
Physiological Markers of Activity-based Anorexia in Sprague Dawley Rats

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Activity-based anorexia is a robust finding in which rats placed on a restricted feeding schedule with access to an activity wheel will increase their running behavior and decrease their food consumption over time, resulting in death if not removed from the experiment. Though previous literature has examined the independent effects of food restriction or wheel running, few have examined these two factors combined and how they alter various physiological markers. The current study used a modified model of activity-based anorexia to examine the effects of wheel running with limited food consumption and limited food consumption alone on blood glucose, urinary ketone levels and weight loss. The current study involved three conditions: 1) food restriction with wheel access, 2) food restriction alone, and 3) an ad libitum control. A significance difference in weight loss and blood glucose levels was observed in both experimental conditions as compared to the control. However, no significant difference was observed in weight loss or blood glucose levels when comparing wheel running with food restriction and the effects of food restriction alone. Changes in ketone levels were only observed in the food restriction with wheel access condition, with all subjects showing trace amounts of ketones by the final day of experimentation.

Introduction

Activity-based anorexia is a phenomenon first founded by Hall and Hanford (1954) and further defined by Routenberg and Kuznesof (1967). Both studies found that when subjects are placed on a restricted feeding schedule of one to two hours of food access, and given the opportunity to run in an activity wheel after food was removed, food consumption would decrease while running activity increased. Left on this schedule for a period of seven to ten days, the subjects would eventually run to the point of death. Researchers have related this finding in rats to the symptoms and behaviors observed in anorexia nervosa within humans. More specifically, many of the core behavioral and physiological components of anorexia are also present in activity-based anorexia. These factors include a restriction of food consumption in the presence of hunger, dramatic weight loss, increase in activity level, and changes in hormone levels.

Physiologically, many of the effects of anorexia nervosa on the human body and endocrine system are also replicated in the animal model. The hypothalamic-pituitary adrenal (HPA) axis has been investigated as a primary factor for the development and maintenance of anorexia nervosa; however its role had yet to be defined (Duclos, Gatti, Bessiere, & Mormede, 2008). One study investigating the role of the paraventricular nucleus of the hypothalamus examined the down-regulation of this area (Rieg & Aravich, 1992). This study indicated that heavy dosage of clonidine, a drug which down-regulates this region of the hypothalamus, actually resulted in increased effects of activity-based anorexia.

Other studies focusing on the physiology of ABA looked at

the effects of corticosteroid, the rat equivalent of cortisol in humans, and its role in the activation of the HPA-axis during periods of starvation (Duclos, Gatti, Bessiere, & Mormede, 2008). In this study, experimenters used adrenalectomized rats and replaced the adrenal glands with pellets which held different concentrations of cortisol and allowed for low to high stimulation of the HPA axis. Cortisol is released in response to stress, and is a key component of fat placement and utilization. This study revealed that the greater the concentration of cortisol, the greater the effects of ABA. Along with previous findings, this study indicates that stress and cortisol may play important roles in the development and maintenance of anorexia nervosa.

Neuropeptide-Y, an appetite hormone that is secreted by the hypothalamus, is increased in both anorexia and ABA. However its effects on the behaviors of anorexia nervosa behaviors are contradictory and therefore are of interest as this hormone's role is to increase food consumption (Nergardh et al, 2007). This study proposed that in contrast to the hypothalamic theory of food intake the increase of neuropeptide-Y would increase running activity and decrease food intake. Neuropeptide-Y was shown to increase running and decrease food intake in rats placed on a restricted feeding schedule, however in the subject given ad libitum food access, food intake increased and wheel running did not change with the supplementation of neuropeptide-Y (Nergardh et al, 2007). This study further validated the importance of hyperactivity and restricted food intake on the development of ABA and the role of neuropeptide-Y may play, yet much remains unknown about how this hormone affects anorexia nervosa behavior maintenance in human subjects.

Though hormonal changes have been investigated, no

current research has investigated the combined effects of the exercise and food restriction on blood glucose and urinary ketone changes. One study examining the effects of food restriction schedules on alteration of glucose levels found that various levels of food restriction result in a decrease in blood glucose levels (Marinkovic et al 2007). There were three schedules of food restriction such that food intake was restricted to 50%, 25%, or 12.5% that of the rats free feeding level. This study found that the greater the food restriction, a greater decrease in observed serum glucose.

Further research examined the independent effects of fasting and exercise on changes in ketone and glucose levels (Balasse & Fery 1989). This study found that when rats are subjected to a restricted feeding schedule, their glucose levels decrease while their level of ketones increase over time. This is because the body began to utilize fat as an energy source as weight loss increased, and one of the by-products of fat metabolism are ketones. Ketone levels were also increased as exercise persisted over days, though a slight decrease was noted in glucose levels over days (Balasse & Fery 1989). This study looked at the effects of fasting and activity independently. This study is interested in the combined effects of these two factors.

The purpose of this study was to examine the changes in certain physiological markers following a modified model of the established activity-based anorexia paradigm. As opposed to other studies that examined hormonal changes and the individual effects of food restriction and wheel running, this study observed the effects of food restriction and food restriction with wheel running on changes in blood glucose, weight loss, and urinary ketone levels. Based upon the findings of previous literature, it is hypothesized that weight loss and blood glucose reduction will be greatest in the food deprived with wheel access condition, followed by an intermediate level in the solely food deprived condition, and little change will be observed in the control condition. Secondly, it is expected that urinary ketone levels will increase the most in the food deprived with wheel access condition, followed by an intermediate level in the solely food deprived condition, with no change in ketones observed in the control condition.

Materials and Methods

All procedures completed in this experiment were approved by the Institutional Animal Care and Use Committee. The subjects in this study were 12 female Sprague Dawley rats (4 individuals per experimental condition), approximately 100 days old, from the University of South Carolina Aiken Psychology Department Animal Vivarium. Subjects were randomly assigned to one of three experimental conditions: 1) food restriction with wheel access, 2) food restriction alone, and 3) an ad libitum control.

Subjects were housed individually in metal homecages for the duration of the experiment. Subjects with access to a running wheel were moved to an operant box with a 12 inch diameter wheel each evening. The operant boxes prevented any external noise from influencing running behavior in adjacent boxes.

The general procedure was a modified version of the established activity-based anorexia model used in the literature (e.g., Routtenberg et al., 1967): A habituation phase (Days 1-4) was followed by food restriction and wheel responding (Days 5-14). During Habituation all subjects received ad lib access to food and water in their individual homecage, with a 12-12hr Light-Dark cycle. Beginning on Day 5, subjects in the Food restriction alone (FR) and food restriction with wheel access (FW) condition were placed on a restricted feeding schedule in which food was administered for a one-and-a-half hour period daily, beginning six hours after the start of the light cycle. Subjects in the control group (CT) remained on an ad libitum food schedule throughout the entire duration of the experiment. Access to the running wheel was available during the 12 hour dark period on Days 5-14 for those subjects in group FW. For all groups on all days weights were recorded manually. Blood collection and urinalysis began on Days 3 and 4 in order to take baseline measurements for the participants. Measurements were subsequently taken every other day thereafter. Blood was collected following NIH recommended procedures, which involved snipping the last 1-2 mm of the tail. This allowed for the obtainment of 1-2 drops of blood which was then analyzed with a One Touch glucose monitor. Urine collection was completed by placing a sterile Petri dish underneath the subjects then rubbing two fingers along the spine of the rat from the shoulders down to stimulate urination. The urine collected was then analyzed for ketone levels using a urinalysis reagent strip. Daily weights were measured in grams using a standard laboratory scale.

A One-way ANOVA was used to test for baseline differences between the three groups. Results were analyzed using a Repeated Measures ANOVA, Tukey's Test and LSD Test to identify a differences were between the groups.

Results

Days 1-4: Habituation

Weight, glucose, and ketone levels observed on Days 3 and 4 were averaged across these two days, and a one-way analysis of variance revealed no significant difference in weight ($F(2,9) = 1.789, p = .222$) or glucose levels ($F(2,9) = .322, p = .733$) between the groups. No ketones were observed in the urine of any of the participants at this time.

Days 5-14: Food Restriction and Wheel Access

On these days subjects in the food restriction with wheel access condition (FW) were placed on food restriction with access to an activity wheel, subjects in the food restriction (FR) condition were placed on food restriction, and subjects in the control condition (CT) remained on ad libitum food access. Weight loss and changes in glucose were analyzed using a mixed model analysis of variance. As observed in Figure 1, weight losses were minimal in group CT, resulting in a fairly constant weight across the duration of the experiment. For groups FR and FW weight dropped steadily over the duration of the experiment with duration being a significant factor ($F(9,81) = 28.297, p < .001$). Weight loss in FR and FW differed significantly from the control group ($F(2,9) = 5.742, p < .025$) but while FR lost more weight overall, this difference

was not significant. A significant Group x Day interaction was also observed ($F(18, 81) = 9.771, p < .001$), revealing that differences from control were not notable until Day 6 of experimentation.

As Figure 2 reveals, group CT's blood glucose readings over the duration of the experiment remained fairly constant, whereas groups FR and FW showed decreasing levels of blood glucose. Blood glucose levels for FR ($p = .010$) and FW ($p = .039$) differed significantly from control, however, there was no significant difference in glucose levels between group FR and FW ($p = .417$).

Ketone levels could register on a nominal scale which indicated either negative, trace, small, moderate, or large levels. All participants began the experiment negative for urinary ketones. The only increase in ketone levels was observed in group FW. A trace amount of ketones was observed on Day 8 for one subject, and all subjects in this condition expressed trace levels of ketones by Day 14. For Groups FR and CT, ketone levels remained negative on all days.

Discussion

The purpose of this study was to examine the combined effects of food deprivation and wheel activity as compared to food deprivation alone and ad libitum on blood glucose, weight loss, and urinary ketones. Significant differences in weight loss did exist between the experimental conditions and control. Unexpectedly, the group that was food deprived alone lost more weight than the group that was food deprived and had wheel access, although this difference was not significant. Although both experimental groups differed significantly from control, the notion that food restriction combined with wheel access would augment the level of weight loss observed in this modified model of activity-based anorexia was not supported. In this study the actual amount of food consumed was not measured. It might be possible that rats with access to exercise increased their food intake during their 90 minute feeding period to meet caloric demands thereby ameliorating weight loss. This could be better controlled in future studies by controlling the amount of food offered rather than just the duration of feeding time.

Glucose levels also decreased significantly in Groups FR and FW, while no reduction in glucose levels was observed for Group CT. Group FR and FW did not differ significantly from each other however. Interestingly, group FR saw a steady decrease in glucose, while, as indicated by Figure 2, it appears a decrease in glucose levels for Group FW was attenuated on Day 10. If the rats with wheel access did consume more food to meet caloric needs they would be consuming more glucose as well. Cartee et al. (1989) found that under strenuous and prolonged exercise there is not enough insulin present in the blood to utilize extra glucose taken in the diet. Blood glucose levels therefore rise as opposed to the expected decrease in glucose levels usually observed as an effect of short term physical activity. This may help to explain the glucose attenuation seen in this group on day 10.

A change in urinary ketone levels was only observed in Group FW, with Group FR and Group CT showing no change in ketone levels. However, Group FW showed trace levels of ketones by Day 8. This indicates that a greater level of fat metabolism was occurring in this group as compared to Group CT and FR. After a longer duration on this schedule it is expected that the FR group would also show trace amounts of urinary ketones as body fat is depleted.

Though the current study found that no significant difference in weight loss or blood glucose levels between rats that have been food deprived alone and those that have been food deprived with access to an exercise wheel, future studies may find significant differences by increasing the amount of wheel access to be nearer to the typical model of activity-based anorexia (24 hours) or increasing the length of the study. Also, future research might examine hormonal changes in association with these experimental conditions. Female subjects were used in this study, but it may be that different results would be obtained using male subjects, as they do not have an estrous cycle that may affect the levels of glucose in the blood. Future studies may also wish to examine the effects of ABA with novel food sources, such as examining the effects of a diet higher in sucrose, fructose, or fat.

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