Fat Pad-Specific Effects of Lipectomy on Foraging, Food Hoarding and Food Intake

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Running Head: Lipectomy and Appetitive Ingestive Behaviors

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ABSTRACT

DAILEY, M. J. and BARTNESS, T. J. Fat pad-specific effects of lipectomy on foraging, food hoarding and food intake. *American Journal of Physiology*, XX: XX-XX, 2007 – Unlike most species, after food deprivation Siberian hamsters increase foraging and food hoarding, two appetitive ingestive behaviors, but not food intake, a consummatory ingestive behavior. We previously demonstrated that increases in food hoarding are triggered by directly decreasing body fat levels through partial surgical lipectomy; however, we did not test if lipectomy affected foraging nor if the magnitude of the lipid deficit affected food hoard size. Therefore, we tested whether varying the size of the lipectomy-induced lipid deficit and/or foraging effort affected foraging, food hoarding or food intake. This was accomplished by housing adult male Siberian hamsters in a foraging/hoarding system and removing both epididymal white adipose tissue (EWATx) pads, both inguinal (IWATx) pads or both EWAT and IWAT pads (EWATx + IWATx) and measuring foraging, food hoarding and food intake for 12 weeks. The lipectomy-induced lipid deficit triggered different patterns of WAT mass compensation that varied with foraging effort. Foraging for food (10 wheel revolutions/to earn a food pellet) abolished the EWATx-induced compensation in IWAT pad mass. The magnitude of the lipid deficit did not engender a proportional change in any of the appetitive or consummatory ingestive behaviors. EWATx caused the greatest increase in food hoarding compared with IWATx or EWATx + IWATx when animals were required to forage for their food. Collectively, it appears that the magnitude of a lipid deficit does not affect appetitive or consummatory behaviors; rather when energy (foraging) demands are increased loss of specific (gonadal) fat pads can preferentially stimulate increases in food hoarding.
**Key Words:** Appetitive Behavior, Carcass Composition, Siberian Hamster, White Adipose Tissue
INTRODUCTION

Goal-oriented behaviors, such as ingestive behaviors, of non-human and human animals consists of the dichotomy of appetitive and consummatory phases (6). The mechanisms underlying the consumption of food (consummatory ingestive behavior) have received considerable attention compared with those underlying the approach, transport, and storage of food (appetitive ingestive behaviors). Both phases contribute to the overall energy strategy and balance of animals. For example, after food deprivation, changes in appetitive and consummatory ingestive behaviors occur, but vary among species (for review see: (3; 4)). Specifically, food deprived laboratory rats increase their foraging for food and, when they find it, eat it (e.g., (22). By contrast, hamster species do not overeat following resumption of feeding after food deprivation (for review see: (3)). Instead, food deprived Siberian and Syrian hamsters increase foraging (7; 15) and food hoarding (1; 2; 7; 15; 26). This separation of appetitive from consummatory ingestive behaviors in Siberian hamsters, as seen after food deprivation but also under other energy demanding conditions (e.g., metabolic blockers, pregnancy, lactation (1; 2)), makes them an ideal model to study the differential expression of each of these phases of ingestive behavior.

The physiological triggers that elicit post food deprivation-induced increases in foraging and food hoarding in Siberian hamsters are not known. One of the myriad of physiological effects of food deprivation is an increase in lipid mobilization from white adipose tissue (WAT) depots [e.g.,(11; 14)]. Food deprivation-induced reductions in body fat are due to the secondary consequences of decreased energy intake and are not the only outcome of negative energy balance [e.g., decreased thermogenesis; (11; 17), decreased reproductive system activity (27)]. Therefore, in order to test the notion that decreases in body fat per se trigger increases in these
appetitive ingestive behaviors by Siberian hamsters, we chose a direct method that at least initially only decreases body fat -- surgical removal of WAT (partial lipectomy referred heretofore as lipectomy). We previously demonstrated that lipectomized Siberian hamsters increase food hoarding, but not food intake (31). Moreover, the enhanced food hoarding returned to normal at a time when there is a complete recovery of lipectomy-induced lipid deficits, a response occurring exclusively in the non-excised WAT pads [(31) for review see: (21)]. In that experiment, however, the housing system did not require the animals to explicitly forage for their food (i.e., the animals only had to traverse a convoluted tube of ~ 1.5 m long to get to the food source which could be accomplished rather easily and quickly) and the size of the surgically-induced lipid deficit was not varied [both inguinal and epididymal WAT pads (IWAT and EWAT) were removed]; thus, no relation between the magnitude of the lipid deficit and the size of the food hoards could be established. Therefore, the purpose of the present experiment was to test the effect of varied lipectomy-induced lipid deficits on appetitive (foraging and food hoarding) and consummatory (food intake) ingestive behaviors with and without a required foraging effort. We have combined the foraging model of Perrigo and Bronson (24) to our previously developed hoarding system (2) where animals are required not only to traverse the tube, but then to meet a prescribed number of revolutions in a wheel to obtain a pellet of food (8). This allowed us to answer the following questions: 1) Does foraging effort (and thus to some degree energy expenditure) affect the compensation of fat pad masses after lipectomy? 2) Does a greater lipid deficit trigger a greater increase in foraging, food hoarding or food intake? This was accomplished by housing male Siberian hamsters in our foraging/hoarding system and removing the pair of EWAT or IWAT pads (EWATx or IWATx, respectively) or both pairs of EWAT and IWAT pads (EWATx + IWATx), or their respective sham surgeries and measuring
foraging, food hoarding and food intake for 12 weeks, a time by which body fat levels return to
control values for lipectomized Siberian hamsters [for review see: (21)].

**METHODS**

*Animals and Housing*

Adult male Siberian hamsters ~3.5 months old and weighing 36-46 g were obtained from
our breeding colony. The colony was established in 1988 and its genealogy was described
recently (5). Hamsters were group-housed and reared from birth in a 16:8h light-dark cycle
(lights-on at 2030). Room temperature was maintained at 21 ± 2 °C and relative humidity was
50 ± 10%. All procedures were approved by the Georgia State University Institutional Animals
Care and Use Committee and were in accordance with the Public Health Service and United
States Department of Agriculture guidelines.

Animals were tested in two identical replicates of 68 animals due to limited
foraging/hoarding apparatuses. Each hamster was acclimated for one wk in our
hoarding/foraging apparatus as previously shown and described (8). Briefly, two cages were
connected with a convoluted polyvinyl-chloride tubing system (38.1 mm inner diameter and
~1.52 m long), with corner and straightways for both horizontal and vertical climbs. The top or
“food cage” was 456 x 234 x 200 mm (length x width x height) equipped with a water bottle and
running wheel. The bottom or “burrow cage” was 290 x 180 x 130 mm and was covered to
simulate the darkness of a natural burrow. The burrow cage contained Alpha-Dri (Specialty
Papers, Kalamazoo, MI) bedding and cotton nesting material. The test diet (75 mg pellets:
Purified Rodent Diet; Research Diets, New Brunswick, NJ) and tap water were available *ad
libitum* during this period.
At the end of the adaptation period, hamsters were then trained to forage for their food based on our previously published procedure (8). In brief, hamsters were given free access to food 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. Wheel revolutions were counted using a magnetic detection system and monitored by a computer-based hardware/software system (Med Associated, Lancaster, NH). On the third day, the free food condition was replaced by a response-contingent condition in which only every 10 wheel revolutions triggered the delivery of a pellet. This condition was in effect for the remaining 5 d of the 1 wk-long training period. The hamsters were then separated into 12 groups (3 foraging groups x 4 lipectomy conditions) that were matched for percent change in body mass and average hoard size. The three foraging groups were 10 revolutions/pellet (10 Revolutions/pellet), Free Wheel/Free Food (Free Wheel; food was available non-contingently [not earned]), but the running wheel was active [locomotor activity control group]) or Blocked Wheel/Free Food (food was available non-contingently [not earned], but the running wheel was blocked [sedentary control group]).

Surgery

Animals within each of the foraging groups underwent one of four lipectomy conditions: 1) bilateral EWATx and sham IWATx (EWATx), 2) bilateral IWATx and sham EWATx (IWATx), 3) bilateral EWATx and IWATx (EWATx + IWATx), or 4) sham EWATx and sham IWATx (sham EWATx + sham IWATx). Hamsters were anesthetized with isoflurane. The dorsal and ventral hindquarters of each hamster were shaved and disinfected with ethanol (10% vol/vol). An incision was made lateral to the ventral midline through the dermis and peritoneum.
Each EWAT pad was externalized and carefully excised without disrupting the neural and vascular provisions of the testes. The peritoneal incision was sutured shut with sterile silk and the dermal incision closed with sterile wound clips. Each IWAT pad was exposed and excised through an incision to the dorsal midline immediately adjacent to each hind leg. The IWAT pads were blunt dissected and the incision was closed with sterile wound clips. Sham lipectomy included making an incision, visualization of the fat pads and closure of the incision.

Nitrofurozone powder was applied to all incisions to minimize infection. Hamsters had a 1 wk post surgery recovery period housed singly in polystyrene shoebox cages (290 x 189 x 130 mm) and given ad libitum food (75 mg pellets) and water. Fresh apple slices were given to facilitate fluid and caloric intake for the first 2-3 d post surgery. The animals also received subcutaneous buprenorphine (0.05mg/kg, s.c.) injections for 2 d after surgery. After recovery, hamsters were returned to their respective hoarding/foraging cages maintaining the same group membership. They subsequently were trained for an additional 1 wk training period as previously described (8) to recover baseline measures of foraging, food intake and food hoarding.

Measurements of Food Hoarding, Foraging 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. Surplus pellets were defined as the number of pellets removed from the top food cage that were earned, but neither eaten nor hoarded. For the 10 Revolutions/pellet group, food intake (pellets eaten) was defined as: pellets earned – surplus pellets – hoarded pellets = food intake. For the
Free Wheel and Blocked Wheel groups, food intake (pellets eaten) was defined as: pellets given (400 pellets) – pellets left in the top cage – hoarded pellets = food intake.

Food hoard size and food intake were measured to the nearest whole pellet by setting the electronic balance to ‘parts’ measurement rather than obtaining fractions of a pellet in mg; thus the smallest unit measured was one 75 mg food pellet and is equal to 1. These measures were made daily before the onset of the dark cycle (between 0830 and lights-off at 1230) for the 1 wk training period and the 12 wks of the experiment that followed the surgical recovery period. Foraging was monitored by the computer system continuously during the experiment. Body mass was measured weekly to the nearest 0.01 g.

**Blood Collection and Hormone Assays**

Blood samples were drawn once a week after the surgical recovery period. Animals were lightly anesthetized with isoflurane, blood samples (~300 µl) were drawn from the retroorbital sinus and animals were returned to their respective housing conditions. The samples were centrifuged at 4 °C for 30 min at 2500 rpm. Serum was recovered and stored at -20 ºC until subsequent measurement of testosterone (Testosterone EIA kit, ALPCO Diagnostics, Salem, NH) and leptin (Mouse Leptin ELISA kit, Millipore, St. Charles, MO) using commercially available ELISA kits according to the manufacturers’ instructions.

**Tissue Harvesting and Carcass Composition**

Twelve wk after surgery, the hamsters were then killed by an overdose of pentobarbital sodium (100mg/kg) and their WAT pads (bilateral EWAT, IWAT, retroperitoneal WAT
[RWAT], dorsal subcutaneous WAT [DWAT]) were removed, blotted dry and weighed to the nearest 0.01g.

Carcass composition was determined according to a modification of the method of Leshner et al. (16). Briefly, the carcass was shaved and the gastrointestinal tract was removed and discarded. The carcass was weighed to establish the carcass wet weights. The carcass was then dehydrated in an oven at 70 °C and weighed every 1-2 d until dry to determine total carcass water content. The carcass was blended and an ~1 g sample of the homogenous material was processed for lipid extraction using petroleum ether to determine lipid content gravimetrically. The remaining dried delipidated carcass sample was termed fat-free dry mass (FFDM). Total carcass lipid was then back-calculated based on the analysis of these samples and their respective carcass wet weights. The lipid, water and FFDM of the dissected WAT pads was estimated according to the estimations of DiGirolamo et al. (23) and added to each carcass component to determine total values.

Statistical Analyses

Body mass, foraging, food hoarding and food intake were analyzed using a three-way analysis of variance (ANOVA) for repeated measures (NCSS v 2000, Kaysville, UT) with a Surgery x Foraging Effort x Time (4 x 3 x 12) design for each behavioral measure. A separate, non-repeated measures two-way ANOVA design (Surgery x Foraging Effort; 4 x 3) was done on cumulative foraging, food hoarding, food intake and final body mass measures. Terminal measures including individual WAT pad mass and carcass composition were analyzed by a two-way ANOVA design (Surgery x Foraging Effort; 4 x 3). Measures of serum hormone concentrations were compared across surgical groups using a one-way ANOVA design. Post
Hoc analyses for significant differences between group means were determined using Duncan’s New Multiple-Range Tests when appropriate. Differences among groups 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

Hormone Assays.

**Leptin.** There were no differences in the percent change in serum leptin of the lipectomized groups relative to their respective sham controls at any of the three time points tested (Fig. 1). One wk after surgery, however, the absolute serum leptin concentrations were significantly decreased in IWATx condition (Mean±SEM = 1.17±0.29) compared with sham control animals (Mean±SEM = 1.90±0.41; Ps<0.05; Fig. 1) likely reflecting the surgery-induced lipid deficit. Seven wk after surgery (the approximate mid-point of the experiment), animals in the EWATx condition still had significantly decreased absolute serum leptin concentrations (Mean±SEM = 0.47±0.36) compared with the sham controls (Mean±SEM = 1.09±0.27; P<0.05; Fig. 1). No differences in the absolute serum leptin concentrations were seen by 14 wk after surgery (the end-point of the study) among the lipectomy conditions.

**Testosterone.** There were no differences in the percent change in serum testosterone concentrations of the lipectomized groups relative to the respective sham controls at the end of the experiment (Fig. 2), nor were there differences in the absolute serum testosterone concentrations.
**Body Mass.** There were no differences in the percent change in the final body mass from that of the respective sham controls among the lipectomy conditions (Fig. 3). Absolute body mass was affected by the foraging effort, however, the final body mass significantly lower in the 10 Revolutions/pellet group (Mean+SEM = 39.5 ± 0.66g) compared with Free Wheel (Mean+SEM = 45.2 ± 0.92g) and Blocked Wheel (Mean+SEM = 43.8 ± 0.85g) animals (Ps<0.05). Whereas the animals in the Free Wheel and Blocked Wheel groups increased their body mass progressively after surgery (~14% and 10%, respectively), animals in the 10 Revolutions/pellet group did not begin to increase their body mass until the 9th wk after surgery (albeit never reaching a statistically significant difference from their post-surgical body mass; data not shown).

**Carcass Composition.** There were no differences in carcass lipid, water content or fat free dry mass among the lipectomy conditions despite removal of various WAT pads (Fig. 4). Animals in the 10 Revolutions/pellet group had significantly increased total water content compared with Free Wheel and Blocked Wheel groups (P<0.05; Fig. 4).

**Fat Pad Mass**

*EWAT.* There was no significant compensatory increase in EWAT mass after EWATx (Fig. 5), as expected from our previous studies e.g.,(18; 28; 31).

*IWAT.* Similar to EWATx, there was no significant compensatory increase in IWAT mass after IWATx, as expected (18; 20; 31). IWAT mass was significantly increased by EWATx in both
the Free Wheel and Blocked Wheel groups compared with animals that were in the 10
Revolutions/pellet group (Ps<0.05; Fig. 5).

**RWAT.** There were no significant compensatory increases in RWAT mass after either EWATx and/or IWATx in any of the foraging groups. Both 10 Revolutions/pellet and Free Wheel groups had significantly decreased RWAT mass after EWATx and/or IWATx compared with animals in the Blocked Wheel group (Ps<0.05; Fig. 5).

**DWAT.** The only statistically significant changes in DWAT mass after lipectomy was a significant increase in mass for the Free Wheel group compared with the 10 Revolutions/pellet group for all lipectomies (P<0.05; Fig. 5).

**Total WAT.** By adding EWAT, IWAT, RWAT, and DWAT to obtain total dissectible WAT, the original graded lipid deficit that was produced by EWATx and/or IWATx was still evident 12 wks after surgery (P<0.05; Fig. 5). That is, EWATx animals show the smallest or no total lipid deficit, IWATx animals show a significantly larger lipid deficit, and the EWATx + IWATx animals show the largest lipid deficit (Ps<0.05; Fig. 5).

**Behavioral Measures**

**Wheel Running.** There was no effect of lipectomy on wheel running (total revolutions, data not shown), suggesting no debilitating effect of the surgery or non-specific stimulation of locomotor activity.
**Foraging.** Consistent with our previous findings (7; 8), animals in the 10 Revolutions/pellet group ran significantly more wheel revolutions than the Free Wheel animals (P<0.05; Mean±SEM = 3,625±101 revolutions and Mean±SEM = 2,555±92 revolutions, respectively).

**Food Hoarding.** Animals in the 10 Revolutions/pellet group hoarded significantly more food than animals in the Free Wheel or Blocked Wheel groups (Ps<0.05; Fig. 6) consistent with our previous findings (8). Among the 10 Revolutions/pellet groups, EWATx hamsters significantly hoarded more food compared with IWATx or EWATx + IWATx animals (P<0.05; Fig. 6).

**Food Intake.** Animals in the 10 Revolutions/pellet group ate significantly less than animals in the Free Wheel or Blocked Wheel groups (P<0.05; Fig. 6). There was no effect of lipectomy on food intake (Fig. 4), a result we also have seen previously [e.g., (18; 31)].

**DISCUSSION**

The major results of the present study were that: 1) compensatory increases in non-excised WAT masses occurred for all lipectomies, regardless of the foraging effort, 2) foraging for food (10 Revolutions/pellet group) abolished the EWATx-induced increase in IWAT mass, 3) the magnitude of the lipid deficit did not elicit a proportional change in appetitive or consummatory ingestive behaviors and 4) although EWATx produced the smallest lipid deficit (~50% of IWATx, ~33% of EWATx + IWATx), it triggered the largest increase in food hoarding when animals experienced the greatest foraging effort (10 Revolutions/pellet group).

The EWATx-specific effects seen in the present experiment, and discussed in detail below, are not likely due to compromised testicular function (i.e., decreased testosterone
synthesis/release). Although EWATx can decrease paired testes wet weight to some extent and did so here, no differences between the lipectomy conditions or circulating testosterone concentrations were found here or previously (19).

The results of the present experiment support previous studies showing that total body fat can be tightly regulated, as evidenced by the lipectomy-induced compensatory increases in non-excised WAT mass, a response that not only occurs in Siberian hamsters [e.g., (18; 28; 31), but in laboratory rats and mice, lambs, ground squirrels and Syrian hamsters (for review see: (21)] including suggestive evidence in humans (25; 33)]. Thus, there was no difference in carcass lipid content of EWATx and/or IWATx hamsters compared to their respective sham lipectomized controls 12 wks after lipectomy. Foraging effort differentially affected the pattern and degree of lipectomy-induced compensatory WAT mass increases. Specifically, the Blocked Wheel hamsters increased IWAT mass after EWATx to approximately the same extent as seen in our previous studies [~50% compared to sham controls; e.g., (18; 28; 31)], as might be expected given essentially the same housing conditions. By contrast, the Free Wheel EWATx hamsters more strikingly increased their IWAT mass (~145% compared to sham controls). Thus, the increased energy expenditure that likely occurred by wheel running, whether voluntary (Free Wheel) or imposed (10 Revolutions/pellet), triggered a fat pad-specific augmentation of the compensatory increase in IWAT mass compared with standard shoebox cage housing as in our previous lipectomy studies [e.g., (18; 28; 31)]. If the exercise-induced increase in energy expenditure was too great, however, as likely occurred with the 10 Revolutions/pellet group that ran significantly more (~42% more) than the Free Wheel group, this response was abolished. Thus, it would appear that in the case for the greater wheel running by the 10 Revolutions/pellet hamsters, the presumed additional increase in energy expenditure apparently curtailed any
supplemental storage of energy in IWAT. Instead, the animals may have utilized this energy to offset the increased energy demands of the required foraging effort.

In the present study, the total lipectomy-induced body fat losses were not associated with proportional increases in the appetitive or consummatory ingestive behaviors measured here. As noted above, the smallest lipid deficit (EWATx) produced the largest increase in food hoarding. Instead of increases in ingestive behaviors being stimulated by the size of the lipid deficit, loss of lipid stores from specific fat pads appears to be more important in triggering some of the changes in these ingestive behaviors. That is, there was no significant correlation between the amount of WAT removed and the degree to which any of these ingestive behaviors were expressed (data not shown). Conversely, hamsters required to forage for their food (10 Revolutions/pellet) hoarded significantly more when only EWAT (EWATx) was removed then when either IWAT (IWATx) alone was removed or when both EWAT and IWAT were removed (EWATx + IWATx). It is unclear why the EWATx-induced increase in food hoarding did not occur when EWAT was removed in the EWATx + IWATx hamsters, although it may be because this maximal lipid deficit overcame any EWATx-specific effects. That is, we previously have seen the elimination of fat pad-specific effects triggered by lipectomy if the lipid deficit became too large (20), as may be occurring with the EWATx + IWATx procedure, implying that at some point the size of the decrease in overall lipid reserves may affect behavior/physiology. This idea that the site of lipid loss may be more important than the size of the total lipid loss (at least until a more extreme level of fat loss occurs) is not unique to the present experiment. For example, in a recent study (Y. Chu, G. Huddleston, R. Bowers, A. Clancy, R. Harris, and T. Bartness, in preparation), EWATx of Syrian hamsters completely inhibits spermatogenesis, as it does in laboratory rats (30); however, IWATx of Syrian hamsters, that produces twice the lipid deficit of
EWATx, does not affect spermatogenesis. Therefore, it may be that lipid loss from fat pads that are most critical to the animal, such as those related to reproduction (EWAT in males, parametrial WAT in females), affects physiology (spermatogenesis) and behavior (hoarding, in 10 Revolutions/pellet group in the present study) more than does the size of the total lipid deficit or lipid deficits in non-reproductive associated WAT pads. If this is the case, then circulating factors such as the largely adipocyte derived cytokine leptin, thought by some to convey body fat levels to the brain [e.g., (32)], cannot be involved in such fat pad-specific responses because leptin released from any WAT pad, or other tissue, would not carry with it specific signaling of its origin. Moreover, we have shown previously that a functioning leptin signaling system is not necessary for lipectomy-induced compensatory increases in the mass of non-excised WAT pads because ob/ob and db/db mice show normal lipectomy-triggered compensation in total body fat for their surgical lipid deficit (12). In the present study there were no differences in serum leptin concentrations among any of the lipectomy groups, regardless of the graded lipid deficit or the specific fat pad removed. Alternatively, the sensory innervation of WAT (9; 10; 28) has the potential to convey such fat pad-specific information. Indeed, selective destruction of sensory nerves in EWAT via locally injected capsaicin, a sensory nerve specific neurotoxin (13), triggers an increase in WAT pad mass by the non-injected WAT pads indistinguishable from the compensatory increases in WAT pad mass triggered by actual EWATx (28).

The lipectomy-induced compensatory increase in RWAT mass seen in our previous studies where hamsters were housed in standard shoebox cages (18; 20; 29) also appears to be affected by increases in foraging effort. That is, RWAT was not increased by any of the lipectomy conditions in the present study, regardless of the foraging group. This may be because all animals had to minimally climb to the top cage to obtain food (Blocked Wheel) and some
animals had the additional energy drains of non-contingently running in the wheel (Free Wheel) or contingently running in the wheel to obtain food (10 Revolutions/pellet) in the present study. If this notion is correct, then it would be predicted that the Blocked Wheel group would expend the least amount of energy (no wheel running) and, therefore, might marshal a greater RWAT compensatory response than the two groups with running wheels (Free Wheel and 10 Revolutions/pellet). Although RWAT mass was not significantly increased by lipectomy in any group, RWAT mass was not decreased in two of the three lipectomy groups in the Blocked Wheel condition, as occurred in all the other groups compared with their respective sham controls. Thus, differences in the pattern of fat pad mass compensation after lipectomy between our current and former studies seems to be most readily explained by the requirement to explicitly forage for food (10 Revolutions/pellet group), or the opportunity to wheel run (Free Wheel group) and necessity to traverse the convoluted tubing connecting the burrow cage to the upper cage for all groups including the Blocked Wheel group.

Collectively, the present data suggest that: 1) appetitive ingestive behaviors are not altered by the total amount of fat mass loss, but instead are altered by lipid deficits in gonadal WAT and 2) apparent increases in energy expenditure due to increases in foraging effort can differentially affect the ability of the non-excised WAT pads to compensate for lipectomy-induced lipid deficits. Understanding the complex interactions among environmental factors affecting food availability and consumption, internal energy reserves (body fat) and external energy reserves (food hoards) is in its infancy, but fully explored should yield important information about the control of appetitive behaviors that are understudied relative to their consummatory counterparts.
ACKNOWLEDGEMENTS

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REFERENCES


**FIGURE CAPTIONS**

Figure 1. Mean + SEM percent change in serum leptin concentrations from sham epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (sham EWATx + sham IWATx) for each lipectomy condition [(bilateral epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (EWATx), bilateral inguinal white adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx)] 1wk (A), 7wk (B), or 14wk (C) after surgical lipectomy. Serum leptin concentrations of sham EWATx + sham IWATx at 1wk (Mean±SEM=1.90±0.41ng/ml), 7wk (Mean±SEM=1.08±0.27ng/ml), and 14wk (Mean±SEM=2.48±0.06ng/ml).

Figure 2. Mean + SEM percent change in serum testosterone concentrations from sham epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (sham EWATx + sham IWATx) for each lipectomy condition [(bilateral epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (EWATx), bilateral inguinal white adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx)]. Serum testosterone concentrations of sham EWATx + sham IWATx at 1wk (Mean±SEM=1.88±0.77ng/ml).

Figure 3. Mean + SEM percent change in body mass from sham epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (sham EWATx + sham IWATx) for each lipectomy condition [(bilateral epididymal white adipose tissue lipectomy and sham...
inguinal white adipose tissue lipectomy (EWATx), bilateral inguinal white adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx) in groups 10 Revolutions/pellet (A), Free Wheel (B), and Blocked Wheel (C). Body mass of sham EWATx + sham IWATx in 10 Revolutions/pellet group (A; Mean±SEM=39.52±1.95g), Free Wheel (B; Mean±SEM=47.58±1.94g), Blocked Wheel (C; Mean±SEM=44.81±2.36g).

Figure 4. Mean + SEM percent change in carcass lipid mass (A), fat free dry mass (B), and carcass water content (C) from sham epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (sham EWATx + sham IWATx) for each lipectomy condition [(bilateral epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (EWATx), bilateral inguinal white adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx)] for each group (10 Revolutions/pellets, Free Wheel, and Blocked Wheel). *P<0.05 compared with Free Wheel and Blocked Wheel. Carcass lipid mass (A) for sham EWATx + sham IWATx in 10 Revolutions/pellet (Mean±SEM=9.71±1.46g), Free Wheel (Mean±SEM=10.35±1.61g), Blocked Wheel (Mean±SEM=10.80±2.36g). Fat free dry mass (B) for sham EWATx + sham IWATx in 10 Revolutions/pellet (Mean±SEM=0.04±0.01g), Free Wheel (Mean±SEM=0.01±0.01g), Blocked Wheel (Mean±SEM=0.03±0.01g). Carcass water content (C) for sham EWATx + sham IWATx in 10 Revolutions/pellet (Mean±SEM=17.62±0.68g), Free Wheel (Mean±SEM=19.75±1.04g), Blocked Wheel (Mean±SEM=18.89±1.22g).
Figure 5. Mean + SEM percent change in fat pad mass from sham epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (sham EWATx + sham IWATx) for each lipectomy condition [(bilateral epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (EWATx), bilateral inguinal white adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx)] in groups 10 Revolutions/pellet (A), Free Wheel (B) and Blocked Wheel (C). Values are means ± SEM. * \( P < 0.05 \) compared with other lipectomy conditions. \( ^a \) \( P < 0.05 \) compared with Free Wheel and Blocked Wheel. \( ^b \) \( P < 0.05 \) compared with 10 Revolutions/pellet and Free Wheel. \( ^d \) \( P < 0.05 \) compared with 10 Revolutions/pellet. Fat pad mass of sham EWATx + sham IWATx in 10 Revolutions/pellet group (A) for IWAT (Mean±SEM=1.45±0.31g), EWAT (Mean±SEM=0.77±0.13g), RWAT (Mean±SEM=0.18±0.06g), DWAT (Mean±SEM=1.23±0.25g) and TOTAL (Mean±SEM=3.68±0.57g). Fat pad mass of sham EWATx + sham IWATx in Free Blocked group (B) for IWAT (Mean±SEM=2.08±0.41g), EWAT (Mean±SEM=0.89±0.12g), RWAT (Mean±SEM=0.21±0.07), DWAT (Mean±SEM=1.59±0.15) and TOTAL (Mean±SEM=4.77±0.64g). Fat pad mass of sham EWATx + sham IWATx in the Blocked Wheel group (C) for IWAT (Mean±SEM=1.56±0.29g), EWAT (Mean±SEM=1.01±0.19g), RWAT (Mean±SEM=0.14±0.04g), DWAT (Mean±SEM=1.48±0.22) and TOTAL (Mean±SEM=4.19±0.66).

Figure 6. Mean + SEM percent change in cumulative foraging from sham epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (sham EWATx + sham IWATx) for each lipectomy condition [(bilateral epididymal white adipose tissue
lipectomy and sham inguinal white adipose tissue lipectomy (EWATx), bilateral inguinal white adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx) in 10 Revolutions/pellet. Cumulative foraging of sham EWATx + sham IWATx in 10 Revolutions/pellet group (Mean±SEM=3788±110),

Figure 7. Mean ± SEM percent change in cumulative food hoard from sham epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (sham EWATx + sham IWATx) for each lipectomy condition [(bilateral epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (EWATx), bilateral inguinal white adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx)] in groups 10 Revolutions/pellet, Free Wheel and Blocked Wheel. *P<0.05 compared with IWATx and EWATx + IWATx. aP<0.05 compared with free Wheel and Blocked Wheel. bP<0.05 compared with 10 Revolutions/pellet and Blocked Wheel. cP<0.05 compared with 10 Revolutions/pellet and Free Wheel. Cumulative food hoard of sham EWATx + sham IWATx in 10 Revolutions/pellet group (Mean±SEM=96±6), Free Wheel group (Mean±SEM=23±1), and Blocked Wheel group (Mean±SEM=11±1).

Figure 8. Mean ± SEM percent change in cumulative food intake from sham epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (sham EWATx + sham IWATx) for each lipectomy condition [(bilateral epididymal white adipose tissue lipectomy and sham inguinal white adipose tissue lipectomy (EWATx), bilateral inguinal white adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx)] in 10 Revolutions/pellet, Free Wheel and Blocked Wheel. *P<0.05 compared with IWATx and EWATx + IWATx. aP<0.05 compared with free Wheel and Blocked Wheel. bP<0.05 compared with 10 Revolutions/pellet and Blocked Wheel. cP<0.05 compared with 10 Revolutions/pellet and Free Wheel. Cumulative food hoard of sham EWATx + sham IWATx in 10 Revolutions/pellet group (Mean±SEM=96±6), Free Wheel group (Mean±SEM=23±1), and Blocked Wheel group (Mean±SEM=11±1).
adipose tissue lipectomy and sham epididymal white adipose tissue lipectomy (IWATx), bilateral epididymal white adipose tissue lipectomy and inguinal white adipose tissue lipectomy (EWATx + IWATx)] in groups 10 Revolutions/pellet, Free Wheel and Blocked Wheel. Values are means ± SEM. aP<0.05 compared with Free Wheel and Blocked Wheel. Cumulative food intake of sham EWATx + sham IWATx in 10 Revolutions/pellet group (Mean±SEM=72±2), Free Wheel group (Mean±SEM=46±0.6), and Blocked Wheel group (Mean±SEM=41±0.6).
Figure 1

**Serum Leptin**

*1wk Post-surgery*

![Graph showing % Change From Sham Control for Serum Leptin 1wk Post-surgery.](image)

*7wk Post-Surgery*

![Graph showing % Change From Sham Control for Serum Leptin 7wk Post-Surgery.](image)

*14wk Post-surgery*

![Graph showing % Change From Sham Control for Serum Leptin 14wk Post-surgery.](image)
Figure 2

Serum Testosterone
Figure 3

Final Body Mass

% Change From Sham Controls

-20 -15 -10 -5 0 5 10

10Revs FW BW

EWATx + Sham IWAT
IWATx + Sham EWAT
EWATx + IWATx
Figure 4

Carcass Lipid Content

Fat Free Dry Mass

Carcass Water Content
Figure 5

**WAT Pad Mass**

10 Revolutions/pellet

- EWATx + Sham IWATx
- IWATx + Sham EWATx
- EWATx + IWATx

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**Free Wheel**

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Figure 6

Cumulative Foraging
10 Revolutions/pellet

% Change From Sham Controls

-20  -10  0  10  20

-20

EWATx + Sham IWAT
IWATx + Sham EWAT
EWATx + IWATx
Figure 7

Cumulative Food Hoard

- EWATx + Sham fWAT
- iWATx + Sham EWAT
- EWATx + iWATx

% Change From Sham Controls

10Revs FW BW

- a
- b
- c
Figure 8

Cumulative Food Intake

- EWATx + Sham IWAT
- IWATx + Sham EWAT
- EWATx + IWATx

% Change From Sham Controls

10Revs  FW  BW

-25  -20  -15  -10  -5  0  5  10

-20  -15  -10  -5  0  5  10