Leptin inhibits food-deprivation-induced increases in food intake and food hoarding

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ABSTRACT

KEEN-RHINEHART, E. AND BARTNESS, T. J. Leptin inhibits food deprivation-induced increases in food intake and food hoarding in Siberian hamsters. American Journal of Physiology XX:XX-XX, 2008. – Food deprivation stimulates foraging, hoarding and to a much lesser extent food intake, in Siberian hamsters. Leptin, the anorexigenic hormone secreted primarily from adipocytes, may act in the periphery, the brain or both to inhibit these ingestive behaviors. Therefore, we tested whether leptin given either intracerebroventricularly (icv) or intraperitoneally (ip), would block food deprivation-induced increases in food hoarding, foraging and intake in animals with differing foraging requirements. Hamsters were trained in a running wheel-based food delivery foraging system coupled with simulated burrow housing. We determined the effects of food deprivation and several peripheral doses of leptin on plasma leptin concentrations. Then hamsters were food deprived for 48 h and given ip leptin (0, 10, 40 or 80 μg), and additional hamsters were food deprived for 48 h and given icv leptin (0, 1.25, 2.5 or 5.0 μg). Foraging, food intake and hoarding were measured post-injection. Food deprivation stimulated food hoarding to a greater degree and duration than food intake. In animals with a foraging requirement, icv leptin almost completely blocked food deprivation-induced increased food hoarding and intake, but increased foraging, whereas peripheral leptin treatment was most effective in a sedentary control group, completely inhibiting food deprivation-induced increased food hoarding and intake at the two highest doses, and did not affect foraging at any dose. Thus, the ability of leptin to inhibit food deprivation-induced increases in ingestive behaviors differs based on foraging effort (energy expenditure) and the route of administration of leptin administration.
**Key Words:** food deprivation, foraging, wheel running, feeding, hypothalamus, Siberian hamsters
INTRODUCTION

Obesity is a disease of both literally and figuratively enormous proportions. As of yet, there currently are no effective treatments for obesity and this disease continues to run rampant throughout developed and underdeveloped countries. Therefore, innovative and alternative lines of basic research are needed to forge the beginnings of pathways to new potential obesity treatments. One critical area of basic research involves determining the neuroendocrine factors that regulate ingestive behavior. Often ingestive behavior is thought of in terms of food intake only, but it is important to consider the entire sequence of events associated with food and this includes two phases: 1) the acquisition and storage of food or the appetitive phase and 2) the actual eating of the food or the consummatory phase (20). The consummatory aspects of ingestive behavior have received the most attention in the quest to understand the energy intake portion of the obesity phenomenon. As for the appetitive phase of ingestive behavior, consisting of foraging and food hoarding, there is comparatively little known about the mechanisms underlying these widely expressed behaviors across animal species (for review see: (32; 60)). Therefore, understanding how both the appetitive and consummatory phases of ingestive behavior are controlled may provide key insights into the etiology of obesity that could lead to new avenues for its treatment.

Siberian hamsters (*Phodopus sungorus*) and other hamster species (for review see: (11)) primarily increase foraging (10; 22) and food hoarding (8; 9; 62) -- that is appetitive behaviors-- in response to energetic challenges. Specifically, Siberian hamsters (and other animals that have the capacity to transport significant amounts of food; for review see: (60)) use food hoarding as a crucial part of their ingestive behavioral repertoire in response to many naturally-occurring energy demands (*e.g.*, food shortages, pregnancy, lactation (8; 10); for review see: (10)). In this
manner, Siberian hamsters are not unlike humans that transport food back to their domiciles in their vehicles and store it in their refrigerators/pantries for later consumption, as evidenced by the finding that 85% of all purchased food being eaten at home (50). Therefore, Siberian hamsters are an ideal model for studying the neuroendocrine factors that regulate both appetitive and consummatory ingestive behaviors compared with other rodents where appetitive behaviors are a smaller part of their naturally-occurring ingestive behavior repertoire (41) or where both appetitive and consummatory ingestive behaviors increase or decrease together, such as in laboratory rats and mice (for review see: (10)).

Food deprivation is a naturally-occurring energetic challenge encountered by Siberian hamsters and triggers a plethora of alterations in peripheral metabolism and signaling peptides as well as in central neurochemicals (for reviews see: (49)). When food is available again, Siberian hamsters markedly increase their appetitive ingestive behaviors (foraging and especially hoarding) occur in Siberian hamsters, with either no increase in food intake or relatively minor increases compared to other species tested (for review see: (10)). The exact mechanisms underlying these food deprivation-induced increases in appetitive ingestive behaviors in Siberian hamsters are just beginning to be uncovered. For example, we now know that food deprivation triggers increases in circulating concentrations of the largely stomach-derived peptide ghrelin in Siberian hamsters (34), as it does in laboratory rats (57) and humans (2), and that peripheral ghrelin treatment stimulates foraging and food hoarding and, to a lesser extent, food intake in Siberian hamsters (34). We also now know that the ability of food deprivation or ghrelin to stimulate appetitive and consummatory ingestive behaviors is impaired by central treatment with anorexigenic agents such as the neuropeptide Y (NPY) a Y1- receptor antagonist, 1229U91 and the melanocortin 3/4 receptor agonist melanotan II (MTII (35; 36)).
A physiological factor that may participate in the termination of appetitive ingestive behaviors is leptin, the product of the obesity gene (Ob) that is synthesized and primarily secreted by white adipocytes (63). Circulating leptin concentrations are decreased by food deprivation and increased by feeding (63). In rodents, central leptin reduces voluntary food intake (25; 54). Leptin treatment following food deprivation abolishes the normal increases in food intake seen in laboratory rats (42) and mice (51). Not surprising, given that food deprivation stimulates ghrelin secretion (see above), leptin blocks the ability of ghrelin to stimulate food intake (3). The possible acute inhibitory/satiety effects of leptin on appetitive consummatory ingestive behaviors have been studied in Siberian hamsters, but under chronic exogenous administration of the cytokine resulting in decreases in food intake (4; 38), or in one case increases in food intake (27) – acute effects of leptin have not been studied nor have there been any tests of leptin on appetitive ingestive behaviors in this species. Leptin does, however, decrease food hoarding by Syrian hamsters (Mesocricetus auratus; 53), but foraging was not assessed, central application of leptin was not done and food deprivation-induced changes in serum leptin concentrations, as well as serum leptin concentrations after its administration were not measured. Therefore, we asked: can either peripheral or central leptin treatment block food deprivation-induced increases in foraging, food hoarding and food intake in Siberian hamsters? This was accomplished by attempting to block 48 h food deprivation-induced increases in foraging and food hoarding by injecting murine leptin either intraperitoneally or intracerebroventricularly into the 3rd ventricle of food deprived, long day-housed male Siberian hamsters housed in a running wheel-based food delivery foraging system that is coupled with simulated burrow-housing (21). Murine leptin was used because of the
unavailability of purified hamster leptin and the ~95% amino acid sequence homology between hamster and mouse leptin (44).

METHODS

Animals

All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with Public Health Service and United States Department of Agriculture guidelines. One hundred-four adult male Siberian hamsters, ~3.5 months old and weighing 35-50 g were obtained from our breeding colony. The lineage of this colony has been described recently (15). In brief, the breeding stock for this colony was donated by Dr. Bruce Goldman (University of Connecticut in 1988) based on founder stock from Dr. Klaus Hoffman (Germany) and interbred with second generation wild-trapped hamsters donated by Dr. Katherine Wynne-Edwards (Queens College) in 1990. In 1995, the colony hamsters were interbred with animals donated by Dr. G. Robert Lynch (University of Colorado) that also had originated from the original Hoffman stock but had been kept isolated for approximately 20 years. Later in 1999, F2 generation wild-trapped hamsters were interbred with the colony through a generous gift from Dr. Stephan Steinlechner (School of Veterinary Medicine (Hannover, Germany). Hamsters were group-housed and raised in a long-day photoperiod (16:8 light: dark; lights on at 0200h) from birth. Room temperature was maintained at 21±2.0 °C.

Experiment 1: Does food deprivation decrease plasma leptin in Siberian hamsters and how is it affected by different doses of peripheral leptin treatment?
Twenty male Siberian hamsters with body masses ranging from 35-50 g kept in standard shoebox cage housing were used in this experiment. Intraorbital blood samples were drawn on each hamster at the onset of the experiment and then again after 48 h of food deprivation. The animals were then given one of the following treatments: saline, 10, 40 or 80 μg of leptin (recombinant murine leptin, Peprotech, Rocky Hill, NJ) intraperitoneally (ip), then refed and blood samples were taken at 4 and 24 h after animals post refeeding/injection. Blood samples were spun in a centrifuge at 3000 RPM for 40 min and the serum was collected. Serum samples were then analyzed in a leptin ELISA as described below.

**Leptin ELISA**

The serum leptin concentrations in plasma obtained from animals in Experiment 1 were determined using a mouse ELISA kit (Linco Research Inc.; St. Charles, MO) according to the manufacturer’s instructions, as reported previously (16; 17; 40). All samples were run in duplicate in the same assay and the limits of the assay were 0.05 to 30 ng/ml.

**General Food Foraging, Hoarding and Food Intake Protocols for Experiments 2 and 3**

**Hoarding Apparatuses:**

Hamsters used in Experiments 2 and 3 were acclimated for two wk in specially designed hoarding apparatuses as previously described (21) that would serve as their housing for the duration of the experiment. More specifically, two cages were connected with a convoluted polyvinylchloride tubing system (38.1 mm id. and ~1.52 m long) with corners and straightways for horizontal and vertical climbs. The diet (75 mg pellets: Purified Rodent Diet; Research Diets, New Brunswick, NJ) and tap water were available ad libitum. A running wheel (524 mm circumference) and magazine type pellet dispenser that holds ~1750 pellets and always delivers
one whole pellet at a time were attached to the food (top) cage. Wheel revolutions were counted using a magnetic detection, response system that produced a switch closure every 360 degrees of wheel rotation that was monitored by a computer based hardware-software program (Med Associates, Lancaster, NH). For Experiment 3 only, hamsters were first trained in these apparatuses (24; 37) and then received a third ventricular cannula (23; 24), as previously described and described in brief below.

*Measurement of Foraging, Food Hoarding and Food Intake.*

Foraging (pellets earned) was defined as the number of pellets delivered upon completion of the requisite wheel revolutions. Food hoarding (pellets hoarded) was defined as the number of pellets found in the bottom ‘burrow’ cage in addition to those removed from the cheek pouches. For the 10 revolutions/Pellet groups, food intake (pellets eaten) was defined as: pellets earned – surplus pellets – hoarded pellets = food intake. For the Free and Blocked Wheel groups, food intake (pellets eaten) was defined as: pellets given – pellets left in the top cage – hoarded pellets = food intake. An electronic balance used to weigh the food pellets was set to ‘parts’ measurement rather than obtaining fractions of a pellet in mg; thus one 75 mg food pellet = 1 and fractions of a pellet were computed by the scale.

*Foraging Training Regimen*

We used a wheel-running training regimen that eases the hamsters into their foraging efforts without changes in body mass or food intake (21). Specifically, hamsters were given free access to food pellets for 2 d while they adapted to the running wheel. In addition to the free food, a 75 mg food pellet was dispensed upon completion of every 10 wheel revolutions. On the
third day, the free food condition was replaced by a response-contingent condition where only
every 10 wheel revolutions triggered the delivery of a pellet. This condition was in effect for 5 d
during which time body mass, food intake, food hoarding, wheel revolutions and pellets earned
were measured daily. During this time there was little or no evidence of changes in food intake
or body weight. Because these animals are outbred from wild caught populations, we did
observe the expected inherent individual variability in food intake, food hoarding and foraging in
this population of animals which has evolved polymorphic and plastic responses due to the
potential for fluctuating food availability in their natural environment. At the end of this
acclimation period (7 d total), animals in the icv leptin experiment were removed from the
foraging apparatuses and temporarily housed in shoebox cages where the same food pellets were
available ad libitum with no foraging requirements. Guide cannulae were then surgically
implanted in these hamsters (see below for details). Following a one wk post surgical recovery
period, all hamsters were returned to the hoarding/foraging apparatus and retrained to the
following schedule: 2 d for adaptation with free access to food pellets and 5 d at 10
revolutions/Pellet.

Experimental Design for Experiments 2 and 3

At the end of training, the hamsters for use in Experiments 2 and 3 were separated into
three groups matched for their current body mass and average hoard size across these last 3 d of
training at 10 revolutions/Pellet (n=14/group). The three groups consisted of 10
revolutions/Pellet foraging requirement, no foraging requirement with an active running wheel
(Free Wheel; exercise control group) or no foraging requirement with a blocked wheel (Blocked
Wheel; sedentary control group), each of the last two with food available non-contingently.
Selection of the 10 revolutions/Pellet foraging effort was based on a previous study in Siberian hamsters using this foraging/hoarding system to maximize hoarding levels (21).

Experiment 2: Does peripheral leptin treatment block the effects of food deprivation on ingestive behaviors in Siberian hamsters?

An additional forty-two male hamsters with a starting average body mass of 39.68 ± 0.71 g were trained in the foraging/hoarding apparatuses for Experiment 2. These animals were separated into three groups: 10 Revolutions/Pellet (foraging) group (n=14), Free Wheel (exercise) group (n=14) and Blocked Wheel (sedentary) group (n=14) as described above, were food deprived at 39.88 ± 0.89 g average body mass for 48 h and then injected ip with one of four solutions at the onset of the dark phase of the light cycle: sterile saline vehicle or 10, 40 or 80 μg of leptin (recombinant murine leptin, Peprotech, Rocky Hill, NJ). Following these ip injections, food intake, wheel running and food hoarding were monitored at 1, 2, 4, 24 and 48 h post-injection. After a two wk recovery/washout period, animals were reassigned to one of the four treatments listed above in a counterbalanced fashion and the same behavioral measurements were performed a second time. In our previous studies of food hoarding, we have used food deprivation periods ranging from 12 to 56 h (IACUC approved) with the latter length appearing somewhat lengthy or ‘non-physiological’ at first blush. In the utopian conditions of the laboratory, however, Siberian hamsters are almost 50% body fat compared to as low as ~25% in nature (61); therefore, short periods of food deprivation in the laboratory of 12-24 h are minimally energetically challenging in these animals and thus stimulation of food hoarding is minimal (Clein and Bartness, unpublished results). Therefore, we selected 48 h food deprivation to trigger the behavior nearly maximally. It also seems reasonable to envision these food
deprivation lengths as on a physiological continuum with the inter-meal intervals occurring
naturally of much shorter lengths in hamsters (~4 h (12)).

Experiment 3: Does icv leptin treatment block the effects of food deprivation on ingestive
behaviors in Siberian hamsters?

An additional 42 male hamsters with a starting average body mass of 39.73 ± 0.81 g were
implanted with 3rd ventricular cannulae for Experiment 3 as described below. These animals
were separated into three groups: 10 Revolutions/Pellet (foraging) group (n=14), Free Wheel
(exercise) group (n=14) and Blocked Wheel (sedentary) group (n=14) as described above, and
after recovery at an average body weight of 39.07 ± 0.84 g, they were food deprived for 48 h and
then injected icv with one of four solutions at the onset of the dark phase of the light cycle:
sterile saline vehicle or 1.25 μg, 2.5 μg or 5.0 μg of leptin (recombinant murine leptin,
Peprotech, Rocky Hill, NJ). Following these icv injections, food intake, wheel running and food
hoarding were monitored at 1, 2, 4, 24 and 48 h post-injection. After a two wk wash-out period,
animals were reassigned to one of the four treatments listed above in a counterbalanced fashion
and the same behavioral measurements were performed a second time.

Cannula Implantation

Cannulae were stereotaxically implanted into the third ventricle of hamsters used in
Experiment 3 as described previously (23). In brief, the animals were anesthetized with
isoflurane and the fur at the top of the head was removed to expose the area to be incised. A hole
was trephined at the intersection of bregma and the midsaggital sinus and the guide cannula (26
gauge stainless steel; Plastics One, Roanoke, VA) was lowered using the following stereotaxic
coordinates (level skull, anterior-posterior from bregma 0, medial-lateral from midsagittal sinus 0, and dorsal-ventral from the top of the skull -5.0 mm) targeted for placement just above the third ventricle. The guide cannula was secured to the skull using cyanoacrylate ester gel, 3/16 mm jeweler’s screws and dental acrylic. A removable obturator sealed the opening in the guide cannula throughout the experiment except when it was removed for the injections. Hamsters received 0.2 mg/kg buprenorphine at 12 and 24 h post-surgery to minimize discomfort and subsequently were allowed one wk to recover fully in the shoebox cage housing before being returned to their simulated burrow housing.

*Intracerebroventricular Injection Protocol*

The inner cannula (33 gauge stainless steel, Plastics One, Roanoke, VA) extended 5.5 mm below the top of the skull and all hamsters were injected with a 0.4 µl volume. All injections were given at the beginning of the dark phase of the photoperiod. Animals were lightly restrained by hand during the 30 s injection and the injection needle remained in place ~30 s before withdrawal as we have done previously (23; 24).

*Cannulae Verification*

Following the last test in *Experiment 3*, an injection of 0.4 µl bromophenol blue dye was given to confirm placement of the cannula in the third ventricle. The animals were anesthetized with an overdose of pentobarbital sodium (100 mg/kg), their brains removed and then postfixfixed in 10% paraformaldehyde for a minimum of two d. Each brain was sliced manually for cannulae verification. Cannulae were considered to be located in the third ventricle if the dye was visible in any part of this ventricle. Only the data from animals with confirmed third ventricle cannulae
placements were included in the analyses (n=42), and there was no incidence of cannula loss during the study.

Statistical Analyses

In experiment 1, leptin concentrations in response to food deprivation were analyzed by a Student’s t test, the plasma leptin concentrations in response to ip leptin injection were analyzed by one way ANOVA and Tukey’s Multiple Comparison tests were used for individual pair-wise comparison. For all measures of food intake, foraging and food hoarding in experiments 2 and 3, the data were analyzed for each time interval in order to identify intervals where the effects were occurring; thus, no statistical comparisons were made across time points (i.e., there was no ‘effect of time’ analysis). Therefore, the data from one time interval only are compared with data within that time interval. Data were analyzed using a two-way ANOVA (Foraging Effort Group x Leptin Treatment: 3 x 4) and Bonferroni’s post-hoc tests were used for individual pair-wise comparison. All statistical analyses were done using GraphPad Prism Software (San Diego, CA). Differences between means were considered statistically significant if P<0.05. Exact probabilities and test values were omitted for simplicity and clarity of the presentation of the results.

RESULTS

Experiment 1: Does food deprivation decrease plasma leptin in Siberian hamsters and how is it affected by different doses of peripheral leptin treatment?

Plasma Leptin. Forty-eight hours of food deprivation significantly decreased serum leptin concentration by ~4-fold (Fig. 1; P<0.05). Peripheral leptin treatment dose-dependently
increased serum leptin concentrations up to 4 h post-injection such that the 10, 40 and 80 µg of ip leptin resulted in ~2-, 4- and 7-fold increases compared with baseline values (Fig. 1; Ps<0.05). By 24 h, post-injection serum leptin concentrations had returned to baseline (Fig. 1).

**Experiment 2: Does peripheral leptin treatment block the effects of food deprivation on ingestive behaviors in Siberian hamsters?**

*Wheel Running.* Leptin did not affect cumulative wheel running after food deprivation, a general measure of locomotor activity, by the Free Wheel group (data not shown).

*Foraging.* Peripheral leptin treatment did not significantly alter foraging (pellets earned) after food deprivation by the 10 Revolution/Pellet groups (data not shown).

*Food Intake.* The hamsters with an explicit foraging requirement (10 Revolutions/Pellet) ate approximately twice as much as the Free Wheel and Blocked Wheel Groups (P<0.05; Fig. 2). Peripheral leptin treatment most effectively inhibited food deprivation-induced increased food intake by the Blocked Wheel hamsters, decreasing the cumulative number of pellets eaten at 24 and 48 h post-injection at all doses compared with saline injections (Fig. 2, Ps<0.05). Leptin also significantly decreased the food deprivation-induced increased cumulative food intake by the Free Wheel group at the two higher doses (40 and 80 µg) at 24 and 48 hours post-injection compared with saline (Fig. 2, Ps<0.05). Peripheral leptin treatment was the least effective at inhibiting food deprivation-induced increased food intake by the 10 Revolutions/Pellet group, only significantly decreasing the cumulative number of consumed pellets at the 80 µg dose at 24 and 48 h post-injection compared with saline (Fig. 2, Ps<0.05).
Food Hoarding. Peripheral leptin treatment significantly blocked the effects of food deprivation on food hoarding in a time-dependent, dose-dependent and foraging effort-dependent manner. Specifically, in the most sedentary groups of hamsters (Blocked Wheel), peripheral leptin treatment at the two highest doses (40 and 80 μg) decreased the cumulative number of pellets hoarded at all times post-injection compared with saline injections (Fig. 3, Ps<0.05). In the Free Wheel group, leptin was moderately effective at counteracting the effects of food deprivation on food hoarding. In this group, the lowest dose of leptin significantly inhibited food deprivation induced food hoarding, but only at the 24 and 48 h post-injection, whereas the 40 μg dose was effective in significantly inhibiting hoarding at 4, 24 and 48 h post-injection compared with the saline vehicle (Fig. 3, Ps<0.05). The highest leptin dose (80 μg) was most effective, inhibiting food deprivation-induced increased food hoarding at all times except for the first h post-injection compared with saline injections in this Free Wheel Group (Fig. 3, P<0.05). In the 10 Revolutions/Pellet groups, leptin was the least effective at blocking the food deprivation-induced increased hoarding versus the other two groups. Thus, only the highest leptin dose (80 μg) significantly decreased the cumulative number of pellets hoarded at 4, 24 and 48 h post-injection compared with saline injections (Fig. 3, Ps<0.05).

Experiment 3: Does icv leptin treatment block the effects of food deprivation on ingestive behaviors in Siberian hamsters?

Wheel Running. Central leptin treatment at the highest dose (5 μg) significantly decreased cumulative wheel running that was not associated with food delivery (Free Wheel Group) at all
time points post-injection compared with saline injections (Fig. 4, Ps<0.05), an apparent non-specific effect not seen with the other two lower central leptin doses (1.25 and 2.5 μg).

**Foraging.** Central leptin treatment significantly increased foraging by the 10 Revolutions/Pellet hamsters at 2, 4 and 24 h post-injection at the lowest dose (1.25 μg), and at the two higher doses (2.5 and 5 μg) at 24 h post-injection compared with saline (Fig. 4, Ps<0.05).

**Food Intake.** Central leptin treatment at all doses significantly inhibited food deprivation-induced increased food intake in the Blocked Wheel group, decreasing the cumulative number of pellets eaten at 2, 24 and 48 h post-injection (Fig. 5, Ps<0.05). For the Free Wheel hamsters, the highest dose of central leptin (5.0 μg) decreased food intake at all times (Fig. 5, Ps<0.05). In addition, 2.5 μg leptin treatment only decreased food intake at 4 and 24 h post-injection, whereas the 1.25 μg leptin dose only decreased food intake 24 h post-injection (Fig. 5, Ps<0.05). All doses of leptin inhibited food deprivation-induced food intake in the 10 Revolutions/Pellet group at 24 and 48 h post-injection, and the two highest doses (2.5 and 5 μg) also inhibited food deprivation-induced food intake at 1, 2 and 4 h post-injection compared with saline treatment (Fig. 5, Ps<0.05).

**Food Hoarding.** Leptin decreased the cumulative food hoarded at all times for the two higher doses (2.5 and 5.0 μg) in the Blocked Wheel group compared with vehicle-injected controls (Fig. 6, Ps<0.05). For both the Free Wheel and 10 Revolutions/Pellet groups, all doses of leptin significantly decreased food deprivation-induced increased food hoarding at all times post-injection compared with their vehicle-injected counterparts (Fig. 6, Ps<0.05).
DISCUSSION

The results of this study show that leptin not only inhibits food deprivation-induced increases in consummatory ingestive behavior, but it also effectively inhibits food deprivation-induced increases in food hoarding when given either intracerebroventricularly or peripherally. These data also indicate that the ability of leptin to inhibit food deprivation-induced increases in appetitive and consummatory ingestive behaviors can differ based on foraging effort (energy expenditure) and the route of leptin administration.

Food deprivation decreases plasma leptin concentrations in humans (14), laboratory rats or mice (28), pigs (7), horses (18) and Siberian hamsters (40; 58). In one report, 24 h food deprived female Siberian hamsters did not exhibit significant decreases in serum leptin concentrations (29), although this may have been due to the relatively short length of the food deprivation. Others find that food-deprived female Siberian hamsters have decreased serum leptin concentrations when food is withheld for 48 (58) or 56 h (40) and here, we found significantly decreased serum leptin concentrations in male Siberian hamsters food deprived for 48 h.

Peripheral leptin injection did not inhibit food deprivation-induced foraging, but it did inhibit food hoarding and intake as discussed below. Central leptin injections at the middle (2.5 µg) and high (5.0 µg) doses, however, significantly increased foraging, whereas it inhibited food hoarding and intake (also discussed below). This appears to be a bona fide specific central leptin-induced increase in foraging because there was not a non-specific increase in wheel running in the Free Wheel group where food was not contingent on this locomotor activity. By contrast, wheel running was decreased instead in these hamsters, at least at the highest central
leptin dose (5 µg). Interestingly, we recently found that parenchymal microinjections of NPY into the hypothalamic paraventricular nucleus (PVH) dose-relatedly decreased foraging (M. E. Daily and T. J. Bartness, in preparation). Given the presence of leptin receptors on Arc NPY/agouti-related protein (AgRP) neurons (45) and that leptin inhibits their activity (55), the ability of central leptin to increase foraging fits with the capability of PVH NPY to inhibit foraging. Why peripheral leptin also does not do so is unclear, although this may reflect the general finding of central leptin being more effective in altering ingestive behaviors than peripheral leptin (discussed directly below).

It is accepted that leptin acts in the CNS to alter most ingestive behaviors (19), with central treatments often being more effective than peripheral. Therefore, as noted above for foraging, central leptin treatment appears considerably more effective inhibiting food deprivation-induced increased food intake, and indeed, significantly decreased food intake within 1-2 h for most doses in most groups, whereas peripheral leptin had unusually long delays in decreasing food intake, becoming significantly much later (24 and 48 h) and often only at the higher doses (Free Wheel and 10 Revolutions/Pellet groups). Similarly, central leptin injections were more effective in significantly decreasing food hoarding, although the rapidity of the central versus peripheral effects was not as disparate as for the leptin-induced decreases in food intake. That is, peripheral leptin decreased food hoarding at 1, 2 and 4 h post-injection for the Blocked Wheel, Free Wheel and 10 Revolutions/Pellet groups, respectively, whereas central leptin began decreasing food hoarding for all groups 1 h after injection. The more rapid effect of central compared with peripheral leptin likely reflects the more direct access to central leptin target sites such as the periventricular hypothalamic region (e.g., (52)) and periventricular brainstem (30) sites of action than peripheral leptin where transport across the blood brain barrier
might not only require longer time to access such targets, but also might deliver less leptin because of transporter saturation properties (6). Indeed, the non-physiological level of peripheral leptin resulting from our exogenous administration which could, as with high concentrations of endogenous leptin (5), saturate the uptake system. For example, both obese humans and Siberian hamsters housed in long-day photoperiod have abundant adipose tissue that elevates peripheral leptin concentrations thereby likely saturating the leptin transporter system potentially diminishing access of leptin into the brain. Obviously, centrally administered leptin does not have to work through this obstruction to its brain sites of action and this may be at least part of the observed greater effectiveness of central versus peripheral leptin on these ingestive behaviors. We also realize, however, that it is impossible to compare the results of central to peripheral injections of any substance in terms of equivalency of doses (47); thus, an alternative hypothesis is that any differences between centrally versus peripherally injected leptin could be that the central dose is higher and this could be independent of penetration to the leptin sites of action.

We previously have used this Siberian hamster foraging/hoarding model to help determine the mechanisms underlying food deprivation-induced increases in the appetitive ingestive behaviors of food foraging and hoarding. The present study continues this work to further understand the ability of food deprivation to stimulate foraging and hoarding with refeeding. Thus, to date, it appears that food deprivation stimulates the release of ghrelin from the stomach, as evidenced by a positive relation between the length of food deprivation and circulating ghrelin concentrations (34). Presumably, ghrelin then stimulates its growth hormone secretagogue receptors in the brain (i.e., GHS-R1α; (31; 59)), especially arcuate nucleus NPY/AgRP neurons that have GHS-R1α (e.g.,(46)) to increase the expression and release of
these peptides into the PVH and other projection sites (e.g., perifornical area) to increase these appetitive ingestive behaviors. In support of this notion is the ability of central NPY (24) or AgRP (23) to trigger impressive food-deprivation-like increases in foraging and food hoarding. With the present data, and the evidence that leptin may be a ‘physiological antagonist’ of ghrelin, decreasing NPY and AgRP expression in the hypothalamus and inhibiting food intake (e.g., (48)), there is continued support for this functional conceptualization of how food deprivation stimulates appetitive ingestive behaviors in this and likely other species.

PERSPECTIVES AND SIGNIFICANCE

How might circulating leptin affect foraging/hoarding and food intake naturally in Siberian hamsters? With food deprivation, circulating leptin levels drop, as seen in the present experiment, likely due to increases in the sympathetic nervous system drive to WAT (16) that is known to inhibit leptin secretion (56). In addition, food deprivation triggers increases in ghrelin release that, in turn, stimulates its GHS-R1a receptors, some of which are located on Arc NPY/AgRP neurons. Stimulation of these neurons can cause the release of these peptides in several sites, primarily the PVH (e.g., (33)). Microinjection of NPY or the NPY Y-1 receptor agonist, BIBO 3304, increases foraging/food hoarding similarly to food deprivation in ad libitum-fed Siberian hamsters (M. E. Dailey and T. J. Bartness, in preparation). With refeeding, however, the physiological milieu is reversed, such that sympathetic drive to white fat is decreased and consequently leptin secretion is increased (e.g., (39)). Foraging and hoarding increase and concomitant with these initial large increases in these appetitive ingestive behaviors comes smaller increases in food intake (e.g., (21); and present experiment).
Understanding the underlying basis for the fundamental behaviors of foraging (for review see: (32)) and hoarding (for reviews see : (10; 60) that are so pervasive across animal taxa, including humans (e.g., (26) has great importance for understanding the development of obesity. For example, as your mother said, do not go to the grocery store hungry because you will bring home more food than if you go after you have eaten, and indeed, hungry people bring home more food than sated people (e.g.,(13; 26; 43)). Obese people bring home more high fat foods and more calories per person than do lean people (50). Therefore, understanding the underlying mechanisms involved in human foraging and food storing (hoarding) behaviors could greatly impact the obesity epidemic, especially because 85% of purchased food is eaten at home (50). Thus, foraging and hoarding of food may provide another point of attack for pharmacological and/or behavioral intervention.
ACKNOWLEDGEMENTS

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Figure Captions

**Figure 1:** Mean ± SEM plasma leptin expressed in ng/ml at baseline (black bar, n=10) and after a 48 hour food deprivation (grey bar, n=10) and mean ± SEM plasma leptin expressed in ng/ml at baseline, after 48 hr food deprivation and after refeeding with 0 μg (black bars, n=5), 10 μg (gray bars, n=5), 40 μg (striped bars, n=5) and 80 μg (white bars, n=5) peripheral leptin treatment.

**Figure 2:** Mean ± SEM cumulative food intake expressed as the number of pellets eaten after 48 hour food deprivation with 0 μg (black bars, n=7), 10 μg (gray bars, n=7), 40 μg (striped bars, n=7) and 80 μg doses (white bars, n=7) of peripheral leptin for hamsters without a foraging requirement and a stationary wheel (Blocked Wheel Group), hamsters with no foraging requirement and a freely moving wheel (Free Wheel Group) and hamsters with a foraging requirement (10 revolutions/Pellet Group) *=p<0.05 compared to the food deprived with 0 μg leptin control condition.

**Figure 3:** Mean ± SEM of cumulative food hoarding expressed as the number of pellets hoarded after 48 hour food deprivation with 0 μg (black bars, n=7), 10 μg (gray bars, n=7), 40 μg (striped bars, n=7) and 80 μg (white bars, n=7) doses of peripheral leptin for hamsters without a foraging requirement and a stationary wheel (Blocked Wheel Group), hamsters with no foraging requirement and a freely moving wheel (Free Wheel Group) and hamsters with a foraging requirement (10 revolutions/Pellet Group) *=p<0.05 compared to the food deprived with 0 μg leptin control condition.
Figure 4: Mean ± SEM cumulative wheel revolutions run after 48 hour food deprivation with 0 μg (black bars, n=7), 1.25 μg (gray bars, n=7), 2.5 μg (striped bars, n=7) and 5 μg doses (white bars, n=7) of central leptin for hamsters with no foraging requirement and a freely moving wheel (Free Wheel Revolutions) and hamsters with a foraging requirement (Foraging).

Figure 5: Mean ± SEM of cumulative food intake expressed as the number of pellets eaten after 48 hour food deprivation with 0 μg (black bars, n=7), 1.25 μg (gray bars, n=7), 2.5 μg (striped bars, n=7) and 5 μg (white bars, n=7) doses of central leptin for hamsters without a foraging requirement and a stationary wheel (Blocked Wheel Group), hamsters with no foraging requirement and a freely moving wheel (Free Wheel Group) and hamsters with a foraging requirement (10 revolutions/Pellet Group). *=p<0.05 compared to the food deprived with 0 μg leptin control condition.

Figure 6: Mean ± SEM food hoarding expressed as the number of pellets hoarded after 48 hour food deprivation with 0 μg (black bars, n=7), 1.25 μg (gray bars, n=7), 2.5 μg (striped bars, n=7) and 5.0 μg (white bars, n=7) doses of central leptin for hamsters without a foraging requirement and a stationary wheel (Blocked Wheel Group), hamsters with no foraging requirement and a freely moving wheel (Free Wheel Group) and hamsters with a foraging requirement (10 revolutions/Pellet Group) *=p<0.05 compared to the food deprived with 0 μg leptin control condition.
Figure 1

Leptin (ng/ml)

Ad lib | Food Dep.

Leptin (0) | Leptin (10 ug) | Leptin (40 ug) | Leptin (80 ug)
Figure 2

**Food Intake**

**Blocked Wheel Group**

- Leptin (0)
- Leptin (10 ug)
- Leptin (40 ug)
- Leptin (80 ug)

**Free Wheel Group**

**10 Revolutions/Pellet Group**
Figure 3

**Food Hoarding**

**Blocked Wheel Group**

- Hours Post-injection: 1 hr, 2 hr, 4 hr, 24 hr, 48 hr
- Pellets Hoarded: 0, 100, 200, 300, 400

**Free Wheel Group**

- Hours Post-injection: 1 hr, 2 hr, 4 hr, 24 hr, 48 hr
- Pellets Hoarded: 0, 100, 200, 300, 400

**10 Revolutions/Pellet Group**

- Hours Post-injection: 1 hr, 2 hr, 4 hr, 24 hr, 48 hr
- Pellets Hoarded: 0, 100, 200, 300, 400

Legend:
- Leptin (0)
- Leptin (10 ug)
- Leptin (40 ug)
- Leptin (80 ug)
Figure 4

**Free Wheel Revolutions**

- Black: Leptin (0 ug)
- Light gray: Leptin (1.25 ug)
- Dark gray: Leptin (2.5 ug)
- White: Leptin (5 ug)

<table>
<thead>
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<th>Hours Post-injection</th>
<th>Revolutions</th>
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<tbody>
<tr>
<td>1 hr</td>
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</tr>
<tr>
<td>2 hr</td>
<td>1000</td>
</tr>
<tr>
<td>4 hr</td>
<td>2000</td>
</tr>
<tr>
<td>24 hr</td>
<td>3000</td>
</tr>
<tr>
<td>48 hr</td>
<td>4000</td>
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**Foraging**

<table>
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<th>Pellets</th>
</tr>
</thead>
<tbody>
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<td>4 hr</td>
<td>200</td>
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<tr>
<td>24 hr</td>
<td>300</td>
</tr>
<tr>
<td>48 hr</td>
<td>400</td>
</tr>
</tbody>
</table>

* * *
Figure 5

**Food Intake**

**Blocked Wheel Group**

- Leptin (0 ug)
- Leptin (1.25 ug)
- Leptin (2.5 ug)
- Leptin (5 ug)

**Free Wheel Group**

**10 Revolutions/Pellet Group**

Pellets Eaten vs. Hours Post-injection
Figure 6

**Food Hoarding**

**Blocked Wheel Group**

- Leptin (0 ug)
- Leptin (1.25 ug)
- Leptin (2.5 ug)
- Leptin (5 ug)

**Free Wheel Group**

**10 Revolutions/Pellet Group**

Hours Post-injection: 1 hr, 2 hr, 4 hr, 24 hr, 48 hr

Pellets Hoarded

- 0
- 50
- 100
- 150
- 200
- 250
- 300

**Legend:**

- *: Significant difference
- **: Very significant difference

Note: The diagrams depict the amount of pellets hoarded by different groups at various hours post-injection.