Social stress and recovery: Implications for body weight and body composition

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ABSTRACT

Social stress resulting from dominant-subordinate relationships is associated with body weight loss and altered body composition in subordinate (SUB) male rats. Here we extend these findings to determine whether stress-induced changes in energy homeostasis persist when the social stress is removed and the animal is allowed to recover. We examined body weight (BW), body composition, and relevant endocrine measures after 1 or 2 cycles of 14 days of social stress, each followed by 21 days of recovery in each rat’s individual home cage. SUB lost significantly more BW during social housing in a visible burrow system (VBS) compared to dominant (DOM) animals. Weight loss during social stress was attributable to a decrease in adipose tissue in DOM and SUB, with an additional loss of lean tissue in SUB. During both 21-day recovery periods, DOM and SUB regained lost BW but only SUB were hyperphagic. Following recovery, SUB had a relatively larger increase in adipose tissue and plasma leptin compared to DOM, indicating that body composition changes were dependent on social status. Control animals that were weight matched to SUB or male rats exposed to the VBS environment without females, and which did not form a social hierarchy, did not exhibit changes in body composition like SUB in the VBS. Therefore, chronic social stress causes social status-dependent changes in body weight, composition and endocrine measures that persist after repeated stress and recovery cycles and that may ultimately lead to metabolic disorders and obesity.

Running head (60 characters): Social stress effects on body weight and body composition
INTRODUCTION

Obesity is a worldwide public health problem and a major contributor to the increased incidence of coronary artery disease, hypertension and type 2 diabetes (13, 37). Psychosocial and socioeconomic challenges or stress in humans activate the hypothalamic-pituitary-adrenal (HPA) axis, and when these occur on a chronic basis they are associated with an increased incidence of visceral obesity and predisposition to develop what has been called the metabolic syndrome (28, 29, 41-43). Although these associations have been documented, it is unclear how stress leads to obesity and symptoms comprising the metabolic syndrome.

Laboratory models have been developed to study the consequences of stress in animals. Psychosocial stress also predisposes non-human primates to visceral obesity, insulin resistance, dyslipidemia, hypertension and coronary artery atherosclerosis (24, 50), and this has been associated with reduced feedback regulation of cortisol secretion, suppression of the hypothalamic-pituitary-gonadal (HPG) axis and reproduction, and depressive behavior (24, 50). The “stress” in the primate experiments is best described as chronic and intermittent. Hence, while the results are intriguing, they do not allow rigorous assessment of whether the metabolic abnormalities arise during the actual period of stress or during interspersed intervals when average stress is reduced.

To address these issues, we have developed a rat model to systematically evaluate the effect of psychosocial stress on susceptibility to metabolic pathology. Specifically, we use an established rodent model of psychosocial stress called the Visible Burrow System (VBS). The VBS is an apparatus that was designed to mimic the natural underground burrow systems that rats normally live in thus providing an ethologially-relevant environment in which to study the consequences of
social stress in the laboratory. In the VBS model, mixed gender colonies of adult rats are housed continuously in a VBS apparatus for up to 2 weeks during which the male rats of the colony spontaneously form dominant-subordinate relationships. The dominance hierarchy is firmly established within a few days and subordinate (SUB) and dominant (DOM) rats can be identified by established behavioral and physiological criteria (4, 5, 56). We and others have documented that SUB display behavioral, physiological, neuroendocrine characteristics consistent with chronic stress (4, 5, 16, 51, 56).

Body weight loss is one of the most pronounced and reliable consequences of subordination stress in the VBS (5, 8, 11, 56). We have previously observed that the body weight decrease in SUB results from loss of both adipose and lean tissue, with preferential retention of visceral adiposity, a characteristic that is not shared by the DOM animals suggesting that this results from subordination stress (54). Once the SUB animals are removed from the stress of the VBS, our preliminary data show that they rapidly regain their lost body weight in an endocrine environment characterized initially by high glucocorticoids and low testosterone levels (44). The long-term consequences of chronic social stress on body weight, body composition, and endocrine parameters have not yet been examined in this model. We hypothesized that this post-stress period of heightened metabolic activity in the context of an altered endocrine environment might predispose SUB to the metabolic profile observed in psychosocially stressed primates including humans, and that the profile would be exacerbated with repeated episodes of stress and recovery.

In the current experiments we also included novel control groups to dissociate the effects of social stress on body weight and body composition from those resulting from weight loss and regain alone and from exposure to the complex VBS environment in the absence of dominance hierarchy
formation. Collectively our data suggest that SUB animals have increased adiposity following intermittent cycles of stress and recovery and that the changes in body composition result from social subordination stress.
MATERIALS AND METHODS.

Experiment 1. Metabolic and endocrine effects of repeated, intermittent social stress.

Subjects. Subjects were Long-Evans rats (Harlan, Indianapolis, IN), 100-120 days of age at the beginning of the experiment. Animals were randomly assigned to 16 rat colonies consisting of 4 males and 2 females each. Animals were weight-matched such that all males in a given colony were within 25 g of body weight prior to the start of the experiment. Controls were 16 additional male rats that were age and weight matched to rats assigned to the colonies. Each control rat was housed with an adult female in a conventional plastic shoebox cage (L x H x W, 46 x 21 x 24 cm) while experimental rats were housed in the VBS.

A digital photo was taken of each animal’s unique pied coloration pattern for identification. Each colony was housed in a visible burrow system (VBS) constructed of opaque black Lexan. The VBS consists of an open, uncovered surface area connected to smaller covered chambers by a series of tunnels (Figure 1). A 15-W light bulb located above the open surface area was maintained on a 12:12-hr light:dark cycle with light onset at 0600 hr and provided illumination to the open surface area only; all tunnels and chambers remained in constant darkness. The ceilings of the tunnels and the chambers were constructed of clear Lexan to enable behavioral monitoring by a digital video camera and infrared light source mounted directly above each VBS apparatus. The activity of animals in each VBS colony was recorded on videotape for 6 hr beginning at lights out (1800 hr), a time when the animals were most active, on Days 0, 1, 2, 4, 6, 8, 10 and 12. Day 0 was defined as the first night of colony housing and formation.

(Insert Figure 1 about here)
The animals were continuously housed in the VBS for 14 days in a temperature and humidity-controlled room. Animals were allowed *ad libitum* access to standard rodent chow (Teklad Sterilizable Mouse/Rat Diet 7012, Harlan Teklad) and water in three areas of the VBS (the small burrow chamber, large burrow chamber, and open surface area) as indicated in Figure 1. Animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (NIH, 1996). All protocols of animal handling and treatment were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

Beginning on Day 1 and on every second day thereafter, all animals were removed from the VBS at 1000 hr and placed in their individual home cages with water but no food available. Body weights were recorded and each animal was surveyed for overall health status. The time period spent outside of the VBS did not exceed two hours and all animals in a given colony were returned to the VBS simultaneously. Control males were also separated from female control rats during the time that colony animals were removed from the VBS. On Day 14 of VBS housing, all animals were removed from the VBS, weighed and returned to their individual home cages for a RECOVERY period. They continued on the same diet throughout the recovery period, and food intake and body weight were recorded daily.

(Insert Figure 2 about here)

A schematic diagram of the experimental design is depicted in Figure 2. Animals were sacrificed on the final day of VBS 1 (COHORT 1, n = 4), RECOVERY 1 (COHORT 2, n = 4 colonies) or RECOVERY 2 (COHORT 3, n = 8 colonies) by decapitation. Trunk blood was
collected into heparinized tubes, cold-centrifuged to collect plasma, and stored at –80 ºC for hormone assays. Each carcass was individually sealed in a plastic bag and frozen at –80 ºC for later body composition analysis. Half of the animals in COHORT 3 (n = 4 colonies) were additionally processed to determine body fat distribution. In this procedure, all of the skin and fat attached to the skin was gently removed from the carcass. The skin and attached subcutaneous fat (i.e., the “pelt”) were then analyzed for fat content separately from the rest of the body, which contained bone, muscle, organs and visceral fat. Fat pads that are included in the pelt include all fat that is attached to the skin and outside the peritoneal cavity (i.e., including dorsosubcutaneous and inguinal fat pads). All fat within the rest of the body (i.e., inside the muscle layer of the body) is non-pelt, and it includes all of the visceral fat and intraorgan (e.g., liver, heart) fat. Since most of this is visceral fat we use that term as a descriptor. This method had been published and accepted (9). The two samples were placed into individual plastic freezer bags and frozen at –80º C until analyzed.

**Determination of dominance.** The dominance hierarchy within each VBS colony was determined at the end of the study by standard criteria as previously described (56). The dominant (DOM) animal in a colony is characterized by the least body weight loss while in the VBS for 14 days and the lowest number of bite wounds. In addition, DOM animals typically have bite wounds localized to the face, head, and neck, whereas subordinate (SUB) rats typically have bite patterns on the back and tail. DOM males spend relatively more time in the uncovered surface area, while SUB males tend to stay in the tunnels and chamber systems.

**Experiment 2. Effects of repeated cycles of food restriction or social housing without hierarchy formation on body composition.** In Experiment 2 we included novel control groups to dissociate the effects of social stress on body weight and body composition from those resulting
from weight loss and regain alone and from exposure to the complex VBS environment in the absence of dominance hierarchy formation.

**Subjects.** Male Long-Evans rats (Harlan, Indianapolis, IN), 100-120 days of age at the beginning of the experiment, were used. **Group 1:** Body weight matched (n = 26 male rats, divided into 6 groups of 6-7 rats each). Animals were divided into 2 groups. One group (ADLIB) was maintained on *ad libitum* access to standard rodent chow and the second group (BW MATCH) was food restricted to match the body weight of the SUB in standard VBS colonies. **Group 2:** All-male VBS colonies (n = 4 colonies). Sixteen male rats were divided into 4 colonies of 4 males each. All rats in a given colony (MALE ONLY VBS) were within 25 g body weight of each other at the start of the experiment. Two control male rats (MALE ONLY CON) were assigned to each colony and was individually housed in a conventional shoebox cage.

**Restraint stress test.** An acute restraint stress test was administered to AD LIB and BW MATCH males on the last day of each food restriction period to assess their ability to respond to a novel stressor. Individual male rats were removed from the VBS at 0900 hr, placed into their individual home cages, and left undisturbed for 1 hr. Each animal was then placed in a Plexiglas restraint tube (length 21.5 cm, inner diameter 6.3 cm) and a small blood sample (~50 µl) was quickly collected into a heparinized 1.5-ml microcentrifuge tube from a small nick at the tail tip for later measurement of basal plasma corticosterone (CORT) concentration. A second blood sample was collected by removing the clot from the original nick after 60 min in the restraint tube to measure the animal’s CORT response to the acute stressor. The animals were then removed from the restraint tubes and replaced in their individual home cages for 60 min with only water available. A final blood sample was then taken, again by removing the clot from the original nick, to determine
plasma CORT concentration after the recovery period. Basal blood samples were taken at 1000 hr when corticosterone secretion is normally at its diurnal trough. Samples were centrifuged at 4 °C to collect plasma and stored at -80 °C until assayed for hormone concentration by radioimmunoassay (RIA).

**General methods.**

**Blood sample collection.** Blood samples were collected from each animal on the last day of each phase of the experiment (i.e. VBS or RESTRICT 1, RECOVERY 1, VBS or RESTRICT 2 and RECOVERY 2). Animals were moved 3 hrs after lights on to a quiet testing room and were left undisturbed for 60 minutes prior to sampling. Animals that were in the VBS were removed from the VBS and placed in their individual home cages prior to being moved. Animals were briefly restrained and two blood samples were quickly collected in heparinized microcentrifuge tubes through a nick at the tip of the tail. The first sample (~50 µL) was taken within the first 2 minutes for baseline plasma corticosterone determination. The second sample (~300 µL) was then collected for plasma testosterone, leptin and insulin measurements. Samples were centrifuged at 4 °C to collect plasma and stored at -80 °C until assayed for hormone concentration by radioimmunoassay (RIA).

**Plasma hormone concentrations.** Total plasma corticosterone was measured by RIA using rabbit antiserum raised against corticosterone (#B3-163, Esoterix, Inc., Austin, TX). Plasma testosterone was measured by a commercially available RIA kit (Diagnostic Products Corporation, Los Angeles, CA). Plasma leptin was determined using a commercial RIA kit (Linco, St. Charles, MO). Plasma insulin was measured by RIA using an antiserum recognizing rodent insulin (12).
Body composition. Frozen individual carcasses and pelts were dried continuously for several days using a high-capacity lyophilizer (Labconco, Kansas City, MO) until individual weights differed by less than 1.0 g from that of the previous day. Total water content of each carcass was determined by the difference in weight before and after lyophilization. Each dried carcass or pelt was then individually wrapped in a protective cotton sac, and placed into a custom-designed Soxlet apparatus (Chemglass, Inc., Vineland, NJ) where it was flushed with at least 2 cycles (8-10 L each) of boiling petroleum ether (Fisher Scientific) every hour for at least 6 hours. We previously determined that this is sufficient to ensure that all lipid is extracted and that additional time in the Soxlet does not have a significant effect. Each carcass or pelt was then removed, dried thoroughly of ether, and reweighed. The difference between the weight before and after lipid extraction was recorded as fat weight. Total initial weight minus water and fat weight is recorded as lean tissue weight.

In experiment 2, body composition was determined by nuclear magnetic resonance (NMR) technology (EchoMRI, Waco, TX) which reports fat, lean and water content of the animal. Live, unanesthetized rats were individually placed into a plastic restrainer and inserted into the NMR machine. Each scan took less than one minute. We and others have found that use of the NMR method to determine body composition has a significant correlation \([r = +0.98, p < 0.01; (9)]\) with adipose content determined by a chemical method (lyophilization and ether extraction) (9, 27, 53, 57).

Statistical analysis. Data were analyzed using Statistica v.6.1 (StatSoft, Tulsa, OK), by one-way analysis of variance (ANOVA), repeated measures ANOVA, or t-tests for independent samples as appropriate. Subsequent comparisons between groups were carried out using Newman-Keuls procedures. Differences were considered statistically significant if \(P < 0.05\). One subordinate rat
from COHORT 2 was sacrificed prior to the end of the study due to unusually severe bite wounds and weight loss. All of the data for this animal have been excluded from the analyses. The dominance hierarchy of the colony from which this animal was removed did not change and remained stable over the remainder of the experiment.
RESULTS

Experiment 1.

Body weight. Body weight is depicted in Figure 3 and is expressed as the percentage of original body weight on Day 0 of VBS 1. SUB lost body weight during the first week of VBS 1 compared to CON and DOM, and this weight loss persisted throughout the second week of VBS 1 ($P < 0.001$). In contrast, DOM initially lost a small amount of weight and then continued to gain comparable weight as CON for the remainder of VBS 1. During RECOVERY 1, SUB immediately started to regain body weight and this continued throughout the 3-week period until Day 14 when body weight was not longer significantly different among the groups.

The same animals were then re-grouped into the same colonies and the same dominance hierarchy formed as discussed above. In VBS 2 SUB lost approximately the same percentage of body weight as they had in VBS 1 and decreased body weight in SUB compared to CON and DOM persisted for the remainder of VBS 2 ($P < 0.001$). During RECOVERY 2, SUB regained lost body weight. SUB body weight remained significantly lower until Day 12 ($P < 0.05$).

Food intake and caloric efficiency. Food intake during recovery periods is shown in Figure 4A. We adjusted food intake to account for the difference in body weight among the groups and therefore express food intake as mg food intake per g body weight. There was an overall effect of social status on food intake during RECOVERY 1 ($P < 0.001$) and RECOVERY 2 ($P < 0.001$). SUB were hyperphagic compared to CON and DOM throughout RECOVERY 1 and the first two
weeks of RECOVERY 2. There was a main effect of status on caloric efficiency (Figure 4B) such that SUB gained more body weight per kcal consumed compared to CON and DOM during both RECOVERY 1 ($P < 0.0001$) and RECOVERY 2 ($P < 0.005$).

Body composition and fat distribution. Body composition was analyzed by two-way ANOVA with social status (CON, DOM, SUB) and time point (RECOVERY 1, RECOVERY 2) as main factors and were analyzed within time point by one-way ANOVA with social status as the main factor. We previously reported that DOM and SUB lose a significant amount of adipose tissue during social stress in the VBS while SUB additionally lose lean tissue and these data are represented by the first group of bars (VBS 1) in Figure 5 (56). As depicted by the second group of bars (RECOVERY 1) in the top panel of Figure 5, SUB that were exposed to one bout of social stress and recovery had a greater percentage of body adiposity compared to CON ($P < 0.05$). SUB that were subjected to 2 cycles of social stress and recovery also had greater percentage of body adiposity compared to CON and DOM following RECOVERY 2 while lean tissue did not differ at the end of either RECOVERY 1 or RECOVERY 2 (Figure 5, bottom panel). Thus, although SUB lost both adipose and lean tissue during social stress in the VBS, they re-gained weight during recovery periods predominantly as adipose tissue.

Adipose tissue distribution between the subcutaneous and visceral areas is depicted in Figure 6. For purposes of this analysis, we have designated all the fat attached to the skin including the dorsosubcutaneous and inguinal fat pads, as the “subcutaneous depot.” The “visceral fat depot”
contained fat located within the carcass (including retroperitoneal, perirenal, mesenteric and epididymal fat pads). After two cycles of social stress and recovery SUB had a significantly higher percentage of fat deposited in the visceral depot compared to CON and DOM ($P < 0.05$).

(Insert Figure 6 about here)

**Plasma hormone concentrations.** Endocrine data were analyzed by two-way repeated measures ANOVA with social status and time as the main factors. Data were further analyzed at each time point by one-way ANOVA.

**Corticosterone.** There was a significant effect of time, but no significant effect of status or time x status interaction. One-way ANOVA at each timepoint revealed that consistent with previous reports, basal corticosterone was higher in SUB than CON or DOM rats after 14 days in a VBS ($P < 0.05$) (Table 1). After 3 weeks of recovery (RECOVERY 1), SUB levels were not different from CON and DOM. Exposure to a second period of social stress during VBS 2 resulted in elevated corticosterone in SUB compared to DOM. There were no differences among the groups after the subsequent three weeks of recovery (RECOVERY 2).

**Testosterone.** Plasma testosterone was significantly decreased in SUB after 14 days in the VBS while levels in CON and DOM did not differ (Table 1). CON had a significant decrease in plasma testosterone at the end of RECOVERY 1 and RECOVERY 2 compared to VBS 1 and VBS 2, respectively ($P < 0.05$). This decrease may be attributed to a change in housing conditions from pair housing with a female during VBS 1 to individual housing during RECOVERY 1. After RECOVERY 1, SUB displayed a reliable increase in plasma testosterone compared to VBS 1 and
both DOM and SUB testosterone levels were significantly higher than those in CON ($P < 0.05$). Following re-grouping in the same VBS colonies, the dominance hierarchy was re-established and plasma testosterone in SUB again decreased below that of CON by the end of VBS 2. After RECOVERY 2, there was no difference among the groups.

**Leptin and insulin.** Consistent with the loss of body fat, plasma leptin was significantly decreased in DOM and SUB after VBS 1 and VBS 2 (Table 1). After RECOVERY 1 and RECOVERY 2, leptin increased in both SUB and DOM, the increase being greater in the SUB males ($P < 0.05$). Plasma insulin after a mild food deprivation (4 hours) during the light cycle was reliably higher in SUB compared to CON and DOM following RECOVERY 2, while plasma glucose did not differ (data not shown).

(Insert Table 1 about here)

**Experiment 2.**

**Body weight.** BW MATCH rats lost approximately 13% of their original body weight over the course of 14 days of food restriction (Figure 7). Daily food allotment was adjusted such that BW MATCH rats lost weight in a temporal pattern that resembled that of SUB in mixed gender colonies in Experiment 1. Following restriction BW MATCH rats were allowed ad lib access to food during which time they became hyperphagic (data not shown) and regained lost body weight over the course of 21 days. At the end of the 21 day recovery period body weight of BW MATCH rats did not differ significantly from that of AD LIB control rats. During the second cycle of food restriction and re-feeding the BW MATCH rats were again food restricted to the body weight of SUB. However, when BW MATCH rats were returned to ad lib feeding, they did not regain their
lost body weight by the end of 21 days recovery and were significantly lighter than AD LIB control rats at the end of RECOVERY 2.

(Insert Figure 7 about here)

Male rats housed in the VBS without females (‘‘MALE ONLY VBS’’) did not show evidence of social hierarchy formation. They did not display aggressive behaviors resulting in wounds as male rats in mixed gender VBS colonies do (56). MALE ONLY VBS rats lost a significant amount of weight during VBS 1 (Figure 7), but not to the same degree as SUB in mixed gender colonies further suggesting the absence of a distinct social hierarchy. There was no significant difference in body weight between MALE ONLY VBS and MALE ONLY CON throughout the remainder of the experiment.

Body composition. Body composition in Experiment 2 was analyzed by the NMR method. We and others have found that use of the NMR method to determine body composition has a significant correlation with adipose content determined by a chemical method (lyophilization and ether extraction) \[ r = +0.98, P < 0.01; (9) \] (9, 27, 53, 57). Body composition of BW MATCH rats following food restriction is depicted in Figure 8. As expected, BW MATCH males lost both adipose and lean tissue during the food restriction period while AD LIB control males continued to gain body weight and fat mass. When allowed to re-feed, BW MATCH rats were hyperphagic and re-gained lost body weight over the 3 weeks of ad libitum feeding and this weight was regained as both adipose and lean tissue. However, fat mass remained significantly lower than that of AD LIB controls at the end of the first recovery period.
Relative to singly housed control males (MALE ONLY CON), body fat of MALE ONLY VBS rats was significantly lower following VBS housing (Figure 9). MALE ONLY VBS rats regained lost adiposity when they were returned to their individual home cages. During the second cycle of VBS and recovery, the MALE ONLY VBS rats showed a similar pattern of adipose tissue loss and regain; however by the end of RECOVERY 2 they continued to have significantly less adiposity compared to MALE ONLY CON ($P < 0.05$).

Plasma hormone concentrations (Table 2). Plasma testosterone was reliably lower in BW MATCH males following 2 weeks of food restriction but did not differ from levels in AD LIB control for the remainder of the experiment. Plasma testosterone did not differ between MALE ONLY CON and MALE ONLY VBS at any timepoint examined.

Plasma corticosterone levels were elevated in BW MATCH compared to AD LIB controls following the first food restriction period. There was a significant increase in corticosterone levels between VBS 2 and RECOVERY 2 that occurred in both AD LIB and BW MATCH, however there were no significant differences between the groups. There were no reliable differences in corticosterone between MALE ONLY CON and MALE ONLY VBS at any of the time points examined.
Acute stress test. In order to determine whether food restriction and consequent body weight loss affected the animals’ capacity to respond to stress, we administered an acute restraint stress test on the last day of food restriction. As depicted in Figure 10, BW MATCH rats had elevated baseline corticosterone compared to AD LIB controls ($P < 0.05$) and responded to 1-hour restraint stress with a significant increase in plasma corticosterone ($P < 0.05$ vs. BASAL) that did not differ in magnitude compared to controls. After the restraint stress was terminated, plasma corticosterone in both groups returned to baseline within 1 hour. Following RESTRICT 2, baseline corticosterone was not different between the groups and both groups had a significant increase in plasma corticosterone in response to 1-hour restraint stress.

(Insert Figure 10 about here)
DISCUSSION

We have confirmed previous reports that social stress results in significant body weight loss in both DOM and SUB rats housed in a VBS (5, 8, 11, 21, 34, 56). We further found that fat distribution changes differentially in SUB and DOM rats. We previously demonstrated that despite losing body weight during social stress, SUB retain more visceral adiposity relative to CON and DOM (54). Following recovery from stress, SUB rats then gain relatively more visceral fat compared to DOM and develop hyperinsulinemia, hyperleptinemia and other physiological and endocrine parameters consistent with a worsening metabolic profile during a recovery period following exposure to chronic social stress. Finally, we demonstrate that repeated, intermittent periods of chronic social stress may exacerbate these changes. Thus, the social status of rats during a period of heightened stress may play an important role in determining susceptibility to developing central adiposity. Together our data suggest that animals that are repeatedly exposed to chronic stress may result in a predisposition to develop symptoms of the metabolic syndrome. The VBS model is a potential animal model to examine the genesis of obesity and its associated comorbidities.

We further determined that the changes in body composition exhibited by SUB cannot be explained by weight cycling (via food restriction and re-feeding) on body composition. Thus, the data suggest that changes in body weight and body composition can be attributed to social stress in the VBS. Male rats that are food restricted to match the body weight loss of SUB (“BW MATCH”) also lose a significant portion of adipose tissue and lean body mass following restriction and regained body weight as lean as well as adipose tissue such that they ultimately did not differ in adiposity compared to their respective AD LIB controls. In addition, they had greater lean mass
relative to controls suggesting that increased adiposity in SUB is not attributable to weight cycling alone.

The VBS apparatus provides a larger area for animals to interact in and is a more complex, social environment compared to the standard shoe box cages the CON rats are housed in. Therefore, increased physical activity and social interaction (in the absence of a social hierarchy) may contribute to the changes that occur in DOM and SUB during and following VBS housing. We previously determined that the male rats in all-male colonies (“MALE ONLY VBS”) do not form dominance hierarchies as mixed gender colonies do (56), and inclusion of this group allowed us to control for housing conditions in the VBS in the absence of social hierarchy and stress. Our data suggest that weight loss in SUB during VBS housing cannot be solely attributed to the opportunity for greater activity or to exposure to the complex environment in the VBS. Furthermore, the data suggest that body composition changes seen in DOM may be attributed in part to increased activity in the VBS. In contrast, increased adiposity following recovery in SUB are attributable to stress derived from social subordination.

We previously documented that weight loss in SUB during social stress was attributed, at least in part to, decreased food intake (55). In other models of social stress such as the resident-intruder model, weight loss in SUB tree shrews is associated with decreased food intake as well as elevated energy expenditure (26). Repeated episodes of acute restraint stress elicit a prolonged decrease in body weight in rats (17-19) that is associated with hypophagia during the stress period but returns to control levels after the stress period has ended (18, 19) suggesting that restraint stress-induced weight loss may result in a lower defended level of body weight that persists for long intervals after the stress has been terminated. In contrast, SUB in our social-stress model were
hyperphagic throughout the recovery period, and this was true both in terms of absolute amount consumed (data not shown) and amount consumed per gram of body weight (Figure 4). SUB also have greater caloric efficiency when they are allowed to recover from social stress. The intriguing aspect of this finding is that caloric efficiency and hyperphagia remain elevated relative to CON and DOM despite having regained lost body weight by the end of 2 weeks and this is consistent with other reports of stress-induced weight loss and regain (23). These data highlight an important difference between social and other kinds of stressors, and they imply that social stress can predispose to rapid weight gain with associated metabolic sequelae. The endocrine status of an animal has a strong influence on body composition and adipose tissue distribution during weight gain. Both visceral and subcutaneous adipocytes express glucocorticoid and androgen receptors, but visceral adipocytes have greater expression (38). Corticosterone activation of the glucocorticoid receptor (GR) has been proposed to result in increased expression of lipoprotein lipase (LPL), a major regulator of lipid uptake in adipose tissue (7). In contrast, androgens inhibit LPL thereby acting to decrease fat accumulation (39). SUB have high levels of glucocorticoids and low levels of testosterone, both within the VBS and immediately upon entering the recovery period. We previously observed that both total corticosterone and total testosterone in SUB does not return to control levels until 7-14 days into the recovery period (44). Therefore, in the context of this endocrine milieu SUB preferentially retain visceral adipose tissue during the stress period (54) and regain lost body weight as additional visceral adipose tissue, resulting in a vicious cycle directed toward development of symptoms associated with the metabolic syndrome. One implication is that repeated exposures to VBS stress, or to other stressors that result in a similar endocrine profile, would produce cumulative effects on the physiology that may have catastrophic effects over the long term.
Other aspects of the endocrine system also need to be considered. Under many conditions, the overall level of active circulating glucocorticoids is controlled by circulating corticosterone binding globulin (CBG) which has a buffering function, preventing free (active) corticosterone from becoming excessively high. However, previous studies using the VBS model have reported that CBG is decreased by approximately 70% in SUB and by 40% in DOM (51). Comparable findings have been observed in primate models of social stress (52). Therefore, the combination of elevated total corticosterone levels and decreased CBG suggests that there is a significant increase in biologically active, free corticosterone in all VBS animals and particularly in SUB. In addition, the target tissues themselves (e.g. adipose tissue) can modulate steroid action at the pre-receptor level by altering the amount of steroid available to bind to receptors. Glucocorticoid action on target tissues is also influenced by the levels of intracellular 11β-hydroxysteroid dehydrogenase (11β-HSD) (36). Mice with adipocyte-specific over-expression of 11β-HSD1 have increased visceral adipose tissue and develop symptoms of the metabolic syndrome (33). In contrast, adipocyte-specific expression of 11β-HSD2, which inactivates glucocorticoids, protects against weight gain on a high-fat diet through reduced adipose tissue fat accumulation (25). Previous studies indicate that SUB have decreased 11β-HSD activity in testis, but reported no change in liver 11β-HSD activity compared to DOM (35). Hence, increased free corticosterone and a likely decrease of 11β-HSD activity would combine to favor increased fat deposition in SUB animals in the VBS model.

The BW MATCH and MALE ONLY VBS groups provide additional insights into the role of the endocrine milieu present during the various phases of stress and recovery. Although BW MATCH had higher corticosterone and lower testosterone levels compared to AD LIB rats they did not show any significant differences in either measure compared to AD LIB controls for the remainder of the experiment. It is unclear why BW MATCH rats failed to regain body weight by
the end of the second 3-week recovery period, however, we have replicated these findings in a second group of animals. Additionally, testosterone and corticosterone did not differ between MALE ONLY VBS and MALE ONLY CON rats thus supporting the hypothesis that body composition changes in SUB may be attributed to stress-associated changes in endocrine parameters. Further examination of these control groups may provide information about how chronic social stress results in greater adiposity in SUB rats.

There are other possible mechanisms to consider. Increases in fat mass to lean mass ratio, similar to that seen in SUB after two VBS episodes, have been documented in adult humans recovering body weight after weight loss resulting from a variety of conditions such as war-related famine and poverty-related undernutrition (10). Humans and animals that undergo catch-up growth accumulate excess fat, and catch-up growth is associated with development of obesity and increased susceptibility to diabetes (14, 31). Animals that regain lost weight also display suppressed thermogenesis in certain organs or tissues and this may play a role in enhancing the efficiency of catch-up growth and fat accumulation. The fact that BW MATCH group did not regain weight as adipose tissue suggests that perhaps the degree of weight loss imposed (~10%) was not sufficient to trigger rapid catch-up growth in these animals. The BW MATCH group also constitutes a critical control group with which comparisons can be made in determining the mechanisms responsible for greater adiposity in SUB.

Leptin is secreted by adipocytes and plasma leptin is reflective of the amount of body adiposity and suppresses food intake in normal animals (45, 58). Plasma leptin levels were consistent with the level of body adiposity after each VBS and recovery period. Leptin levels in SUB were not different from CON or DOM at the end of the RECOVERY 1 and RECOVERY 2,
however food intake and caloric efficiency remained elevated, particularly in RECOVERY 1. 
These data suggest that with repeated exposures to chronic social stress, SUB may also be 
developing leptin resistance. Further studies are required to determine the time course of changes in 
leptin relative to weight gain, body composition and other endocrine parameters as well as to 
determine the animals’ sensitivity to exogenous leptin.

Summary and perspectives.

Several characteristics of SUB rats in the VBS paradigm conspire to make them susceptible 
to a worsening metabolic profile. First, while in the VBS, SUB lose a significant amount of body 
fat and a relatively large percentage of this is from the subcutaneous region, thus sparing visceral fat 
(54). Second, when given the opportunity, as during a recovery period, they are able to eat more 
food, have greater caloric efficiency, and regain weight rapidly. Third, during the recovery phase 
when SUB rats are hyperphagic, they have an endocrine profile that favors adding newly formed fat 
into the visceral depots; i.e., the combination of high glucocorticoid and low testosterone levels 
characteristic of SUB is known to predispose to visceral obesity in humans (2). Finally, repeated 
episodes of social stress and recovery increase the percentage of body fat contained in visceral 
depots and would be anticipated to result in further metabolic consequences such as increased 
plasma lipids and insulin insensitivity. Visceral adiposity has been suggested to be a critical 
determinant in the development of metabolic syndrome (3).

Housyar et al. recently demonstrated that chronic stress results in decreased fat stores, lower 
leptin, insulin and testosterone in male rats following 8-days of morphine treatment (22). 
Consistent with our findings in SUB in the VBS, the authors of that study found that when the rats
were subsequently allowed to recover, food intake and caloric efficiency was greater than that of controls. In addition, lost adipose stores were regained and not different from controls by the end of the 8-day recovery period despite significantly lower body weight suggesting that percent adiposity was higher than that of controls. Houshyar and colleagues further report that their observations were attributed to elevated arcuate nucleus neuropeptide Y mRNA expression with no concomitant change in expression of the catabolic neuropeptide pro-opiomelanocortin during the withdrawal recovery period. Studies to examine these systems in VBS animals are currently being completed.

Although acute exposure to social stress may produce physiological changes that are reversible, a sufficiently long chronic or intermittent stress situation can lead to irreversible adverse consequences to the organism. As an example, Henry et al. studied mice kept in a social housing paradigm similar to the VBS for durations varying from 2 days to 15 months. Blood pressure progressively rose as the duration of social housing increased (20). Sustained hypertension was associated with increases in heart weight that were reversible when the mice were placed in isolation to recover. However, once the social housing duration reached 9 months, the changes in heart weight were fixed and many of the animals developed significant atherosclerotic changes. The present data are consistent with the hypothesis that SUB males intermittently exposed to the VBS situation slowly progress toward a condition of impaired metabolism because they develop higher adiposity that is concentrated in the visceral depot.

Studies in non-human primates and humans have identified associations among stress, glucocorticoid secretion and the deposition of intra-abdominal fat (32, 40). Subordinate non-human primates, when colony-housed in a manner analogous to rats in the VBS, have a similar pattern of fat deposition (24). Female cynomolgus monkeys stressed by social subordination have more
abdominal fat (30, 47, 48). Furthermore, subordinate females have hypercortisolemia (46),
dyslipidemia (15), tachycardia in response to stress (46), impaired ovarian function (1) and
enhanced coronary and carotid atherosclerosis (1, 49), all symptoms being consistent with the
metabolic syndrome profile.

A recent study of healthy young men exposed to long-term stress revealed that long-term
stress results in increased abdominal obesity and early signs of metabolic syndrome suggesting that
stress may play an important role in the genesis of metabolic abnormalities in humans (6). Subjects
in that study lost both fat and lean body mass during the first stressful episode and subsequently
regained body weight as fat resulting in an overall decrease in protein mass. Other studies provide a
growing body of evidence that chronic exposure to stressful conditions can be linked to
disturbances of energy homeostasis. Stress is becoming more widely recognized as a major risk
factor for metabolic and cardiovascular complications. Visceral obesity, type 2 diabetes and
metabolic syndrome appear to be associated with the hyperactivation of the stress system (2, 28).
Although, the precise mechanism through which stress contributes to increased abdominal fat
accumulation and development of metabolic pathophysiology is unclear, our findings suggest that
the VBS model may provide a physiologically and ethologically relevant means to study stress-
related metabolic disease.
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35. **Monder C, Sakai RR, Miroff Y, Blanchard DC, and Blanchard RJ.** Reciprocal changes in plasma corticosterone and testosterone in stressed male rats maintained in a visible burrow.
system: evidence for a mediating role of testicular 11 beta-hydroxysteroid dehydrogenase.


FIGURE LEGENDS

Figure 1. Schematic diagram of a visible burrow system. The entire apparatus measures 1.0 m².

Figure 2. Experimental timeline for Experiment 1. Animals were grouped into mixed gender colonies prior to VBS 1 and remained in the same groupings throughout the experiment. During the RECOVERY 1 & 2 animals were placed in individual shoebox cages and allowed access to food and water ad libitum.

Figure 3. Body weight during two cycles of VBS housing and recovery. Data are expressed as mean ± S.E.M. * P < 0.05 vs. CON; † P < 0.05 vs. CON, DOM.

Figure 4. Food intake (A) and caloric efficiency (B) during recovery. During RECOVERY 1 SUB consumed significantly more food per g body weight throughout the 3-week recovery period compared to CON and DOM. In RECOVERY 2, SUB again had higher food intake per g body weight than CON and DOM for Weeks 1 and 2. The difference in food intake by Week 3 was not significant among the groups. Caloric efficiency was greater in SUB compared to both CON and DOM. Data are expressed as mean ± S.E.M. * P < 0.05 vs. CON; † P < 0.05 vs. DOM; ‡ P < 0.05 vs. CON and DOM.

Figure 5. Body composition by soxlet method. Both DOM and SUB lost adipose tissue over 14 days of VBS housing (top panel) [Adapted from Tamashiro et al. 2004]. SUB additionally lost lean tissue (bottom panel). During recovery, SUB regained body weight and had a higher percentage of body weight as adipose tissue and this was further enhanced after a second cycle of stress and
recovery. Data are expressed as mean ± S.E.M. * $P < 0.05$ vs. CON; † $P < 0.05$ vs. CON and DOM; ‡ $P < 0.05$ vs. SUB RECOVERY 1.

**Figure 6.** Adipose tissue distribution. SUB had a greater relative distribution of adipose tissue in the visceral depots after RECOVERY 2 than CON and DOM after two cycles of stress and recovery. Data are expressed as mean ± S.E.M. * $P < 0.05$ SUB vs. CON and DOM.

**Figure 7.** Body weight for weight matched (top panel) and male only VBS colonies (bottom panel) and controls. * $P < 0.05$ vs. AD LIB CON or MALE ONLY CON.

**Figure 8.** Body adipose and lean tissue in rats matched to SUB body weight. Data are expressed as mean ± S.E.M. * $P < 0.05$ vs. AD LIB CON.

**Figure 9.** Body adipose (top panel) and lean tissue (bottom panel) in MALE ONLY VBS colonies. Data are expressed as mean ± S.E.M. * $P < 0.05$ vs. MALE ONLY CON.

**Figure 10.** Corticosterone response to 60-min restraint stress following first (A) and second (B) 14-day food restriction. Food restriction and weight loss alone does not affect the corticosterone response to acute restraint stress. * $P < 0.05$ vs. AD LIB CON.
Figure 1.
Figure 2.

<table>
<thead>
<tr>
<th>VBS 1</th>
<th>RECOVERY 1</th>
<th>VBS 2</th>
<th>RECOVERY 2</th>
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<tr>
<td>14 days</td>
<td>21 days</td>
<td>14 days</td>
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COHORT 1  
n = 4 colonies  

COHORT 2  
n = 4 colonies  

COHORT 3  
n = 8 colonies
Figure 3.
Figure 4.

A.

B.
Figure 5.
Figure 6.
Figure 7.
Figure 8.

![Graph showing % NMR fat and % NMR lean over time for different conditions.](image-url)
Figure 9.
Figure 10.

A.

![Graph A showing Plasma corticosterone (µg/dL) across Basal, Stress, and Recovery periods for AD LIB and BW MATCH conditions.]

B.

![Graph B showing Plasma corticosterone (µg/dL) across Basal, Stress, and Recovery periods for AD LIB and BW MATCH conditions.]

Table 1. Endocrine parameters for experiment 1. Data are presented as mean ± S.E.M. * P < 0.05 vs. CON; † P < 0.05 vs. DOM; ‡ P < 0.05 vs. CON, DOM; § P < 0.05 vs. prior VBS period.

<table>
<thead>
<tr>
<th>Status</th>
<th>Corticosterone (µg/dl)</th>
<th>Testosterone (ng/ml)</th>
<th>Leptin (ng/ml)</th>
<th>Insulin (ng/ml)</th>
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<tbody>
<tr>
<td></td>
<td>VBS 1</td>
<td>RECOVERY 1</td>
<td>VBS 2</td>
<td>RECOVERY 2</td>
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<td>CON</td>
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<td>7.9 ± 3.6</td>
<td>9.3 ± 1.3</td>
<td>3.4 ± 1.0</td>
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<tr>
<td>DOM</td>
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<td>5.8 ± 1.5</td>
<td>4.4 ± 1.7</td>
<td>5.4 ± 2.0</td>
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<tr>
<td>SUB</td>
<td>3.8 ± 1.2 ‡</td>
<td>9.7 ± 2.0</td>
<td>10.8 ± 2.7 †</td>
<td>3.0 ± 0.9</td>
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<table>
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<tr>
<th></th>
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<th>VBS 2</th>
<th>RECOVERY 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>--</td>
<td>1.79 ± 0.43</td>
<td>--</td>
<td>1.33 ± 0.11</td>
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<tr>
<td>DOM</td>
<td>--</td>
<td>1.31 ± 0.24</td>
<td>--</td>
<td>1.30 ± 0.18</td>
</tr>
<tr>
<td>SUB</td>
<td>--</td>
<td>1.66 ± 0.22</td>
<td>--</td>
<td>1.68 ± 0.19 ‡</td>
</tr>
</tbody>
</table>
Table 2. Endocrine parameters for experiment 2. Data are presented as mean ± S.E.M. * P < 0.05 vs. AD LIB CON; † P < 0.05 vs. VBS 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Corticosterone (µg/dl)</th>
<th>Testosterone (ng/ml)</th>
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<tbody>
<tr>
<td></td>
<td>VBS 1</td>
<td>RECOVERY 1</td>
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<tr>
<td>AD LIB CON</td>
<td>1.2 ± 0.4</td>
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<tr>
<td>BW MATCH</td>
<td>3.0 ± 0.7 *</td>
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<tr>
<td>MALE ONLY CON</td>
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<tr>
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<tr>
<td>AD LIB CON</td>
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<td>2.0 ± 0.3</td>
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<tr>
<td>BW MATCH</td>
<td>1.3 ± 0.3 *</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>MALE ONLY CON</td>
<td>3.2 ± 0.6</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>MALE ONLY VBS</td>
<td>3.6 ± 0.6</td>
<td>3.7 ± 0.4</td>
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