2	0.8 ± 0.6
	20.0 μg	0.4 ± 0.2	0.5 ± 0.4	0.4 ± 0.2	0.4 ± 0.2
	Veh	0.7 ± 0.5	0.3 ± 0.2	1.5 ± 0.6	1.1 ± 0.6

Table 3
Male Preference in Experiment 3 (time with male-time with food)/total time (mean ± s.e.m.).

	E dose	Length of restriction		Length of re-feeding	
		4 days	8 days	4 days	8 days
Food-restricted	1.5 μg	0.14 ± 0.10	0.10 ± 0.07	0.40 ± 0.05	0.43 ± 0.04
	2.5 μg	0.37 ± 0.16	0.35 ± 0.13	0.53 ± 0.04*	0.62 ± 0.02*
	20.0 μg	0.44 ± 0.12	0.04 ± 0.10	0.48 ± 0.06*	0.53 ± 0.07*
	Veh	0.55 ± 0.06	0.16 ± 0.15	0.64 ± 0.01	0.63 ± 0.02
Ad libitum-fed	1.5 μg	0.36 ± 0.09	0.22 ± 0.10	0.09 ± 0.13	0.15 ± 0.12
	2.5 μg	0.55 ± 0.05	0.51 ± 0.13	0.62 ± 0.02	0.59 ± 0.05
	20.0 μg	0.41 ± 0.12	0.37 ± 0.17	0.46 ± 0.09	0.47 ± 0.06
	Veh	0.55 ± 0.05	0.60 ± 0.02	0.57 ± 0.04	0.63 ± 0.01

doses of E + P (Fig. 8). Food restriction for 8 days, but not 4 days significantly decreased the number of females that showed lordosis, and fewer females showed lordosis in response to the restrained male compared to the number that showed lordosis in response to the unrestrained male (P < 0.05). Almost all of the females failed to show lordosis at 12 days after the start of food restriction (Fig. 8). The frequency of females that showed lordosis in response to E + P in response to a male was significantly lower after 8 days of restriction in the group treated with the middle dose of estradiol (2.5 μg) than the number that showed lordosis in the other two groups (Fig. 8).

Discussion

These experiments demonstrate that ovarian hormones increase appetitive sex behaviors and decrease appetitive ingestive behaviors, but hormonal effects differ as a function of the availability of food and potential mating partners.

First, when males were absent and energy was abundant, female food hoarding was highly nocturnal but did not vary over the estrous cycle, and food restriction did not reveal or unmask fluctuations over the estrous cycle in food hoarding (Experiment 1). Next, when access to adult males was coupled with limited energy availability, nocturnal food hoarding increased on 3 out of 4 days of the cycle, but, food hoarding began to decrease the night before ovulation in association with high endogenous levels of estradiol, and food hoarding ceased as sexual motivation peaked on the night of ovulation. This effect of estradiol was masked in females with unlimited energy availability (Experiment 2). Third, in ovariectomized females, exogenous treatment with estradiol and progesterone delayed the onset of food restriction-induced hoarding and reversed the preference for food that was typical of the food-restricted, vehicle-treated hamsters. Estradiol plus progesterone countered the effects of food restriction in the early stages of restriction but failed to do so in the later stages of food restriction (Experiment 3). Last, when the previously food-restricted females were returned to *ad libitum* food intake, ovarian hormone-treated females rapidly decreased hoarding and increased male preference relative to oil-treated females (Experiment 3). These results are consistent with the idea that the hormones of the estrous cycle modulate ingestive and sex behaviors according to the availability of energy and potential mates. This role of estradiol was not obvious in previous experiments because ingestive behaviors were measured in females isolated from males and under conditions of artificial energy abundance and minimal energy demands. The
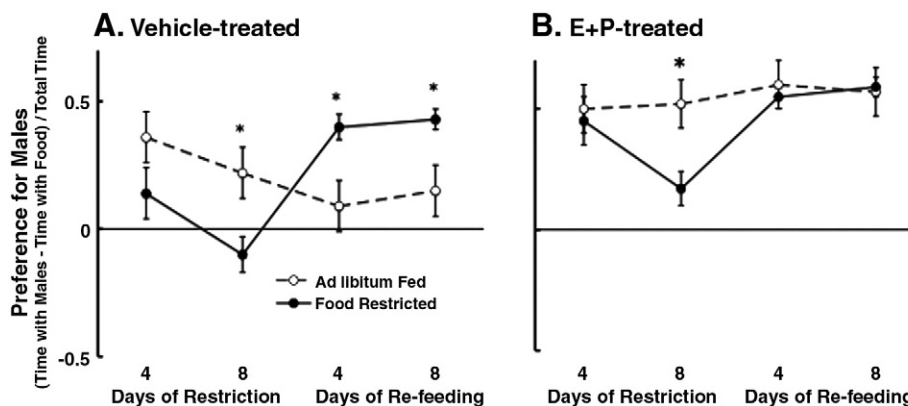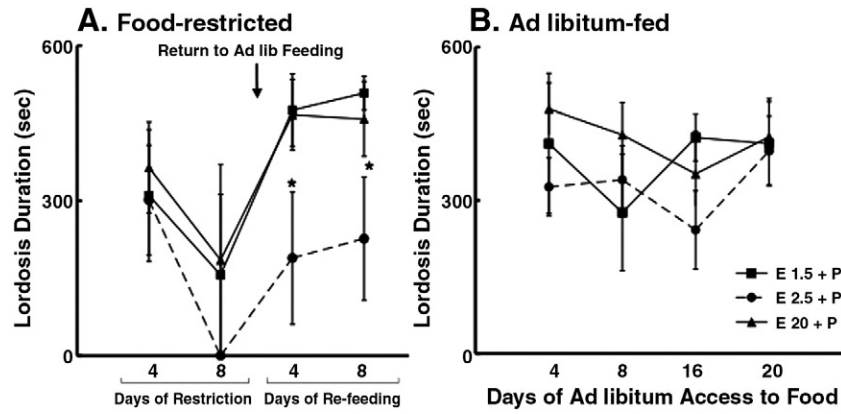

Fig. 6. Mean and standard error of the mean for male preference in hamsters A) tested at 4 and 8 days of food restriction or *ad libitum* feeding, and B) after 4 and 8 days of return to *ad libitum* feeding. Preference was calculated as the (time spent with males − the time spent with food) / the total time. Hamsters were treated by s.c. injection with 500 μg progesterone 6 h before testing and with different doses of estradiol (1.5, 2.5 or 20 μg) or the oil vehicle 48 h before testing. *Significantly different from *ad libitum* at P < 0.05.

Fig. 7. Mean plus standard error of the mean for lordosis duration in females either A) restricted to 75% of their baseline daily food intake for 4, 8 or 12 days and then returned to *ad libitum* food access for 4 and 8 days, or B) fed *ad libitum* during the entire experiment. All females were ovariectomized and treated with 500 μg progesterone 6 h before testing and one of three doses of estradiol benzoate: 1.5, 2.5 or 20 μg 48 h before testing. Vehicle-treated hamsters did not show lordosis and data are not shown. *Significantly different from other two estradiol dose groups at $P<0.05$.

present experiments are rare in that they examined the choice between food and sex under conditions that mimic important aspects of the natural habitat. When females were energetically challenged and offered a choice between food and males, energy restriction unmasked the function of ovarian hormones, i.e., to ensure that females risk an investment in reproduction during the fertile period despite the conflicting motivation to hoard food in anticipation of future food shortages.

In Experiment 1, the nocturnal pattern of food hoarding was consistent with previous experiments in which male Syrian hamsters hoarded more during the dark phase of the photoperiod; it is unfortunate that females were not included in these previous studies (Charlton, 1984; Toates, 1978; Waddell, 1951). Other investigators have reported a nadir in food hoarding on the night of proestrous in singly housed females when hoarding was measured for 30 min per day at the onset of the dark period (Estep et al., 1978); in contrast, in the present study, day–night differences in hoarding dwarfed any differences among days of the estrous cycle (Fig. 1). The fluctuations in food hoarding in the earlier study by Estep et al. (1978) and in Experiment 1 of the present study were modest compared to those seen in Experiment 2, in which females had the option of visiting a male. Food restriction combined with the presence of the male brought to light the role of ovarian steroids at the time of highest fertility.

In Experiment 1, food intake, in contrast to food hoarding, was not strictly nocturnal, and in Experiment 2, food intake varied far less than food hoarding over the estrous cycle. This is typical of the species under study as Syrian hamsters eat small meals throughout the light

and dark periods, consuming between 40 and 45% of their food during the light period, and fail to increase food intake after a metabolic challenge such as food restriction (Schneider et al., 1986; Silverman and Zucker, 1976; Zucker and Stephan, 1973). It is interesting in this regard that food purchases increase with hunger levels in human beings, and food purchases are affected differentially by relative body weight (Mela et al., 1996; Tom, 1983).

In Experiment 2, energy-restricted female hamsters with access to males showed marked fluctuations in food hoarding over the estrous cycle compared to *ad libitum*-fed females (Fig. 2) and time-limited hamsters fed *ad libitum* (Table 1). Decreases in food hoarding at proestrous have been reported in both Syrian hamsters and rats when hoarding was measured at one time point on every day of the estrous cycle in the absence of males (Estep et al., 1978; Herberg et al., 1972). The present experiment makes the important distinction that energy restriction (but not time restriction) increases food-related behaviors on nonestrous days and decreases food-related behaviors during the periovulatory period, thus creating marked fluctuations in hoarding over the estrous cycle (Fig. 2).

Appetitive, but not consummatory behaviors were affected by mild food restriction. Appetitive behaviors are defined as those involved in the motivation to seek out and come in contact with food or mates, and are, to some degree, independent of the consummatory acts of eating or copulating (Ball and Balthazart, 2008; Beach, 1976; Craig, 1917; Everitt, 1990; Lorenz, 1950; Sherrington, 1906). Depending on the species under study, appetitive behaviors are expressed as specific paracopulatory behaviors that occur before or after copulation (vaginal marking is one example), or as other
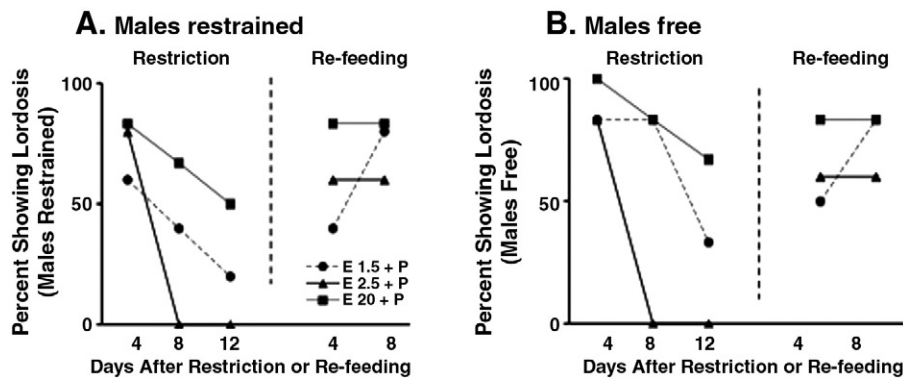

Fig. 8. Percent of food-restricted hamsters showing lordosis when tested with A) restrained males or B) with males restrained behind a wire cage. In the former group, females received gentle flank stimulation by the experimenter. All females were ovariectomized and treated with 500 μg progesterone 6 h before testing and one of three doses of estradiol benzoate: 1.5, 2.5 or 20 μg 48 h before testing. Vehicle-treated hamsters did not show lordosis and data are not shown.

expressions of sexual motivation that can overlap in time with copulation, such as the amount of time spent with males versus food. The distinction between appetitive and consummatory behaviors can be useful when these behaviors are controlled by different mechanisms or are differentially responsive to environmental stimuli (reviewed by Ball and Balthazart, 2008). For example, in Experiment 2, the appetitive ingestive behavior, food hoarding, increased markedly in food-restricted females (Fig. 2), whereas the consummatory ingestive behavior, food intake, did not increase significantly after food restriction (Fig. 2). In a similar vein, the appetitive sex behaviors, male preference (Fig. 3) and vaginal scent marking (Table 1), were decreased in the early stages of food restriction prior to any significant effects on the consummatory sex behavior, lordosis duration (Fig. 4). Consistent with these results, separable mechanisms governing appetitive versus consummatory sex and ingestive behavior were shown in rats and Syrian hamsters (Ammar et al., 2000; Buckley and Schneider, 2003; Schneider et al., 2007a) and in ingestive behavior in Siberian hamsters (Bartness, 1997). Together these results suggest we should examine the mechanisms whereby hormones prioritize the motivations that control potentially conflicting behaviors.

In contrast to food availability, time availability had no effect when hamsters had *ad libitum* food intake in Experiment 2. We had postulated that limited time availability might increase food hoarding, and that ovarian hormones might ameliorate this effect of limited time. In the wild, Syrian hamsters are thought to be subject to environmental pressures that might limit the time they spend outside their burrows to particular times of day, e.g., pressures such as nocturnal predators (e.g., carnivorous birds, reptiles or mammals), intense mid-day heat, or low nighttime ambient temperatures (Gattermann et al., 2008). If time for daily hoarding is limited by these pressures, an important role for periovulatory increases in circulating estradiol might be to divert energy toward courtship and mating even though this might mean missing the day's only opportunity for foraging and food hoarding. If time were a limiting factor that interacts with ovarian hormone levels, it would be predicted that hamsters with limited time for both mating and hoarding would show more dramatic fluctuations over the estrous cycle in hoarding than those with unlimited time. To the contrary, this hypothesis was not supported, since time-restricted females with unlimited access to food did not differ from females with unlimited time and food availability in their sex and ingestive behaviors (Table 1). It was noted that the time-limited females hoarded significantly more than the *ad libitum*-fed females prior to testing and during training, when subject females did not have access to males (data not shown). Time-limited females learned to use their limited hoarding time to gather food during the training period, yet they did not retain this behavior once males became available. Instead, they chose to spend their time in close proximity to males, perhaps because there was no energetic consequence to this decision (the experimenter provided food after the end of the test). The lack of fluctuation in hoarding over the estrous cycle in the time-limited group reinforces the notion that ovarian steroids interact with *energy availability* to determine the choice between food and sex.

Food hoarding in restricted females fluctuated in association with well-known changes in circulating estradiol and progesterone. The amount of food hoarded was lowest during the proestrous periovulatory period, subsequent to increases in circulating estradiol concentrations in female Syrian hamsters (Baranczuk and Greenwald, 1973). The amount of food hoarded increased the day after ovulation (when circulating progesterone levels increase), remained high during follicular day 1 (when both progesterone and estradiol are at their nadir), began to decline on follicular day 2 (when estradiol concentrations rise). To test whether ovarian hormones account for these fluctuations across the estrous cycle, the effects of exogenous E and P on hoarding in food-restricted females were examined in Experiment 3.

In Experiment 3, in oil-treated, OVX controls, four days of food restriction increased food hoarding, whereas treatment with E + P delayed the effects of food restriction on food hoarding to 8 days (Fig. 5). This recapitulates the suppression of food restriction-induced increases in hoarding at estrous in Experiment 2, and is the first evidence that the hormonal regime routinely used in this field of research to induce estrous behavior decreases food hoarding. Experiments in progress suggest that decreased hoarding in the presence of males is due primarily to estradiol-induced sexual motivation because food-restricted, ovariectomized females treated with estradiol alone decrease hoarding when males are present but increase food hoarding when the males are removed, whereas females treated with progesterone alone increase food hoarding whether or not males are present (Klingerman and Schneider, unpublished observations). Furthermore, food hoarding increases during late pregnancy along with rising levels of circulating progesterone in hamsters and rats (Bartness, 1997; Coling and Herberg, 1982; Fleming, 1978), and treatment with estradiol alone decreases food hoarding in rats (Coling and Herberg, 1982). Further testing is underway to differentiate between the role of estradiol, progesterone or estradiol + progesterone, hormones with clear effects on body weight, food intake, activity and thermogenesis in hamsters and other species (Bhatia and Wade, 1989; Fessler, 2003; Swanson, 1968; Zucker et al., 1972).

In Experiment 3, the importance of energy availability was underscored when previously food-restricted females were returned to *ad libitum* food access. In re-fed, OVX females, E + P treatment rapidly increased male preference ((time with males minus time with food) / total time) (Fig. 6). Even in the vehicle-treated hamsters, return to *ad libitum* feeding significantly increased male preference (Fig. 6), cautioning against assuming that increased male preference relative to food preference is a strictly "sexual" behavior.

When restriction was increased to 8 and 12 days, reproductive behaviors were inhibited, confirming previous research in Syrian hamsters (Morin, 1975, 1986; Schneider and Wade, 1989), including paracopulatory appetitive behaviors, such as preference for males versus food (Schneider et al., 2007b). Experiment 3 showed that the longer the food restriction period, the more likely reproductive behaviors would be eliminated, even in animals treated with exogenous doses of E and P. After the restriction period extended to 8 and 12 days, even those females treated with E + P decreased lordosis duration and male preference and increased food hoarding and time spent in the food box (Figs. 5–8). In addition, as food restriction became more severe, the number of females that failed to show the lordosis reflex during testing increased significantly (Fig. 8). This effect of restriction was significantly higher when the males were restrained than when the males had full access to the females and were able to make tactile contact, lick and mount the females (Fig. 8). These data show that the appetitive aspects of sex behavior, those that reflect sexual motivation, are more sensitive to energy availability than sexual performance. Similarly, the appetitive ingestive behavior, food hoarding, is more sensitive to energy availability than the consummatory ingestive behavior, food intake. This work might also be the first to document the ability of prolonged food restriction to negate the ability of exogenous E + P to suppress food hoarding (Fig. 5).

In the present study, energetic inhibition of sexual motivation likely involves changes in sensitivity to hormones, rather than changes in circulating concentrations of hormones, because all females in Experiment 3 showed inhibitory effects of food restriction even though they had the same exogenous doses of E + P as *ad libitum*-fed females. Previous work has demonstrated that the levels of food deprivation that decrease lordosis duration in OVX females treated with E + P have measurable effects on estradiol receptor alpha immunoreactivity (ERαIR) while at the same time, increasing, not decreasing circulating concentrations of ovarian steroids (Li et al., 1994; Panicker et al., 1998). In food-deprived females, ERIR decreases

in the ventromedial hypothalamus and significantly increases in the medial preoptic area, the arcuate nucleus, and the posterior parvicellular paraventricular nucleus of the hypothalamus (Li et al., 1994; Panicker et al., 1998). The relevance of these findings with regard to food deprivation-induced decreases in lordosis duration, the HPG system and estrous cyclicity have been noted (Wade and Jones, 2004), and now should be explored with regard to food hoarding, male preference, vaginal scent marking and other behaviors that reflect behavioral motivation rather than ability.

The relation between behaviors and estradiol dose was not linear at the doses used in our experiment. Lordosis duration has been shown to increase with increasing doses of E in other studies with the same estradiol doses but when females did not have a choice between food and sex (Jones et al., 2002). In the present experiment, all three doses of estradiol delayed the effects of food restriction on food hoarding and the time spent with food when compared to vehicle treatment.

There was a discrepancy between the intact (Experiment 2) and ovariectomized females treated with E + P (Experiment 3) in the efficacy of food restriction. In Experiment 2, 8 days of restriction had no effect on lordosis duration or food hoarding in intact females during the periovulatory period (Figs. 2 and 4), whereas in Experiment 3, 8 days of restriction significantly inhibited lordosis duration and increased food hoarding (Figs. 5 and 7). Differences between experiments might be due to the inability of E + P injections to duplicate hormonal fluctuations in intact females or to unknown differences between the animals used in the two experiments. Both groups of females were between 3 and 4 months of age and between 100 and 150 g in body weight.

Experiment 3 used the hormone regimen that has been most commonly used to induce estrous behavior, E + P, and showed, for the first time, that it significantly attenuated food hoarding and the preference for food under energetically demanding conditions (Figs. 5 and 6). This energy-hormone interaction suggests a mechanism whereby the circulating concentrations of E and P that are required for the lordosis reflex can also attenuate the drive to hoard food. Estradiol, in particular, might interact with energy availability at the level of intracellular metabolism, and this interaction might involve fuel oxidation, lipolysis or both. White adipose tissue estrogen receptors mediate estradiol-induced decreases in lipoprotein lipase activity that are a prerequisite to lipid storage (Wade and Gray, 1979; Wade et al., 1985). In addition, liver and skeletal muscle are likely sites for estradiol-induced increases in thermogenesis and fatty acid oxidation that is thought to account for decreases in body weight that cannot be accounted for by decreases in food intake (Jones et al., 1991; Schneider et al., 1986). Thus, one possibility is that estradiol produces increases in the flow of oxidizable fuels away from storage in adipose tissue and into tissue where they are oxidized, which, in turn, might generate signals in brain or periphery that lead to either decreased motivation for eating/hoarding or more motivation for reproductive activities, or both. Preliminary evidence suggests that estradiol alone acts mainly by increasing sexual motivation (Klingerman et al., unpublished observations), a result that does not preclude a hormonal effect via the disposition of metabolic fuels. The effects of estradiol were masked under conditions of energy abundance and were not unmasked when time was the only limiting factor in Experiment 2, suggesting that the availability of oxidizable metabolic fuels interacts with estradiol to determine the choice between food and sex.

One role of ovarian steroids, especially estradiol, is to increase the probability of risky sexual behaviors, exploration and novelty in females of many species, including women. Sex behavior incurs substantial risks, due to increased chance of predation, injury, sexually-transmitted disease, parasites, other diseases and the disruption of social hierarchies (Wallen, 2000). In the relative safety of the laboratory, when female primates are housed in small enclosures with only one male, mating occurs throughout the menstrual cycle, and the role of estradiol is obscured. In contrast, when sex behavior is studied in the contexts in which these behaviors occur in nature, in large, open spaces with opportunities to hide or escape, it becomes apparent that the hormones of the ovulatory period are essential for overcoming the risks involved in sexual liaisons (Wallen, 2001). In field studies and in large seminatural habitats that allow individuals to escape or hide from their peers, primate sex behavior clusters in association with high circulating concentrations of estradiol. The unnatural, small, enclosed chambers used in the early years of primate laboratory research obscured the importance of estradiol in control of female sexual motivation. The present studies highlight energetic context as another important factor that can either mask or reveal the effects of ovarian hormones on both sexual and hunger motivation. The ability of estradiol to increase sexual motivation and reduce ingestive motivation were most apparent in the early stages of food restriction and immediately after re-feeding, perhaps reflecting that reproductive success is increased when sex drive is immune to mild energetic challenges and when sex drive is quick to rebound as food becomes abundant. More detailed studies are under way to examine whether energy-restricted females will mate directly after meals or bouts of food hoarding, whether mated females will increase food hoarding in anticipation of pregnancy and lactation and whether males will provision females with nuptial gifts of food (Schneider and Klingerman, unpublished observations).

All females treated with E + P spent significantly less time in the home compared to vehicle-treated females, consistent with the notion that estradiol is associated with increased exploration, novelty and risk-taking (reviewed by Fessler, 2003). These inhibitory effects on hoarding and stimulatory effects on exploration and courtship might have functional significance at the onset of puberty, during adult estrous cycles, in the interim of parturition and the next pregnancy and during seasonal fluctuations in ovarian steroids. Hamsters are seasonal breeders and become gonadally regressed during the short days of winter. Decreases in ovarian steroids might play an important role in autumn increases and spring decreases in food hoarding (Lynch et al., 1973; Mull, 1970), and interaction between food availability, day length and the availability of potential mating partners might be imperative for optimizing reproductive success.

One implication of these results in relation to the increase in worldwide obesity in human beings is that ovarian hormones, as well as other so-called 'satiety peptides,' might only influence ingestive behavior temporarily under specific energetic contexts. Ovarian hormones might attenuate the appetite for food under moderate calorie restriction, but according to Figs. 5 and 6 would not be expected to have significant effects on appetite or food hoarding under more extreme calorie restriction or under excessive energy abundance coupled with little or no energetic demand. It has been noted that some so-called 'satiety' peptides are less effective at curtailing food intake under prolonged energy restriction (Henry et al., 2001). The rise in obesity might, in itself, constitute evidence that endogenous satiety peptides are largely ineffective under conditions of food abundance in organisms with minimal requirements for energy expenditure. If the adaptive function of estradiol and other anorectic hormones during millions of years of evolution was to temporarily decrease appetite in favor of sexual ventures, perhaps it is too much to expect of these hormones to maintain body weight within some healthy and fashionable range of our own modern invention.

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