THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF NUTRITIONAL SCIENCES

EFFECTS OF VARIOUS INTERVENTIONS ON BINGE-TYPE BEHAVIOR IN RATS

MAGDALENA JURCZYK
Spring 2012

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Nutritional Sciences
with honors in Nutritional Sciences

Reviewed and approved* by the following:

Rebecca Corwin
Associate Professor of Nutritional Neuroscience
Thesis Supervisor

Jill Patterson
Assistant Professor of Nutrition
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

When non-food-deprived rats are given intermittent access to vegetable shortening (a semi-solid fat used to make baked goods), consumption of the shortening is significantly greater than when daily access is provided. This binge-type behavior has been observed in several studies from this laboratory. The goal of the present study was to examine various interventions on binge-type behavior in rats. Four groups of male Sprague-Dawley rats were used, all of which had continuous access to a standard rodent diet and water. The D (daily access) group had 1-hr unlimited access to shortening every day throughout the duration of the study. The MU, MD, and ML (intermittent) groups all had 1-hr unlimited access to shortening on Monday, Wednesday, and Friday when on protocol. During intervention 1, the MU group remained on the protocol schedule with 1-hr unlimited access to shortening on Monday, Wednesday, and Friday, the MD group was placed on the daily schedule (1-hr unlimited access to shortening every day), and the ML group was given 1-hr "clamped" access (2.6-2.8 grams) to shortening on Monday, Wednesday, and Friday. Intervention 1 lasted 6 weeks. After intervention 1, the MD and ML groups were returned to the original protocol for 3 weeks to determine any long term effects of the intervention. After this period of time, intervention 2 was implemented. During intervention 2, all three intermittent groups were provided 1-hr access to a limited amount of shortening (2.6-2.8 g) on Sunday, Tuesday, Thursday, and Saturday and 1-hr access to unlimited shortening on Monday, Wednesday, and Friday. Intervention 2 lasted 6 weeks. Finally, after intervention 2 the MD, ML, and MU rats were returned to the original protocol for the remaining 2 weeks of the study. During intervention 1, the MD group consumed significantly less shortening on the daily schedule than when they were on the intermittent schedule. Furthermore, it took longer for the MD group to return to bingeing after the intervention was removed compared to the ML group.

Another finding of this study is that intervention 2 seemed to work better than intervention 1 in that the MU and MD rats continued to not binge for an entire week during protocol 3 after intervention 2 compared to protocol 2 after intervention 1, in which the rats (MD group) went back to bingeing within 3 days. These results suggest that consuming a small amount of palatable food every day, while controlling one's own intake, has a slight "protective effect" against bingeing behavior. Furthermore, this study demonstrated that interventions are successful while in place; however, once the intervention is removed and rats are subjected to their initial bingeing environment, the binge behavior returns.

TABLE OF CONTENTS

INTRODUCTION

A *binge* is defined as eating in a discrete period of time an amount of food that is definitely larger than what most individuals would eat under similar circumstances. A "discrete period of time" refers to a limited period, usually less than 2 hours. More specifically, an episode of binge-eating is characterized by both of the following (DSM-IV-TR, 2000):

1. Eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances
2. A sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating)

The binge eating behavior is one that can have several origins. It may be induced by a variety of factors including stress and emotional hurt in humans or an intermittent schedule in rats (Corwin, 2011). If the behavior is severe enough it can develop into several disorders including Bulimia Nervosa (BN), and Binge-eating Disorder (BED), and the binge-eating/purging type of Anorexia Nervosa (AN).

The essential features of AN are that the individual refuses to maintain a minimally normal body weight, is intensely afraid of gaining weight, and exhibits a significant disturbance in the perception of body shape or size. The subtype of binge-eating/purging AN is additionally characterized by regular binge-eating, which is followed by inappropriate compensatory behavior, such as self-induced vomiting (purging) or the misuse of laxatives, diuretics, or enemas, as well as excessive exercise and self-induced starvation (DSM-IV-TR, 2000). As a result of these compensatory behaviors, the energy consumed during the binge does not promote weight gain.

Similarly, BN is influenced by body shape and weight perception and is also characterized by binge eating followed by inappropriate compensatory methods, which prevent weight gain (DSM-IV-TR, 2000). However, bulimics do not meet the reduced body weight criterion that defines anorexia nervosa.

BED is the most common of the eating disorders. This disorder is characterized by recurrent episodes of binge eating that are not followed by the inappropriate compensatory behaviors that characterize BN. As a result, obesity is far more common in those with binge-eating disorder. Bingeing episodes are associated with at least three of the following (DSM-IV-TR, 2000):

1. Eating much more rapidly than normal
2. Eating until feeling uncomfortably full
3. Eating large amounts of food when not feeling physically hungry
4. Eating alone because of feeling embarrassed about how much one is eating
5. Feeling disgusted with oneself, depressed, or very guilty afterwards

The lifetime prevalence of anorexia nervosa, bulimia nervosa, and binge-eating disorder are estimated to be: 0.9%, 1.5%, and 3.5% among women, and 0.3%, 0.5%, and 2.0% among men, respectively (Hudson et al., 2007). As is true for the other eating disorders, binge-eating disorder can have profound long-lasting effects on the overall mental and physical health of the individual affected. Prevalence of BMI greater than or equal to 40 is higher in individuals with a lifetime diagnosis of binge-eating disorder compared to respondents without any eating disorder (Hudson et al., 2007). Obesity is a huge problem in the United States and can lead to various life-threatening conditions including type 2 diabetes, cardiovascular disease, hypertension, stroke, and certain forms of cancer. However, only 35% of all those who regularly binge (BED, BN and

those with subthreshold (ST) BED) are overweight or obese; the other 65% are not (Corwin, et al., 2011) and therefore, it is crucial to address and understand the problems associated with individuals who binge but maintain an acceptable weight.

Binge behavior of any type is associated with comorbidities that can have a dramatic impact on both the physical and mental state of an individual. In adolescents, for instance, 53.0% with BN, 34.4% with binge-eating disorder BED, and 18.3% with subthreshold binge-eating disorder ST-BED have suicide ideation during their lifetime compared to only 11.2% of adolescents with no eating disorders. Furthermore, 25.9% of adolescents with BN, 5.1% with BED, and 5.1% with SBED plan to commit suicide compared to 3.6% of adolescents with no eating disorder, which is of great importance to know because those who formulate a plan for suicide are more likely to follow through and attempt it. Finally, 35.1% of adolescents with BN, 15.1% with BED, and 5.3% with SBED attempt to kill themselves compared to 3.0% of adolescents with no eating disorders (Swanson et al., 2011). These are striking statistics and suggest that "abnormal" or unhealthy eating behaviors accompany most of the teen suicides within the U.S. today.

Adolescents are not the only age group experiencing unhealthy eating behaviors and their associated misery. Hudson, et al. (2007) studied the prevalence and correlates of eating disorders and of overall binge eating behavior in adults. According to this research, lifetime prevalence of BN, BED, SBED, and any binge eating in adults were 1.0%, 2.8%, 1.2%, and 4.5%, respectively. In regards to comorbidity status, 94.5% with BN, 78.9% with BED, 63.6% with SBED, and 76.5% with any binge eating met criteria for at least 1 of the following: panic disorders, generalized anxiety disorder, post-traumatic stress disorder, obsessive compulsive disorder, mood disorders, impulse control disorders, and/or substance use disorders.

As depicted above, BN, BED, ST-BED, and any binge eating, all of which have the common feature of bingeing on food, are part of a constellation of disturbances that has a significant impact on the life of an individual and on our health care system. For these reasons, developing a better understanding of binge eating behavior, and determining effective prevention and treatment strategies for this behavior are crucial to both science and medicine.

In humans, the leading evidence-based treatment for eating disorders has been cognitive behavioral therapy. This therapy is designed to treat eating disorder psychopathology rather than an eating disorder diagnosis, with its exact form depending on an individualized formulation of the processes maintaining the disorder. During the course of the treatment, patients are helped to recognize the effect that their rigid dietary rules and dieting habits have on their lifestyle. With patients who binge, particular attention is paid to "food avoidance" (the avoidance of specific "forbidden" foods, such as cake, cookies, fried foods, bread, ice cream, butter, snacks, pizza, candy, and chocolate) as this is thought to be a major contributory factor to their binge behavior (Kales, 1990). According to this cognitive behavioral therapy treatment, these patients need to systematically re-introduce these forbidden foods" into their diet for successful recovery (Murphy et al., 2010).

This idea of re-introducing "forbidden foods" into one's daily diet in a manner that does not permit bingeing is the foundation of the present study. Previous research has shown that limiting access to an optional source of dietary fat induces a binge-type pattern of eating in rats (Corwin, et al., 1998; Dimitriou et al., 2000; Thomas et al., 2002). In this rat model, non-food-deprived rats that are given intermittent (Mon, Wed, Fri) brief (1-2 hr) access to hydrogenated vegetable shortening (Crisco ® brand All-Vegetable Shortening J.M Smucker Co., Orrville, OH) consumed significantly greater amounts than those rats that were provided more frequent (daily)

brief access to the shortening. This rat model is consistent with the human "forbidden foods" hypothesis (Kales, 1990). "Forbidden foods" are typically high in fat, and individuals trying to lose weight often restrict access to these foods. These foods, however, are consumed in large quantities during a binge. This suggests that foods upon which people binge are those to which they have limited their own access.

This lab, as well as others studying binge eating, uses an animal model, and more specifically a rat model, to address important clinical questions. Animal models are used instead of human subjects for several reasons, including ethical and financial reasons. Using animal models is much less limiting than doing experiments on humans and one can study a lot more with animals. For example, with research animals one can monitor a given study at any time of day, whereas with people doing so is invasive and unrealistic. Also, studying animals is much more inexpensive and time-saving compared to human studies. The research animals used in the present study were Sprague Dawley rats.

There are several rat models that can be used when studying binge eating. One such model is the sugar-addiction model. In this model rats are maintained on daily 12-hr food deprivation, followed by 12-hr access to a 25% glucose or 10% sucrose solution and rodent chow. After a few days on this schedule, the rats begin to escalate their daily intake and binge on the sugar, unlike the control animals fed the sugar *ad libitum*. Another rat model for binge-eating is the history of dieting + stress (HD + stress) model. This model promotes bingeing by pre-exposing rats to a history of dieting and to stress, much like what individuals who clinically binge-eat experience. A history of dieting is stimulated by subjecting the rats to cycles of food restriction and re-feeding. Stress is administered with 3 s of 0.6 mA foot shock just prior to the feeding test. After a few restriction/feeding and stress cycles, the HD + stress group eats

statistically more of the palatable food than the control group (Corwin, et al., 2011).

The model that was used for the present study is the limited access model. To stimulate binge-type eating in this model, rats are given sporadic (generally 3 times per week), time-limited (generally 1-2hr) access to palatable food in addition to continuously available chow. Unlike the HD + stress and sugar overeating models, the limited access model does not make use of previous or current food deprivation to stimulate bingeing. Rats in this model are never food deprived, as they have continuous access to chow and water at all times. Two groups of rats are used in this model, one that has brief, time-limited access to the palatable food (in the present study it is vegetable shortening) every day (daily access control group), and another that has brief time-limited access to the palatable food a few times a week (in the present it is Monday, Wednesday, and Friday) (intermittent access binge group). The result of this type of intermittent schedule is that the intermittent access group consumes more shortening within the brief shortening access period relative to the rats with daily brief shortening, i.e. the intermittent group binges on the shortening (Corwin, et al., 2011).

The current study used the limited access model, described above, to examine ways in which binge-type eating in rats can be attenuated or reversed once it is established. Doing so would provide preclinical information as to how bingeing in humans might be treated. A study conducted in the Corwin lab already examined behavioral methods to prevent bingeing (Wojnicki et al., 2008). However, there has been little behavioral research on how to reverse the binge behavior once it is already established. Most research on reversing bingeing has focused on pharmaceuticals. The research for this thesis took a different approach by attempting to attenuate binge behavior, simply through behavioral interventions.

The prevention method in the Wojnicki, et al. (2008) study was to limit the quantity (or

"clamp" the intake) of shortening before rats had a chance to binge. In that study, two groups of rats (I, I-QL) were placed on an intermittent schedule of access (Monday, Wednesday, and Friday) to shortening while two other groups (D, D-QL) were placed on a daily schedule of access. For the I and D groups the quantity of shortening provided for the 1-hr period of availability was unlimited, as is typical in the studies using this model. For the I-QL and D-QL groups the quantity of shortening provided during the 1-hr period was 2.0-2.2 grams, which was based upon the average amount that was typically consumed by groups with a daily access schedule in other studies. Rats were on these feeding protocols for 5 weeks. At the end of the 5 weeks intake of shortening in the I (intermittent binge) group exceeded that of the Daily control group, i.e. the I group binged. In the next part of the study, all four groups (I, I-QL, D, D-QL) were given an unlimited amount of shortening during the 1-hr period of shortening availability under their respective schedules of access. Thus, I and I-QL were now maintained on the standard intermittent protocol, and D and D-QL were on the standard daily protocol. The results of this study showed that the manipulation of limiting the quantity was successful. That is, in the second part of the study, binge size in the I-QL group was smaller than that of the I group, even though both groups could now eat as much as the wanted during the shortening access period. This demonstrates that quantity-limited shortening intake reduces subsequent quantity-unlimited (binge) intake under an intermittent schedule of access in rats, and suggests that such "clamped" intakes can possibly be used as successful prevention methods for binge-eating behavior. In other words, a quantity-limited history attenuated subsequent binge size.

The results of the prevention study described above encouraged us to try the "clamped" intake method as an intervention. The goal of doing so was to determine if quantity-limited shortening intake could reduce quantity-unlimited (binge) intake once the binge behavior had

already been established. Three interventions were tested within two intervention periods. In the first intervention period, the MD group went from an intermittent schedule (M, W, F) of 1-hr unlimited shortening access to a daily schedule of 1-hr unlimited access and the ML group went from an intermittent schedule (M, W, F) of 1-hr unlimited shortening access to an intermittent schedule of 1-hr limited or "clamped" access (2.6-2.8 grams). Furthermore, after the intervention period, the regular binge protocol was reintroduced. Our hypothesis was that the MD rats would consume less shortening (i.e., not binge) during the intervention, when having access to the palatable food every day as opposed to only three days a week. This hypothesis was based on the results of the previous "clamp" study, which showed that the shortening intakes of the daily group were always significantly less than the intakes of both intermittent groups (Wojnicki, et al., 2008). Furthermore, we hypothesized that in the ML group binge size would decrease after the intervention, i.e., be attenuated, when put back on an unlimited intermittent schedule after having limited access to the palatable food. This is because in the "clamp" study the intermittent rats who had limited access to shortening (I-QL) consumed significantly less shortening than the intermittent group who had unlimited access to shortening (I) when put back on the intermittent schedule (Wojnicki, et al., 2008).

In the second intervention period, all of the intermittent groups were given 1-hr "clamped" shortening intake (2.6-2.8 g) on Tuesday, Thursday, Saturday, and Sunday and 1-hr unlimited shortening intake on Monday, Wednesday, and Friday. This method was one in which the rats had access to the palatable food every day, but had a limited intake on four days of the week and unlimited access on the other three days of the week. Our hypothesis was that the rats would consume less shortening (i.e., not binge) with this intervention on the basis of previous results, which showed that rats with daily unlimited access don't binge. We hoped that providing

the rats with some amount of shortening every day would attenuate binge behavior even though some of the days provided only limited amounts. Furthermore, we hoped that limiting intake on some days of the week (Tues, Thurs, Sat, Sun) would allow the rats to implement an element of self-control when faced with unlimited amounts of shortening on the other days of the week (Mon, Wed, Fri).

Ultimately, the present study aimed to explore intervention methods that would reduce binge intake using preclinical testing in rats. It asked and attempted to answer the questions: Can binge behavior be reversed or attenuated by a behavioral intervention? If the binge eating behavior is already learned will any intervention, and if so what intervention, allow the bingeing behavior to remain suppressed even if the original "binge-inducing" environment is reintroduced?

Cognitive behavioral therapy recommends the reintroduction of avoided or forbidden food into the diet of patients who binge eat. However, to our knowledge the effects of limiting a palatable food along with allowing daily consumption of this food on binge behavior once bingeing has been established (i.e., as an intervention) has never before been scientifically tested in either humans or rats. The present research was designed to explore possible intervention methods for the binge eating behavior in rats using the limited-access model of binge-type eating.

MATERIALS AND METHODS

Animals

Forty eight male Sprague Dawley (Harlan, Indianapolis, IN) rats, 60 days of age and weighing 274-303g (287.5 +/- 0.85 g) at the start of the study, were individually housed in

hanging stainless steel wire cages in a temperature- and humidity-controlled environment placed on a 12:12 light:dark cycle. All rats had continuous access to tap water and to a nutritionally complete commercial laboratory rodent diet (Laboratory Rodent Diet 5001, PMI Feeds, Richmond IN; percent of calories as protein: 28.05%, fat: 12.14%, carbohydrate: 59.81%; 3.3 kcal/g) placed in hanging metal food hoppers at the front of the cage. All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

Bingeing Procedure

After seven days of adaptation to the vivarium, body weights were recorded and solid vegetable shortening (Crisco® All-Vegetable shortening, J.M Smucker Co., Orrville, OH) was provided during a single overnight period. Four groups of 12 rats each were then matched by body weight and the amount of overnight shortening consumed [p NS, $F < 0.20$ for both measures].

For the next six weeks chow and water were available ad libitum to all groups. Shortening was provided for 1h in glass jars clipped to the front of the cage, starting 2 hours prior to the start of the dark cycle. For three groups of rats shortening was provided on an intermittent basis (Mondays, Wednesdays, and Fridays), while for the fourth group (designated as the "D" group) shortening was provided on a daily basis. During the sixth week of the study 24 hr chow intake was also measured so that total daily energy intake could be assessed.

After the sixth week of the study, the three intermittent groups were recombined into one group and then matched for body weight and the average amount of shortening intake during weeks 5 and 6. The goal of recombining the 3 intermittent groups was to have identical groups with respect to shortening intake and body weight to be able to assess the effects of the intervention procedure. Three of the 36 intermittent rats failed to consistently consume more

than 1 gram during the access periods and were eliminated from the study. In order to recombine the three intermittent groups such that there were no differences among them, an additional 3 rats were eliminated from the study. After reassigning rats to the three intermittent groups (n=10 each) there were no differences among any of the intermittent groups and the daily group with respect to body weight [$F(3,38) = 0.19$, $p<0.9040$]. In addition, there were no differences among the intermittent groups with respect to the average amount of shortening intake for week 6 [$F(2,27) = 0.0$, $p < 0.9983$], but there was a significant difference between the intermittent groups and the daily group [$F(3,38) = 4.45$, $p<0.0090$], as expected.

1A Intervention 1

After reassigning the intermittent rats to groups, one of the intermittent groups (MU) was provided 1 hr access to an unlimited amount of shortening on Mondays, Wednesdays, and Fridays, i.e. this group continued to be maintained on the same intermittent protocol that was in place during the first 6 weeks. A second intermittent group (ML) was provided 1 hr access to a limited amount of shortening (2.6-2.8 grams) on Mondays, Wednesdays, and Fridays. The third intermittent group (MD) was provided daily 1 hr access to an unlimited amount of shortening as was the daily (D) group. These conditions were in effect for 6 weeks, and during the sixth week of the study 24 hr chow intake was also measured so that total daily energy intake could be assessed.

1B Bingeing Procedure

Following the above intervention, the MD and ML groups were returned to the intermittent bingeing protocol, and MU continued on that protocol, for 3 weeks to assess the effects of the intervention. The D group continued to be maintained on the daily protocol. During

the third week of the study 24 hr chow intake was also measured so that total daily energy intake could be assessed.

2A Intervention 2

Following the third week of the return to the bingeing procedure all three intermittent groups were provided 1 hr access to a limited amount of shortening (2.6-2.8 grams) on Sundays, Tuesdays, Thursdays and Saturdays and 1 hr access to an unlimited amount of shortening on Mondays, Wednesdays, and Fridays. These environmental arrangements combined and capitalized on the histories of the MD and ML groups as well as being a novel condition for the M group. These conditions were in effect for 6 weeks, and during the sixth week 24 hr chow intake was also measured so that total daily energy intake could be assessed. The D-group continued to have 1-hr access to an unlimited amount of shortening every day.

2B Bingeing Procedure

Following the above, the intermittent groups were returned to the intermittent bingeing protocol for 2 weeks to assess the effects of the intervention.

Statistics

Average 1-hr shortening intakes on Mondays, Wednesdays and Fridays were analyzed. To assess changes in intake across time, 1-hr intakes were analyzed via 2-way analysis of variance (ANOVA) (Group X Time) with time as the repeated measure. For analyses across the entire study, energy intakes were normalized to body weight$^{0.67}$ (Heusner, 1985), in order to determine if changes in intake were independent of changes in body weight. Duncan's Multiple Range Test was used for post-hoc follow-up tests.

RESULTS

Bingeing Procedure (Protocol)

As stated above the intermittent groups were re-grouped at the end of week 6 such that there were no differences among them, but each intermittent group consumed significantly more shortening than the D group [1-way ANOVA $F(3,38) = 4.45$; $p < 0.01$; Duncan's post-hoc ps < 0.05] (Fig. 1, top panel). Total energy intake (chow + shortening) during week six was analyzed via 2-way ANOVA (group x day). There was no main effect of access schedule [$F(3,38) = 0.46$, p < ns], but there was a main effect of day [$F(6,228) = 67.48$, $p < 0.0001$], and a day x access schedule interaction [$F(6,228) = 7.52$, $p < 0.0001$]. The effect of day was due to the increased energy intake on the days that the INT groups got shortening and chow (Monday, Wednesday, and Friday), and the reduced energy intakes on the days that they only got chow (Sunday, Tuesday, Thursday, and Saturday), with intakes being significantly different from D on most days (ps < 0.05, Duncan's Multiple Range Test). However, neither the average energy intake for the week (Fig 1, bottom panel), nor body weight (not shown) were different among the groups (ps = ns). That is, the intermittent rats effectively compensated for the excess energy consumed during the binge by undereating on non-binge days.

1A Intervention 1

With respect to shortening intake for the 6 weeks of the first intervention period there was an effect of intervention [$F(3,38) = 5.19$, $p < 0.0042$], an effect of week [$F(5,190) = 16.43$, $p < 0.0001$] and a week x intervention interaction [$F(5,190) = 2.96$, $p < 0.0003$] (data not shown). These results were expected for the ML group which was by design limited in the amount of shortening available on Mon.,Wed. &Fri. Comparison among the groups for week 6 showed an

effect of intervention [$F(3,38) = 6.87$, $p < 0.0008$] (Fig 2, top panel). Specifically, the MU group consumed more shortening than any other groups; MD and ML did not differ from the D group, although they did differ from each other (MD > ML). In short, the MD and ML interventions successfully returned shortening intake to control (D) levels, in rats with a binge history.

With respect to total energy intake (chow + shortening) during each day of week 6, there was no effect of access schedule [$F(3,38) = 2.06$, $p < 0.1221$], but an effect of day [$F(6,228) = 19.05$, $p < 0.0001$], and a day x access schedule interaction [$F(6,228) = 12.49$, $p < 0.0001$]. The effect of day was primarily due to increased energy intake by the MU group on the days that shortening was provided (M,W, F) and decreased energy intake on the other days of the week. The day x schedule interaction was due to differences among the groups on the different days. The MU group consistently consumed more total energy than the D control group on the days that shortening was available (MWF) and less total energy than the D group on the other days of the week (ps < 0.05, Duncan's Multiple Range Test). Neither of the other groups was consistently different from D. Although day to day differences were present, average energy intake across the week did not differ among the groups [$F(3,38) = 2.06$, $p < 0.1214$] (Fig 2, bottom panel).

Throughout this period body weights did not differ among the groups as there was no effect of intervention [$F(3,38 = 0.58$, $p < 0.6321$], an effect of week [$F(5,190) = 772.99$, $p < 0.0001$], but no effect of week x intervention interaction [$F(5,190) = 1.06$, $p < 0.3997$]. The effect of week was due to an increase in the body weights of all rats across time (data not shown).

1B Bingeing Procedure

The second bingeing protocol period followed the first intervention and lasted 3 weeks.

Shortening intake was affected by access schedule [$F(3,38) = 5.14$, $p < 0.0044$], and week [$F(2,76) = 10.60$, $p < 0.0001$]. Furthermore, week and access schedule interacted [$F(6,76) = 5.92$, $p < 0.0001$] due to the somewhat reduced intake in the MD group during the first two weeks (Fig. 3 top panel).

With respect to total energy intake (chow + shortening) during each day of week 3, there was no effect of access schedule [$F(3,38) = 0.83$, $p < 0.4850$], but an effect of day [$F(6,228) = 87.55$, $p < 0.0001$], and a day x access schedule interaction [$F(18,228) = 15.96$, $p < 0.0001$]. These effects are similar to those described for the first bingeing protocol period. That is, the effect of day was due to the increased energy intake on the days that the INT groups got shortening and chow (Monday, Wednesday, and Friday), and the reduced energy intakes on the days that they only got chow (Sunday, Tuesday, Thursday, and Saturday), with intakes being significantly different from D on most days ($ps < 0.05$, Duncan's Multiple Range Test). Again, with respect to the average total energy intake for the week, there were no differences among the groups [$F(3,38) = 0.84$, $p < 0.4830$] (Fig 3, bottom panel).

Body weights also did not differ among the groups during any of the three weeks. There was no effect of schedule of access [$F(3,38) = 0.69$, $p < 0.5632$], an effect of week [$F(2,76) = 239.28$, $p < 0.0001$] due to all of the rats gaining weight, but no week by access schedule interaction [$F(6,76) = 1.51$, $p < 0.1872$] (data not shown).

2A Intervention 2

With respect to shortening intake for the 6 weeks of the second intervention period there was no main effect of intervention [$F(3,38) = 1.96$, $p < 0.1367$], an effect of week [$F(5,190) = 10.10$, $p < 0.0001$] and a week x intervention interaction [$F(15,190) = 1.82$, $p < 0.0343$]. The absence of an effect of intervention was due to an absence of differences in shortening intake

among the groups collapsed across the 6-wk intervention period. The effect of week and interaction of week by intervention were due to initial differences among the groups during the first week and the gradual convergence of intake by weeks 5 and 6 (Fig. 4, top panel for week six data). Specifically, during week 1, the MU, MD, and ML groups all consumed significantly more shortening during the Monday, Wednesday, and Friday access period than the D group (Duncan's Multiple Range Test, ps < 0.05). These differences gradually disappeared due to increases in intake by the D group; by weeks 5 and 6 there were no differences among the groups.

With respect to total energy intake (chow + shortening) during each day of week 6, there was no effect of access schedule [$F(3,38) = 1.09$, $p < 0.9627$], but an effect of day [$F(6,228) = 12.69$, $p < 0.0001$], and a day x access schedule interaction [$F(18,228) = 2.53$, $p < 0.0008$], due to differences among the groups during individual days. However, with respect to the average total energy intake for the week, there were no differences among the groups [$F(3,38) = 0.09$, $p < 0.9635$] (Fig 4, bottom panel).

Throughout this period body weights did not differ among the groups as there was no main effect of intervention [$F(3,38) = 0.35$, $p < 0.7868$]. There was an effect of week [$F(5,190) = 255.64$, $p < 0.0001$], but no effect of week x intervention interaction [$F(15,190) = 0.98$, p ns]. The effect of week was due to an increase in body weight in all rats across weeks (data not shown).

2B Bingeing Procedure

The third bingeing protocol followed the second intervention and lasted 2 weeks. There was an effect of access schedule [$F(3,38) = 3.48$, $p < 0.0251$], an effect of week [$F(1,38) = 30.99$, $p < 0.0001$], and a week x access schedule interaction [$F(3,38) = 3.03$, $p < 0.0411$] (Fig. 5, top panel). These effects were because during the first week only the ML group consumed

significantly more shortening than the D group (Duncan's Multiple Range Test; $p < 0.05$), but during week 2, all the intermittent groups consumed significantly more shortening than the D group (Duncan's Multiple Range Test; ps < 0.05).

Body weights did not differ among the groups. There was no effect of schedule of access [$F(3,38) = 0.27$, $p < 0.8491$] nor was there a week by access schedule interaction [$F(3,38) = 1.81$, $p < 0.1619$]. There was, however, a main effect of week [$F(1,38) = 22.06$, $p < 0.0001$] due to all of the rats gaining weight.

Shortening intake normalized to body weight$^{0.67}$ during the last week of each of the three binge protocols was analyzed in order to assess behavioral stability across time, and to determine any possible long-term effects of the different interventions. There was an effect of access schedule [$F(3,38) = 5.39$, $p < 0.0034$], and an effect of week [$F(2,76) = 3.54$, $p < 0.0340$], but no week by access schedule interaction [$F(6,76) = 1.11$, $p < 0.3624$]. The effect of week was due to small increases in shortening intake that occurred across the study, even when normalized to body weight. However, when each group's data were analyzed via 1-way repeated measures ANOVA, no differences among weeks were detectable for any of the groups. Thus, the increases were relatively small and only became significant when all rats were included in the larger 2-way ANOVA. During each of the three bingeing protocols, all of the intermittent groups consumed significantly more shortening than the daily group (Duncan's Multiple Range Test ps < 0.05, Fig. 5, bottom panel).

DISCUSSION

Two new findings are reported in this study. First, rats that received vegetable shortening every day (the D group, the MD group during intervention 1, and all groups during intervention

2) consumed less during a binge opportunity than when the intermittent protocol was in place. Second, intervention 2 (in which the rats consumed something every day with restricted access to shortening on Tuesday, Thursday, Saturday, and Sunday, but unlimited access on Monday, Wednesday, and Friday) had a greater subsequent intake reducing effect than intervention 1, in which the MD group received unlimited access to shortening every day and the ML group received a limited amount on Monday, Wednesday, and Friday. These findings introduce critical points that may prove to be beneficial in the treatment of binge behavior in the clinical setting.

The first key finding from this research is that having the opportunity to consume a palatable fatty food every day appears to offer some "protection" against bingeing, even after a history of bingeing has been established. Evidence for this is provided by the D and MD groups. The D group did not have a binge history and consistently ate less than the intermittent groups during each protocol, as has been shown in previous research in which the limited access binge model was used (Corwin, et al., 1998; Wojnicki, et al., 2008). The present study extends this research by showing that during intervention 1 both the D group (which stayed on the daily schedule) and MD group (which went from the intermittent binge schedule to the daily schedule) consumed less shortening than the MU group, which was kept on the intermittent binge schedule. In short, when rats with a M,W,F binge history were allowed to consume shortening every day, they no longer binged. This was evident not only during the shortening access period (Fig 1 top), but also during the 24-hr intake period (Fig 1 bottom). Twenty-four hour energy intake in the MD and MU groups approximated that of the D group during intervention 1. In contrast, the MU group overate on binge days and underrate on non-binge days (relative to the D group), as has been shown in previous studies (Corwin, et al., 1998; Corwin, 2004).

These findings extend those of a previous report (Corwin, et al., 1998). In that study one

group of rats was maintained on the Monday, Wednesday, Friday intermittent binge schedule during the first two weeks of the study (2-hr access to shortening on Monday, Wednesday, and Friday), then switched to a daily access condition (2-h access to shortening every day) for 2 weeks, and then switched back to the binge schedule for the last 2 weeks of the study. Another group (LHL) was maintained on the daily access schedule during the first 2 weeks of the study, then switched to the intermittent binge schedule for 2 weeks, and then switched back to the daily access schedule for the final 2 weeks of the study. The findings of this study showed that rats from both groups ate less shortening when switched from the intermittent binge schedule to the daily schedule. The present study extends these results by showing that this is true even for extended periods, as the current study had 6 weeks of binge protocol, while the previous study had only 2 weeks.

When the intermittent binge protocol was reintroduced after intervention 1, the MD group took longer to return to bingeing than did the ML group. This suggests that consuming a small amount of a "forbidden" food every day, particularly when the individual is able to control the amount, introduces an element of self-control. This finding is consistent with what is suggested in cognitive behavioral therapy in humans, i.e., that individuals should reintroduce their "forbidden" foods into the diet so that they do not end up bingeing on these foods when faced with them (Murphy,2010). Consuming some shortening every day, while controlling their own intake, had a slight "protective effect" against subsequent bingeing in the MD rats. In the ML group (which went from unlimited access to shortening on M, W, F to clamped access to shortening on M, W, F) however, the "clamping" intervention did not seem to have any protective effect. The ML rats returned to bingeing quickly once placed back on protocol with unlimited shortening access on M, W, F.

One explanation for this may be that restriction is what established the binge behavior in the first place and so once the binge behavior is established, further restriction may further exacerbate bingeing. That is, rather than attenuating the binge through limitation and control, the further restriction actually did the opposite and caused the ML rats to return to bingeing more quickly. This suggests that once an individual has learned to binge, it may not be the best idea to further restrict or eliminate "forbidden foods" from their diet in order to prevent future bingeing because that may result in worsening the binge behavior once the intervention is removed. A better option may be to introduce a small amount of a preferred fatty food into the diet every day, therefore implementing a quantity-limited restriction but without the intermittent schedule. Future research is warranted to determine the effects of daily "clamped" shortening access on subsequent binge behavior in rats before such an intervention can be applied clinically in humans.

The second finding reported here is that intervention 2, in which intake was clamped during the 1-hr access period on Tues, Thurs, Sat, Sun and was not limited during the access period on Mon, Wed, Fri appeared to be more effective than the other interventions that were tested. During the intervention, even though the rats could consume as much as they wanted on M,W, F, they did not binge on the shortening starting with week 2 of the intervention. This further supports the idea that having a "treat" every day decreases the likelihood of bingeing on that "treat," even when consuming a large amount would be possible. In addition, during the binge protocol after the intervention, the MU and MD rats continued not to binge during the first week. This is different from what happened the first week after intervention 1: intake of the MD group, although somewhat decreased during the first week, was not statistically different from that of the MU group, which continued to binge.

The ML group, which had a previous history of having clamped shortening access on an intermittent schedule, consumed slightly more shortening on M, W, F than did the other groups during intervention 2, although this did not achieve statistical significance. In addition, as mentioned above, the ML group was the only group that was different from the other groups in week 1 during protocol 3. In other words, the ML group went back to bingeing immediately after the intervention was removed, while the other groups consumed the same as the daily group and did not binge for the first week back on protocol. This suggests that, for individuals with a history of clamping, later restriction of the amount that can be consumed within a discrete period may actually predispose them toward more bingeing at a later time. This is consistent with Ventura's and Birch's work (2008), which showed that restrictive feeding practices can increase the intake and preference for palatable foods. In similar work, Fisher and Birch (1999) found that restriction was also associated with higher levels of eating in the absence of hunger.

However, the present results are different from previous research in this laboratory in which restriction in the form of intermittent "clamping" or limiting rats' intake was used as a preventative method for future binge behavior, as described above (Wojnicki, et al., 2008). Yet, note what happened to the group of rats in which shortening intake was clamped every day, instead of intermittently, in that study. When the daily clamped group was subsequently allowed to eat as much as they wanted during the daily shortening access period, intake was slightly greater than that of the standard D group. That is, the daily "clamped" history had a slight stimulatory effect on subsequent shortening consumption in rats under the daily access schedule. The results of the prevention study suggest that occasionally (intermittently) eating small quantities of preferred fatty foods may protect against subsequent bingeing to some degree in those who have not yet established the binge behavior. The present study, on the other hand,

suggests that once the binge behavior has already been established, limiting or restricting access to the palatable fatty food even more may actually stimulate rats to eat more once the palatable food becomes readily available once again.

These results have potential relevance to clinical recommendations. For instance, once an individual learns to binge, intermittent clamping (e.g. "You can only have one cookie.") may not be such a good idea. However, if bingeing is not yet established, intermittent clamping may work well as a prevention method for high-risk individuals, such as those with a family history of binge behavior or those who are emotionally unstable. Still, if the goal is to reverse binge behavior once it has already started, daily incorporation (rather than occasional incorporation) of preferred fatty foods into the diet may reduce craving for and bingeing on these foods, particularly if the circumstances allow the individual to control how much is consumed, at least on some days each week.

The above reports along with the present findings indicate that daily consumption of a preferred fatty food may offer some protection against bingeing, while clamping or limiting intake may do the opposite and promote further bingeing once the binge behavior has previously been established. Furthermore, bingeing can be attenuated while an intervention is in place, but once that intervention is removed and the original binge-inducing circumstances are back in place, bingeing eventually returns. Whether the effects of the intervention methods shown in the present study apply to humans is not known. Future research is warranted to determine the effects that daily incorporation of "forbidden foods" into the diet would have on bingeing behavior in humans.

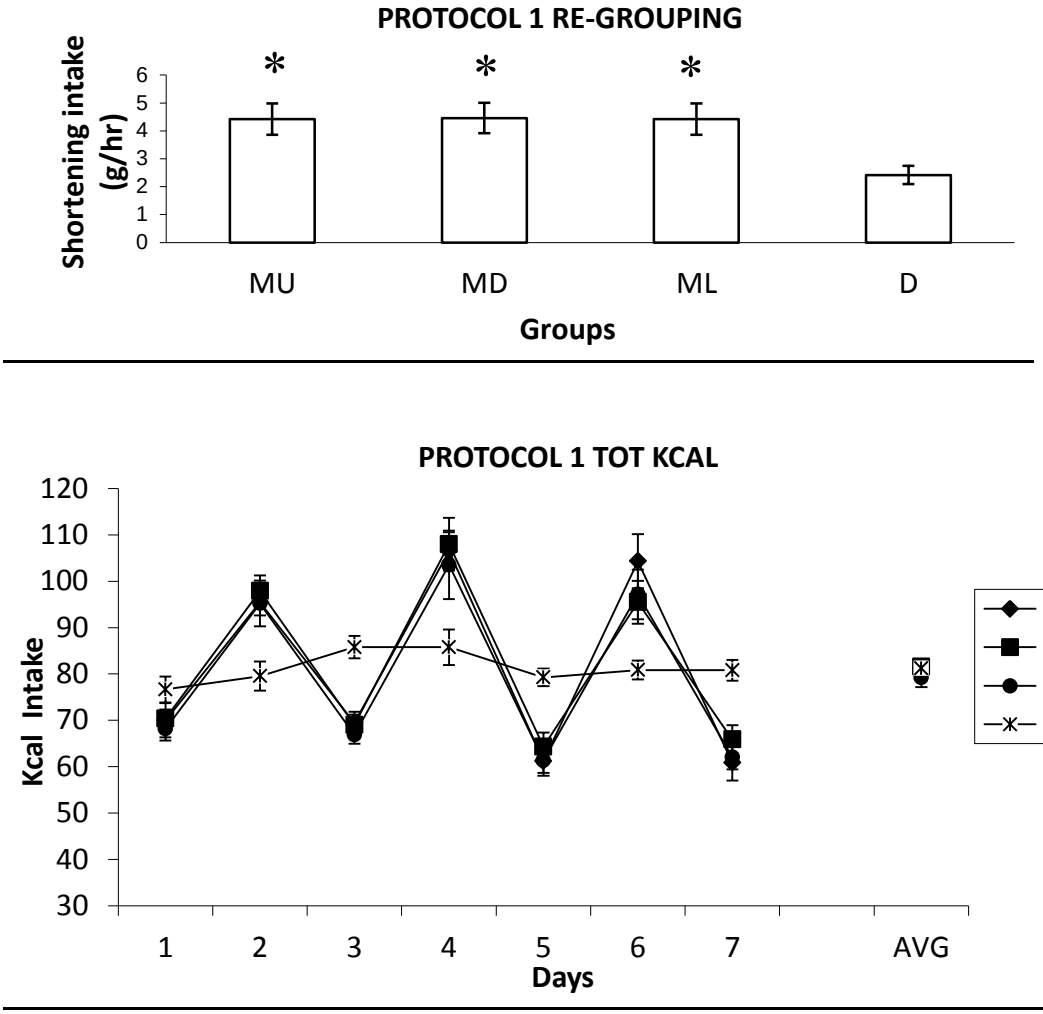

FIG 1

Fig 1. Top. Average shortening intake during week 6 of the first bingeing protocol period. Rats in the three groups (MU, MD, ML) with intermittent (Monday, Wednesday, Friday) 1-hr access to an unlimited amount of shortening consumed significantly more than rats with daily 1-hr access to shortening (D). * indicates significantly greater than D. M= Monday, Wednesday, Friday; U = unlimited access to shortening during the 1-hr access period; D = Daily; L = limited access to shortening during the 1-hr access period. Note that all three M groups were on the MU schedule during this portion of the study.

Bottom. Daily total 24-hr energy intake during week 6 of the first bingeing protocol. Group designations are as described for Fig 1, top panel. Indications of significance not shown for clarity. See text for further details.

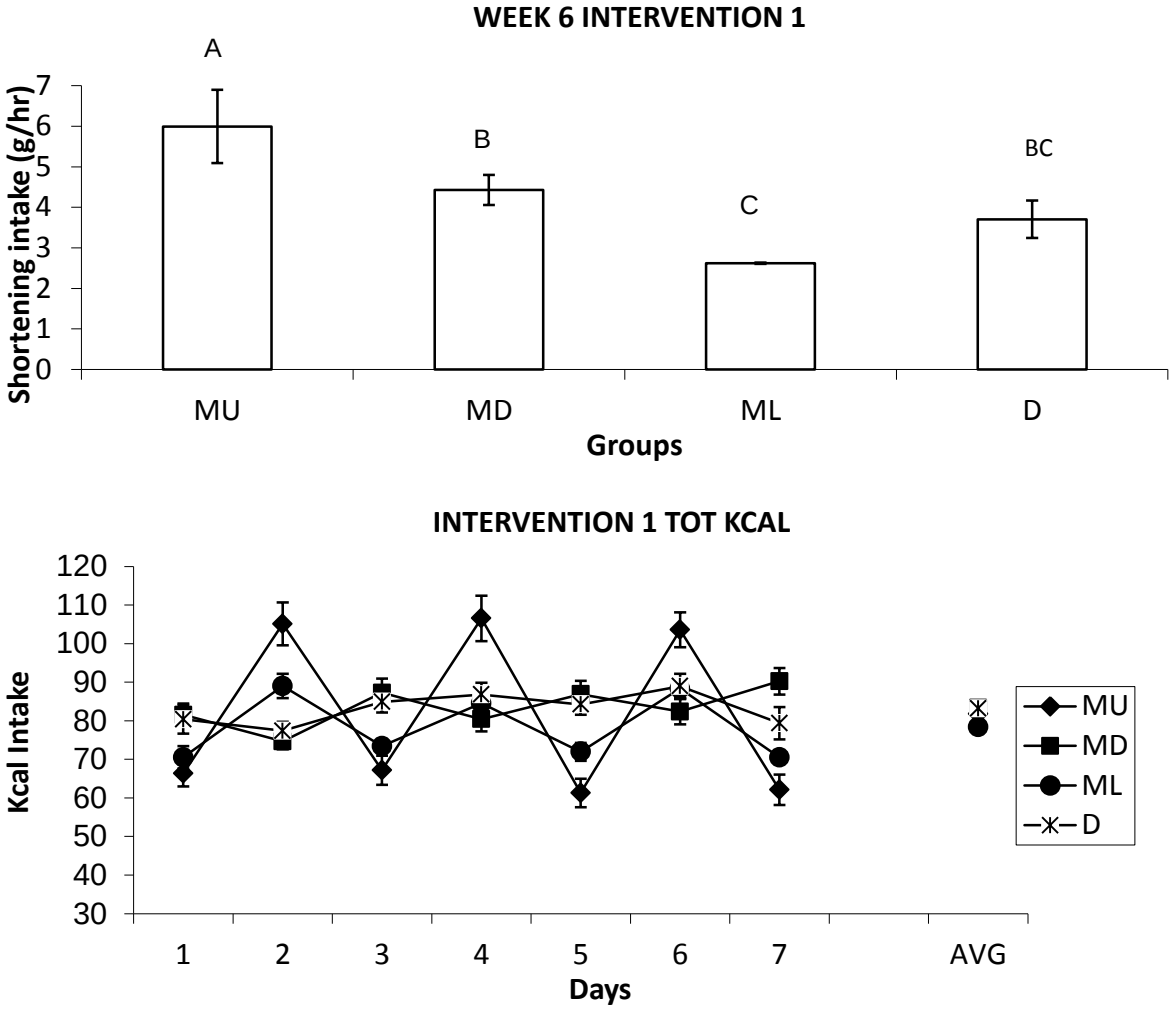

FIG 2

Fig 2. Top. Average shortening intake during week 6 of the first intervention period. Rats in the MU group, with intermittent (Monday, Wednesday, Friday) 1-hr access to an unlimited amount of shortening, consumed significantly more than rats in the other three groups. Both the MD group with daily 1-hr access to unlimited shortening and the ML group with intermittent 1-hr access to limited (2.6-2.8g) shortening were not significantly different from the D group with daily 1-hr access to shortening. Groups that do not share the same letter are significantly different. M= Monday, Wednesday, Friday; U = unlimited access to shortening during the 1-hr access period; D = Daily; L = limited access to shortening during the 1-hr access period. Note that the MD group was on the same schedule as the D group during this part of the study.

Bottom. Daily total 24-hr energy intake during week 6 of the first intervention period. Group designations are as described for Fig 2, top panel. Indications of significance not shown for clarity. See text for further details.

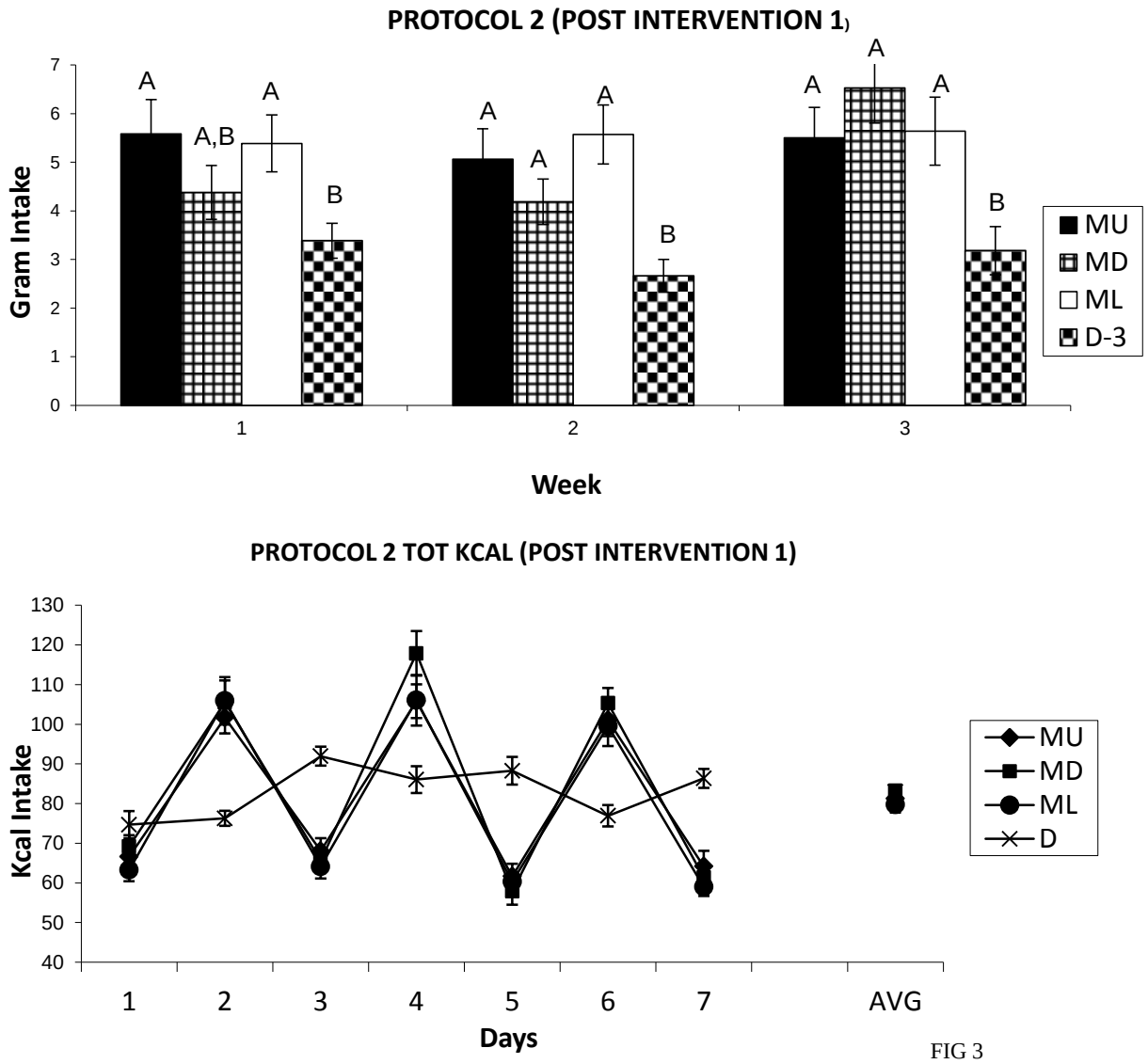

FIG 3

Fig 3. Top. Average shortening intake during the 1-hr access period for the three weeks of protocol 2. During week 1, rats in the MD group, with intermittent (Monday, Wednesday, Friday) 1-hr access to unlimited shortening were not significantly different than rats with daily 1-hr access to shortening (D). The MU and ML groups, with intermittent 1-hr access to shortening, however, consumed significantly more than both the D and MD groups. During week 2 all three groups of rats (MU, MD, ML) with intermittent 1-hr access to unlimited shortening consumed more than rats with daily 1-hr access to shortening (D). During week three, the same trend as was observed in week 2 continued. Groups that do not share the same letter are significantly different. M= Monday, Wednesday, Friday; U = unlimited access to shortening during the 1-hr access period; D = Daily; L = limited access to shortening during the 1-hr access period. Note that all three M groups were on the MU schedule during this portion of the study.

Bottom. Daily total 24-hr energy intake during week 6 of the second bingeing protocol. Group designations are as described for Fig 3, top panel. Indications of significance not shown for clarity. See text for further details.

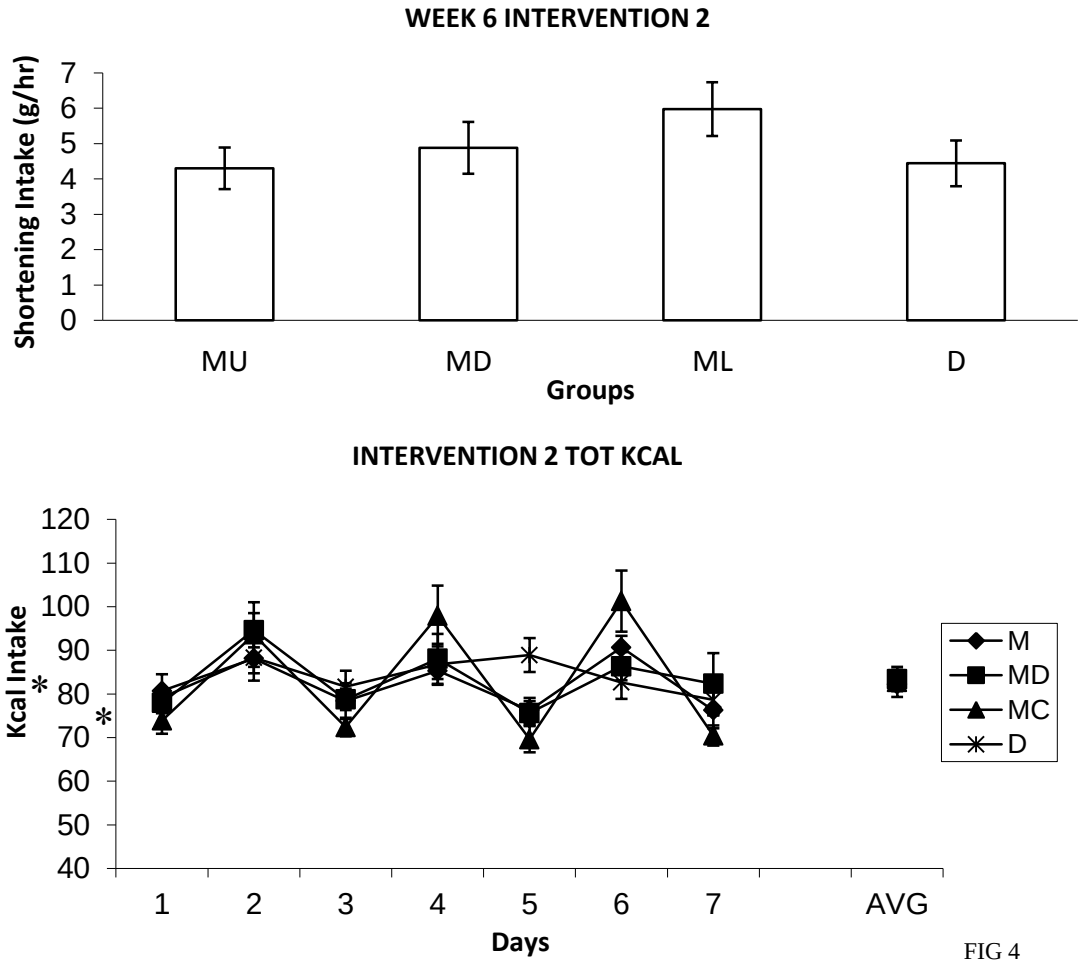

FIG 4

Fig 4. Top. Average shortening intake during week 6 of the second intervention period. Rats in all three intermittent groups (MU, MD, ML) had 1-hr access to a limited amount of shortening (2.6-2.8g) on Tuesday, Thursday, Saturday, and Sunday and 1-hr access to unlimited amount of shortening on Monday, Wednesday, Friday. M= Monday, Wednesday, Friday; U = unlimited access to shortening during the 1-hr access period; D = Daily; L = limited access to shortening during the 1-hr access period. Note that all three M groups were on the same schedule of limited access T, Th, Sat, Sun and unlimited access on M, W, F.

Bottom. Daily total 24-hr energy intake during week 6 of the second intervention period. Group designations are as described for Fig 4, top panel. Indications of significance not shown for clarity. See text for further details.

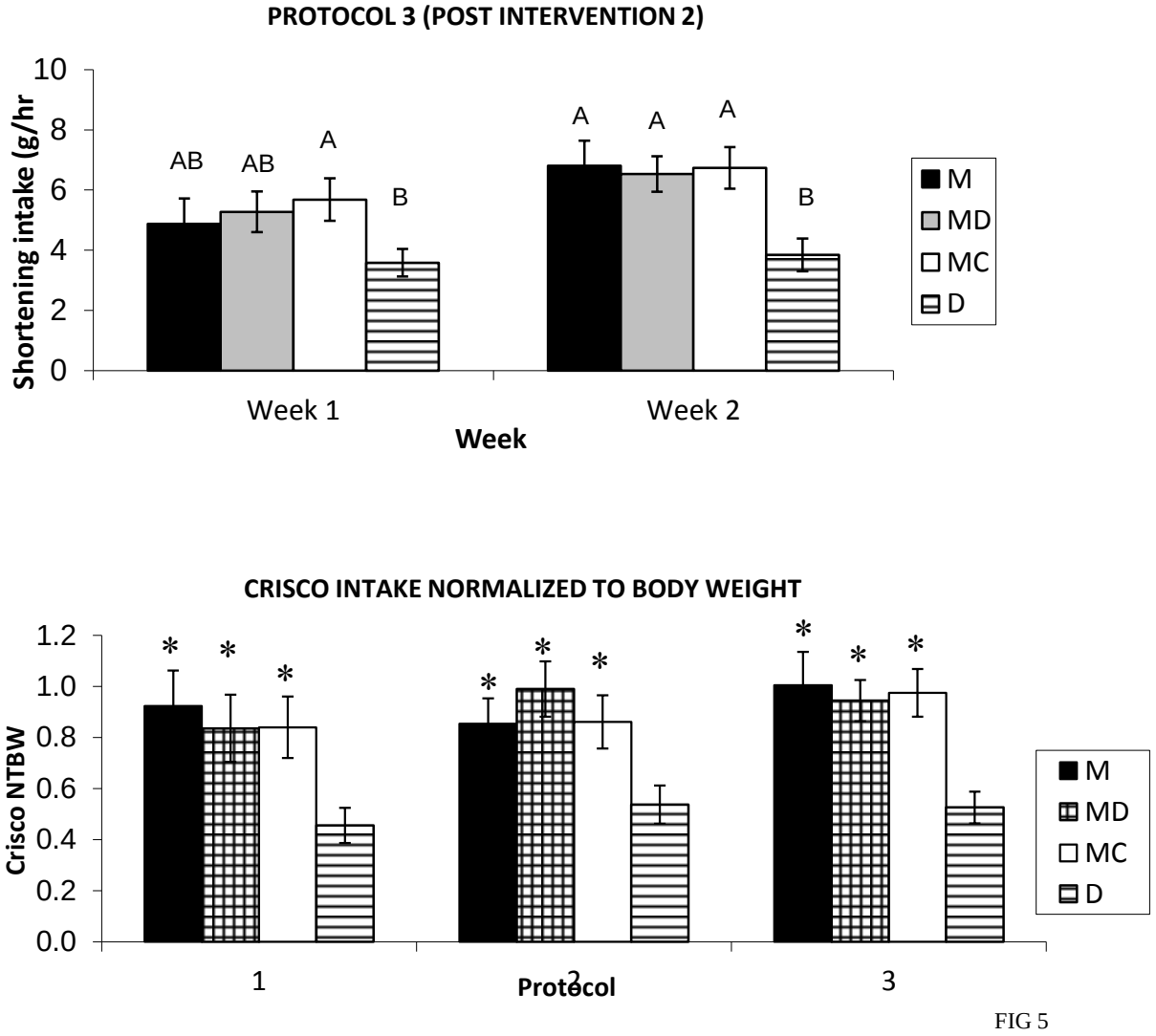

FIG 5

Fig 5. Top. Average shortening intake during the 1-hr access period for the two weeks of the last binge procedure (protocol) period. During week 1, rats in the ML group with intermittent (Monday, Wednesday, Friday) 1-hr access to an unlimited amount of shortening consumed significantly more than rats with daily 1-hr access to shortening (D), but not more than the other rats on the same intermittent access schedule (MU, MD). Groups that do not share the same letter are significantly different. During week 2 all three groups of rats (MU, MD, ML) with intermittent 1-hr access to unlimited shortening consumed more than rats with daily 1-hr access to shortening (D). Groups that do not share the same letter are significantly different. M= Monday, Wednesday, Friday; U = unlimited access to shortening during the 1-hr access period; D = Daily; L = limited access to shortening during the 1-hr access period. Note that all three M groups were on the MU schedule during this portion of the study.

Bottom. Shortening intake normalized to body weight$^{0.67}$ during the last week of each of the three binge protocols. Group designations are as described for Fig 5, top panel. * indicates significantly greater than D. See text for further details.

References:

1. Corwin RL. Binge-type eating induced by limited access in rats does not require energy restriction on the previous day. Appetite. 2004; 42:139-42.
2. Corwin RL. The face of uncertainty eats. Curr Drug Abuse Rev. 2011; 4:174-81.
3. Corwin RL, Avena NM, Boggiano MM. Feeding and reward: perspectives from three rat models of binge eating. Physiol Behav. 2011; 104:87-97.
4. Corwin RL, Wojnicki FE, Fisher JO, Dimitriou SG, Rice HB, Young MA. Limited access to a dietary fat option affects ingestive behavior but not body composition in male rats. Physiol Behav. 1998; 65:545-53.
5. Dimitriou SG, Rice HB, Corwin RL. Effects of limited access to a fat option on food intake and body composition in female rats. Int J Eat Disord. 2000; 28:436-45.
6. DSM-IV^{TM}-TR. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. Washington, DC: American Psychiatric Association, 2000, pp. 583-95, 785-7.
7. Fisher JO, Birch LL. Restricting access to foods and children's eating**.** Appetite 1999, 32**:**405-419.
8. Heusner AA. Body Size and Energy Metabolism. Ann Rev Nutr. 1985; 5:267-93.
9. Hudson JI, Hiripi E, Pope Jr HG, Kessler RC. The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. Biol Psychiatry 2007;61:348–58.
10. Kales EF. Macronutrient analysis of binge eating in bulimia. Physiol Behav 1990; 837-40.
11. Murphy R, Straebler S, Cooper Z, Fairburn CG. Cognitive behavioral therapy for eating disorders. Psychiatr Clin North Am. 2010; 33:611-27.
12. Swanson SA, Crow SJ, Le Grange D, Swendsen J, Merikangas KR. Prevalence and correlates of eating disorders in adolescents: results from the National Comorbidity Survey Replication.
13. Thomas MA, Rice HB, Weinstock D, Corwin RL. Effects of aging on food intake and body composition in rats. Physiol Behav. 2002; 76:487-500.
14. Ventura AK, Birch LL. Does parenting affect children's eating and weight status? Int J Behav Nutr Phys Act. 2008; 5-15.
15. Wojnicki FH, Johnson DS, Corwin RL. Access conditions affect binge-type shortening consumption in rats. Physiol Behav. 2008; 95:649-57.

ACADEMIC VITA
Magdalena Jurczyk

Magdalena Jurczyk

409 HAMILTON HALL, UNIVERSITY PARK, PA 16802
Cell: 201-328-2943 Email: maj5122@psu.edu

Education

PENNSYLVANIA STATE UNIVERSITY, 2012
UNIVERSITY PARK, PA, USA
NUTRITIONAL SCIENCES
HONORS IN NUTRITIONAL SCIENCES
THESIS TITLE: EFFECTS OF VARIOUS INTERVENTIONS ON BINGE-TYPE BEHAVIOR IN RATS
THESIS SUPERVISOR: DR. REBECCA CORWIN

Accomplishments

Pennsylvania State University:
- Evan Pugh Award (top 0.5% of class)
- Dean's List (Fall 2008 – Fall 2011)
- Member of the Schreyer Honors College

Related Experience and Training

Researcher in the Department of Anesthesiology May 2011 to August 2011
St. Luke's-Roosevelt Hospital- New York, NY (Summer Internship)
Research in Animal Lab (Fall 2010 – current) *adviser contact info*: fhw3@psu.edu
Adult Tutoring Internship – 150 hours (Spring 2010)
Observed laparoscopic surgeries in a hospital in Poland (Summer 2009)

Activities

- THON Committee member (2008 – 2011): THON is the largest student-run philanthropy in the world! It is a year-long effort to raise funds and awareness for the fight against pediatric cancer.
- Competitive Ballroom Dancer (1998-current)
- Traveling
  ~ Ballroom Dance
  ~ London Theatre
  ~ Poland, Greece, Spain, France
  ~ Surgeries in Poland