Social defeat and footshock increase body mass and adiposity in male Syrian hamsters

Matia B. Solomon,1,3 Michelle T. Foster,2,3 Timothy J. Bartness,1,2,3 and Kim L. Huhman2,3

1Department of Psychology, 2Department of Biology, and 3Center for Behavioral Neuroscience, Georgia State University, Atlanta, Georgia

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OBESITY IS A WORLD-WIDE EPIDEMIC with comorbidities such as cardiovascular disease, type 2 diabetes, hypertension, depression, and certain forms of cancer (8, 20, 43). Because obesity is associated with these and other pathologies, investigation of the underlying psychosocial and physiological factors that may account for this growing trend is critical. The underlying mechanisms for the increases in obesity involve increases in energy intake and/or decreases in energy expenditure, which have, as their bases, genetic and/or environmental influences. Stress is one environmental influence that has been associated with obesity (9), although it receives considerably less scientific attention than the more frequently studied environmental factor, high-calorie diets.

A variety of animal models incorporating both nonsocial and social stressors have been used to reveal the relations among stress, food intake, and adiposity. Mild, nonsocial stressors, such as tail pinch, brief restraint, and handling, increase food intake in laboratory rats (2, 33). By contrast, chronic footshock, chronic restraint, and chronic noise stress decrease food intake and body mass (1, 22, 30, 42, 46). The extent of the decrease in food intake and body mass depends on several factors, including the intensity of the stressor (36), time of day (23, 44, 46), and diet composition (24). Nonsocial stressors, such as restraint and footshock, often are preferred in experimental studies over more complex social stressors, such as agonistic behavior (i.e., behavior that is exhibited during social conflict between conspecifics), because greater control of the intensity, duration, and frequency of the nonsocial stressor yields less variable responses to stress and, hence, more easily replicable results.

One naturally occurring form of social stress typically observed after an agonistic interaction in rodents, humans, and nonhuman primates is social defeat (7, 45, 47, 48). In the laboratory, social defeat typically is induced using the resident-intruder model, whereby an intruder conspecific is placed into the home cage of a larger resident and the intruder usually is defeated by the resident aggressor. The effects of social defeat on food intake and body mass have been studied extensively in laboratory rats and mice. In most studies, subordinate rats and mice decrease their food intake and either decrease their body mass or have an attenuated body mass gain compared with their nonstressed control and/or dominant counterparts (3, 10, 14, 21, 28, 29, 37, 47). In some isolated cases, however, body mass is increased in subordinate laboratory rats and mice compared with their nonstressed control or dominant counterparts (6, 50). The reason for these discrepant body mass and food intake responses after social defeat is unknown, but they could be due to differences in rat strains (i.e., Wistar vs. Long Evans rats) or the duration and intensity of the defeat episodes. Overall, however, the predominant response of laboratory rats and mice to social stress is inhibition of food intake and growth of body and lipid mass.

In contrast to most laboratory rats and mice, in Syrian hamsters (Mesocricetus auratus), social stress appears to trigger increases in food intake and body and lipid masses. This was first noted in group-housing conditions, where female Syrian hamsters increase their food intake and body and lipid mass compared with their singly housed counterparts (11, 18, 38). Because Syrian hamsters naturally are a solitary species and are highly territorial (40), it may be that group housing is a social stressor for these animals. In the earlier studies of group-housed hamsters, no attempt was made to distinguish...
between dominant and subordinate animals. It is possible that there is a difference in body and lipid mass and food intake depending on social status in these group-housing conditions.

Recently, we demonstrated significant increases in food intake and body and fat pad masses in subordinate male hamsters after agonistic encounters compared with nonstressed controls (17); however, we did not test whether the stress-induced increases in food intake, body mass, and adiposity also occurred in dominant hamsters. Therefore, the purpose of the present study was to determine 1) whether dominant and subordinate hamsters increase food intake, body mass, and adiposity after an agonistic encounter and 2) whether exposure to a nonsocial stressor, footshock, increases these measures as well. In experiment 1, we measured food intake and body and lipid masses in hamsters after exposure to social defeat or footshock stress as well as in their nonstressed controls. In experiment 2, we measured food intake and body and lipid masses in hamsters after exposure to social defeat or footshock stress as well as in their nonstressed controls. In experiment 1, we asked, do dominant male hamsters increase food intake, body mass, and adiposity after an agonistic encounter? In experiment 2, we asked, does footshock stress increase food intake, body mass, and adiposity in male Syrian hamsters?

METHODS

Animals and Housing Conditions

Male Syrian hamsters (Charles River Laboratories) were ~10 wk old and weighed 120–125 g at the beginning of the study. On arrival from the supplier, hamsters were individually housed in polycarbonate cages (20 × 40 × 20 cm) with wire mesh tops and Alpha Dri bedding (Shepherd Specialty Papers, Kalamazzo, MI) in a temperature-controlled vivarium with a 14:10-h light-dark cycle (lights off at 1100 Eastern Standard Time). Hamsters were given food (PMI Rodent Diet no 5001, St. Louis, MO) and water ad libitum, except during the stress procedure. Briefly, the defeat group was placed in the home cage of trained resident aggressors for 7 min over 4 consecutive days. The resident aggressors were larger animals that were known to be aggressive, and, in all cases, the resident aggressors rapidly became dominant over the experimental animals. The resident-intruder model was used to reliably induce social conflict: one hamster (the intruder) was placed into the home cage of its opponent (the resident). The purpose of the experiment was to examine the effects of the agonistic encounter on body mass, food intake, and adiposity in subordinate (i.e., losers) and dominant (i.e., winners) animals; therefore, the animals were divided into winners and losers irrespective of territorial status. All encounters occurred in a 15 × 20 cm (length × height × width) arena at the bottom. Animals were subjected to six intermittent, 5-s scrambled footshocks over 7 min via the electrified metallic floor of the box for a total footshock duration of 30 s. After each session, the footshock box was cleaned with a 70% (vol/vol) ethanol solution to minimize remaining odors. We used a 1-mA footshock, which was shown in previous research from our laboratory to be sufficient to elicit a hormonal stress response in male Syrian hamsters (26).

Experiment 2: Does Footshock Stress Increase Food Intake, Body Mass, and Adiposity in Male Syrian Hamsters?

Twenty male hamsters were matched by body mass and then assigned to the defeat (n = 7), footshock (n = 7), and control (n = 6) groups. Testing for the defeat and footshock groups took place within 2 h after dark onset as in experiment 1. On the day of testing, all animals were transported from the vivarium to the behavioral testing rooms. The control groups remained in their home cages during the stress procedure. Briefly, the defeat group was placed in the home cage of trained resident aggressors for 7 min over 4 consecutive days. The resident aggressors were larger animals that were known to be aggressive, and, in all cases, the resident aggressors rapidly became dominant over the experimental animals.

The footshock group was placed in a standard shock box: 91 × 15 × 20 cm (length × height × width) at the top and 6.4 cm wide at the bottom. Animals were subjected to six intermittent, 5-s scrambled 1-mA footshocks over 7 min via the electrified metallic floor of the box for a total footshock duration of 30 s. After each session, the footshock box was cleaned with a 70% (vol/vol) ethanol solution to minimize remaining odors. We used a 1-mA footshock, which was shown in previous research from our laboratory to be sufficient to elicit a hormonal stress response in male Syrian hamsters (26).

Blood collection and fat pad and testes harvesting. Trunk blood was collected and fat pads and testes were harvested as described in experiment 1.

Radioimmunoassay of leptin and insulin. Assays for serum leptin and insulin were performed in duplicate by the Endocrine Core Laboratory, Yerkes Regional Primate Research Center of Emory University, using commercially prepared kits for murine leptin and rat insulin (Diagnostic Systems Laboratories). Sensitivities were 0.05 and 0.07 ng/ml for the leptin and insulin assays, respectively. Intra-assay variability was <10% for both assays, and all samples were run in the same assay.
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Carcass composition. Carcass composition was determined as described in Experiment 1.

Statistical Analyses

Data were analyzed using SPSS for Windows (release 11.5.0, SPSS, Chicago, IL). For experiments 1 and 2, the effect of experimental treatment on body mass and food intake was determined by repeated-measures ANOVA, with experimental treatment as the between-subjects factor and time (i.e., day) as the within-subjects factor. The effects of experimental treatment on hormones, fat pad masses, and carcass composition were analyzed by one-way ANOVA, with experimental treatment as the fixed factor, followed by least significant difference post hoc tests. Differences among the groups were considered statistically significant if \( P < 0.05 \). Exact probabilities and test values are omitted for simplicity and clarity in the presentation of the results.

RESULTS

Experiment 1: Do Dominant Male Hamsters Increase Food Intake, Body Mass, and Adiposity After an Agonistic Encounter?

Body mass, food intake, and feed efficiency. At \( \sim 1 \) wk after the last agonistic encounter and throughout the experiment, body mass was significantly increased in subordinate hamsters compared with the nonstressed controls (\( P < 0.05 \); Fig. 1A). There was no significant difference in absolute body mass between subordinate and dominant animals across the experiment (Fig. 1A). Although there was no significant difference in absolute body mass between dominant and nonstressed control hamsters, cumulative body mass gain was significantly greater in dominant than in nonstressed control animals (\( P < 0.05 \); Fig. 1B; cumulative body mass gain = final body mass − baseline body mass).

One nonstressed control and one subordinate animal were eliminated from the food intake analysis because of excessive shredding of food, which made it difficult to gather accurate food intake measurements. At \( \sim 1 \) wk after the last agonistic encounter (day 16), subordinate male hamsters began eating significantly more food than nonstressed controls (\( P < 0.05 \); Fig. 2). There was no significant difference in cumulative food intake between dominant and nonstressed control animals; similarly, there was no difference in food intake between subordinate and dominant groups (Fig. 2). Feed efficiencies (cumulative body mass gain ÷ cumulative amount of food consumed during entire experiment) were significantly higher in subordinate male hamsters than in nonstressed control and dominant hamsters (\( P < 0.05 \) for each; Fig. 3). Feed efficiency was significantly higher in dominant than in nonstressed control hamsters (\( P < 0.05 \); Fig. 3).

Agonistic behavior. Throughout the study, once a dominant-subordinate relationship was established, it remained stable throughout the study. In approximately half (4 of 9) of the pairs, the intruders became dominant over the residents. These data indicate that, in studies where hamsters are matched by body mass, resident status is not a reliable predictor of dominance (data not shown).

Fat pad and testes mass. MWAT, IWAT, RWAT, and EWAT masses were significantly increased in subordinate hamsters compared with the dominant hamsters (\( P < 0.05 \) for each; Fig. 4). RWAT and EWAT masses were significantly increased in subordinate male hamsters compared with the nonstressed controls (\( P < 0.05 \) for each; Fig. 4). WAT masses did not differ between nonstressed control and dominant male hamsters. Finally, there was no effect on testes mass among the groups (Table 1).

Serum \( T \) and leptin concentrations. Serum \( T \) concentrations did not differ among the groups (Table 1). Serum leptin concentrations were significantly greater in subordinate than in nonstressed control and dominant male hamsters. Finally, there was no effect on testes mass among the groups.

Carcass composition. There was no significant difference among any of the groups in absolute carcass FFDM or water content (Fig. 5A); however, absolute carcass lipid content was significantly greater in subordinate than in nonstressed control and dominant male hamsters (\( P < 0.05 \) for each; Fig. 5A). Dominant hamsters had a higher percent carcass water content and a lower percentage of carcass lipid content than nonstressed control and subordinate male hamsters (\( P < 0.05 \) for each; Fig. 5B). There was no difference in percentage of carcass FFDM among any of the groups.
Experiment 2: Does Footshock Stress Increase Food Intake, Body Mass, and Adiposity in Male Syrian Hamsters?

Body mass, food intake, and feed efficiency. One animal from the control group was eliminated from statistical analyses because of an unexplainable loss (i.e., 10 g) of body mass. From day 15 until the end of the study, footshock stress induced a significant increase in body mass compared with nonstressed controls (P < 0.05 for each; Fig. 6A), whereas social defeat did not until day 19. The cumulative body mass was significantly greater in subordinate and footshock groups than in the nonstressed controls (P < 0.05 for each; Fig. 6B). Despite the increase in body mass in the footshock and subordinate groups, there was no significant difference in food intake among any of the groups (data not shown). Accordingly, feed efficiency was significantly higher in the footshock and subordinate groups than in the nonstressed controls (P < 0.05; Fig. 7).

Fat pad and testes masses. MWAT mass was significantly increased in footshock and subordinate groups compared with nonstressed controls (P < 0.05; Fig. 8). In addition, EWAT mass was significantly increased in subordinate hamsters compared with nonstressed controls (P < 0.05; Fig. 8). There was no difference in IWAT and EWAT mass among any of the groups (Fig. 8). As in experiment 1, testes mass was not different among the groups (Table 2).

Serum leptin and insulin concentrations. Serum leptin concentration was significantly greater in subordinate than in footshock and nonstressed control animals (P < 0.05 for each; Table 2); moreover, serum leptin concentration was significantly greater in the footshock than in the nonstressed control animals (P < 0.05; Table 2). Serum insulin concentration was also significantly greater in subordinate than in nonstressed control animals (P < 0.05). There was no significant difference in serum insulin levels between footshock and nonstressed control animals (Table 2).

Carcass composition. There were no significant differences in absolute carcass water or FFDM mass among any of the groups (Fig. 9A). However, carcass lipid content was significantly increased in subordinate hamsters compared with nonstressed controls (P < 0.05; Fig. 9A). There was no difference in carcass lipid between footshock and nonstressed control animals nor was there an affect of any experimental treatment on relative carcass composition for any component (data not shown).

DISCUSSION

The present experiments support and extend our previous finding (17) that subordinate Syrian hamsters exhibit increases

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<td>Testosterone, ng/ml</td>
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Values are means ± SE. *P < 0.05 vs. other groups.
in food intake and body mass, as well as increases in several fat pad masses, compared with nonstressed controls. The results of the present study show persistently increased body mass and WAT pad masses, increased total carcass lipid, and increased serum leptin concentrations in Syrian hamsters exposed to social defeat or footshock compared with nonstressed controls. Subordinate animals also ate more and had greater feed efficiencies, suggesting decreased energy expenditure, than the dominant or nonstressed control hamsters. Although feed efficiencies were higher and increases in body mass gain were greater in the dominant than in the control animals, the dominant hamsters did not have significantly larger WAT pad masses, increased total carcass lipid, or larger increases in carcass lipid than the controls. Thus we have demonstrated, for the first time, social stress-induced increases in WAT pad masses as well as carcass lipid specifically in subordinate hamsters, and not in dominant hamsters, after agonistic encounters. Moreover, we have demonstrated that a nonsocial stressor, footshock, similarly increased body mass, feed efficiency, and some WAT pad (i.e., MWAT) masses but not total carcass lipid. By contrast, with footshock stress, it seems that social defeat produces a more marked increase in adiposity, with increases in RWAT and EWAT, as well as overall body fat, as reflected in the significantly increased carcass lipid compared with nonstressed controls.

Animals exposed to defeat or footshock stress exhibited a significant increase in body mass. This increased body mass was largely reflected as increased fat pad mass growth; however, the stress-induced increases in fat pad mass growth were not specific or consistent in all instances. For example, in experiment 1, animals exposed to social defeat exhibited significantly increased RWAT and EWAT fat pad masses relative to nonstressed controls; in experiment 2, however, defeat triggered increases in MWAT and EWAT fat pad mass growth. On the other hand, only MWAT was significantly increased in

Fig. 5. A: absolute carcass components of control (n = 9), subordinate (n = 8), and dominant (n = 9) hamsters in experiment 1. FFDM, fat-free dry mass. B: relative percent carcass component of control (n = 9), subordinate (n = 8), and dominant (n = 9) hamsters in experiment 1. Values are means ± SE. Nonshared letters (a, b) indicate statistically significant differences (P < 0.05).

Fig. 6. A: absolute body mass of control (n = 5), subordinate (n = 7), and footshocked (n = 7) hamsters in experiment 2. Arrows, days of introduction of stress for subordinate and footshock groups only (i.e., days 7, 8, 9, and 10). B: cumulative body mass increase relative to baseline of control (n = 5), subordinate (n = 7), and footshocked (n = 7) hamsters in experiment 2. Values are means ± SE. *P < 0.05, subordinate vs. footshock vs. control. #P < 0.05, footshock vs. control. Nonshared letters (a, b) indicate statistically shared significant differences (P < 0.05).
animals exposed to footshock compared with nonstressed controls. The variation in defeat- and footshock-induced fat pad increases may be due to subtle differences in the intensity of the stressful encounters between experiments. In addition, although the increase in body mass in the subordinate animals relative to the nonstressed controls was more consistently due to increased carcass lipid, footshock stress did not induce a similar increase. This finding suggests that the stress-induced changes in adiposity depend on the nature of the stressor. Nonetheless, the defeat-induced increases in body mass and adiposity in the present study are consistent with our previous findings of increases in body and fat pad mass and overall adiposity in Syrian hamsters with acute exposure (7 min over 4 days) to social stress (17).

Perhaps an even more notable finding of this study is the marked difference in distribution of adiposity depending on social status. For instance, although the dominant and subordinate animals did not differ in their absolute body mass, there were significant differences in fat pad mass growth and carcass composition between the two groups. More specifically, defeat triggered increases in all fat pad (i.e., MWAT, IWAT, RWAT, and EWAT) masses in subordinate hamsters compared with their dominant counterparts. Thus, although thoroughly involved in the agonistic encounter, the dominant hamsters had body fat distributions (i.e., fat pad masses) that were most similar to those of nonstressed controls. In addition, although dominant and subordinate hamsters had agonistic encounter-related increases in body mass, dominant hamsters appeared to achieve this increase by a redistribution of their carcass composition, significantly decreasing carcass lipid while increasing their percentage of carcass water, whereas subordinates increased carcass lipid only, although this could be definitively known only if the initial carcass composition had been assessed. One possible explanation for these differences in distribution of carcass composition might be increased activity levels of dominant hamsters compared with subordinate hamsters. Consistent with this notion, dominant mice have increased physical activity within an open field arena (5) and during an agonistic encounter (4). In the present study, although activity levels were not directly measured during the agonistic encounter, we did not observe any differences in activity during the agonistic encounter; however, activity might have been increased after the encounter. The underlying mechanism for this differential effect on carcass composition depending on territorial status is unknown.

Although subordinate animals in experiment 1 significantly increased their food intake compared with nonstressed controls, this stress-induced increase in food intake was not consistent across experiments. For example, in experiment 2, although animals exposed to footshock or defeat had a significant increase in body mass relative to nonstressed controls, the increase in food intake was not significant. This finding sug-

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<th>Table 2. Serum hormone concentration and testes mass: experiment 2</th>
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<td>Controls</td>
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<td>Leptin, ng/ml</td>
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Values are means ± SE. Nonshared symbols (*, †, ‡) indicate statistical significance (P < 0.05).
gests that increased food intake is not necessary to achieve the stress-induced increases in body mass. Although we did not directly measure energy expenditure, feed efficiency reflects the relation between energy intake and energy stored and, therefore, can reflect changes in energy expenditure. The significantly higher feed efficiencies in animals exposed to social defeat or footshock stress suggest a decrease in energy expenditure, especially given the increases in lipid deposition, the energy cost of which is twice that of similar increases in lean mass, in this group. Thus the increased body and lipid masses observed in animals that were exposed to social defeat or footshock might be due to a decrease in energy expenditure, although the mechanism for this apparent stress-induced decrease in metabolic activity is unknown.

The relation between serum T concentrations and aggression is varied in the literature. For example, many studies report a positive correlation between T concentrations and aggression in a number of species (15, 19, 35). The data from the present study, along with others (13, 27, 39), do not support this association. The conflicting findings on whether aggression is dependent on increased circulating T concentrations might be due to species differences as well as the duration, intensity, and type of the agonistic encounters.

Leptin is primarily synthesized by WAT approximately in proportion to the amount of body fat (for review see Ref. 32), although there are increasing numbers of exceptions (41). Consistent with the notion that leptin serves as an adiposity signal, in both experiments, circulating serum leptin concentrations were significantly elevated in subordinate hamsters, in which total carcass lipid was significantly greater and mass of several WAT pads was increased compared with nonstressed control or dominant hamsters. In experiment 2, insulin [which is also hypothesized to be an adiposity signal (for review see Ref. 32)] was significantly increased only in the subordinate hamsters, a finding that is consistent with their greater adiposity. That is, total carcass lipid only was significantly increased in subordinates compared with nonstressed controls and was reflected as significantly increased MWAT and EWAT. On the other hand, the total carcass lipid of the shocked hamsters was not significantly different from that of the nonstressed controls, and only MWAT mass was increased. It may be that less adiposity of the shocked than the subordinate hamsters did not result in as robust a synthesis/secretion of insulin relative to their body fat.

In the present study, we did not measure glucocorticoids (i.e., cortisol or corticosterone), because the terminal measures their body fat.

Because chronic stress has been associated with an accumulation of excess body fat, particularly in the abdominal region in humans (49), and because excess abdominal tissue is highly correlated with the deleterious comorbidities associated with obesity (34), the use of these stressors with Syrian hamsters may be a useful model for the human condition that is not offered by other rodent models.

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