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Sex Differences in Motivational Responses to Dietary Fat in Syrian Hamsters

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Abstract

Women are more likely than men to exhibit motivational disorders (e.g., anhedonia and anxiety) with limited treatment options, and to overconsume high-fat “comfort foods” to improve motivational disruptions. Unfortunately, neurobiological underpinnings for sex differences in motivational disruptions and their responses to dietary fat are poorly understood. To help bridge these fundamental knowledge gaps, we assessed behavioral and neurobiological responses to dietary fat in a hamster model of female-biased motivational lability. Relative to social housing, social separation reduced hedonic drive in a new behavioral assay, the reward investigational preference (RIP) test. Fluoxetine or desipramine treatment for 21, but not 7, days improved RIP test performance. Pharmacologic specificity in this test was shown by non-responsiveness to diazepam, trazolone, propranolol, or naltrexone. In the anxiety-related feeding/exploration conflict (AFEC) test, social separation worsened latency to eat highly palatable food under anxiogenic conditions, but not in home cages. Social separation also reduced weight gain, food intake, and adiposity while elevating energy expenditure, assessed by caloric efficiency and indirect calorimetry. Furthermore, chronic high-fat feeding improved anhedonic and anxious responses to separation, particularly in females. In the motivation-influencing nucleus accumbens, females, but not males, exhibited a separation-induced anxiety-related decrease in *Creb1* mRNA levels and an anhedonia-related decrease in *Fosb* mRNA levels. Consistent with its antidepressant- and anxiolytic-like effects on behavior, high-fat feeding elevated accumbal *Creb1* and *Fosb* mRNA levels in females only. Another accumbal reward marker, *Tlr4* mRNA, was elevated in females by high-fat feeding. These results show that social separation of hamsters

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provides a novel model of sex-dependent comorbid anhedonia, anxiety, and anorexia, and implicate accumbal CREB, FosB, and TLR4. Moreover, the results validate a new assay for chronic anti-depressant efficacy.

Keywords

anhedonia; anorexia; antidepressant; anxiety; anxiolytic

INTRODUCTION

Beneficial effects of high-fat foods on motivational disruptions in humans presumably contribute to their designation as “comfort foods”, but the extent to which sex plays a role has not been reported extensively. Interestingly, sex differences in the motivation and preference to eat high-fat foods are thought to contribute to the prevalence of obesity in women more than it does in men [1, 2]. In studies that controlled for genetic background with monozygotic twins, obesity was associated with restrictive eating, frequent snacking, and attempted restriction of high-fat foods [3, 4]. Furthermore, obese women have self-reported more vulnerability to eating “comfort foods” than normal-weight women [4]. Paradoxically, women have been found to exhibit more dietary restraint, emotional eating, and binge eating than men [5]. Investigation in this area is important because female biases are established for eating disorders and overconsumption of high-fat foods [6, 7], as well as motivational disorders [8, 9], but their biological underpinnings are not. Anhedonia and anxiety are thought to represent neural dysfunctions in positive and negative motivation, respectively [10, 11], and they are frequently comorbid with eating disorders [12, 13].

Previous rodent studies have shown that dietary fat influences depression- and anxiety-related behaviors. In adult Sprague Dawley rats, subjected to maternal separation as pups, high-fat feeding exerted anxiolytic effects, particularly in males [14, 15]. Similar anxiolytic effects of dietary fat have been demonstrated in male Sprague Dawley rats without maternal separation [16]. Additionally, binge eating of high-fat food prevented anxiety after its discontinuation in rats [17]. Interestingly, chronic high-fat feeding increased the immobility of male and female Wistar rats in the forced swim test, which is related to psychomotor inhibition, a core symptom of depression in humans [18]. In male C57BL/6 mice, high-fat feeding attenuated stress-induced anxiety and immobility, but not anhedonia [19]. In male CD-1 mice, however, high-fat feeding exerted antidepressant-like effects, but also anxiogenic effects [20]. Discontinuation of high-fat diet worsened anxious and reward behaviors in mice, and it increased their willingness to endure anxiogenic conditions to regain access to high-fat chow [21]. Species, strain, and sex differences notwithstanding, these findings suggest that dietary fat can improve emotionality under certain experimental conditions.

There is mounting evidence that high-fat consumption exerts rewarding effects through actions in the nucleus accumbens, which is an integral component of the mesolimbic reward system and important for bioenergetic and emotional integration. This limbic structure influences depression-related, anxious, and reward states in rats, mice, and humans [22–24],

as well as reward behaviors and food preferences in hamsters [25, 26]. In rats, a high-fat meal induced greater release of accumbal dopamine, which is a potent reward signal [27]. Studies assessing the correlation to FosB expression or effects of FosB overexpression suggest that this transcription factor increases motivation for high-fat reward, and promotes energy conservation and adiposity [28–30]. Mice overexpressing FosB in the nucleus accumbens also exhibited an enhanced reward response and anxiety following high-fat withdrawal [31]. Furthermore, chronic high-fat feeding in Long Evans rats reduced the accumbal activation of an anxiogenic signal, phosphorylated CREB [32], and in mice FosB-overexpression prevented a decrease in accumbal CREB phosphorylation [31]. In the latter two studies, however, reward behavior was not assessed.

Hypothalamic expression of the cytokine, TLR4, has been implicated as a mediator in the stimulatory effects of high-fat diet on neuronal inflammation, apoptosis, and insulin resistance [33–35]. We have shown that the elevation of hypothalamic *Tlr4* mRNA levels is associated with separation-induced anxiety and anorexia in hamsters [36]. Additionally, TLR4 activation has been shown to mediate drug reward [37–40], but the neuroanatomic site of action has not been established. Because CREB and FosB play opposing roles in the accumbens versus other brain regions [41–44], it is plausible that accumbal *Tlr4* expression increases with reward. Therefore, we explored that possibility in the present study.

The studies cited above collectively illustrate the need for a clinically relevant model to test the hypothesis that modulation of dietary fat contributes to sex differences in motivational states. We have shown previously that, relative to social housing, social separation induced anorexia and anxiety with female biases in Syrian hamsters [36, 45, 46]. In the present study, we sought to determine whether social separation also induces anhedonia with female bias using a new assay, the reward investigational preference (RIP) test. Importantly, we also sought to determine whether dietary fat reduces anhedonic and anxious behaviors with sex differences.

METHODS AND MATERIALS

Subjects

Syrian golden hamsters (*Mesocricetus auratus*) of the Crl:LVG(SYR) strain (Charles River, Kingston, NY) were purchased for use at Weill Cornell Medical College, or they were bred for up to three generations in the Kleberg Laboratory Animal Research Facility at Texas A&M University. A 14h:10h light-dark schedule (lights on at 0600h) and temperature of 23 ± 3 °C were kept. Low-fat diet, LabDiet 5001 (Purina Mills, Richmond, IN), high-fat diet, D12451 (Research Diets, New Brunswick, NJ), and tap water were provided *ad libitum*. Procedures used in this study were approved by the Institutional Animal Care and Use Committee.

Experimental designs

Hamsters were housed 2–4/cage during same-sex social housing (SH) after weaning. Some were housed 1/cage during social separation (SS) at age 10 weeks (see Fig. 1 for timelines of the studies and time courses for drug treatments). Territorial aggression was minimized by

using littermates for SH, and screening for over-aggressiveness daily from age 6 weeks. It was not necessary to separate any cage mates.

Study 1—Female and male hamsters ($n = 8$) were assessed for anxiety and anhedonia between 1000h-1600h in a dedicated behavior room during SH or SS. Experiment 1.1. Anxiety was assessed in the anxiety-related feeding/exploration conflict test (described below) after 8 weeks of SS. We have previously determined this time point to be near the peak for anxiety [36]. Experiment 1.2. Hamsters were assessed for anhedonia in the reward investigational preference test (described below) immediately after experiment 1.1.

Study 2—The second and third cohorts, containing females ($n = 6$), were assessed after 8 weeks of SS for responses to standard doses of antidepressants during SS in the RIP test. Experiment 2.1. The prototypical selective serotonin reuptake inhibitor, fluoxetine (0 or 10 mg/kg/day, po), was administered in the drinking water, beginning after 5 weeks of separation, and behaviors were assessed after 7 and 21 days. Experiment 2.2. The prototypical tricyclic antidepressant, desipramine (0 or 20 mg/kg/day, po), was administered and behaviors were assessed as above. We have reported previously that desipramine at the same dose reduced anxious behavior in hamsters [46]

Study 3—The fourth and fifth cohorts, containing females ($n = 6$), were assessed after 8 weeks of SH or SS for responses to non-antidepressants in the RIP test during SS. Experiment 3.1. Hamsters were injected with vehicle, beta-adrenergic receptor antagonist, propranolol (2 mg/kg, ip), GABAA receptor modulator, tracazolate (1 mg/kg, ip), or opioid receptor antagonist, naltrexone (4 mg/kg, ip), 30 min before testing. Experiment 3.2. Hamsters were injected with prototypical benzodiazepine diazepam (0 or 1 mg/kg, ip) as above. We have reported previously that propranolol and diazepam, but not naltrexone, used at the same doses reduced anxious behavior in hamsters [46]. Tracazolate is a putative anxiolytic [47].

Study 4—The sixth and seventh cohorts, containing females and males, were assessed for anorexia after 8 weeks of SS. We previously determined this time point to be near the peak for near the peak values for SS-induced anorexia [36]. Experiment 4.1. Hamsters ($n = 8$) and food were weighed at the time of regular cage changes during the light phase to the nearest 0.1 g. Food intake was assessed by weighing chow removed from the hopper, bedding, and cheek pouches as necessary. During SH, food intake per cage was divided by the number of hamsters per cage. Caloric efficiency was calculated as weight gain (g) normalized to caloric intake (kcal). Experiment 4.2. Metabolic rates, respiratory exchange ratios, and skin temperatures were measured in females ($n = 6-10$) and males ($n = 6-8$) by indirect calorimetry (described below).

Study 5—A subset of hamsters from study 4 ($n = 5$) was assessed after 10 weeks of SS for gene expression and tissue weights. Experiment 5.1. Accumbal gene expression was assessed by RT-PCR (see Table 1 for primer sequences and amplification conditions). Experiment 5.2. Tissues were excised *post mortem*, and weighed to the nearest 0.1 g (adipose) or 1 mg (all others). Body and tibia lengths were measured *post mortem* to the nearest 1 cm and 1 mm, respectively. Adrenal and spleen weights provided indices of

chronic corticosteroid activity [36, 48]. Body and tibia lengths provided indices of linear growth. Tibia weight and length provided a crude index of bone density. Anterior tibialis weight provided an index of muscularity. White adipose tissue weights provided indices of adiposity. Brown adipose tissue weights provided an index of the capacity for energy depletion through thermogenesis.

Anxiety-related feeding/exploration conflict (AFEC) test

As we have described [36, 46], individuals were transferred to clean test cages, and graham cracker (Nabisco, East Hanover, NJ) was presented immediately. Intervals to sniff the test food (approach latency) or bite it (feed latency) were timed by a treatment-blind scorer. To control for non-emotional factors, approach and feed latencies were also measured after return to home cages. Food was removed from home cages 90 minutes before testing. For 7 days before testing, subjects were habituated daily with ~30 mg of test food.

Reward investigational preference (RIP) test

To minimize novelty-induced anxiety, individuals were assessed immediately after the AFEC test, which allowed approximately 10 minutes of acclimation to the test cages. Reward (graham cracker) and blank (empty) cassettes were placed in the overhead food hopper for 5 minutes. The cassettes were clear plastic containers of MiniDV camcorder videotape (Maxell, Woodland Park, NJ). Cumulative time spent investigating (i.e., sniffing, scratching, and biting) each cassette was measured using a stopwatch. Increases in reward investigation and reward investigational preference (reward investigation / by total investigation x 100) were used as indices of hedonic drive.

Indirect calorimetry

Individuals were assessed in an Oxymax System (Columbus Instruments, Columbus, OH) as we have described [36, 45]. Sampled air was passed through O₂ and CO₂ sensors, and heat production as the metabolic rate (kcal/h/kg^{2/3}), and lipid catabolism as the respiratory exchange ratio (CO₂/O₂) were determined.

Accumbal microdissection

Frozen brains were excised *post mortem*, frozen in isopentane on dry ice, and stored at -80 °C. They were later blocked, mounted on a freezing microtome stage, and sectioned at 150 µm through the striatum, using the hamster brain atlas [49]. Tissue sections were placed on glass slides onto a freezing plate and the ventral striatum containing the nucleus accumbens was dissected under a surgical microscope using 0.75 mm-diameter metal core punches (Ted Pella, Redding, CA).

RNA extraction

We used a modification of the phenol, isoamyl alcohol, guanidinium isothiocyanate, β-mercaptoethanol (PIG-B) method [50]. Briefly, tissue samples were homogenized in PIG-B by repeatedly passing them through 1-cc syringes fitted with 18-G needles. Monophasic homogenates were separated into 2 layers with one-fifth volume of chloroform, followed by centrifugation. The aqueous phase was extracted twice using one volume of 5:1

phenol:chloroform (pH 4.5), and once with one volume of chloroform by followed by isopropanol precipitation and washing with 75% ethanol. RNA quality was assessed by agarose gel electrophoresis and spectrophotometry.

Reverse transcription PCR

Reverse transcription was performed as described [46, 51], using M-MLV reverse transcriptase (New England Biolabs, Ipswich, MA). PCR reactions were performed using Taq 2X Master Mix (New England Biolabs). Amplifications were performed with primers listed in Table 1. We performed PCR with a hot start at 91°C, denaturation at 94°C for 50s, and cycling at melting temperatures listed in Table 1 for 20s. Hybridization occurred at 60°C for 20s, with extension at 72°C for 30s using cycle numbers listed in Table 1. Amplicons were visualized on 1.4% agarose, 5 mM sodium tetraborate gels, and stained with ethidium bromide (ISC Bioexpress, Kaysville, UT). Band intensities were analyzed using Kodak 1D Image Analysis Software (Eastman Kodak, Rochester, NY). Data from genes of interest are means of triplicate reactions normalized to the median. Beta-actin standards were run in triplicate, and correlated with one another ($r^2 > 0.9$). Target signals were normalized to means of the standards.

Drugs

Fluoxetine-HCl and desipramine-HCl were administered in drinking water as described [46]. Propranolol-HCl, trazolone-HCl, and naltrexone were dissolved in 0.9% saline. Diazepam was dissolved in ethanol and 0.9% saline. Drugs were purchased from Sigma (St. Louis, MO).

Statistical analyses

One-way ANOVA (experiment 3.1), Student's *t*-test (experiment 3.2), and two-way ANOVA followed by Bonferroni's *t*-test (remaining experiments) were used to determine differences between means, which were considered significant at *p* values less than 0.050. ANOVA results are presented in Tables 2–10. Analyses were performed using Prism 5.04 and InStat 3.00 (GraphPad, San Diego, CA).

RESULTS

Sex differences in hedonic drive

We first assessed effects of low- or high-fat feeding on female hamsters ($n = 8$) subjected to social housing (SH) or social separation (SS) using the RIP test. Effects of SS and high-fat diet are described relative to those of SH and low-fat diet, respectively. ANOVA results are presented in Table 2. SS reduced reward cassette investigation by females on low-fat diet, and high-fat diet abolished this effect (Fig. 2A). Blank cassette investigation was unaltered by these treatments (Fig.2B). SS reduced reward investigational preference on low-fat diet, and high-fat diet blocked this effect (Fig. 2C). Reward investigation by males was unaltered by housing or dietary treatments (Fig. 2D). Blank investigation was also unaltered by these treatments (Fig. 2E). Reward investigational preference by males was reduced by combining SS and high-fat diet (Fig. 2F).

Antidepressant effects in the RIP test

Treatment with fluoxetine, a selective serotonin reuptake inhibitor, for 21 days, but not 7 days, increased reward investigation (Fig. 3A). Blank investigation was not influenced by fluoxetine (Fig. 3B). Chronic, but not acute, fluoxetine treatment increased reward investigational preference (Fig. 3C). Treatment with desipramine, a selective norepinephrine reuptake inhibitor, for 21 days, but not 7 days, increased reward investigation (Fig. 3D). Blank investigation was not influenced by desipramine (Fig. 3E). Chronic, but not acute, desipramine treatment increased reward investigational preference (Fig. 3F). ANOVA results are presented in Table 3.

Pharmacologic specificity of the RIP test

One-way ANOVA showed that treatment with beta-adrenergic receptor antagonist, propranolol, GABA_A receptor agonist tracazolol, or opioid receptor antagonist naltrexone did not alter reward investigation (Fig. 4A), blank investigation (Fig. 4B), or reward investigational preference (Fig. 4C). Student's unpaired *t*-test showed that anxiolytic treatment with the benzo-diazepine receptor agonist, diazepam, did not alter reward investigation (Fig. 4D), blank investigation (Fig. 4E), or reward investigational preference (Fig. 4F). ANOVA results are presented in Table 4A. The *t*-test results are presented in Table 4B.

Sex differences in anxious behavior

We also assessed female hamsters represented in Fig. 1 in the anxiety-related feeding/exploration conflict (AFEC) test. Results for SS and high-fat feeding are described relative to those for SH and low-fat feeding, respectively. SS increased latency to feed in the anxiogenic test cage on low-fat diet, and high-fat feeding attenuated this effect (Fig. 5A). Feed latency in the non-anxiogenic home cage was unaltered by housing or diet in females (Fig. 5B). SS increased the feed latency (test/home) ratio on low-fat diet, and high-fat feeding attenuated this effect (Fig. 5C). We also assessed male hamsters represented in Fig. 1 in the AFEC test. SS increased test cage feed latency on low-fat, and high-fat tended to elevate it further (Fig. 5D). Feed latency in the home cage was increased overall by housing and diet in the males (Fig. 5E). SS increased the feed latency ratio for males to comparable levels on either diet (Fig. 5F). ANOVA results are presented in Table 5.

Sex differences in accumbal mRNA levels

We used RT-PCR to assess signals related to reward and anxiety in female and male hamsters (*n* = 5) subjected to SH or SS during low- or high-fat feeding. Accumbal CREB signaling in the nucleus accumbens exerts anxiolytic effects [43, 44], FosB, a truncated splice variant of FosB, is crucial for reward [25, 42, 52], and toll-like receptor-4 (*Tlr4*) is an additional index of reward [36, 40, 53–59]. Results for SS and high-fat feeding are described relative to those for SH and low-fat feeding, respectively. In females, *Creb1* mRNA levels were reduced by SS during low-fat feeding, and elevated by high-fat feeding, particularly during social separation (Fig. 6A). SS reduced *Fosb* mRNA levels in females during low-fat feeding, and elevated them during high-fat feeding (Fig. 6B). SS did not alter *Tlr4* mRNA levels in females during low-fat feeding, and high-fat feeding elevated them,

particularly during social separation (Fig. 6C). In males on low-fat diet, housing did not alter *Creb1* mRNA levels, but high-fat feeding reduced them (Fig. 6D). Housing did not alter *Fosb* mRNA levels in males on low-fat diet, but they were reduced by the combination of SS and high-fat feeding (Fig. 6E). Neither housing nor diet altered *Tlr4* mRNA levels in males (Fig. 6F). ANOVA results are presented in Table 6.

Sex differences in energy balance

We have previously established that the peak of separation-induced anorexia occurs after approximately 8 weeks [36, 46]. Here we assessed effects of 8 weeks of high-fat feeding on female and male hamsters subjected to SH or SS. ANOVA results are presented in Table 7. Results for SS and high-fat feeding are described relative to those for SH and low-fat feeding, respectively. SS induced weight loss in females ($n = 8$) during low- or high-fat feeding (Fig. 7A). SS reduced energy intake in females on either diet, and high-fat feeding increased energy intake in both housing conditions (Fig. 7B). SS induced negative caloric efficiency in females on either diet (Fig. 7C). SS reduced weight gain, without net weight loss, in males ($n = 8$) during low- and high-fat feeding (Fig. 7D). SS did not alter energy intake in males, and the levels were higher overall during high-fat feeding (Fig. 7E). SS decreased caloric efficiency without inducing a negative net caloric efficiency (Fig. 7F).

Additional cohorts of females ($n = 6-10$) and males ($n = 6-8$) were assessed for metabolism. ANOVA results are presented in Table 8. The female metabolic rate was elevated by SS, and unaltered by dietary fat (Fig. 7G). Consistent with elevated lipid catabolism, SS and high-fat feeding lowered the respiratory exchange ratio (RER) in females (Fig. 7H). The combination of SS and high-fat feeding elevated skin temperature in females (Fig. 7I). The male metabolic rate was unaltered by SS or high-fat feeding (Fig. 7J). High-fat feeding, but not SS, lowered the RER in males (Fig. 7K). Overall, high-fat feeding, but not SS, elevated skin temperature in males (Fig. 7L).

Sex differences in growth

We assessed females and males ($n = 5$) for indices of growth after 20 weeks of exposure to SH or SS. SS did not alter female adrenal weights, but they were larger after high-fat feeding (Fig. 8A). Female spleen weight was suppressed by SS, but unaltered by high-fat feeding (Fig. 8B). Female body length was suppressed by SS, but unaltered by high-fat feeding (Fig. 8C). Female tibia length was unaltered by housing or diet (Fig. 8D). Female tibia weight was suppressed by SS, but unaltered by high-fat feeding (Fig. 8E). Anterior tibialis muscle weight in females was suppressed by SS (Fig. 8F). Male adrenal weight was lowered by SS, but unaltered by diet (Fig. 8G). Male spleen weight was unaltered by housing or diet (Fig. 8H). Male body length was reduced by SS combined with high-fat feeding (Fig. 8I). Male tibia length was unaltered by housing or diet (Fig. 8J). Male tibia weight was unaltered by SS, but suppressed by high-fat feeding (Fig. 8K). Male anterior tibialis weight was unaltered by housing, but elevated by diet (Fig. 8L). ANOVA results are presented in Table 9.

Sex differences in adiposity

In the hamsters represented in Fig. 8, we also assessed fat depot weights. SS reduced the white adipose tissue (WAT) weight of females in the inguinal depot (Fig. 9A), perigonadal depot (Fig. 9B), and omental depot (Fig. 9C), but not that of interscapular brown adipose tissue (iBAT) (Fig. 9D). SS of males reduced the weights of iWAT (Fig. 9E), pgWAT depot (Fig. 9F), oWAT depot (Fig. 9G), and iBAT (Fig. 9H). ANOVA results are presented in Table 10.

DISCUSSION

Although there are strong female biases in the prevalence rates and comorbidities of motivational and eating disorders [6, 7, 60], the underlying mechanisms are poorly understood. An important contributor to this knowledge gap is the scarcity of relevant animal models. The four principal findings of the present study help to bridge this gap. First, social separation of hamsters suppressed investigational responses to food reward with female bias. Second, consistent with our previous findings [36, 46, 61], social separation induced anxiety and anorexia with female biases. Third, high-fat feeding reduced the reward deficit and anxiety in females, and tended to worsen them in males. Fourth, molecular changes in the nucleus accumbens are implicated in underpinnings for these social and dietary influences on the motivational state.

Our finding of a separation-induced reward deficit in female hamsters during low-fat feeding, but not in their male counterparts, suggests clinical relevance. Interestingly, high-fat feeding abolished two behavioral indices of the reward deficit in females, but worsened one index in socially separated males. Chronic, but not acute, antidepressant treatment improved reward investigation. Additionally, the antidepressant-induced improvement of investigational preference, partly due to the stability of blank investigation, demonstrated behavioral specificity in the RIP test. Furthermore, the non-responsiveness to non-antidepressants demonstrated pharmacologic specificity. Importantly, anorexigenic effects of fluoxetine are conserved in vertebrates [62–65], and this antidepressant can exert conditioned taste aversion [66]. Additionally, we have reported that desipramine treatment of hamsters appeared to exert a transient hypophagic effect that was not statistically significant [46]. For these reasons, and because consummatory drive in hamsters does not increase with exposure to various orexigenic stimuli [67–69], it is unlikely that high-fat feeding elevated it. It is noteworthy that hamsters express appetitive responses to food by cheek-pouching it rather than exhibiting hyperphagia [70–72]. Thus, prevention of feeding and pouching by presenting test food in cassettes eliminated the display of consummatory and appetitive behaviors in the RIP test.

The reward deficit of female hamsters, under conditions that we have reported to reveal emotional lability [36], suggests face validity for the RIP test as an assay of hedonic drive. The responses to prototypical SSRI and tricyclic antidepressants suggest predictive validity for this assay. In addition, the requirement for chronic antidepressant treatment for therapeutic efficacy provides etiological validity. Moreover, given the roles of FosB and TLR4 in reward [40, 42], our results show potential construct validity. To improve the ability to study neurobiological underpinnings for anhedonia, recent efforts have resolved

hedonic drive into consummatory, appetitive, decisional, and motivational components [73–76]. Our findings suggest that it is unlikely that social separation, high-fat feeding, or antidepressant treatment exerted major effects on consummatory anhedonia (i.e., liking an activity), appetitive anhedonia (i.e., reward anticipation), or decisional anhedonia (i.e., use of reward information for decision making). Accordingly, we deduce that the RIP test assesses motivational anhedonia (i.e., wanting to engage in pleasurable activity).

In addition to female biases in their prevalence rates [8, 77], anxiety and anorexia are frequently comorbid with depression [78, 79], and losses of appetite and body weight are neurovegetative symptoms in up to forty percent of depressed persons [80, 81]. Our results show that social separation increased indices of anxiety in the AFEC test, which we have previously validated [36, 46]. During low-fat feeding, socially separated females exhibited high levels of anxiety, but socially separated males were most anxious during high-fat feeding. As we have shown previously, separation-induced anorexia consisted of hypophagia, weight loss, and hypermetabolism [36, 45]. We also report here that lower skin temperatures in males potentially contributed to greater transdermal heat loss [82, 83].

Accumbal CREB overexpression has been shown to exert anxiolytic effects in rats [44, 84]. Thus, the reduction of *Creb1* mRNA levels in female hamsters in the present study is consistent with our finding of separation-induced anxious behavior. The non-responsiveness of accumbal *Creb1* mRNA levels to the social separation of males during low-fat feeding is perhaps consistent with their mild increase in anxious behavior. Nonetheless, the female increase and male decrease in this signal by high-fat feeding are consistent with the clear sex difference in the effects of dietary fat on anxious behavior. Given the role of FosB in responses to natural reward, including in hamsters [28, 85], the reduction of accumbal *FosB* mRNA levels in females, but not males, is in agreement with the opposite effects of social separation and dietary fat on reward behavior in females, as well as their sex differences. We have previously implicated hypothalamic TLR4 in separation-induced anxiety and anorexia [36]. Given its potential role in reward in the nucleus accumbens [37–40], an increase in this signal further supports the idea of rewarding effects of high-fat feeding in female hamsters, but not in their male counterparts.

High-fat feeding did not impact physiological indices of growth. Social separation suppressed linear growth, assessed by body length and tibia length, particularly in females. Tibia weight, a putative index of bone density, was reduced by social separation of females, and by the combination of social separation and high-fat feeding in males. Likewise, social separation alone suppressed muscularity and adiposity with female biases. Consistent with greater bioenergetic depletion [86, 87], social separation reduced the sizes of brown adipose depots in males, but not in females. Social separation decreased adrenal weights in females only, and we have reported that this effect was more robust in females than in males [36].

To improve the study of mechanisms underlying antidepressant effects, there is a need for expedient tests that respond to chronic, but not acute, antidepressant treatment in animal models [88]. The commonly used tail suspension and forced swim tests assess behavioral changes after less than 60 minutes post-injection of antidepressants, [89]. Although the sucrose preference test requires chronic antidepressant treatment for improved performance,

it also requires subjection to chronic stress models [90–92], which help to make this test labor- and time-intensive, and difficult to establish [93]. Moreover, in the progressive ratio operant task, chronic stress models do not readily alter the break point, which is an index of reward motivation [94]. Due to these issues, the RIP test compares favorably with these assays for depression-related behavior.

In conclusion, social separation of hamsters worsens anorexia, as well as behavioral and molecular indices of motivational pathology with female biases. Accumbal CREB, FosB, and TLR4 potentially contribute to sex differences in the social and dietary control of anhedonia and anxiety. In addition, high-fat feeding attenuates behavioral and molecular indices of motivational disruption in females, but worsens some of them in males. In contrast, high-fat feeding does not cause major alterations in bioenergetic changes that are induced by social separation. To our knowledge, female-biased benefits of dietary fat on motivated behaviors have not been reported extensively. Overall, our findings suggest that socially separated hamsters are useful as a model of sex differences in comorbid motivational and eating disorders, as well as their modulation by dietary fat. They further suggest the utility of the RIP test for assessing anhedonic behavior.

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HIGHLIGHTS

- Social separation induces female-biased motivational disruption and anorexia
- High-fat feeding reduces motivational disruption in females only
- Novel assay, reward investigational preference (RIP) test, for anhedonia
- Improved performance in the RIP test requires chronic antidepressant treatment
- RIP test distinguishes depressive, anxious, appetitive, and consummatory, behaviors

Figure 1. Timelines for Studies

Timelines are shown for the social manipulations and administration of drugs, as well as assessments of motivated behaviors, anorexia, and accumbal expression of target genes.

Figure 2. Sex differences in housing and dietary control of reward behavior

Hamsters ($n = 8$) were subjected to social housing (SH) or social separation (SS) for 8 weeks, and assessed in the reward investigational preference (RIP) test. Subjects investigated reward (graham cracker) and blank (empty) cassettes for 5 minutes after acclimation to test chambers for 5 minutes. Cumulative times spent investigating reward and blank cassettes were measured, and reward investigational preference (reward investigation / total investigation $\times 100$) was calculated. Effects of SS and HF are described, relative SH and LF, respectively. **(A–C) Female treatments.** (A) SS reduced reward investigation during LF feeding, and HF feeding blocked this effect. (B) Neither housing nor diet altered blank investigation. (C) SS reduced investigational preference for the reward cassette during LF feeding, and HF feeding blocked this effect. **(D–F) Male treatments.** (D) Neither housing nor diet altered reward investigation. (E) Neither housing nor diet altered blank investigation. (F) SS and HF feeding combined reduced reward investigational preference. Means $\pm$ SEM were compared by two-way ANOVA: SH vs SS, $*p < 0.050$, $**p < 0.010$; LF vs HF, $\dagger p < 0.050$ and $\dagger\dagger p < 0.010$.

Figure 3. Antidepressant improvement of reward behavior

Female hamsters ($n = 6$) subjected to social separation for 5 weeks, were administered fluoxetine (0 or 10 mg/kg/day, po) or desipramine (0 or 20 mg/kg/day, po) in the drinking water for the terminal 21 days, and assessed in the RIP test on days 7 and 21. Antidepressant effects are described, relative to vehicle treatment. (A–C) **Fluoxetine (Flu) treatment.** (A) Flu increased reward investigation after 21, but not 7, days. (B) Flu did not alter investigation of the blank cassette. (C) Flu increased reward investigational preference. (D–F) **Desipramine (DMI) treatment.** DMI increased reward investigation after 21, but not 7, days. (E) DMI did not alter blank investigation. (F) DMI increased reward investigational preference. Means $\pm$ SEM were compared by two-way ANOVA: drug vs vehicle, $**p < 0.010$.

Figure 4. Pharmacologic specificity of reward behavior

Female hamsters ($n = 6$) were assessed 30 minutes after intraperitoneal drug injections. Drug effects are described, relative to those of vehicle. (A–C) Subjects were injected with vehicle (Veh, 0.9% saline), 2 mg/kg of pro-pranolol (Pro), 1 mg/kg tracazolate (Trac), or 4 mg/kg naltrexone (Nal). Neither propranolol, tracazolate, nor naltrexone altered: (A) reward investigation; (B) blank investigation; or (C) reward investigational preference. (D–E) Subjects were injected with vehicle ethanol and then 0.9% saline (Veh) or 1 mg/kg of diazepam (Dia). Diazepam did not alter: (D) reward investigation; (E) blank investigation; or (F) reward investigational preference. Means $\pm$ SEM were compared by one-way ANOVA (Pro, Trac, and Nal) or Student's t -test (Dia).

Figure 5. Sex differences in housing and dietary control of anxiety

Hamsters ($n = 8$) represented in Fig. 1 were assessed in the anxiety-related feeding/exploration conflict (AFEC) test. Effects of SS and HF are described, relative SH and LF, respectively. **(A–C) Female treatments.** (A) SS increased latency to feed in the test cage during LF feeding, and HF feeding attenuated this effect. (B) Home cage feed latencies were not altered by housing or diet. (C) SS increased the feed latency (test/home) ratio during LF feeding, and HF feeding attenuated this effect. **(D–F) Male treatments.** (D) SS increased test cage feed latency during LF and HF feeding. (E) Home cage feed latencies were not altered by housing or diet. (F) SS increased the feed latency (test/home) ratio during LF and HF feeding. Means $\pm$ SEM were compared by two-way ANOVA: SH vs SS, $*p < 0.050$, $**p < 0.010$; LF vs HF, $\dagger p < 0.050$ and $\dagger\dagger p < 0.010$.

Figure 6. Sex differences in housing and dietary control of motivation-related gene expression

Hamsters were subjected to social housing (SH) or social separation (SS) for 8 weeks.

Accumbal mRNA levels of target genes were assessed by RT-PCR and normalized to those of beta-actin (*Actb*). Effects of SS and HF are described, relative SH and LF, respectively.

(A–C) **Female treatments** ($n = 5$). (A) *Creb1* mRNA levels were reduced by SS during LF feeding, and elevated by HF feeding, particularly during SS. (B) SS did not alter *FosB* mRNA levels during LF feeding, but HF feeding elevated them, particularly during SS. (C) SS did not alter *Tlr4* mRNA levels during LF feeding, but HF feeding elevated them, particularly during SS. (D–F) **Male treatments** ($n = 5$). (D) SS did not alter *Creb1* mRNA levels, but HF feeding reduced them. (E) SS did not alter *FosB* mRNA levels during LF feeding, and HF feeding reduced them, particularly during HF feeding. (F) SS did not alter *Tlr4* mRNA levels, but HF feeding elevated them. Means $\pm$ SEM were compared by two-way ANOVA: SH vs SS, $*p < 0.050$, $**p < 0.010$; LF vs HF, $\dagger p < 0.050$ and $\dagger\dagger p < 0.010$.

Figure 7. Sex differences in housing and dietary control of bioenergetics

(A–F) Hamsters ($n = 8$) represented in Fig. 1 were subjected to social housing (SH) or social separation (SS) and low-fat (LF) or high-fat (HF) feeding, and assessed for biometrics after 10 weeks. Effects of SS and HF are described, relative SH and LF, respectively. (A–C) **Female biometrics.** (A) SS induced weight loss during LF and HF feeding. (B) Energy intake was reduced by SS during LF and HF feeding, and it was higher during HF feeding. (C) SS reduced feed efficiency during LF and HF feeding. (D–F) **Male biometrics.** (D) SS reduced weight gain during LF and HF feeding. (E) Energy intake was higher during HF feeding. (F) SS reduced feed efficiency during LF and HF feeding. (G–L) Hamsters were subjected to SH or SS and LF or HF feeding, and assessed for metabolic activity after 8 weeks. (G–I) **Female metabolic activity** ($n = 6$ –10). (G) SS increased the metabolic rate (MR) assessed as heat production/surface area. (H) SS decreased the respiratory exchange ratio (RER), carbon dioxide production/oxygen consumption (V_{CO_2}/V_{O_2}). (I) Neither SS nor HF feeding altered abdominal skin temperature. (J–L) **Male metabolic activity** ($n = 6$ –8). (J) SS did not alter the MR. (K) HF feeding decreased the RER. (L) HF feeding increased abdominal skin temperature. Means $\pm$ SEM were compared by two-way ANOVA: SH vs SS, * $p < 0.050$, ** $p < 0.010$; LF vs HF, † $p < 0.050$ and †† $p < 0.010$.

Figure 8. Sex differences in housing and dietary control of growth indices

Hamsters represented in Fig. 1 were subjected to SH or SS and LF or HF feeding, and subset ($n = 5$) were assessed *post mortem* for tissue weights after 12 weeks. **(A–F) Effects on females.** (A) Adrenal weights were greater during HF feeding. (B) Spleen weights were reduced by SS. (C) Body length was reduced by SS. (D) Tibia length was reduced by SS. (E) Tibia weight was reduced by SS. (F) Anterior tibialis weight was reduced by SS. **(G–L) Effects on males.** (G) Adrenal weights were reduced by SS. (H) Spleen weights were lower during HF feeding. (I) Body length was reduced by SS. (J) Tibia length was unaltered by SS. (K) Tibia weight was reduced by SS. (L) Anterior tibialis weight was greater during HF feeding. Means $\pm$ SEM were compared by two-way ANOVA: SH vs SS, * $p < 0.050$, ** $p < 0.010$; LF vs HF, † $p < 0.050$ and †† $p < 0.010$.

Figure 9. Sex differences in housing and dietary control of adiposity

Hamsters represented in Fig. 1 were subjected to SH or SS and LF or HF feeding, and subset ($n = 5$) were assessed *post mortem* for white or brown adipose tissue (WAT or BAT) fat pad weights after 12 weeks. **(A–D) Effects on females.** (A) SS reduced inguinal fat pad (iWAT) weight. (B) SS reduced perigonadal fat pad (pgWAT) weight. (C) SS reduced omental fat pad (oWAT) weight. (D) Neither housing nor diet altered interscapular brown fat pad (iBAT) weight. **(E–H) Effects on males.** (E) SS reduced iWAT weight. (F) SS reduced pgWAT weight. (G) SS reduced oWAT weight. (H) SS reduced iBAT weight. Means $\pm$ SEM were compared by two-way ANOVA: SH vs SS, $*p < 0.050$, $**p < 0.010$; and $\dagger\dagger p < 0.010$.

Table 1

PCR Primers and Conditions

Gene:	Primer Sequence	Conditions
<i>Actb</i>	Fwd: GGTATGGAATCCTGTGGCATCCATGA Rev: ACTCCTGCTTGCTGATCCACATCT	24 cycles, T _M = 93°C
<i>Creb1</i>	Fwd: ACAGATTGCCACATTAGNCCAGGTA Rev: TCCACAGACTCCTGTGAATCTTCACT	33 cycles, T _M = 92°C
<i>Fosb</i>	Fwd: GGAGGGTTCGCAGAGAGAGAAACAA Rev: CCGAGGACTTGAACCTCACTCGG	35 cycles, T _M = 94°C
<i>Tlr4</i>	Fwd: CTCCCTGAGACCTGAAAGCTTGGAT Rev: GGTGTAGACCCTGATATGCCTTGTCTT	35 cycles, T _M = 91°C

Table 2

RM ANOVA for the RIP Test

Females	F-Statistic	p-Value
Fig. 2A. Reward Investigation		
Housing	$F_{1,28} = 3.16$	0.086
Diet	$F_{1,28} = 4.22$	0.049
Housing x Diet	$F_{1,28} = 2.80$	0.105
Fig. 2B. Blank Investigation		
Housing	$F_{1,28} < 0.01$	0.971
Diet	$F_{1,28} = 0.02$	0.886
Housing x Diet	$F_{1,28} = 2.81$	0.105
Fig. 2C. Reward Investigational Preference		
Housing	$F_{1,28} = 7.83$	0.009
Diet	$F_{1,28} = 14.69$	<0.001
Housing x Diet	$F_{1,28} = 1.79$	0.192

Males	F-Statistic	p-Value
Fig. 2D. Reward Investigation		
Housing	$F_{1,28} = 2.35$	0.137
Diet	$F_{1,28} = 0.50$	0.484
Housing x Diet	$F_{1,28} = 0.02$	0.900
Fig. 2E. Blank Investigation		
Housing	$F_{1,28} = 2.87$	0.103
Diet	$F_{1,28} = 0.85$	0.365
Housing x Diet	$F_{1,28} = 0.41$	0.528
Fig. 2F. Reward Investigational Preference		
Housing	$F_{1,28} = 6.50$	0.017
Diet	$F_{1,28} = 2.52$	0.124
Housing x Diet	$F_{1,28} = 0.89$	0.355

Table 3

RM ANOVA for Antidepressant Effects in the RIP Test

	F-Statistic	p-Value
Fig. 3A. Reward Investigation		
Fluoxetine	$F_{1,20} = 11.84$	0.003
Time	$F_{1,20} = 1.76$	0.199
Fluoxetine x Time	$F_{1,20} = 1.58$	0.224
Fig. 3B. Blank Investigation		
Fluoxetine	$F_{1,20} = 0.04$	0.844
Time	$F_{1,20} = 0.28$	0.605
Fluoxetine x Time	$F_{1,20} = 1.14$	0.298
Fig. 3C. Reward Investigational Preference		
Fluoxetine	$F_{1,20} = 9.31$	0.006
Time	$F_{1,20} = 2.33$	0.143
Fluoxetine x Time	$F_{1,20} = 0.01$	0.914
Fig. 3D. Reward Investigation		
Desipramine	$F_{1,20} = 10.10$	0.005
Time	$F_{1,20} = 1.71$	0.205
Desipramine x Time	$F_{1,20} = 2.43$	0.134
Fig. 3E. Blank Investigation		
Desipramine	$F_{1,20} = 0.20$	0.557
Time	$F_{1,20} = 0.29$	0.598
Desipramine x Time	$F_{1,20} = 0.70$	0.412
Fig. 3F. Reward Investigational Preference		
Desipramine	$F_{1,20} = 7.91$	0.011
Time	$F_{1,20} = 6.08$	0.023
Desipramine x Time	$F_{1,20} = 1.36$	0.256

Table 4

Table 4A . Propranolol, Tracazolate, and Naltrexone Effects in the RIP Test		
1-Way ANOVA	F-Statistic	p-Value
Fig. 4A. Reward Investigation	$F_{3,23} = 0.147$	0.931
Fig. 4B. Blank Investigation	$F_{3,23} = 0.244$	0.865
Fig. 4C. Reward Investigational Preference	$F_{3,23} = 0.110$	0.954

Table 4B. Diazepam Effects in the RIP Test		
t-Test	t-Statistic	p-Value
Fig. 4D. Reward Investigation	$t_{10} = 0.229$	0.823
Fig. 4E. Blank Investigation	$t_{10} = 0.897$	0.391
Fig. 4F. Reward Investigational Preference	$t_{10} = 0.866$	0.407

Table 5

RM ANOVA for the AFEC Test

Females	F-Statistic	p-Value
Fig. 5A. Feed Latency Test Cage		
Housing	$F_{1,28} = 46.55$	< 0.001
Diet	$F_{1,28} = 22.66$	< 0.001
Housing x Diet	$F_{1,28} = 17.31$	< 0.001
Fig. 5B. Feed Latency Home Cage		
Housing	$F_{1,28} = 1.90$	0.179
Diet	$F_{1,28} = 1.82$	0.188
Housing x Diet	$F_{1,28} = 0.03$	0.855
Fig. 5C. Feed Latency Ratio (Home Cage / Test Cage)		
Housing	$F_{1,28} = 25.39$	< 0.001
Diet	$F_{1,28} = 13.86$	< 0.001
Housing x Diet	$F_{1,28} = 10.89$	0.003

Males	F-Statistic	p-Value
Fig. 5D. Feed Latency Test Cage		
Housing	$F_{1,28} = 34.21$	< 0.001
Diet	$F_{1,28} = 4.11$	0.052
Housing x Diet	$F_{1,28} = 1.22$	0.279
Fig. 5E. Feed Latency Home Cage		
Housing	$F_{1,28} = 5.63$	0.025
Diet	$F_{1,28} = 7.27$	0.012
Housing x Diet	$F_{1,28} = 0.04$	0.853
Housing	$F_{1,28} = 12.14$	0.002
Diet	$F_{1,28} = 0.48$	0.495
Housing x Diet	$F_{1,28} = 0.01$	0.909

Table 6

RM ANOVA for Accumbal mRNA Levels

Females	F-Statistic	p-Value
Fig. 6A. Creb1		
Housing	$F_{1,28} = 22.41$	< 0.001
Diet	$F_{1,28} = 192.27$	< 0.001
Housing x Diet	$F_{1,28} = 40.17$	< 0.001
Fig. 6B. FosB		
Housing	$F_{1,28} = 0.11$	0.742
Diet	$F_{1,28} = 26.11$	< 0.001
Housing x Diet	$F_{1,28} = 18.98$	< 0.001
Fig. 6C. Tlr4		
Housing	$F_{1,28} = 13.14$	0.002
Diet	$F_{1,28} = 156.09$	< 0.001
Housing x Diet	$F_{1,28} = 16.85$	< 0.001
Males	F-Statistic	p-Value
Fig. 6D. Creb1		
Housing	$F_{1,28} = 0.02$	0.898
Diet	$F_{1,28} = 6.17$	0.024
Housing x Diet	$F_{1,28} = 0.27$	0.608
Fig. 6E. FosB		
Housing	$F_{1,28} = 5.88$	0.028
Diet	$F_{1,28} = 22.72$	< 0.001
Housing x Diet	$F_{1,28} = 2.52$	0.132
Fig. 6F. Tlr4		
Housing	$F_{1,28} = 2.18$	0.160
Diet	$F_{1,28} = 3.22$	0.092
Housing x Diet	$F_{1,28} = 0.99$	0.334

Table 7

RM ANOVA for Energy Balance

Females	F-Statistic	p-Value
Fig. 7A. Weight Gain		
Housing	$F_{1,28} = 133.96$	< 0.001
Diet	$F_{1,28} = 0.13$	0.717
Housing x Diet	$F_{1,28} = 1.43$	0.242
Fig. 7B. Energy Intake		
Housing	$F_{1,28} = 53.69$	< 0.001
Diet	$F_{1,28} = 24.51$	< 0.001
Housing x Diet	$F_{1,28} = 0.16$	0.695
Fig. 7C. Caloric Efficiency		
Housing	$F_{1,28} = 128.49$	< 0.001
Diet	$F_{1,28} = 0.01$	0.939
Housing x Diet	$F_{1,28} = 4.73$	0.038

Males	F-Statistic	p-Value
Fig. 7D. Weight Gain		
Housing	$F_{1,28} = 23.16$	< 0.001
Diet	$F_{1,28} = 5.08$	0.032
Housing x Diet	$F_{1,28} = 0.14$	0.711
Fig. 7E. Energy Intake		
Housing	$F_{1,28} = 4.17$	0.051
Diet	$F_{1,28} = 6.22$	0.019
Housing x Diet	$F_{1,28} = 0.69$	0.413
Fig. 7F. Caloric Efficiency		
Housing	$F_{1,28} = 22.57$	< 0.001
Diet	$F_{1,28} = 3.10$	0.089
Housing x Diet	$F_{1,28} = 0.10$	0.752

Table 8

RM ANOVA for Resting Energy Expenditure

Females	F-Statistic	p-Value
Fig. 7G. Metabolic Rate		
Housing	$F_{1,27} = 13.34$	0.001
Diet	$F_{1,27} = < 0.01$	0.983
Housing x Diet	$F_{1,27} = 0.22$	0.643
Fig. 7H. Respiratory Exchange Ratio		
Housing	$F_{1,27} = 4.72$	0.039
Diet	$F_{1,27} = 40.92$	< 0.001
Housing x Diet	$F_{1,27} = 12.39$	0.002
Fig. 7I. Skin Temperature		
Housing	$F_{1,27} = 0.12$	0.734
Diet	$F_{1,27} = 3.35$	0.078
Housing x Diet	$F_{1,27} = 3.98$	0.056

Males	F-Statistic	p-Value
Fig. 7J. Metabolic Rate		
Housing	$F_{1,25} = 0.23$	0.634
Diet	$F_{1,25} = 0.03$	0.874
Housing x Diet	$F_{1,25} = 0.23$	0.634
Fig. 7K. Respiratory Exchange Ratio		
Housing	$F_{1,25} = 1.98$	0.171
Diet	$F_{1,25} = 35.68$	< 0.001
Housing x Diet	$F_{1,25} = 0.19$	0.669
Fig. 7L. Skin Temperature		
Housing	$F_{1,25} = 0.19$	0.669
Diet	$F_{1,25} = 5.42$	0.028
Housing x Diet	$F_{1,25} = 0.49$	0.491

Table 9

RM ANOVA for Growth

Females	F-Statistic	p-Value
Fig. 8A. Adrenal Weight		
Housing	$F_{1,16} = 1.10$	0.311
Diet	$F_{1,16} = 28.20$	< 0.001
Housing x Diet	$F_{1,16} = < 0.01$	0.961
Fig. 8B. Spleen Weight		
Housing	$F_{1,16} = 67.44$	< 0.001
Diet	$F_{1,16} = 3.70$	0.073
Housing x Diet	$F_{1,16} = 1.00$	0.331
Fig. 8C. Body Length		
Housing	$F_{1,16} = 47.61$	< 0.001
Diet	$F_{1,16} = 1.44$	0.248
Housing x Diet	$F_{1,16} = 0.20$	0.659
Fig. 8D. Tibia Length		
Housing	$F_{1,16} = 7.18$	0.017
Diet	$F_{1,16} = 0.15$	0.707
Housing x Diet	$F_{1,16} = < 0.01$	1.000
Fig. 8E. Tibia Weight		
Housing	$F_{1,16} = 10.96$	0.004
Diet	$F_{1,16} = < 0.01$	0.951
Housing x Diet	$F_{1,16} = 0.81$	0.382
Fig. 8F. Anterior Tibialis Weight		
Housing	$F_{1,16} = 10.29$	0.006
Diet	$F_{1,16} = 2.27$	0.152
Housing x Diet	$F_{1,16} = 0.11$	0.748
Males	F-Statistic	p-Value
Fig. 8G. Adrenal Weight		
Housing	$F_{1,16} = 6.47$	0.022
Diet	$F_{1,16} = 0.02$	0.900
Housing x Diet	$F_{1,16} = 1.04$	0.324
Fig. 8H. Spleen Weight		
Housing	$F_{1,16} = 4.09$	0.060
Diet	$F_{1,16} = 6.18$	0.024
Housing x Diet	$F_{1,16} = 0.01$	0.914
Fig. 8I. Body Length		

Males	F-Statistic	p-Value
Housing	$F_{1,16} = 7.47$	0.015
Diet	$F_{1,16} = 3.18$	0.094
Housing x Diet	$F_{1,16} = 1.41$	0.252
Fig. 8J. Tibia Length		
Housing	$F_{1,16} = 1.52$	0.235
Diet	$F_{1,16} = 0.38$	0.546
Housing x Diet	$F_{1,16} = < 0.01$	1.000
Fig. 8K. Tibia Weight		
Housing	$F_{1,16} = 15.90$	0.001
Diet	$F_{1,16} = 1.41$	0.252
Housing x Diet	$F_{1,16} = 2.02$	0.175
Fig. 8L. Anterior Tibialis Weight		
Housing	$F_{1,16} = 1.97$	0.179
Diet	$F_{1,16} = 10.74$	0.005
Housing x Diet	$F_{1,16} = 1.06$	0.318

Table 10

RM ANOVA for Adiposity

Females	F-Statistic	p-Value
Fig. 9A. Inguinal WAT Weight		
Housing	$F_{1,16} = 120.91$	< 0.001
Diet	$F_{1,16} = 3.87$	0.067
Housing x Diet	$F_{1,16} = 2.13$	0.164
Fig. 9B. Perigonadal WAT Weight		
Housing	$F_{1,16} = 61.72$	< 0.001
Diet	$F_{1,16} = 2.38$	0.142
Housing x Diet	$F_{1,16} = 0.35$	0.561
Fig. 9C. Omental WAT Weight		
Housing	$F_{1,16} = 80.27$	< 0.001
Diet	$F_{1,16} = 0.16$	0.692
Housing x Diet	$F_{1,16} = 0.10$	0.884
Fig. 9D. Interscapular BAT Weight		
Housing	$F_{1,16} = 4.47$	0.051
Diet	$F_{1,16} = 0.05$	0.831
Housing x Diet	$F_{1,16} = 0.02$	0.884

Males	F-Statistic	p-Value
Fig. 9E. Inguinal WAT Weight		
Housing	$F_{1,16} = 6.52$	0.021
Diet	$F_{1,16} = 1.48$	0.241
Housing x Diet	$F_{1,16} = 0.22$	0.644
Fig. 9F. Perigonadal WAT Weight		
Housing	$F_{1,16} = 24.03$	< 0.001
Diet	$F_{1,16} = 0.92$	0.352
Housing x Diet	$F_{1,16} = 0.01$	0.913
Fig. 9G. Omental WAT Weight		
Housing	$F_{1,16} = 26.46$	< 0.001
Diet	$F_{1,16} = 0.42$	0.525
Housing x Diet	$F_{1,16} = 0.56$	0.465
Fig. 9H. Interscapular BAT Weight		
Housing	$F_{1,16} = 8.15$	0.012
Diet	$F_{1,16} = 0.15$	0.700
Housing x Diet	$F_{1,16} = 0.03$	0.861