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Metabolic consequences of chronic intermittent mild stress exposure

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HIGHLIGHTS

- Chronic intermittent mild stress (CIMS) transiently decreased body weight gain.
- CIMS transiently increased high-fat diet preference.
- CIMS did not alter total caloric intake, adiposity or glucose tolerance.
- Metabolic effects of chronic stress are likely related to its intensity/frequency.

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ABSTRACT

Chronic stress in humans has divergent effects on food intake, with some individuals reporting increased vs. decreased food intake during stress. This divergence may depend in part on stress intensity, with higher-intensity stressors preferentially promoting anorexia. Consistent with this idea, rodents given a high-intensity chronic variable stress paradigm have robustly decreased food intake and body weight gain. However, the metabolic effects of a less intense chronic stress paradigm are not clear. Thus in the present study, adult male rats were given chronic intermittent mild stress (CIMS) exposure (3 cycles, in which each cycle consists of once daily mild stress for 5 days/week for 2 weeks, followed by 2 weeks of no stress) vs. non-stress controls, combined with ongoing access to a palatable diet (PD; choice of chow, high-fat diet, 30% sucrose drink, and water) vs. control diet (chow and water). As expected, access to PD increased caloric intake, body weight gain, and adiposity, and impaired glucose tolerance. CIMS decreased body weight gain only during the first cycle of stress and did not affect body weight gain thereafter, regardless of diet. Moreover, CIMS did not alter total food intake, adiposity or glucose tolerance regardless of diet. Lastly, CIMS transiently increased high-fat diet preference in PD-fed rats during the first stress cycle. Collectively, these results suggest that CIMS has relatively modest metabolic effects that occur primarily during initial stress exposure. These results support the hypothesis that the metabolic consequences of chronic stress vary with stress intensity and/or frequency.

1. Introduction

There are complex interactions among stress, food intake and obesity, with stress often defined as real or perceived threats to homeostasis or well-being (reviewed in [1]). Stress exerts numerous effects on behavior, and also activates physiological stress responses. These physiological responses include activation of the hypothalamic–pituitary–adrenocortical (HPA) axis, resulting in increased circulating glucocorticoids, as well as increased sympathetic nervous system (SNS) tone (reviewed in [2]). Glucocorticoids work together with elevated SNS tone to exert numerous effects throughout the brain and body. Many of these effects (e.g., increased liver glucose output, increased release of fatty acids from white adipose tissue, and reduced insulin secretion) are focused on increasing the mobilization of energy to provide ready fuel for appropriate behavioral and physiological responses that maintain homeostasis and promote survival.

Given the profound effects that physiological stress responses have on behavior and metabolism, it is not surprising that stress is an important factor contributing to the regulation of food intake and energy balance. For instance, stress is linked with increased total food intake and the development of obesity in some groups of people, whereas stress decreases food intake and body weight in others [3–5]. The reasons for the discrepant effects of stress on food intake and energy balance are not clear, but one factor that might contribute is stressor intensity.

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For instance, high-intensity stressors that involve real threats to homeostasis (e.g., military combat, recently being the victim of violence) are often linked with anorexia, decreased appetite, and decreased body weight [6–8]. In contrast, hyperphagia often occurs with stressors that are likely less intense and involve more psychological threats to well-being — so called daily life stressors like school, work and interpersonal relationships [9–13]. Consistent with this idea, we and others have seen that rodents given a high-intensity chronic variable stress (CVS) paradigm (consisting of twice-daily stressors that include warm and cold water swims, restraint, hypoxia, and cold room exposure) have markedly decreased food intake and body weight gain throughout the duration of the chronic stress paradigm [14–16].

To test the hypothesis that the metabolic consequences of stress vary with the intensity of the chronic stress paradigm, the present work characterizes the metabolic (e.g., food intake, body weight, adiposity, glucose tolerance) effects of a low-intensity chronic stress paradigm. More specifically, a chronic intermittent mild stress (CIMS) paradigm is developed that modifies CVS in 3 important ways. First, it utilizes mild stressors that do not pose direct threats to homeostasis (e.g., cage tilt, dampened bedding, placement of a novel object into the home cage). Second, the stressors are given less often (e.g., 3 cycles in which each cycle consists of once daily stressors for 5 days/week for 2 weeks, followed by 2 weeks of recovery). Recovery periods were included to decrease the total number of stressors, and because intermittent stress-free periods have been implicated as important contributors to the metabolic effects of chronic stress [17]. Moreover, as some evidence suggests that the metabolic effects of stress interact with the consumption of highly-palatable foods [18,19], the effects of CIMS are studied in rats concurrently eating a palatable diet (PD; free access to high-fat diet (HFD), 30% sucrose drink, chow and water) or control diet (free access to chow and water).

2. Materials and methods

2.1. Subjects

Adult male Long-Evans rats (~250 g body weight) were purchased from Harlan Laboratories (Indianapolis, IN). Rats were singly-housed in a temperature- and humidity-controlled room with a 12–12 hour light cycle (lights on at 06:00 h; lights off at 18:00 h). Rats acclimated to the facility for at least 11 days (d) before experiment onset. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati and are compliant with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Diet treatments

At 4 days before experiment onset (denoted as day −4; Fig. 1), all rats were weighed and body composition was determined by NMR (EchoMRI, Echo Medical Systems, Houston, TX). Rats were then divided into 4 treatment groups (n = 12–13/group) that were matched for body weight and percent body fat (in order to ensure that all groups began the study with a similar metabolic status). Two of these treatment groups then began continuous access to PD, consisting of ad libitum access to HFD (15% calories from protein, 40% calories from fat, 46% calories from carbohydrate; D03082706, Research Diets, New Brunswick, NJ), normal chow (25% calories from protein, 17% calories from fat, 58% calories from carbohydrate; LM-485, Harlan-Teklad, Indianapolis, IN), 30% sucrose (MP Biomedicals, Solon, OH) drink, and water. The other 2 treatment groups were maintained on ad libitum normal chow and water as a control diet.

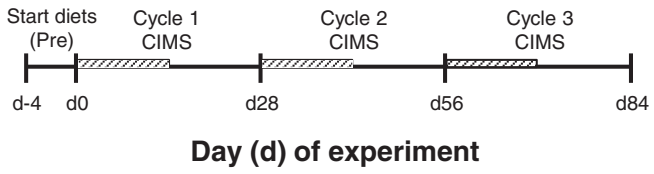

Fig. 1. Schematic of experimental timeline. At 4 days prior to experiment onset (day −4), rats began continuous access to either palatable diet (PD; ad libitum access to high-fat diet, 30% sucrose drink, normal chow and water) or control diet (ad libitum access to normal chow and water). The experiment (onset on day 0) consisted of 3 cycles of chronic intermittent mild stress (CIMS), in which each cycle comprised once daily mild stress for 5 days/week for 2 weeks (*denoted as hatched bars*), followed by 2 weeks of recovery (no stress). Non-stressed control rats did not receive stress exposure and instead remained undisturbed in their home cages.

2.3. CIMS paradigm

At four days after initiation of the diet treatments (experiment day 0; Fig. 1), one of each dietary treatment groups was randomly selected to receive CIMS, while the other group remained in their home cages as non-stress controls. CIMS consisted of 3 cycles of stress, in which each cycle comprised one daily mild stress exposure for 5 days/week for 2 weeks, followed by 2 weeks of recovery (no stress). Mild stressors were presented in an unpredictable order and included: overnight housing in home cage with water-dampened bedding; overnight housing in home cage tilted to ~30°; being transported (while in home cage) on a wheeled cart up and down the halls of the animal facility for 5 min; being placed in an open field for 5 min; placement of a novel object (e.g., plastic duplo block, nylabone) into the home cage for 30 min; and exposure to white noise at a moderate volume (similar to volume of human conversation) for 30 min.

2.4. Metabolic measures

Food intake (from all nutrient sources) and body weight were monitored throughout the study. In addition, adiposity (% body fat measured by NMR) and oral glucose tolerance were assessed at the end of each cycle of CIMS. For the oral glucose tolerance test, rats were fasted overnight and the following morning basal blood glucose (0 min) was measured by tail-clip using Precision Xtra glucometers and test strips (Abbott, Alameda, CA). After completing measurement of the basal time point, rats were immediately given an orogastric gavage of 50% glucose solution (Fisher Scientific, Pittsburgh, PA) at a final dose of 1.5 g glucose per kg of body weight. At 15, 30, 60, and 120 min after glucose gavage, tail blood glucose was re-measured.

2.5. Statistical analyses

Data are shown as mean ± SEM. Statistical differences were determined by ANOVA (with repeated measures when appropriate) with protected Fisher's post-hoc analysis using GB-STAT software (Dynamic Microsystems, Inc., Silver Spring, MD). Statistical significance was taken as $p < 0.05$.

3. Results

3.1. Body weight and body weight gain

Body weight (Fig. 2A) was monitored throughout the experiment and analyzed by 3-way ANOVA with repeated measures comparing Diet, Stress, and Day (repeated factor). This analysis showed main effects of Diet ($p = 0.001$) and Time ($p < 0.001$) with a Diet × Time interaction ($p < 0.001$), but no main effect of Stress ($p = 0.381$) and no other interactions (all $p > 0.05$). Post-hoc analysis identified increased body weight by PD beginning on experiment day 28. These data suggest that ongoing access to PD increased overall body weight and that this was not affected by exposure to CIMS.

In order to determine whether CIMS and PD affected body weight gain, this measure was determined for each cycle of CIMS and analyzed by 3-way ANOVA with repeated measures comparing Diet, Stress, and

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