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Opposing Effects of Chronic Stress and Weight Restriction on Cardiovascular, Neuroendocrine and Metabolic Function

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Abstract

Chronic stress is associated with dysregulation of energy homeostasis, but the link between the two is largely unknown. For most rodents, periods of chronic stress reduce weight gain. We hypothesized that these reductions in weight are an additional homeostatic challenge, contributing to the chronic stress syndrome. Experiment #1 examined cardiovascular responsivity following exposure to prolonged intermittent stress. We used radio-telemetry to monitor mean arterial pressure and heart rate in freely moving, conscious rats. Three groups of animals were tested: chronic variable stress (CVS), weight-matched (WM), and controls. Using this design, we can distinguish between effects due to stress and effects due to the changing body weight. WM, but not CVS, markedly reduced basal heart rate. Although an acute stress challenge elicited similar peak heart rate, WM expedited the recovery to baseline heart rate. The data suggest that CVS prevents the weight-induced attenuation of cardiovascular stress reactivity. Experiment #2 investigated hypothalamic-pituitary-adrenal axis and metabolic hormone reactivity to novel psychogenic stress. WM increased corticosterone area under the curve. CVS blunted plasma glucose, leptin, and insulin levels in response to restraint. Experiment #3 tested the effects of WM and CVS on PVN oxytocin and corticotrophin-releasing hormone mRNA expression. CVS increased, while WM reduced PVN CRH mRNA expression, whereas both CVS and WM reduced dorsal parvocellular PVN oxytocin mRNA. Overall, the data suggest that weight loss is unlikely to account for the deleterious effects of chronic stress on the organism, but in fact produces beneficial effects that are effectively absent or indeed, reversed in the face of chronic stress exposure.

Keywords

heart rate; glucocorticoids; corticotropin-releasing-hormone; oxytocin; leptin; insulin; glucose

INTRODUCTION

Humans, as well as animals, experience random, unpredictable bouts of stress throughout their daily lives due to a myriad of psychogenic and systemic threats to their livelihood. In reaction to these challenges, their bodies have evolved two main physiological responses to

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stress: the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenocortical (HPA) axis responses. Within seconds of stress onset, catecholamines are released into the bloodstream from sympathetic ganglia and adrenal medulla, triggering the SNS (“fight or flight”) response. Concurrently, corticotropin-releasing hormone (CRH)-containing neurons within the paraventricular nucleus of the hypothalamus (PVN) are activated, which evokes a neuroendocrine cascade culminating in the synthesis and release of glucocorticoids from the adrenal cortex. The temporal differences between these two responses produce very different means by which the SNS and HPA axis can influence present and future stress reactivity. Peak SNS responses can be seen within seconds of stress onset, but it takes minutes to yield an increase in plasma glucocorticoid levels. **However**, SNS responses terminate more expeditiously and do not typically produce lasting changes in whole-organism physiology. Glucocorticoids can produce lasting effects because they act on steroid receptors throughout the body, which can have delayed and persistent changes in gene expression [1] and cellular plasticity [2].

Repeated activation of physiological stress responses modulates future stress reactivity. For example, following chronic stress, corticosterone responses to novel stressors are significantly exaggerated, likely due to enhanced adrenal sensitivity [3] and/or reduced glucocorticoid negative feedback [4]. In addition, following chronic stress, PVN CRH neurons exhibit electrophysiological [5], genetic [6, 7], and morphological changes [8] that lead to enhanced central drive of the HPA axis [9]. While adaptations of physiological responses can be seen as adaptive, dysregulated stress responses are a facet of mood and anxiety disorders, which underscores the importance of proper stress control.

Stress responses can affect numerous central and peripheral systems that impact physiological status. Both the SNS and HPA axis release glucose from energy stores, which could affect energy balance. In rodents, chronic stress regimens, such as social subordination [10], variable stress [11], or homotypic stress [12], reduce food intake, body weight gain, and adiposity. As a result, plasma leptin and insulin are also reduced [13, 14]. In turn, metabolic state can also influence stress responding. Metabolic challenges activate both the HPA axis and SNS [15, 16]. Furthermore, leptin blunts HPA axis and behavioral responses to stress [17-19], but can shift heart rate variability toward sympathetic outflow [20]. Following the cessation of the stress regimen, body weight eventually recovers, but is delayed relative to food-restricted animals [21], indicating that stress attenuates weight gain independent of effects on metabolic parameters.

Our group has demonstrated that chronic stress (using a chronic variable stress (CVS) regimen) reduces food intake, body weight gain, and adiposity [11]. Given that there are clear connections between metabolism and stress responding, modulations in metabolic state due to chronic stress-induced weight loss may represent a substantial component of the physiological response to chronic stress exposure. Therefore, the present study tested the hypothesis that weight loss alone would contribute to chronic stress-related pathology and chronic stress facilitation of HPA axis and sympathetic responses to a novel stressor.

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats from Harlan (Indianapolis, IN) weighing 250-275 g upon arrival were group-housed two per cage for the duration of the experiment in clear polycarbonate cages containing granulated corncob bedding, with food and water available *ad libitum*. The colony room was temperature- and humidity-controlled with a 12 h light cycle (lights on 6:00 am; lights off 6:00 pm). Rats acclimated to the colony facility for one week prior to experimental manipulations. All experimental procedures were conducted in accordance

with the National Institutes of Health Guidelines for the Care and Use of Animals and approved by the University of Cincinnati Institutional Animal Care and Use Committee. Hopper and body weights were collected once per day prior to stress exposure in all animals. In experiment 1, rats (CVS (n=7), WM (n=7), and Control (n=5)) were implanted with radio-telemetry devices and allowed to recover from surgery. Following recovery, one group of animals was placed through two weeks of CVS, and their **cardiovascular response (for details see Cardiovascular recording after novel (footshock) stress.)** to a novel stressor (acute footshock) was analyzed. In experiments 2 and 3, rats were exposed to CVS immediately following the acclimation period to the colony facility. At the end of each experiment, the rats were perfused. In experiment 2, the rats (CVS (n=8), WM (n=9), and Control (n=8)) were exposed to novel restraint test and perfused two hours following the onset of stress. In experiment 3, the rats (CVS (n=8), WM (n=9), and Control (n=8)) were sacrificed without acute stimulus to assess basal hypothalamic mRNA analyses.

Chronic Stress Procedure

Subjects were randomly assigned to either CVS, weight-matched (WM), or control groups. The chronic stress protocol consisted of twice-daily (morning and afternoon) exposure to randomly assigned stressors for two weeks. Morning stressors were conducted between 8:00 am and 11:30 am and afternoon stressors were administered between 1:30 pm and 5:00 pm. Stressors consisted of rotation stress (1 h at 100 rpm on a platform orbital shaker), warm swim (20 min at 31°C); cold swim (10 min at 18°C), cold room stress (kept in 4°C for one hour) and hypoxia (8% O₂ 92% N₂). In the morning following the last afternoon stressor of experiment 2 and 3, rats received an overdose of sodium pentobarbital and were perfused with phosphate buffered saline, followed by 4% paraformaldehyde. Brains were post-fixed overnight in 4% paraformaldehyde and transferred to 30% sucrose at 4°C until they were cut on a freezing microtome.

Weight-matching

Animals were fed a sufficient amount of chow to produce a similar reduction in weight gain compared to the chronically stressed animals. In order to provide a less stressful means of food restriction, animals were fed 5 grams of chow in the morning at a random time between 7am and 11am and the rest between 5:30 and 6pm. Feeding the animals just before lights off does not shift their circadian rhythm. The morning feeding was added in to limit the total amount of time without food (since rats typically eat small amounts during the light period of the circadian cycle). Feeding them in this manner did not shift their circadian pattern of heart rate and blood pressure (data not shown). WM and CVS animals did not differ in body weight on any day of either experiment (Figure 1A).

Blood Collection

In experiment 2, blood was collected in EDTA at 0, 30, 60, and 120 minutes following the onset of 30 minutes of restraint stress. The animals were placed into a plastic restraint tube. Following collection, blood was spun for 15 minutes at 6000 rpm. Plasma was collected and stored at -20 °C. Corticosterone was quantified on these samples by radioimmunoassay using kit from MP Biomedicals. Area under curve was calculated using equation for a trapezoid as previously described [22, 23]. Additional aliquots were analyzed using a luminex assay for insulin and leptin analysis, with 2 ul used to calculate plasma glucose concentration via Freestyle glucometer (Abbot Laboratories, Alameda Ca)

Telemetry surgery

The use of radiotelemetry to measure cardiovascular parameters and activity allows the continuous recording of mean arterial pressure (MAP), heart rate (HR), and activity in

conscious freely moving animals. Rats were anesthetized with isoflurane and implanted with a radiotelemetry transmitter (TA11PA-C40, Data Sciences International (DSI), St Paul, MN, USA). The descending aorta was exposed via an abdominal incision, and a catheter extending from the transmitter capsule was placed into the descending aorta and secured with tissue adhesive (Vetbond; St Paul, MN, USA) and a cellulose patch. The capsule was sutured to the abdominal musculature, the abdominal musculature was sutured, and wound clips were applied to the skin. Following surgery, animals were monitored for two weeks to insure that the animals recovered properly. Animals were removed from the study if they did not recover their pre-surgery body weight. Baseline cardiovascular parameters were recorded for two prior days the beginning of experimentation. Animals were divided into groups such that there was no difference in starting body weight, MAP, and HR. The radiotelemetry system produced data points at 10 second intervals throughout the day. For the daily time points, data was averaged throughout the 24 hour period, excluding an hour following the termination of each stressor. MAP, HR, and activity were determined using A.R.T. Platinum software (DSI, St. Paul, MN)

Cardiovascular recording after novel (footshock) stress

After 14 days of CVS in experiment #1, the cardiovascular response to footshock stress was monitored. Baseline values were defined as the average measure taken over the hour immediately prior to placement in a Gemini Shock Apparatus (San Diego Instruments). The animals were allowed to explore for five minutes, after which they were given a series of five 1.5mA shocks over a period of two and a half minutes. The shocks were administered at five minutes, six minutes, six minutes and 10 seconds, seven minutes, and seven minutes and 30 seconds following exposure to the shock chamber. The animals were then returned to their homecage, where cardiovascular recordings were collected. Average MAP and HR were calculated across five minute time bins over the 120 minute post-stress period.

Adipose tissue analysis

The carcasses of the animals were placed into a plexiglass tube and inserted into a EchoMRI whole body composition analyzer system (Echo Medical Systems, Houston, TX). The EchoMRI provides estimations of fat and lean mass. After completion, the animals were pelted in order to separate subcutaneous from visceral fat and subsequently re-analyzed in the same EchoMRI. The “pelt” contains inguinal white adipose tissue fat pads and any other subcutaneous fat. The remaining carcass contained all internal fat pads and intramyocellular adipose tissue (Table 1).

In Situ Hybridization

Animals were overdosed with Pentobarbital and perfused 0.9% saline followed by 4% phosphate buffered formaldehyde. The brains were sectioned on a microtome at 35 micrometers in a one in twelve series. Two wells of one in twelve series of tissue were used for analysis of oxytocin (OT) and CRH mRNA expression. Sections were immersed in 0.25% acetic anhydride [suspended in 0.1 M triethanolamine (pH 8)] for 10 min, rinsed twice in 2x saline sodium citrate buffer (SSC) for 5 min, then dehydrated through graded alcohols. Antisense cRNA probes complementary to CRH (765 bp) and OT (477 bp) were generated by *in vitro* transcription using ³⁵S-UTP. The CRH probe was synthesized as previously described [11, 22, 23]. The OT fragment was cloned into a BSSK vector, linearized with *Hind*III, and transcribed with T7 RNA polymerase (Fisher Scientific Co., Pittsburgh, PA). Each transcription reaction (15 µl) consisted of 1x transcription buffer, 62.5 µCi ³⁵S-UTP, 330 µM ATP, 330 µM GTP, 330 µM CTP, 10 µM cold UTP, 66.6 mM dithiothreitol, 40 U ribonuclease inhibitor, 20 U T7 RNA polymerase, and 2.5 µg linearized DNA. The transcription reaction was incubated at 37°C for 60 min, and labeled probe separated from free nucleotide by ammonium acetate precipitation. ³⁵S-probes were diluted

in hybridization buffer [50% formamide, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 335 mM NaCl, 1x Denhardt's solution, 200 µg/ml herring sperm DNA, 100 µg/ml yeast tRNA, 20 mM dithiothreitol, and 10% dextran sulfate] at an activity count of 1,000,000 cpm per 50 µl buffer. 50 µl of buffer was added to each slide. Slides were coverslipped then incubated overnight at 55°C in humidified chambers containing 50% formamide. The coverslips were removed in 2x SSC and the slides incubated in 100 µg/ml ribonuclease A for 30 min at 37°C. Slides were rinsed in 2x SSC, incubated in 0.2x SSC (65°C) for 1 h then dehydrated through graded alcohols. Slides were exposed to Kodak Biomax MR-2 film (Eastman Kodak, Rochester, NY) for 7 days for CRH and 8 hours for OT. Scion Image software (Scion, Frederick, MD) was used for semiquantitative analyses of autoradiographs. The anatomical regions of interest (CRH: PVN; OT: dorsal parvocellular PVN (dpPVN), lateral parvocellular PVN (lpPVN), medial parvocellular PVN (mpPVN), magnocellular PVN (mgPVN), and supra optic nucleus (SON)) were determined based on Paxinos and Watson's rat brain atlas [24]. Gray level units were collected for specified brain regions and the background signal over a non-hybridized area within the same section subtracted to obtain corrected gray level units (CGL). With each film, ¹⁴C radioactive standards (ARC) were included to assure that all signal intensities were within the linear range of detection. Analysis of autoradiographs was completed by a researcher blind to experimental groups.

Statistical Analyses. Statistics were analyzed using Sigma Stat (Systat Software, San Jose, California)

Data are expressed as mean ± standard error. Outliers were determined if the value exceeded both 1.96 times the standard deviation and 1.5 times the interquartile range [25]. The data in figures 1A, 2, 3A, and 4 were analyzed by two way repeated measure ANOVA and Fisher's LSD post-hoc test with group (CVS, WM, and control) as between subject factors and time as repeating within subjects factor. The data in figure 3B, 5, table 1, and table 2 were analyzed by one way ANOVA with a Fisher's LSD post-hoc test with group (CVS, WM, and control) as a between subject factor. Since the food intake values of the WM animals did not differ from animal to animal, Figure 1B was analyzed by one way ANOVA on rank. When necessary, the data went under log transformation and then re-analyzed.

RESULTS

Experiment #1

To test the hypothesis that the reductions in weight gain produced by CVS are sufficient to drive the **physiological** effects of chronic stress, we used three different groups: CVS, WM, and controls. WM animals were fed a sufficient amount of food to produce similar reductions in weight gain to CVS animals. Notably, we fed the WM animals significantly less food to produce similar reductions in body weight gain to the CVS group (Figure 1B) { $H(25,1)=109.166$, $p<.01$ } suggesting that CVS elevates energy expenditure to a greater degree than would be predicted by weight loss alone.

The animals' mean arterial pressure, heart rate, activity, body weight, and 24 hour food intake were monitored during recovery from telemetry surgery. Animals were assigned to CVS, WM and control groups on the basis of these parameters to normalize baseline values. On the first day of CVS, 24 hour HR (Figure 2A), MAP (Figure 2B), and activity did not differ between groups. Starting at day six, **24 hour average** heart rate was reduced in WM animals relative to CVS and control animals { $F(284,27)=4.303$, $p<.01$ } (Figure 2A). Reduced heart rate persisted through the end of the experiment (Figure 2A). However, neither MAP (Figure 2B) nor activity differed between the groups over the course of the study.

On day 14, we tested cardiovascular parameters following exposure to a brief novel stressor (footshock). Footshock elicited an increase in heart rate that did not differ between the groups (Figure 2C). However, the WM animals recovered to their baseline heart rate more quickly than unstressed controls $\{F(208,19)=1.65, p=.047\}$ (Figure 2C), indicating that weight loss **affects** the recovery of cardiovascular responses to psychogenic stress. Despite a similar reduction in body weight gain, CVS did not reduce the duration of the HR response to novel stress to similar degree than WM animals (Figure 2C), indicating that chronic stress negates the effects of weight loss alone on return to baseline HR.

Experiment #2

In experiment #2, we tested the impact of CVS or WM on post-stress facilitation of HPA axis responses to psychogenic stress. Groups of CVS, WM, and control animals were prepared using the same stress and weight-matching protocol outlined in experiment #1. As expected, novel restraint elicited a significant increase in plasma corticosterone in all groups (Figure 3B). Food restriction increased the area under the curve of the corticosterone response, whereas there was no main effect of chronic stress $\{F(24,1)=6.335, p<.01\}$ (Figure 3B). These results suggest that weight restriction may be sufficient to facilitate HPA axis responses to psychogenic stress. Despite elevated plasma corticosterone, it is important to note that food restriction reduced adrenal weight, whereas chronic stress induced adrenal hypertrophy $\{F(32,2)=7.614, p<.01\}$ and thymic involution $\{F(32,2)=4.297, p=.013\}$ (table 1) (as previously noted ([8, 22, 23])). These data suggest that chronic stress has long-term HPA axis effects above and beyond those of weight restriction alone.

Since weight restriction modulated both the cardiovascular and glucocorticoid responses to psychogenic stress, we also assessed secretion of leptin and insulin, two major factors that are known to be modulated by energy stores. Importantly, chronic stress and food restriction reduced adiposity in both the subcutaneous $\{F(25,2)=5.387, p=.01\}$ and visceral $\{F(25,2)=3.728, p=.036\}$ depots of the rats (table 1), suggesting a possible connection between changes in stress responsiveness and altered adipose signaling. We examined plasma leptin and insulin in the same samples used for corticosterone measures. Despite a similar amount of fat to that of WM animals, chronic stress reduced plasma leptin $\{F(98,5)=5.325, p<.01\}$ and insulin $\{F(96,5)=3.342, p<.01\}$, suggesting that chronic stress reduces release/storage of leptin/insulin independent of the effects of weight loss (Figure 4). Thus, we predicted that chronic stress would facilitate stress-induced hyperglycemia, but CVS attenuated stress-induced hyperglycemia $\{F(96,2)=62.873, p<.01\}$ (Figure 4), suggesting either an enhancement in leptin/insulin sensitivity or reduction in hepatic glucose output.

Experiment #3

Additional groups of CVS (n=8), WM (n=9) and control (n=8) animals were prepared to test the impact of CVS and WM on key neuropeptidergic systems involved in stress excitation (CRH) [26] and body weight regulation (OT) [27]. Indeed, CVS (n=8) increased and WM (n=9) reduced PVN CRH mRNA (Figure 5A) $\{F(19,2)=3.482, p<.032\}$. PVN OT was not altered within the SON, mgPVN, mpPVN, or lpPVN (Table 2). However, both CVS and WM reduced dpPVN OT mRNA $\{F(22,1)=6.143, p<.01\}$, suggesting a weight-specific effect on OT regulation in this pre-autonomic cell group (Figure 5B).

DISCUSSION

Our data indicate that body weight alone is not sufficient to account for the deleterious physiological effects of chronic stress. In fact, reductions in weight gain generally reduced resting heart rate, suggestive of a beneficial effect on the organism. Basal heart rate was

reduced in WM animals beginning at day six, and was accompanied by a more expeditious recovery of baseline heart rate following a psychogenic challenge. The therapeutic properties of dieting for hypertensive patients have long been accepted, but the mechanism is not known [28-30]. Currently published reviews would presume that the connection is due to a change in circulating hormones, that alters sympathetic/parasympathetic reactivity. Since previously published work utilized months of intermittent food deprivation [31], it is somewhat surprising that our food restriction model attenuated heart rate. In addition, our ~20% food restriction is mild compared to that of other groups (usually employing 40% restriction) [32].

Interestingly, CVS did not attenuate cardiovascular responses like WM despite a similar reduction in body weight gain. This suggests that chronic stress exposure blocks the cardiovascular effect of reduced body weight, or alternatively, that weight loss protects against a more severe cardiovascular effect of chronic drive. Since chronic unpredictable stress can produce hypertension in rats without altering body weight [33], we assume that the latter possibility is more likely. Future studies will **specifically test these two possibilities**.

Chronic stress-induced regulation of metabolic parameters appears to go above and beyond the effect of weight alone. For instance, chronic stress reduced insulin and leptin levels during the restraint challenge to a greater degree than weight restriction alone, indicating that chronic stress-specific regulation of insulin and leptin transcends that driven by negative energy balance alone. Other studies have shown reductions in leptin and insulin following chronic stress regimens [13, 18, 34], but those studies did not include a weight-matched control. These reductions in leptin and insulin could have subsequent effects on stress responsiveness, since they both can modulate HPA axis reactivity [19, 35], but we do not know the current state of leptin and insulin sensitivity following CVS. Dysregulation in leptin and insulin action [28-30] is proposed to mediate obesity-associated cardiovascular disease, but our data suggest that these hormones are not critical components in differentiating the cardiovascular impact of weight restriction and stress. We should note that for the purposes of studying the HPA axis endpoints, animals were not fasted prior to leptin and insulin measures, which may have some bearing on the observed basal hormone levels. For example, some of these changes could possibly be due to different feeding schedules of the animals, which limits the overall interpretation of the basal values. True 'basal' levels require fasting of the animals for a substantial time prior to testing, which could not be performed within the context of a stress paradigm (food depriving animals clearly influences stress responses [36, 37]). The current studies queries whether stress-related changes in leptin and insulin may influence the differential regulation of stress responses, which does not appear to be the case in our paradigm.

Both CVS and WM reduced PVN OT mRNA in the dpPVN, suggesting a weight-related regulation of OT mRNA. This effect was not observed in other PVN subregions or the SON. The dpPVN neurons send pre-autonomic projections to either the brainstem (e.g., nucleus of the solitary tract (NTS) or the interomediolateral division of the spinal column [38]. OT is known to have anorectic actions at the NTS [27], and thus reductions in OT mRNA may occur as a reaction to negative energy balance in both WM and CVS animals.

Food restriction reduced PVN CRH mRNA. Current data suggests that PVN CRH is both anorectic [39, 40] and stimulatory for sympathetic activity [41, 42]. The weight-related reduction in CRH may, like OT, be involved in reducing satiation and curtailing sympathetic activity, perhaps in concert with decrements in OT. This stands in contrast to the effects of CVS, which enhance PVN CRH mRNA expression (see also [11, 22, 23]) and are associated with anorexia and, relative to WM animals, increased HR responsiveness to stress. Thus,

stress-induced changes in CRH may at least in part, drive the dissociation in cardiovascular responsiveness that are observed between CVS animals and their weight-matched (but otherwise unstressed) counterparts.

Food restriction elevated resting corticosterone levels (as previously observed [16, 31]) and increased the overall HPA axis response to restraint stress (area under the curve). These data are not consistent with reduced adrenal weight and decreased PVN CRH mRNA expression observed in the WM group relative to controls, suggesting that the increase in corticosterone is due to enhanced drive of central or pituitary limbs of the HPA axis, and may be adrenal in origin. In line with this interpretation, previous studies indicate that ACTH responses are blunted, but glucocorticoids are increased following 24 hour food deprivation [36], which is sufficient to reduce body weight. These observed changes in HPA axis responsiveness to a novel challenge are correlated with reduced clearance of glucocorticoids [37], consistent with peripheral enhancement of adrenal hormone availability. With the addition of the data collected within this manuscript, the evidence suggests that these post-pituitary changes in HPA responsivity are not unique to food deprivation, but sensitive to changes in metabolic state. Overall, the data suggest peripheral enhancement of both basal and post-stress corticosterone under mild weight restriction that could be a compensation for reduced energy availability. This compensatory mechanism does not appear to be in place in CVS animals, perhaps as a result of enhanced central HPA axis and cardiovascular activation. It is important to note that the CVS animals did not exhibit an exaggerated **glucocorticoid** response to acute novel restraint. However, Dallman defines facilitation as a maintained or enhanced response despite a history of negative feedback signals caused by intermittently elevated plasma glucocorticoids [43]. By this definition, the maintained response to a novel stressor after CVS is consistent with HPA axis facilitation.

The corticosterone increases seen in the weight-matched animal suggests that animals may be under some degree of chronic stress. However, whereas glucocorticoid hypersecretion can be seen during chronic stress, it does not define the condition, as elevated glucocorticoid levels can be driven by negative energy balance [31, 36, 37], which is the case with the WM group. Indeed, CRH mRNA levels and adrenal weights are both reduced in the WM group, whereas these endpoints are consistently increased in a variety of chronic stress models [3, 6, 7, 44]. These data suggest a reduced central HPA axis drive in the WM group, inconsistent with a state of chronic stress. Moreover, the spectrum of data in the WM group are not consistent with a habituated response to stress [45], given that habituating models typically show normal adrenal weights and CRH expression, within the context of HPA axis facilitation (not observed in our WM group). However, it is important to note that the weight loss of CVS animals is due to voluntary means, while it is involuntary in WM animals. This may have ramifications independent of altered body weight, as reward-aversion related pathways may modulate both HPA axis and sympathetic responses to novel stressors [46].

In conclusion, our data demonstrate that reductions in weight gain produce changes in cardiovascular tone and reactivity. The observed changes **could be** beneficial to the organism, and **possibly** reversed during chronic stress. In addition, reduced body weight decreased adrenal weight and attenuated PVN CRH expression, indicative of blunted central HPA drive, whereas chronic stress has the opposite effects. Overall, the data suggest that weight loss is unlikely to account for the deleterious effects of chronic stress on the organism, but in fact produces beneficial effects that are effectively absent or indeed, reversed in the face of chronic stress exposure. Additional experiments will be required to fully understand the interplay between metabolism and stress in regulation of physiological responses to chronic adversity.

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Research Highlights

- Chronic stress prevents weight-induced attenuation of cardiovascular stress reactivity.
- Weight restriction reduced, while chronic stress increased PVN CRH mRNA.
- Chronic stress and weight restriction reduced dorsal parvocellular PVN oxytocin mRNA.
- Chronic stress blunted plasma glucose, leptin, and insulin following restraint.

Figure 1.

Experiment #1: Body weight and Food Intake. Figure 1A demonstrates the body weight gain throughout experiment 1 and Figure 1B displays total food intake over the two week experiment. CVS exposure reduced body weight (A), but not food intake (B). WM animals did not differ from CVS animals on any day of the experiments. We fed the WM animals significantly less food than the control and CVS animals despite a similar trajectory in weight gain. * denotes $p < .05$ compared to control animals.

Figure 2.

Experiment #1: *Basal cardiovascular parameters*. Figures 2A and 2B summarize data on basal cardiovascular activity. The “0” time point refers to the day prior to chronic stress exposure/ weight restriction. WM reduced heart rate beginning at day 6 and continuing through the rest of the experiment (B). Despite a similar body weight, CVS did not exhibit recapitulate this effect. However, mean arterial pressure did not differ between groups (A). * denotes p < .05 compared to control animals.

Figure 3.

Experiment #1: Cardiovascular responses to acute psychogenic stress. Figures 3A and 3B illustrate cardiovascular responses to acute psychogenic stress. “0” time point refers to the period before anyone entered the animal housing room. The arrow indicates the time where the animals experienced footshock. WM expedited the heart rate recovery to baseline following acute shock exposure (B) without altering mean arterial pressure (A). In this regard, the CVS animals displayed an intermediate recovery to baseline heart rate. * denotes $p < .05$ compared to control animals.

Figure 4.

Experiment #2: Corticosterone responses to acute psychogenic stress. Figure 3A illustrates glucocorticoid responses to acute psychogenic stress. The animals were restrained for 30 minutes and then released from the restraint tube after this time period. Blood samples were collected prior to restraint, after 30 minutes of restraint, after 30 minutes of recovery, and after 90 minutes of recovery. The 30 minute time point refers to peak glucocorticoid levels. Figure 3B summarizes area under the curve analysis. There was a main effect of WM to increase plasma corticosterone (A), as well as the integrated stress response (area under the curve) (B). * denotes $p < .05$ compared to control animals.

Experiment #2: Metabolic Responses to Acute Psychogenic Stress

Figure 5.

Experiment #2: Metabolic responses to acute psychogenic stress. Figure 4 summarizes plasma leptin (A), insulin (B), and glucose (C) responses to acute psychogenic stress. CVS reduced leptin 0 and 60 minute timepoint following 30 minutes of restraint stress, but WM only reduced leptin at the 0 minute timepoint (A). CVS reduced plasma insulin at the 0, 30, and 60 minute timepoints (B). WM only increased plasma insulin at the 0 minute timepoint (B). There was a main effect of CVS to attenuate stress-induced hyperglycemia (C). * denotes $p < .05$ compared to control animals.

Figure 6.

Experiment #3: Basal PVN peptide mRNA. Figure 5 illustrates PVN CRH (A) and OT (B) expression. CVS increased, while WM reduced PVN CRH mRNA (A). Both CVS and WM reduced dpPVN OT mRNA (B). * denotes $p < .05$ compared to control animals.

Table 1
Experiment 2 body, organ, and adipose weight

	Control	CVS	WM
Body Weight (Pre) (grams)	335.26 ± 3.03 g	336 ± 3.09 g	335.3 ± 2.95 g
Body Weight (Post) (grams)	378.6 ± 6.38 g	348.61 ± 3.72 g *	348 ± 1.57 g *
Adrenal Weight (mg)	70 ± 3.35 mg	77.43 ± 2.37 mg *	60.9 ± 1.79 mg *
Thymus Weight (mg)	400.01 ± 32.56 mg	331.78 ± 12.88 mg *	403.58 ± 17.65 mg
Total Lean (grams)	298.86 ± 2.11 g	272.22 ± 1.66 g *	284.4 ± 3.94 g *
Total Fat (grams)	27.71 ± 1.17 g	22.7 ± .9 g *	22.49 ± .84 g *
Subcutaneous Fat (grams)	13.56 ± .89 g	10.86 ± .65 g *	9.86 ± .7 g *
Visceral Fat (grams)	31.25 ± 1.03 g	27.09 ± 1.34 g *	29.04 ± .65 g *

Data are expressed as mean ± SEM.

* group is significantly different from control at P = 0.05.

Table 2
PVN and SON oxytocin mRNA expression

	Control	CVS	WM
IpPVN (CGL % Control)	100 ± 10.22%	83.95 ± 12.46%	91.14 ± 13.73%
mpPVN (CGL % Control)	100 ± 8.51%	94.46 ± 9.42%	84.12 ± 9.77%
mgPVN (CGL % Control)	100 ± 3.6%	76.32 ± 5.11%	80.48 ± 12.69%
SON (CGL % Control)	100 ± 3.28%	90.54 ± 4.75%	103.36 ± 11.07%

Data are expressed as mean ± SEM.