Short communication

Inaccessible food cues affect stress and weight gain in calorically-restricted and ad lib fed rats

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ABSTRACT

Research suggests that caloric restriction (CR) is beneficial; however, the effects of CR in the context of food cues are unclear. A 2 (food cue vs. no cue) × 2 (CR vs. ad lib) between-subjects design was employed to test these effects in 40 rats. It was predicted that cue exposure and CR would induce stress, and that these factors might interact synergistically. The results demonstrated that cue-exposed CR rats weighed less than did non-exposed CR rats. A blunted stress response was evident in CR rats relative to ad lib rats. Finally, cue-exposed rats had higher corticosterone levels and body weight during ad lib feeding than did non-cued rats. These results suggest that both CR and chronic food-cue exposure can be stressful, and the implications of this research are discussed in the context of humans’ ‘obesigenic’ environment.

Introduction

Caloric restriction (CR) in animals has various physiological benefits, including increased longevity, postponement of age-related declines in learning ability, and delays in the onset of cancer and diabetes (Pinel, Assanand, & Lehman, 2000). On the basis of this research, some have suggested that humans should undertake CR (Weindruch & Walford, 1988). In fact, the ‘CR for longevity’ movement has entered mainstream culture with the publication of dieting books (e.g., The Longevity Diet, Delaney & Walford, 2005). Despite prolific research supporting the benefits of CR, there is debate about whether CR could increase human longevity (e.g., Le Bourg & Rattan, 2006). Vitousek and colleagues (Vitousek, 2004; Vitousek, Manke, Gray, & Vitousek, 2004) also questioned the benefits of CR and its application in humans, and point to research in the eating disorders field, which shows detrimental effects of restriction and weight loss. The study by Keys and colleagues (Keys, Brozek, Henschel, Mickelson, & Taylor, 1950), which demonstrated extreme, long-lasting negative effects of CR on emotions and behavior, is particularly noteworthy. Vitousek et al. (2004) also highlight the limited research into the behavioral and psychological outcomes of CR, including a lack of research into how restricted animals respond to stressors.

CR animals appear to have higher baseline corticosterone (CORT) than do non-restricted controls, suggesting that CR leads to a chronic activation of the hypothalamic–pituitary–adrenal axis (e.g., Jahng et al., 2007). Chronic exposure to stress does not further raise CORT in CR rats, leading some researchers to conclude that CR leads to stress resistance and adaptation (e.g., Gursoy, Cardounel, Hu, & Kalimi, 2001). However, there appear to be detrimental effects of chronically elevated CORT: induction of chronic elevations in CORT lead to increased aggressive behavior, decreased foraging efficiency, and slower learning in chicks (Kitaysky, Kitaiskaia, Piatt, & Wingfield, 2003). Chronic stress is also associated with increased aggression in rats (Wood, Young, Reagan, & McEwen, 2003).

The study of possible interactions between CR and other stressors is new, and only a limited number of stressors have been examined. For the hungry animal, one potent stressor might be exposure to inaccessible palatable food. Typically, CR animals are provided with a limited amount of food, with no additional food cues present. As a result, it is difficult to use animal research to draw inferences about how humans will respond to CR. Unlike animals subjected to CR, humans are bombarded with food cues, making over 200 food-related decisions daily (Wansink & Sobal, 2007). Food-cue exposure in the context of restriction therefore represents an important, yet understudied research area.

Most research on food cues has focused on their role as conditioned cues that elicit eating (e.g., Weingarten, 1985). Weingarten developed a theory of hunger that proposes that food-related stimuli become associated with subsequent intake, leading to incentive-induced hunger. Similarly, environmental factors that are reliably associated with intake elicit cephalic responses which prepare organisms for food intake (Woods, 1991). Yet, the effects of chronic, unconditioned food-cue exposure have not been widely investigated. Presumably, chronic food-cue exposure in the absence of intake would extinguish cephalic phase
responses. Drawing from research on humans, chronic food-cue exposure appears to be associated with increased intake and body weight (Wang, Kim, Gonzalez, MacLeod, & Winkleby, 2007). Furthermore, Rosen (1981) reported that obese individuals who were on a diet reported increased anxiety after repeated exposures to diet-prohibited food, supporting the postulation that food-cue exposure may be stressful for food-restricted organisms. Recent evidence has emerged that food-cue exposure can counteract the beneficial effects of CR in drosophila – olfactory exposure to yeast decreased life span in CR flies compared to non-exposed flies (Libert et al., 2007). The authors suggest that the beneficial effects of CR may be partly due to a perception of a lack of food availability; therefore, cues suggesting food availability may reduce the benefits of CR. However, the potential role of food cues as a stressor, and the impact of such a stressor during CR, remains to be determined.

The literature suggests that both CR and exposure to unattainable food are stressors; however, it is not clear whether food cues act as a stressor independently, or whether exposure to cues is stressful only in combination with CR. We set out to test the effects of CR in the presence versus absence of inaccessible food cues. We predicted that CR would lead to increased baseline CORT and a blunted stress response. We further predicted that exposure to inaccessible food would lead to increased physiological indices of stress (i.e., CORT), in addition to increasing intake when food was available. Finally, we sought to investigate how CR and food-cue exposure would interact in their effects on physiology (i.e., CORT, body weight) and behavior (i.e., intake), both during the CR period as well as after the restriction was lifted. We predicted that cue-exposed CR animals would have higher CORT levels than would non-exposed CR animals during restriction, and that cue-exposed CR animals would eat and weigh more during a re-feeding period than would non-exposed CR animals.

Method

Animals

Forty male Sprague–Dawley rats (Charles River, Quebec) were used. Animals were 53–55 days of age at arrival, and weighed 300–372 g (M = 334 g) at the start of testing. Animals were housed individually in Plexiglas cages (47 cm × 26 cm × 20 cm) with wood shaving bedding. Tap water and food (Purina Rat Chow) was provided ad lib except as noted. The laboratory was maintained with a light–dark cycle of 12:12 (lights on at 07:00), a room temperature of 22 ± 1 °C and humidity at 45–55%. Procedures were approved by the University of Toronto at Mississauga Animal Care Committee.

Materials

An Ohaus Compact Scale (Model CS2000; Pine Brook NJ) was used to measure animals’ weights and food intake. Serum CORT was determined using enzyme immunoassay kits (MP Biomedicals ImmuChem Double Antibody Corticosterone RIA kit; Orangeburg NY).

Procedure

All animals were given a 1-week habituation period. At the end of habituation, their mean baseline intake of chow was assessed over 3 days (M = 32 g/day). After completion of baseline intake measurements, all animals were provided with 10 Froot Loops® (to ensure that all rats were familiar with the food cue that would be used). Froot Loops® were chosen as they are palatable to rats and have strong, distinctive odor cues (Ahn & Phillips, 1999). Animals were then quasi-randomly assigned to each of the groups (CR/no cue; CR/cue; Ad Lib (AL)/no cue; and AL/cue, n = 10 per group), ensuring that there were no group differences in baseline body weight. Animals assigned to CR were fed 80% of average baseline for 2 weeks and then were reduced to 60% of baseline for 10 weeks. CR animals were fed thrice weekly (3 h after light onset), with 2/7th of their weekly allotment of food on Mondays and Wednesdays, and 3/7th of their weekly allotment on Fridays. This procedure, also used by Dhambi, Kim, Mote, Beaver, and Spindler (2004), was chosen to minimize staff time spent servicing the rats. Animals assigned to the food-cue condition were exposed to visual and olfactory food cues throughout the study – an inaccessible wire mesh container filled with Froot Loops® was suspended approximately 10 cm above their cages. Animals in the CR/no-cue condition were housed separately (to prevent extraneous food-cue exposure), while animals from the other groups were housed together. The food hoppers of AL rats were monitored daily and kept full.

Measurements of rats’ body weight and 6- and 24-h chow intake were taken weekly. At the end of the 12-week CR, all animals were subjected to 1-h of restraint stress in a Plexiglas tube in their home cages, with tail blood samples taken immediately pre- and post-stress to assess CORT. Restraint stress was staggered across 3 days, beginning between 1130 and 1430, to limit the influence of circadian variation in CORT. CR rats were then returned to AL feeding. During the 14-week re-feeding period, weekly measurements of body weight and intake (6- and 24-h) continued. All rats were euthanized with carbon dioxide at the conclusion of the study.

Statistics

Analyses were conducted using SPSS version 15 for Windows, with restriction and food-cue condition entered as between-subjects factors. Effect sizes were computed as partial Eta-squared values.

Results

Body weight

Rats’ percentage of baseline body weight was calculated, and analyzed separately during the restriction and re-feeding periods (Fig. 1) using a repeated-measures ANOVA (Bonferroni-corrected, p ≤ .025). The Greenhouse–Geiser correction was used when sphericity was violated.

Restriction period

There was an interaction between time and restriction condition on percentage of baseline body weight, F(2,20,72.43) = 303.8, p < .001, η² = 0.89. No other within-subjects effects emerged. Between-subjects analyses demonstrated a main effect of restriction condition on body weight (F(1,36) = 401.1, p ≤ .001, η² = 0.92), with AL rats weighing more during restriction than CR rats. There was also an interaction between restriction and food-cue condition (F(1,36) = 7.3, p ≤ .02, η² = 0.17). T-tests (Bonferroni-corrected, p ≤ .01) were conducted for the last 5 weeks of the CR period, to test differences between the food-cue groups for the CR and AL conditions separately. The last 5 weeks were chosen for these analyses to allow sufficient time for potential weight differences to emerge. CR/cue rats weighed less than did those in the CR/no-cue condition at weeks 10, 11, and 14 (p ≤ .005). Differences between AL/cue and AL/no cue were not significant (p’s > .13).

Re-feeding period

There was an interaction between time and restriction on body weight over the re-feeding period, F(2,23,80.11) = 14.99, p < .001, η² = 0.35. There was also an effect of the food cue on weight throughout re-feeding, F(2,23,80.11) = 6.57, p < .005, η² = 0.15. T-tests on the last 5 weeks of re-feeding were conducted (Bonferroni-
CR/cue versus CR/no cue rats did not reach significance during the study duration of both restriction (p's > .08). 

Between-subjects analyses (collapsed across the re-feeding period) demonstrated main effects of both restriction (F_{1,36} = 53.39, p < .001, \eta^2 = 0.60) and food cue (F_{1,36} = 7.89, p < .01, \eta^2 = 0.18) on weight. AL rats weighed more than did CR rats, and rats exposed to a food cue weighed more than did non-exposed rats. No other effects reached significance.

Corticosterone

A repeated-measures ANOVA (with food-cue and restriction conditions entered as between-subjects factors, and time of measurement entered as a within factor) demonstrated a significant interaction between restriction and time on CORT, F_{1,36} = 18.64, p < .001, \eta^2 = 0.34 (Fig. 2). To follow-up on this interaction, t-tests were conducted (Bonferroni-corrected, p < .0125). Although there was a significant increase in CORT from pre- to post-stress for both AL rats (t_{19} = 14.4, p < .001) and CR rats (t_{19} = 3.55, p < .005), there were significant differences between CR and AL rats at post-stress (t_{18} = 3.59, p < .001), but not at pre-stress (t_{18} = 0.88, p = ns), with AL rats having higher post-stress CORT than did CR rats. This suggests that the AL rats were more responsive to the stress. An ANOVA conducted on the proportional change in CORT ([post – pre]/[pre × 100] supports this, as a main effect of restriction emerged (F_{1,36} = 4.37, p < .05, \eta^2 = 0.108), with AL rats having a higher proportional change in CORT (M = 426.1, SD = 487.8) than CR rats (M = 163.3, SD = 261.9).

ANOVA were also conducted on pre- and post-stress CORT, with food-cue and CR conditions entered as between-subjects factors. These analyses demonstrated a main effect of food cue on CORT, with food-cue-exposed rats exhibiting higher pre-stress CORT than non-exposed rats (F_{1,36} = 13.88, p < .001, \eta^2 = 0.30), and a trend towards higher CORT at post-stress (F_{1,36} = 2.97, p < .095, \eta^2 = 0.08). No significant interaction between food cue and CR emerged.

Intake

Values for chow intake were collapsed across the restriction and re-feeding periods respectively, and separate ANOVAs were conducted (Bonferroni-corrected, p ≤ .025). During the CR period, there was a main effect of restriction on intake for both 6-h (F_{1,36} = 406.08, p < .001, \eta^2 = 0.92; subjected to a log transform due to a homogeneity of variance violation) and 24-h intake (F_{1,36} = 58.84, p < .001, \eta^2 = 0.62). CR rats ate more in 6 h (M = 18.19, SD = 4.10) than did AL rats (M = 3.08, SD = 0.91). CR rats also ate more in 24 h (M = 36.21, SD = 2.43) than did AL rats (M = 30.65, SD = 2.25), reflecting the fact that CR rats were eating nearly all (approximately 95%) of their 2-day allotment of food within 24 h (leaving them with practically no food until their next feeding, whereas AL rats had unlimited food access).

During re-feeding, a significant effect of restriction on 6-h (but not 24-h) intake remained (F_{1,36} = 12.52, p < .001, \eta^2 = 0.26; subjected to a log transform due to a homogeneity of variance violation), with CR rats eating more (M = 8.76, SD = 2.08) than AL rats (M = 6.89, SD = 0.98). There was also a main effect of food-cue condition on 24-h intake (F_{1,36} = 6.17, p < .02, \eta^2 = 0.15, subjected to a cubic transform due to a homogeneity of variance violation), with cued rats eating more (M = 29.86, SD = 2.78) than non-cued rats (M = 27.43, SD = 3.05). No other significant effects emerged.

Discussion

We initially hypothesized that both CR and food-cue exposure would be stressful, and found partial support for our hypotheses. As expected, CR animals exhibited a blunted stress response relative to AL rats, with AL rats having a higher proportional CORT change than CR rats. Exposure to chronic stress has been found to attenuate the stress response (e.g., Cohen et al., 2006; but see Bhatnagar & Dallman, 1998), suggesting that CR may represent a chronic stress. Contrary to previous research (Jahng et al., 2007), CR rats did not exhibit higher baseline CORT compared to AL rats; however, increased baseline CORT has not been found ubiquitously, with some reports that CR leads to decreased CORT (Philippps, von Mayersbach, & Scheving, 1977). One possible reason for the discrepancies is the timing of food provision during CR, as there is evidence that the feeding schedule in relation to the light–dark schedule changes rats' circadian rhythms (e.g., Belda, Ols, Carrasco, & Armarrio, 2005). Given the relatively novel feeding schedule employed in this study, it remains unclear how the circadian rhythms of the CR rats were affected by this schedule; however, this presents a possible explanation for the lack of higher baseline CORT in CR rats.

We also predicted that chronic food-cue exposure would represent a stressor, leading to higher CORT and increased intake. Indeed, cued rats exhibited higher CORT than did non-cued rats. Although no interaction between CR and food-cue exposure emerged, and the difference in CORT did not reach statistical significance for the AL and CR groups individually (possibly due to a lack of power), the main effect suggests that even when food is freely available (and therefore hunger is not a concern), food-cue exposure may be stressful for ad lib fed animals. Further potency of the food cue is evidenced by the increased intake (and corresponding higher weight) of cued animals relative to non-cued animals during re-feeding. This is one of the first studies to show that chronic food-cue exposure has physiological and behavioral effects in rats.
An interaction between food-cue exposure and restriction emerged on body weight during the restriction period, with CR/cue rats weighing less during restriction than CR/no-cue rats. This pattern reversed during the re-feeding period: a main effect of cue condition on intake and body weight emerged, in which cued rats ate and weighed more than did non-cued rats (although the effect was not statistically significant for CR cued vs. non-cued rats alone). There is evidence that chronic stress leads to lower body weights in rats (Faraday, 2002). Therefore, the fact that CR/cue rats weighed less than the CR/no cue rats during restriction (though they received the same amount of food) is suggestive that CR/cue rats were more stressed. Research suggests that food-restricted “gorging” mice had a significantly lower body mass than did restricted non-gorging mice, with the authors suggesting that gorging mice are somehow less able to compensate for food restriction (Hambly et al., 2007). It is therefore possible that the lower weight in the CR/cue rats was the result of food cues increasing gorging behavior. Inspection of the means suggests that CR/cue rats ate somewhat more within 6 h of feeding (19.1 g) than did CR/no cue rats (17.3 g). Although this difference was not significant, these measurements were taken after 6 h of feeding, on limited occasions. Future research would benefit from more regular assessment of intake patterns to determine whether they account for body weight differences.

During the re-feeding period, CR rats continued to eat more than AL rats after 6 h (during the day), although their 24-h intake was not elevated. We infer that the feeding times of the CR rats shifted in comparison to AL rats. Rats are normally nocturnal eaters; however, during restriction, CR rats were fed after light onset. It appears that CR rats continued to eat more during the day, even after being returned to AL feeding. Although the idiosyncratic nature of the feeding schedule probably contributed to this pattern of increased daytime feeding, there are interesting implications of a shift away from nocturnal feeding. For example, the development of obesity leads to disruptions in nocturnal feeding patterns in rats (Fukagawa, Sakata, Yoshimatsu, Fujimoto, & Shiraishi, 1988). Further investigation into the feeding patterns of previously restricted rats by extending the re-feeding period, and studying meal parameters more closely across the light-dark cycle may be important. Future research should also take into consideration the fact that single-housing conditions and a laboratory temperature of 22 °C can represent a stressor to food-restricted animals (Gutiérrez, Baysari, Carrera, Whitford, & Boakes, 2006). The blunted stress response evident in CR rats can be viewed as a detrimental effect of CR. Although some researchers have conceptualized a blunted response to additional stressors as adaptive (Gursoy et al., 2001), there is evidence that a blunted cortisol response in humans who are chronically stressed can increase vulnerability to the development of a variety of disorders (Heim, Ehlert, & Hellhammer, 2000). This research has implications for the ‘obesogenic’ environment to which humans are exposed. Humans who would undergo a CR regime would undoubtedly be exposed to food cues, which could be associated with a variety of problems. Further research on the effects of food-cue exposure in the presence of CR on feeding patterns and gorging behavior is particularly warranted. The relatively short-term nature of this study makes it impossible to determine whether CR in the presence of food cues would reduce health and longevity benefits. The present study does, however, provide support for Vitousek’s (2004) assertion that additional research on the effects of CR is needed prior to applying it as a panacea for humans.

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References