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Peptides that Regulate Food Intake

Food hoarding is increased by food deprivation and decreased by leptin treatment in Syrian hamsters

Carolyn A. Buckley and Jill E. Schneider
Department of Biological Sciences, Lehigh University, Bethlehem, Pennsylvania 18015

Buckley, Carolyn A., and Jill E. Schneider. Food hoarding is increased by food deprivation and decreased by leptin treatment in Syrian hamsters. *Am J Physiol Regul Integr Comp Physiol* 285: R1021–R1029, 2003. First published May 1, 2003; 10.1152/ajpregu.00488.2002.—Compensatory increases in food intake are commonly observed after a period of food deprivation in many species, including laboratory rats and mice. Thus it is interesting that Syrian hamsters fail to increase food intake after a period of food deprivation, despite a fall in plasma leptin concentrations similar to those seen in food-deprived rats and mice. In previous laboratory studies, food-deprived Syrian hamsters increased the amount of food hoarded. We hypothesized that leptin treatment during food deprivation would attenuate food-deprivation-induced increases in hoarding. Baseline levels of hoarding were bimodally distributed, with no hamsters showing intermediate levels of hoarding. Both high (HH) and low hoarding (LH) hamsters were included in each experimental group. Fifty-six male hamsters were either food deprived or given ad libitum access to food for 48 h. One-half of each group received intraperitoneal injections of leptin (4 mg/kg) or vehicle every 12 h during the food-deprivation period. Within the HH group, the hoarding score increased significantly in food-deprived but not fed hamsters ($P < 0.05$). Leptin treatment significantly decreased hoarding in the food-deprived HH hamsters ($P < 0.05$). The LH hamsters did not increase hoarding regardless of whether they were food deprived or had ad libitum access to food. These results are consistent with the idea that HH hamsters respond to energetic challenges at least in part by changing their hoarding behavior and that leptin might be one factor that mediates this response.

food intake; hunger; ob protein

THE PHYSIOLOGICAL CONTROL of ingestive behavior involves both consummatory and appetitive aspects of behavior, and yet most research has focused only on the consummatory aspect. Most investigators have measured the amount of food ingested in response to treatment with various hormones, neuropeptides, and metabolic conditions (reviewed in Refs. 1, 14, 32, 33, 41), whereas fewer have examined the appetitive aspects of ingestion, such as the approach to food, the consumption of unpalatable substances, or foraging and hoarding (e.g., 3–6, 11). Treatment with putative anorectic hormones, such as the adipocyte protein leptin, decreases the amount of food eaten in a wide variety of species, including chickens (10, 12), mice (9, 15, 18, 26), rats (34), Syrian hamsters (39), ground squirrels (25), dogs (20), sheep (16), primates (36), and marsupials (17). In many species, food deprivation results in decreased plasma concentrations of leptin, increased central release of neuropeptide Y (NPY), and increased food intake relative to that of ad libitum-fed controls (reviewed in Refs. 14, 32, 33, 41). This phenomenon is termed postfast hyperphagia and is common in laboratory animals, such as rats, mice, and monkeys and is also observed in most human beings.

However, not all species show compensatory postfast hyperphagia, although plasma leptin concentrations fall rapidly after the start of food deprivation. For example, in Syrian hamsters (*Mesocricetus auratus*) housed in summerlike conditions, food deprivation leads to significant decreases in plasma leptin concentrations (29) without concomitant increases in daily food intake (28, 31, 35). Additionally, hamsters (Turkish, Syrian, and Siberian) accomplish dramatic seasonal changes in adiposity and plasma leptin concentrations with little or no change in food intake (4, 6). Furthermore, NPY and leptin treatment do not have consistent effects on the amount of food consumed. When sucrose solution is administered via intraoral infusion, leptin-treated rats show increased rather than decreased passive consumption of sucrose compared with saline-treated rats (3). NPY treatment increases appetitive aspects of ingestion, such as approach to the source of a palatable sucrose solution, whereas the same treatment significantly decreases passive (intraoral) consumption of sucrose (3). These and other results suggest that leptin and other factors are more than simply satiety peptides or starvation signals that govern consumption.

An alternative perspective is that the function of these putative "feeding" hormones and neuropeptides is to prioritize behaviors related to energy intake and expenditure by changing the motivation to engage in different types of species-specific behavior. During food shortages, some species might respond to a fall in plasma leptin concentrations by overeating when food becomes available. For those species that live in harsh

Address for reprint requests and other correspondence: C. A. Buckley, Dept. of Biological Sciences, Lehigh Univ., 111 Research Dr., Bethlehem, PA 18015 (E-mail: caba@lehigh.edu).

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environments and are in danger of predation, those individuals that hoard rather than overeat might gain a selective advantage because they are able to maintain their species-specific meal pattern and daily energy intake in the relative safety and less harsh environ of the burrow. Syrian hamsters, for example, are thought to live and hoard food in underground burrows (23) and rarely change their daily food intake to match energetic demands (28, 31, 35). Thus, when plasma concentrations of leptin are low, Syrian hamsters might be expected to engage in behaviors such as foraging and hoarding that increase the likelihood of surviving future food shortages, whereas they might be less likely to show postfast hyperphagia. Conversely, high plasma concentrations of leptin might decrease the likelihood of hoarding or foraging, giving priority instead to behaviors related to reproductive success.

Consistent with this perspective, Lea and Tarpy (19), Wong (40), and Phillips et al. (27), using only minor modifications of laboratory cages, found that Syrian hamsters increase hoarding behavior but not food intake in response to food restriction. In most studies, however, the food source was no more than 20 cm from the cage to which it was hoarded. Using a different hamster species, Siberian hamsters (*Phodopus sungorus sungorus*), Bartness and Clein (5) used a simulated burrow system designed to duplicate important characteristics of a natural habitat, such that climbing and descending were required for foraging from a distant source (1.52 m), and food was hoarded to the same cage in which the hamster was housed. Siberian hamsters increase hoarding behavior, but not food intake, after a period of food restriction or deprivation (5). These efforts to duplicate the natural conditions under which the experimental animal obtains and stores food are critical to understanding biological responses to energetic challenges within the context in which they evolved (4). The hoarding response of Syrian hamsters to energetic challenges has not been tested in such a system. In this experiment, we used the naturalistic burrow system based on the design of Bartness and Clein (5) to confirm reports that hoarding behavior is increased after a period of food deprivation. In addition, we hypothesized that the changes in leptin that occur with fasting might be involved in this response and predicted that replacing leptin in food-deprived hamsters would attenuate the increase in hoarding.

EXPERIMENT 1 AND REPLICATE: EFFECTS OF FOOD DEPRIVATION AND LEPTIN TREATMENT ON HOARDING AND SHORT-TERM FOOD INTAKE

Materials and Methods

Subjects, housing, and hoarding apparatus. Experiments were conducted according to the American Physiological Society guiding principles for research, the National Institutes of Health, and the Lehigh University Institutional Animal Care and Use Committee. This experiment was conducted in April and again in August 2002, with 28 new animals for each replicate. Male Syrian hamsters (*Mesocricetus auratus*) purchased from Charles River Breeding Laboratories (Wilmington, MA), 85–90 g in body weight on arrival, were housed on a 16:8-h reversed light-dark photoperiod, with "lights on" at 1600, at 23 ± 1°C, and with food (Purina Laboratory Rodent Chow #5001) and water available ad libitum. They were housed in standard 33 × 20 × 18-cm opaque Nalgene cages until the hamsters were 2 mo of age.

To begin the introduction to the hoarding apparatus, the hamsters were transferred to 25 × 20 × 24-cm clear plastic cages (CritterTrail by SuperPet), referred to as "home cages." At the time of transfer, each hamster weighed between 105.8 and 139.2 g and was given ad libitum access to food and water and two cotton balls to encourage nesting within the home cage. Each home cage setup included ~80 cm of translucent plastic tubing (30 cm vertical, 50 cm horizontal, 4 cm minimum internal diameter) available for attachment, leading from the home cage to a 13 × 6 × 6-cm clear plastic box, the "food source box" (FSB), designed using CritterTrail Puzzle Boxes (SuperPet). At first, the FSBs remained detached from home cages for several days to promote nesting within the home cage. By attaching the FSBs to the home cage, we allowed hamsters access for periods of 45–90 min, each beginning at dark onset. All dark period procedures were performed under red illumination. Some tests included food in the FSB, whereas others did not; however, all animals were treated equally in this regard. Of the 56 hamsters used in this study, all visited the FSBs and less than four moved cotton to the FSB more than once. The cotton was moved back to the home cage by the experimenter. No hamster was ever observed to nap in the FSB or to move food from the home cage to the FSB; therefore, those few that moved nesting material to the FSB remained in the study. These initial observations confirmed that the hamsters were nesting in their home cages and were using the FSBs to forage, but not to nest.

General procedures. Each replicate of the experiment was conducted in four phases: acclimation (6 days), baseline (4 days), experimental treatment (2 days), and testing (1 day). Acclimation to daily measurements of food intake and hoarding behavior began ~3 wk after hamsters were initially placed in the home cages. In the baseline phase, the same procedures continued for 4 more days, and individual means for food intake and hoard weight across these 4 days were used as baseline data. The treatment phase consisted of leptin or vehicle treatment during either food deprivation or ad libitum access to food. The procedures that accompany measurement of hoarding and food intake continued during the treatment period so that the animals would remain on the same schedule until the time of testing. For the testing phase, all animals received the same procedure used in acclimation and baseline.

The experiment was performed in two replicates. Within each of the two replicates, baseline hoarding tendency was bimodal, such that 16 hamsters hoarded considerably more food than the other 12, with an identical distribution in both replicates. Hamsters that

hoarded more than an average of 13 g of food per day during the 4-day baseline phase were designated as "high hoarding" (HH) hamsters, whereas hamsters that hoarded <5 g/day were designated as "low hoarding" (LH) hamsters. There were no intermediates. Both LH and HH hamsters were represented within each experimental group as described below. The sample sizes of the subgroups did not differ between replicates. There were no significant differences in mean body weight between the LH (120.92 ± 1.79) and HH hamsters (121.32 ± 1.91) at the start of the experiment.

Sample sizes (replicates combined). Sample sizes were as follows: HH, food deprived (FD), leptin treated (L) (n = 8); HH, FD vehicle-treated (V) (n = 8); HH, fed, L (n = 8); HH, fed, V (n = 8); LH, FD, L (n = 6); LH, FD, V (n = 6); LH, fed, L (n = 6); LH, fed, V (n = 6).

Acclimation and baseline. ONE-HOUR FOOD INTAKE. In the hour after the end of a food-deprivation period, rodents show a tendency to consume, rather than hoard, a meal. Thus, in some experiments, rats (22), gerbils (24, 40), and Syrian hamsters (40) are given a brief meal at the end of the food-deprivation period before testing the hoarding response to reduce confounds in the hoarding scores due to individual and group differences in time spent eating. Previous data confirm that plasma leptin concentrations are not increased until 36–48 h after the start of refeeding (29). Thus 1-h food intake was measured at the end of the treatment phase (food deprivation or feeding) and before the hoarding period during the last hour of the light cycle by placing hamsters into a clean holding cage (28 × 18 × 13 cm Nalgene, wire top) with a single preweighed food pellet (4–5 g) weighed to the nearest 0.1 g. Beginning at dark onset, hamsters were returned to home cages, and any pouched food was removed and weighed together with food remaining in holding cages. One-hour food intake was determined by subtraction of the weight of food remaining from the weight of food placed in the holding cage.

HOARDING BEHAVIOR AND FOOD INTAKE DURING THE HOARDING PERIOD. While hamsters were in holding cages, plastic tubing was attached to the home cages to provide access to FSBs, each containing 150–155 g of uniformly small food pellets (chow pellets cut to average weight of 1.5 g/pellet). The weight of food placed in each FSB was recorded to the nearest 0.1 g. Hoarding trials began at dark onset, when hamsters were moved back to home cages and given access to the FSB for 90 min. Additional preweighed food was added if the FSB was emptied. After 90 min, hamsters were moved back to holding cages, and pouched food was removed and added to hoards. While hamsters were in holding cages, all hoarded and pouched food was weighed and returned to home cages. Hamsters were then moved back to home cages, and ~12 g of food was placed in the cages of those that had hoarded little or no food so that no animals would be deprived of food before the treatment phase. Hoarded food was determined directly by the weight of the hoarded and pouched food. The amount ingested during the hoarding period was determined by subtracting the weight of hoarded and pouched food plus that remaining in the FSB from the total amount placed in the FSB. The transfer of all hamsters between cage types required 12–15 min and was accomplished in seven sets of four, with one animal from each treatment group randomly assigned to each set.

EXPERIMENTAL TREATMENT. In each of two replicates, hamsters were divided into four groups by block randomization, with three LH and four HH hamsters in each group. There were no significant differences among these four groups in body weight. Starting 1 h before dark onset (on transfer to holding cages), two groups were food deprived and two groups continued to be fed ad libitum for 48 h. All procedures used in the acclimation and baseline phases were continued throughout the treatment period, except that food-deprived animals received no food in holding cages or FSBs. One food-deprived group and one fed group received intraperitoneal injections of murine leptin (4 mg/kg in 0.01 M Tris buffer, pH 7.4), whereas all other hamsters received intraperitoneal vehicle injections of equal volume (5 ml/kg body wt). Injections began 12 h into the treatment period and were administered every 12 h, based on body weights taken within the previous hour. Each animal received four injections, with the last one given ~1 h before the testing period (refeeding) began.

Testing. On conclusion of the treatment phase, 1-h food intake and 90-min hoarding behavior were again measured for all animals on the same schedule as described for acclimation and baseline procedures.

Statistical analysis. Baseline hoarding behavior was highly variable. To minimize individual differences in hoarding scores, hoard weights during testing were divided by the average baseline hoard weights for each animal to arrive at a "hoard ratio," reflecting the proportion of change in hoarding behavior from baseline to testing. Because several of the LH had baseline hoarding scores of zero, a 2-g constant was added to each baseline hoarding score in all groups before calculating the hoard ratio. Hoard ratio data did not meet the assumption of homogeneity of variance required for the ANOVA; thus they were analyzed using the Mann-Whitney U-test for planned comparisons. Differences were considered significant if P was <0.05.

Average 1-h posttreatment food intake (g) and food intake during the 90-min hoarding period were analyzed by a four-way ANOVA with these main effects: replicate, metabolic condition (fed or food deprived), drug treatment (leptin or vehicle), and hoarding tendency (HH or LH). These tests were followed by post hoc analyses by Duncan's multiple range test and planned comparisons when the main effects were significant.

EXPERIMENT 2: HOARDING TENDENCY AND LONG-TERM FOOD INTAKE

In *experiment 2*, food intake was measured in standard laboratory cages at several time points over a 24-h period to examine whether the HH and LH ham-

sters differed in their food intake response to food deprivation when they were not allowed to hoard.

Materials and Methods

Twenty-eight new animals of the same age and mean body weights as in *experiment 1* were housed in identical home cages and given food and water ad libitum. Hoarding behavior during the first 90 min of the dark period was measured as previously described for 7 days. Hoarding tendency (low or high) was determined, and 10 LH and 10 HH hamsters were selected for *experiment 2*. Five of each type were randomly assigned to a food-deprived or ad libitum-fed group. All animals were moved to 33 × 20 × 18-cm opaque Nalgene cages when the treatment phase began. Food-deprived animals received no food in these cages for a total of 48 h, whereas all other animals were fed ad libitum throughout. On completion of this treatment phase, all hamsters were given a preweighed surplus of food and intake was recorded at 2, 4, 8, 12, and 24 h. Cumulative food intake and cumulative food intake per gram body weight were compared across groups using a two-way ANOVA.

RESULTS

Experiment 1: Effects of Food Deprivation and Leptin on Hoarding and Short-Term Food Intake

Hoarding. Hoard ratios (posttreatment hoarding divided by baseline hoarding + 2 g) were not significantly different between replicates ($U = 374.50$, $P = 0.78$); mean hoard ratios were, therefore, collapsed across replicates. Mean hoard ratios for the LH were near zero and significantly lower than those of the HH ($U = 233.50$, $P < 0.05$), demonstrating that the LH did not change their hoarding behavior after treatment, whereas the HH did (Fig. 1). The mean hoard ratio for the LH in *replicate 1* was 0.323 ± 0.31 and in *replicate 2* was 2.4 ± 2.10 when all LH hamsters were included. The higher variance and mean in the LH of the second replicate were due to a single outlier, as determined by statistical analysis: an LH, food-deprived, vehicle-treated hamster with a hoard ratio of 25. This outlier was not included in the means in Fig. 1. All other food-deprived, vehicle-treated LH hamsters in both replicates hoarded 0 g during testing. Without this outlier, the mean hoard ratio for the LH, food-deprived, vehicle-treated group was 0.347 ± 0.23 and the final sample size was for this group was 5 (Fig. 1).

Twenty of the twenty-four LH in both replicates either decreased or did not change their hoarding behavior, regardless of food availability or drug treatment. Thus further comparisons were limited to the HH hamsters. The HH hamsters significantly increased their hoarding in response to food deprivation, compared with ad-libitum fed controls ($U = 33.0$, $P < 0.05$). The mean hoard ratio for the food-deprived HH hamsters was 4.51 ± 0.89 and for the ad libitum-fed HH hamsters was 0.14 ± 0.06 (Fig. 1). With fed and food-deprived HH hamsters combined, leptin-treated

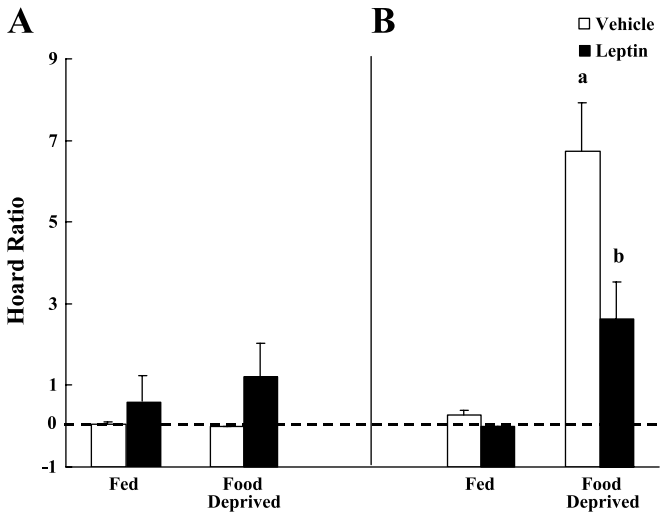

Fig. 1. Mean ± SE of hoard ratios (posttreatment hoarding/baseline hoarding + 2 g) for Syrian hamsters with either high (*B*) or low (*A*) tendency to hoard food in a 90-min test. Hamsters were either food deprived or fed ad libitum, and one-half of each of these groups received intraperitoneal injections with either leptin (4 mg/kg) or vehicle. *A*: in the low hoarding (LH) hamsters, $n = 6$ in the food-deprived vehicle-treated, fed leptin-treated, and fed vehicle-treated groups, and $n = 5$ in the food-deprived leptin-treated group due to the removal of one outlier (see text). *B*: in the high hoarding (HH) hamsters, $n = 8$ per group. There was no significant effect of replicate on food hoarding. LH hamsters had hoard ratios near zero, and thus the *y*-axis begins at −1 so that the LH bars are visible. [a]Significantly different from HH fed groups, $P < 0.05$. [b]significantly different from HH food-deprived vehicle-treated group, $P < 0.05$.

hamsters had a significantly lower hoard ratio (means ± SE = 1.27 ± 0.54) than vehicle-treated hamsters (means ± SE = 3.37 ± 1.00, $U = 69.00$, $P < 0.05$).

Within the ad libitum-fed HH hamsters, the effect of leptin treatment on hoarding was not significant (Fig. 1). However, within the food-deprived HH group, the mean hoard ratio for leptin-treated hamsters (2.55 ± 0.89) was significantly lower than for vehicle-treated hamsters (6.48 ± 1.22, $U = 11.00$, $P < 0.05$). Even in the ad libitum-fed HH group, mean hoard ratios for leptin- and vehicle-treated animals were 0.002 ± 0.002 and 0.27 ± 0.09, respectively ($U = 13.5$, $P = 0.056$), suggesting a trend toward lower hoard ratios in the leptin-treated, fed, HH group. No significant metabolic condition × drug treatment interaction effect was observed. The lack of interaction term and the trend toward a lower hoard ratio in the leptin-treated, fed HH group suggest that leptin treatment decreased hoard ratio in a fashion that was additive rather than synergistic with metabolic condition.

One-hour food intake. There were no significant main effects or interaction terms in the four-way ANOVA for baseline 1-h food intake (baseline food intake data not shown).

Four-way ANOVA on 1-h food intake posttreatment showed a significant main effect of replicate ($P < 0.01$, Fig. 2). When the replicates were analyzed separately by a three-way ANOVA (hoarding tendency × metabolic condition × drug treatment), there were signifi-

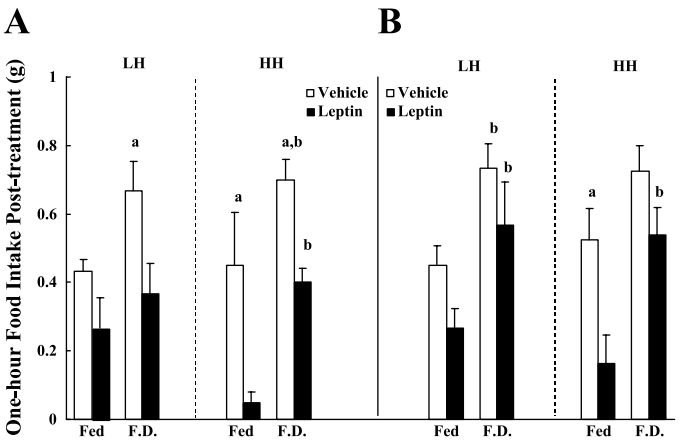

Fig. 2. Mean ± SEM 1-h food intake for HH and LH Syrian hamsters either food deprived (F.D.) or fed ad libitum (Fed). Food intake was measured in a separate holding cage before hamsters were given access to the hoarding tubes. One-half of each of these groups received intraperitoneal injections with either leptin (4 mg/kg) or vehicle. *A*: first replicate; *B*: second replicate. Within *replicates 1* and *2*, in the HH hamsters, $n = 4$ per group, and in the LH hamsters, $n = 3$ per group. [a]Significantly different from leptin-treated within metabolic condition (F.D. or Fed); [b]significantly different from ad libitum-fed [within hoarding group (HH or LH)].

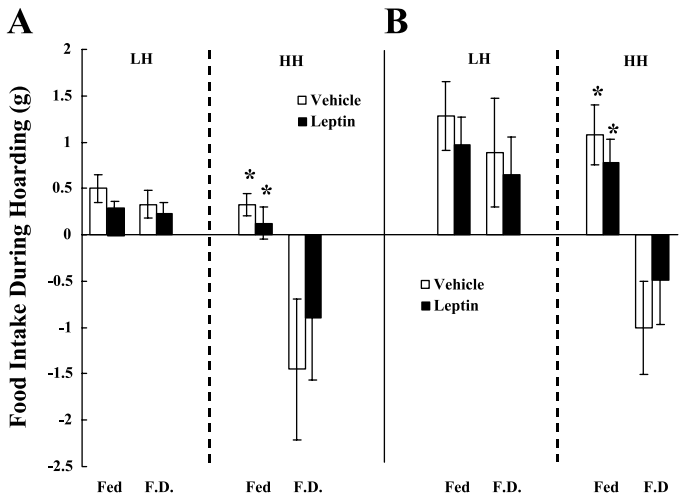

Fig. 3. Mean ± SEM food intake during the 90-min hoarding period for HH and LH Syrian hamsters either food deprived (F.D.) or fed ad libitum (Fed). One-half of each of these groups received intraperitoneal injections with either leptin (4 mg/kg) or vehicle. *A*: first replicate; *B*: second replicate. Within *replicates 1* and *2*, in the HH hamsters, $n = 4$ per group, and in the LH hamsters, $n = 3$ per group. *Significantly different from food-deprived HH within drug treatment (leptin or vehicle) at $P < 0.05$.

cant main effects of metabolic condition ($P < 0.001$) and drug treatment ($P < 0.001$), but no effect of hoarding tendency and no interactions in both replicates. In both replicates, food-deprived hamsters ate significantly more than fed hamsters, and leptin-treated hamsters ate significantly less than vehicle-treated hamsters (Fig. 2). The same pattern of main effects and interactions in both replicates suggests that the significant main effect of replicate in the four-way ANOVA was due to lower food intake in one of the replicates (Fig. 2).

Food intake during the hoarding period. For baseline food intake during the hoarding period, the main effect of replicate was not significant, and replicates were combined for analysis. Before the food-deprivation and drug treatment period during baseline testing, LH hamsters had significantly higher food intake than HH hamsters during the hoarding period ($P < 0.001$, data not shown). Before the food-deprivation and drug treatment period, there were no significant main effects due to metabolic condition or drug treatment or interaction terms in the four-way ANOVA for baseline food intake during the hoarding period (data not shown).

Four-way ANOVA on posttreatment food intake during the hoarding period showed a significant main effect of replicate ($F_{1,40} = 41.265$, $P < 0.0001$, Fig. 3). When the replicates were analyzed separately by three-way ANOVA (hoarding tendency × metabolic condition × drug treatment), there was a significant main effect of metabolic condition in both replicates such that food-deprived hamsters ate significantly less than fed hamsters during the hoarding period ($P < 0.01$ in both replicates). In *replicate 1*, there were no other significant main effects and no significant interaction terms. In *replicate 2*, there was a main effect of hoarding tendency ($P < 0.01$) and a significant hoarding tendency × metabolic condition interaction ($P < 0.03$) such that food deprivation-induced decreases in food intake were exaggerated in the HH group (Fig. 3).

Experiment 2: Hoarding Tendency and Long-Term Food Intake

Cumulative food intake (not shown) and cumulative food intake per gram body weight at all time intervals up to 24 h were not significantly different for low and high hoarders before or after food deprivation, i.e., the main effect of hoarding tendency was not significant at any time point (Fig. 4). Ad libitum-fed hamsters ate significantly more than food-deprived hamsters starting 4 h after refeeding ($P < 0.05$). This difference

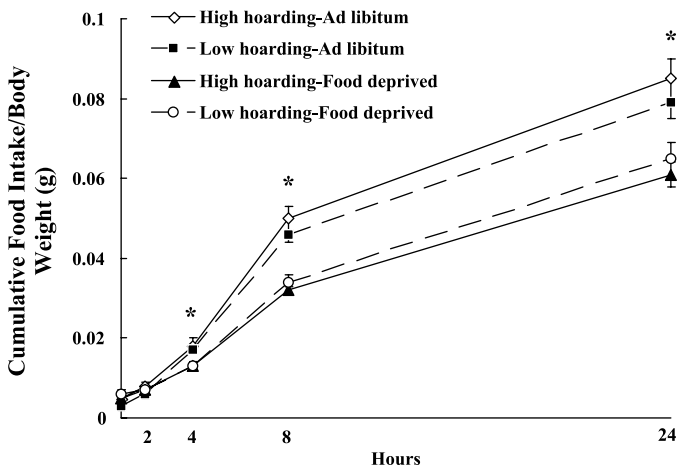

Fig. 4. Mean ± SEM cumulative food intake per gram body weight at 2, 4, 8, 12, and 24 h after the beginning of refeeding for HH and LH Syrian hamsters either food deprived or fed ad libitum. $n = 5$ per group *Beginning 4 h after refeeding, food deprivation significantly decreased food intake for both LH and HH hamsters, $P < 0.05$.

persisted throughout the 24 h of food intake measurement ($P < 0.05$).

DISCUSSION

The primary results of this study were *1*) a significant increase in hoarding in food-deprived HH but not food-deprived LH Syrian hamsters and *2*) a significant attenuation of the hoarding response by systemic treatment with leptin (Fig. 1). The increase in hoarding after food deprivation in our simulated burrow system is consistent with previous research on Siberian hamsters under similar conditions (5) and Syrian hamsters under less naturalistic conditions (19, 27, 40). This was, to the best of our knowledge, the first demonstration of an effect of leptin treatment on hoarding behavior in Syrian hamsters. In two replicates of *experiment 1* and in *experiment 2* we observed that approximately one-half of each population showed a tendency toward high levels of hoarding, whereas the other one-half showed a tendency toward low levels of hoarding. We are not aware of other reports of both high and low hoarding members of any species that consistently appear in approximately equal proportions.

Other experiments from this laboratory have shown that plasma leptin concentrations fall precipitously within 6 h after the start of food deprivation and continue to drop to the limit of detection of the leptin assay by 48 h after the start of food deprivation (29). It is widely believed that falling leptin concentrations are a trigger for increased food intake, and yet food-deprived hamsters do not exhibit postfast hyperphagia to the extent seen in rats, mice, and other species (Fig. 4). Food deprivation failed to increase food intake in Syrian hamsters whether they have a high or low hoarding tendency or whether they had the opportunity to hoard food. It has been demonstrated that food-deprived hamsters experience increased hunger motivation relative to hamsters that were fed ad libitum. For example, fed hamsters avoid an unpalatable diet, whereas food-deprived hamsters will eat significantly more of the same unpalatable diet than fed hamsters (11, 31). Furthermore, food-deprived Syrian hamsters show decreased latency to the first meal and larger initial meals than fed hamsters (11). These data are consistent with the increase in 1-h food intake in our food-deprived hamsters (Fig. 2) and also consistent with the lack of increase in long-term food intake in our food-deprived hamsters (Fig. 4). In food-deprived hamsters, hoarding behavior was increased, and in hamsters that hoarded a great deal, food intake was decreased. For example, HH hamsters showed lower food intake during the baseline hoarding period compared with LH hamsters ($P < 0.001$), and HH hamsters that were food-deprived ate less than fed HH hamsters during the hoarding period (Fig. 3). These and the present results support the notion that the metabolic and hormonal sequelae of food deprivation create signals that increase hunger motivation, which, in this species, leads to increased food hoarding but not consumption.

In addition, our results confirm the well-known effects of systemic leptin injections on food intake in Syrian hamsters (7, 30, 39). Leptin injections decrease daily food intake, and yet do not support a conditioned taste aversion in Syrian hamsters (7). It is not known why increased leptin decreases food intake, whereas the falling concentrations of plasma leptin fail to increase food intake in this species. Our results suggest that leptin and other metabolic factors might control food hoarding, whereas food intake remains relatively constant in Syrian hamsters.

Mechanisms that change food procurement, but not food consumption, might confer a selective advantage in prey animals faced with energetic challenges, such as seasonal decreases in food supply. We are not aware of data that support this speculation; however, animals that have food hoarded in their burrows or nests need not venture out of the burrow when environmental conditions are harsh. When they find food outside the burrow, they might be able to minimize their exposure to harsh weather and predators if they are inclined to take food to their burrows rather than ingest it immediately. Syrian hamsters are thought to live and hoard food in underground burrows in the desert where ambient temperatures vary drastically from day to night and with the seasons. They subsist on resources that are not available year round. Thus it might be adaptive to respond to a food shortage and the hormonal and metabolic events that accompany food shortage by increasing foraging and hoarding and then consume their food at the usual species-specific rate in the relative safety and less harsh environ of the burrow.

Our results suggest that changes in plasma leptin might be one factor involved in this putative adaptation. Thus when plasma concentrations of leptin are low, hamsters might be expected to eat only enough to survive and then engage in food procurement behaviors and other responses that increase survival during subsequent food shortages. High plasma concentrations of leptin that occur in fattened hamsters (and the associated metabolic sequelae) might decrease the likelihood of hoarding or foraging, giving priority instead to behaviors related to reproductive success. It is interesting to note that leptin treatment reverses the effects of food deprivation on estrous cyclicity and facilitates some aspects of estrous behavior in Syrian hamsters, provided that they are not excessively energetically challenged (30, 39). Hamster species respond to energetic challenges with a variety of adaptations that include increased nest building, decreased resting metabolic rate, pelage changes, hibernation, and/or daily torpor. Inhibition of reproduction and decreased metabolic rates are two other adaptations correlated with low plasma leptin concentrations (reviewed in Refs. 2, 13, 32). Two of these responses, hibernation and daily torpor, are incompatible with increased food consumption. Thus the lack of postfast hyperphagia might also be linked to the tendency to engage in hibernation (Syrian hamsters) or daily torpor (Siberian hamsters), as first suggested by Bartness (4). Together, these results argue against the idea that leptin is

simply a satiety hormone or a trigger for the brain mechanisms that control the amount of food consumed. Rather, they suggest that leptin might function within an array of metabolic mechanisms that prioritize behaviors related to food procurement, energy expenditure, and reproduction.

Leptin is probably not the only factor involved, because leptin treatment attenuated but did not reverse the effects of food deprivation on hoarding (Fig. 1). Similarly, leptin treatment cannot fully reverse the effects of severe metabolic challenges on aspects of reproductive physiology and behavior (reviewed in Refs. 32, 33, 39). It is likely that hoarding is directly influenced by a variety of other factors, such as the availability of oxidizable metabolic fuels, other hormones (such as those that rise during pregnancy), photoperiod, and ambient temperature, as has been shown for Siberian hamsters (reviewed in Ref. 4). If hoarding increases before winter in Syrian hamsters, it occurs despite increases in adiposity and plasma leptin concentrations.

The role of hormones and neuropeptides in prioritizing behaviors is not restricted to leptin or to hamsters. If orexigenic peptides were strictly acting on mechanisms that control the amount of food ingested, it would be predicted that such peptides would increase intraoral ingestion. To the contrary, some orexigenic and anorexigenic substances influence the number of approaches to a bottle of sucrose solution, without the expected changes in consumption of food delivered intraorally. For example, treatment with the putative anorectic agent, leptin, decreases the amount of sucrose that rats consume from a bottle (presumably a measurement of both appetitive and consummatory aspects of ingestive behavior), while actually increasing the amount of sucrose consumed via intraoral infusion (presumably a measure of strictly consummatory ingestive behavior) (3). The same investigators examined appetitive behavior, defined as the number of approaches to a sucrose bottle in NPY-treated vs. vehicle-treated rats. NPY treatment significantly increased the number of visits to the bottle, even when the bottle was empty, while simultaneously decreasing the amount of fluid ingested by intraoral infusion compared with vehicle treatment. The effects of leptin and NPY treatment on appetitive aspects of ingestive behavior were exaggerated when the rats had the opportunity to engage in sex behavior. Thus leptin, other peptides, and metabolic events involved in motivation do not always decrease food intake per se. Rather, they are chemical messengers that prioritize and change the likelihood of occurrence of different behaviors by changing motivational states or by diverting attention to or away from food-related stimuli. This, in turn, results in changes in some combination of behaviors such as food consumption, foraging, hoarding, reproductive behaviors, nest building, or metabolic rate, depending on the species, season, ambient temperature, and genetic predisposition.

The mechanism by which falling leptin increases food hoarding is unknown. Leptin might act directly on mechanisms that influence hunger motivation and hoarding or indirectly by its well-known effects on energy expenditure and fuel oxidation (21, 30, 32, 33). Our data suggest that food deprivation and leptin treatment had independent, rather than synergistic, effects on hoarding and on 1-h food intake (the drug $\times$ metabolic condition interaction was not significant). Leptin's influence on hoarding might include leptin binding to ObRb receptors on NPY-containing neurons. ObRb receptors on NPY-containing neurons have been implicated in control of food intake (reviewed in Refs. 8, 14, 41). In Siberian hamsters, hoarding behavior is increased by central treatment with NPY, or NPY agonists, and decreased by treatment with NPY antagonists (D. Day and T. J. Bartness, unpublished data). The fact that leptin treatment attenuated, but did not fully reverse, the effects of food deprivation on hoarding suggests that either leptin is not the only factor that influences hoarding, and thus hoarding behavior might be sensitive to changes in other hormones or more directly to changes in the availability of oxidizable fuels, or we did not use a mode or dose of leptin treatment that sufficiently mimics the effects of endogenous leptin, or both. Due to the small decreases in hoarding behavior in leptin-treated, ad libitum-fed HH hamsters (Fig. 1), it might be assumed that leptin treatment caused some general malaise incompatible with hoarding behavior. This difference was not significant, however, and we have shown that this leptin treatment does not support a conditioned taste aversion in Syrian hamsters (7). It is therefore unlikely that leptin caused malaise, nausea, or aversion in HH hamsters. Additionally, all subjects were observed to visit the FSB at least once during each hoarding period, and most leptin-treated hamsters visited the FSB several times throughout the hoarding period. Subjective observations in this and other experiments suggested that leptin treatment had either no effect on or increased general activity.

To our knowledge, this is the first report to document and investigate a bimodal distribution of hoarding scores among male Syrian hamsters. Furthermore, LH hamsters (averaging <5 g/90 min) did not increase hoarding behavior in response to 48-h food deprivation, whereas HH hamsters (averaging >15 g/90 min) increased their hoard weights by a factor of at least 2.1 and, at most, 11.6, after a 48-h period without food (vehicle treated). The LH hamsters did not appear to have difficulty maneuvering through the tubes with full pouches. Most LH hamsters climbed through the tubes to the FSB, but never attempted pouching while in the FSB. Other LH hamsters pouched and hoarded small quantities of food successfully. These hamsters did not make further attempts at hoarding, despite having ample time to do so. The reliable finding that about one-half of the hamsters we tested did not hoard before or after food deprivation is intriguing and raises at least two important questions. Why does the low hoarding phenotype exist, and might these animals exhibit alternative compensatory strategies for dealing with energetic challenges?

After the completion of the first replicate of *experiment 1*, it seemed reasonable to hypothesize that low hoarders represent an alternative genotype that survives energetically challenging conditions by increasing food intake rather than hoarding behavior. The food intake data do not support this hypothesis. Both the LH and HH hamsters showed a significant increase in 1-h food intake due to food deprivation [consistent with earlier data showing that food-deprived hamsters show a shorter latency to the first meal and eat larger initial meals compared with fed hamsters (11)], and the hoarding tendency × metabolic condition interaction was not significant. Thus HH and LH hamsters did not differ significantly in their 1-h food intake response to food deprivation (Fig. 2). During the hoarding period, LH hamsters did not respond to food deprivation by increased hoarding or food intake (Figs. 1 and 3). During the postfast hoarding period, there was a significant decrease in food intake in HH food-deprived hamsters, which might be postulated to be due to the increased amount of time spent exploring the tubes and visiting the FSB (Fig. 3). Negative food intake scores might be due to moisture in the hoarded food after it was carried in cheek pouches to the home cage. The 1-h food intake and food intake during hoarding are viewed with caution due to the fact that the replicates were analyzed separately, leading to a smaller sample size. However, despite the differences between replicates, the food-deprived LH hamsters did not eat more than the fed LH hamsters during the hoarding period. Small food deprivation-induced decreases (not increases) in cumulative food intake and cumulative food intake per gram body weight were observed in both LH and HH hamsters in *experiment 2* when animals were in standard laboratory cages and thus had no opportunity to hoard. When food intake was measured in these cages, no significant difference was observed between the LH and HH hamsters in food intake or in food intake per gram body weight. As expected, no postfast hyperphagia was observed, and food-deprived animals ate significantly less per gram body weight than their ad libitum-fed controls. Thus differences in hoarding tendency are not related to innate differences in the food intake response to deprivation. Alternatively, differences in hoarding tendency might result from some aspect of social experience, such as dominant-subordinate relationships that develop when the hamsters are group housed before weaning or during shipping. Whether differences in hoarding tendency result from genetic or environmental factors is unknown and worthy of further exploration.

In summary, the significant effects of food deprivation on hoarding and the attenuation of these effects by leptin support the idea that the low plasma leptin concentrations that are characteristic of food deprivation might increase behaviors related to hunger motivation and procurement of food without necessarily increasing the consumption of food. These and other studies on sex behavior and estrous cyclicity suggest that increased plasma leptin concentrations might decrease hunger motivation, which might result in or accompany increased interest in activities related to reproductive success.

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DISCLOSURES

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