Published in final edited form as:

Physiol Behav. 2007 December 5; 92(5): 924–930.

Comparison of hydrogenated vegetable shortening and nutritionally complete high fat diet on limited access-binge behavior in rats

Jon F. Davis^{*}, Susan J. Melhorn^{*}, Justin U. Heiman, Matthias H. Tschöp, Deborah J. Clegg, and Stephen C. Benoit

University of Cincinnati, Department of Psychiatry, ML # 0506, Reading, OH 45237

Abstract

Previous studies have suggested that intermittent exposure to hydrogenated vegetable shortening yields a binge/compensate pattern of feeding in rats. The present study was designed to assess whether rats would exhibit similar patterns of intake when given intermittent access to a nutritionally complete high-fat diet. Four groups of rats received varying exposure to either hydrogenated vegetable shortening or high-fat diet for 8 consecutive weeks. Animals were given daily and intermittent access to determine if the binge/compensate pattern of feeding was frequency dependent. At the conclusion of the study, body composition and plasma leptin levels were assessed to determine effects of diet and binge/compensate intake on endocrine alterations. As predicted, animals receiving intermittent access to high-fat diet displayed the binge/compensate pattern of feeding and appeared to compensate as a result of the caloric overload accompanying a particular binge episode. In addition, exposure to either shortening or high-fat diet led to alterations in body composition, while only exposure to shortening altered plasma leptin levels. These results suggest that binge-intake behavior occurs on a nutritionally complete high-fat diet and that this regimen is capable of altering both body composition and endocrine profile.

Keywords

binge-intake; behavior; binge/compensate; high fat diet; meal feeding; eating disorders

1. Introduction

Binge eating behavior represents a loss of control over the ability to terminate a meal when satiated and is a core symptom of disordered eating. According to the Diagnostic and Statistical Manual-IV, human binge-eating disorder is characterized by 'recurrent episodes of binge eating' including, but not limited to: eating a large amount of food in two hours, lacking control over food intake, hurried intake, and eating when not physically hungry (1). Stress and dieting are two factors known to contribute to this type of disordered eating in both humans and animals. In particular, when stressed, animals with histories of caloric restriction will engage in "binge" like intake behavior when presented with a high fat diet (2,3,4). However, recent work suggests that limited access to an optional source of dietary fat alone, irrespective of

To whom correspondence should be addressed: voice: (513) 558-6468, fax: (513) 558-4800. Electronic mail: Jon.Davis@uc.edu.

^{*}Denotes that both authors contributed equally to the preparation of this manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

caloric restriction or stress, can lead to a binge/compensate pattern of feeding in rodents (5,6,7). This feeding pattern can be maintained for extended periods of time and is not dependent on prior bouts of food deprivation or stress. It should also be noted that this particular binge pattern has only been brought out after exposure to a diet comprised exclusively of fat, that is, a diet which is devoid of carbohydrates and proteins. In this model, animals maintained on an *ad libitum* standard rodent chow diet and given intermittent (approximately every 3 days) access to hydrogenated vegetable shortening (*Crisco*) display caloric over consumption relative to chow fed control animals and animals that receive a daily or constant access to the shortening (5). Importantly, “binge” in this case is defined as the increased caloric intake of the intermittent-access group above and beyond the caloric intake of control group with access to the same shortening for the same amount of time.

An important factor contributing to the development of this feeding pattern is the frequency in which animals have access to the optional dietary source. In particular, animals given daily limited access to vegetable shortening do not display the binge/compensate pattern of feeding; this feeding pattern only emerges when animals are given intermittent access to the shortening (5,8). In this way, animals given limited access to the vegetable shortening not only consume more calories than chow fed control animals, but also more than animals receiving daily access to vegetable shortening during the restricted feeding period. Furthermore, animals given intermittent access to vegetable shortening will under-consume their standard chow diet to compensate for the augmented caloric load obtained during the restricted feeding period. Because animals in this paradigm continually engage in bouts of hyperphagia that result in a binge/compensate behavioral pattern of feeding, it has been suggested that this is a sufficient model to study binge eating behavior.

Although it is clear that this type of feeding regimen yields a behavioral pattern similar to that reported in human eating disorders, it is unclear if nutritionally complete high fat foods are sufficient to induce this behavior or if altering the frequency and duration of exposure to the optional source of fat will yield differences in the binge/compensate pattern of feeding. Previous studies have only described the binge/compensate model of feeding when animals had been given intermittent access to an optional fat source comprised exclusively of fat (*Crisco*), but have not investigated nutritionally complete sources of dietary fat and their ability to induce this behavior (5,7). Because humans rarely if ever binge on pure fat, it is important to determine if a nutritionally complete diet that is high in fat is also capable of eliciting this feeding pattern without the confounds of stress or caloric restriction. In addition, few studies have investigated the impact this particular feeding regimen has on body weight, body composition, and the endocrine profile of animals after prolonged exposure to this feeding regimen (4). Therefore, an additional goal of this study was to describe both the temporal feeding pattern as well as the metabolic consequences after exposure to this model system. In particular, it is not clear if animals will display this type of feeding behavior when given limited access to an alternative nutritionally complete high fat diet. Because most western diets are rich in macronutrients in addition to fat, it is important to determine if limited access to a mixed nutrient high fat diet is sufficient to produce this pattern of feeding behavior. Furthermore, previous reports using this limited access protocol to vegetable shortening report no significant differences in body weight relative to chow fed control animals (9), which could be due in part to the composition of the dietary fat used. Moreover, although this limited access protocol produces no differences in overall body weight, it is unclear if this regimen is capable of altering body composition or adiposity signals critical for the central control of homeostatic feeding, or how frequency and duration of exposure to this regimen may affect the compensation pattern that accompanies an increased caloric load. Thus another goal of the current study was to assess this binge/compensate pattern of feeding by extending the duration of the limited access feeding regimen for a total of 60 days, altering the frequency in which animals were exposed to the limited access protocol, as well as testing an alternate source of the dietary fat used to produce

this behavioral pattern of feeding. Because both body composition as well as circulating plasma hormones are known to be important predisposing factors for the metabolic syndrome, another goal of this study was to determine if the binge/compensate behavior alters body composition or plasma leptin levels. Here we are testing the hypothesis that a diet high in fat but also replete with proteins and carbohydrates will be sufficient to produce a binge/compensate pattern of feeding, while also altering the animals body weight and body composition. Additionally, we predict that animals given limited access to a high fat diet will have increased body adiposity and body weight in addition to displaying a binge/compensate pattern of feeding.

2. Methods

2.1 Subjects

Forty-two male Long-Evans rats (Harlan, IN) weighing 200-250 g were housed individually in a vivarium with a 12:12 light/dark schedule lights on 4am, lights off 4pm (n = 8-9 / group). The temperature of the room was maintained at 25° C. All animals had *ad libitum* access to water and standard chow. All experiments were conducted in accordance with the guidelines set forth by the University of Cincinnati's institutional animal care and use committee (IACUC) for the proper care and welfare of laboratory animals.

2.2 Procedure

Animals were allowed to acclimate to their housing environment for one week prior to experimental manipulation. All animals (minus controls) were given 48-hour access to Crisco (Crisco All-Vegetable Shorting, Procter and Gamble, Cincinnati, OH; percent of calories as fat: 100%; 9.2 kcal/g) or High Fat Diet (HFD; Dyets, inc., Bethlehem, PA, 4.41 kcal/gm, 1.71 kcal/gm from fat) to reduce neophobia. Rats were then matched based on weight and divided into five groups (n=8-9 per group) and assigned to one of the following dietary regimens for the remainder of the eight week study.

Control animals (CNTRL; n=8) had continuous access to standard rodent chow (Teklad, 3.41 kcal/gm, 0.51 kcal/gm from fat), and water throughout the study. Control animals received hoppers filled with standard chow each day during the two hour access period to control for the effect of novelty on feeding.

Sixteen animals were given two hour access (12pm-2pm) each day to either Crisco (CrisED; n=8) or HFD (HFD3D; n=8) for the entire eight week study. Each high calorie diet was put in a regular food hopper and placed in the back left corner of their home cage for two hours everyday, totaling 60 limited access feeding sessions throughout the study. This group had *ad libitum* access to food and water for the duration of the study.

A separate group of animals were given access to Crisco (Cris3D; n=9) or HFD (HFD3D; n=9) every third day for the duration of the study. This group had *ad libitum* access to regular laboratory chow at all times throughout the study and was presented with Crisco or HFD in the back left corner of their cage every third day of the study. On all other days, this group received a hopper filled with the regular chow in the same cage location

The test diet (HFD and *Crisco*) was presented for 2-hrs during the light cycle (beginning 4 hours before lights out). The presentation of the hopper filled with Crisco or HFD during this time attempted to initiate feeding when the rat would not normally be ingesting food. The test diet was exchanged with a fresh source 1-2 times per week. Total kilocalories (kcal) consumed in 24-hrs, kcal consumed during the 2-hr feeding period, and kcal from the specific sources (Crisco, HFD diet, regular chow) were tracked during the study. Energy intake was calculated by multiplying the total amount of diet ingested in the feeding period by the kilocalories present in each diet (chow=3.4, HFD=4.5, *Crisco*=9.16). Both 2 and 24 hr intake from chow and the

test diets were summed to determine total energy intake per day. Body weights were assessed every four days.

2.3 Body Composition Analysis

Body composition was assessed using a whole body NMR instrument (Echo-MRI, Waco, TX) to determine percent fat, lean and water content for each animal. To determine body composition, each animal was placed into a clear Plexiglas tube and subsequently scanned for 45 seconds. Body composition was assessed at the beginning and on day 59 of the study.

2.4 Plasma Leptin

At the conclusion of the experiment, one day after the final restricted feeding session, all animals were euthanized during the middle of their light phase by Carbon Dioxide asphyxiation. Subsequently, trunk blood was collected and the plasma isolated by centrifugation and stored at -80°C until analyzed by radioimmunoassay for plasma leptin using a rat leptin radioimmunoassay (RIA) kit (Linco Research, St. Charles, Missouri). This assay is able to detect leptin in 100 μl samples of plasma with intra and interassay coefficients of variation of 4.6% to 5.7% for leptin according to the manufacturer's specifications.

2.5 Statistics

Data were analyzed using STATISTICA version 6.0 for PC's. All data were analyzed using analysis of variance (ANOVA) and LSD post-hoc comparisons were used to determine differences among groups.

3. Results

3.1 Food intake

Restricted access to Crisco or HFD diet produced a binge/compensate pattern of eating relative to control animals. This pattern developed in both groups (Cris3D and HFD3D) within the first week of the restricted access protocol and persisted for up to two months following the initiation of the study. In both the Cris3D and HFD3D groups, this pattern began as early as day 3 in animals receiving Crisco (Cris3D vs. CNTRL, $p < 0.05$) and day 6 in animals receiving HFD (HFD3D vs. CNTRL, $p < 0.05$) and persisted to day 61 (Figure 1). There were no differences in energy intake between the control and every day access groups (CrisED or HFDED) at any time point tested. The average kcals consumed each day from Crisco or HFD did not differ between groups that received the test diet every day (CrisED, HFDED) or those that received the diet every third day (Cris3D, HFD3D). In addition, the cumulative kcals consumed over the 60 day feeding regimen did not differ between groups (Figure 2).

The groups that received either Crisco or HFD every third day compensated for their high calorie intake by under consuming kcals from chow immediately after each binge session in comparison to control animals. This affect was apparent within the first week of the study, shown in Figure 1. Figure 3 represents the total amount of kilocalories consumed during the two hour restricted feeding session obtained from the test diet only (HFD or Crisco) on the last "binge" session of the two month feeding regimen. ANOVA revealed a main effect of group ($F(1, 37) = 17.86$, $p < .05$). In particular, animals receiving daily two hour access to Crisco or HFD as well as intermittent access displayed increased caloric intakes during the two hour feeding regimen relative to control animals (all p 's $< .05$, LSD post-hoc tests). Furthermore, animals given daily exposure to Crisco (CrisED) consumed significantly more calories during the two hour feeding period in comparison to animals receiving Crisco every third day ($p < 0.05$). Although animals receiving limited access to HFD every third day (HFD3D) appeared to

consume more kilocalories during the two hour restricted feeding regimens in comparison to their daily counterparts (HFDED), this effect was not statistically significant.

3.2 Bodyweight

Figure 4 depicts the total bodyweight for each group measured at the conclusion of the study. After 60 days of the restricted access protocol there were no differences in bodyweight among the five groups employed in the study.

3.3 Body Composition Analysis

Figure 5 depicts the percentage of body fat from all four groups as measured by NMR at the conclusion of the study. There was a main effect of group ($F(1, 37) = 6.83, p < 0.01$) in relation to body composition. Specifically, both groups that received *ad lib* access to Crisco (CrisED) or high-fat diet (HFDED) everyday had a greater percentage of body fat relative to control animals at the conclusion of the study (CrisED vs. CNTRL, $p < 0.05$; HFDED vs. CNTRL, $p < 0.05$). In addition, the HFD3D displayed a greater percentage of body fat relative to control animals ($p < 0.05$). The group receiving restricted access to Crisco did not differ from controls in relation to the percentage of overall body fat as measured by NMR.

3.4 Plasma Leptin

Figure 6 depicts plasma leptin levels in each group of animals obtained at the conclusion of the experiment. ANOVA yielded a main effect of group ($F(1, 16) = 4.47, p < 0.01$). Only animals receiving restricted access to Crisco each day displayed elevated plasma leptin levels compared to control animals ($p < 0.05$).

4. Discussion

There are three significant findings to report from the current study. The first is that a binge/compensate type of feeding behavior can be elicited by restricted access to a nutritionally complete high fat diet as well as to vegetable shortening. Although the vegetable shortening contained significantly more energy than the high fat diet, both diets led to a similar binge intake behavior relative to the chow fed control animals. This is significant due to the fact that western diets consumed during human bingeing are comprised of mixed nutrients in addition to fat, and suggests that high energy diets are not necessary to elicit binge intake behavior. Thus, the high fat diet regimen used here may represent a more clinically relevant model to study human binge eating. Another important finding of this study is that altering the frequency of the limited access protocol produces changes in the temporal pattern of the binge/compensation phenomenon. In particular, animals in our study appeared to under-eat as a consequence of bingeing the day before rather than in anticipation of a future opportunity to binge. The third significant finding of this study is that extending the length of time of the restricted feeding regimens used here produced significant alterations in body composition as well as circulating adiposity signals without altering overall body weight. Because fat content directly correlates with circulating leptin levels, the changes reported here represent a significant set of metabolic consequences for individuals engaging in ‘binge-type’ feeding behavior. Thus, it is possible that the model described could be used to examine dissociations in body weight from endocrine disruptions and body composition.

It should be noted that although these results, in large part, reproduce those reported previously using limited access feeding regimens, this study differed in several ways compared to the limited access protocol developed and previously reported by Corwin and colleagues (5,6,7). First, male Long-Evans rats were used rather than the Sprague-Dawley strain and the 2-hour access period was given in the middle of the light cycle rather than two hours prior to lights out. When using our 2-hour access regimen, a researcher would enter the room to provide the

test diet during the quiescent period, which triggered small feeding bouts. This may account for the increased variability in ED and control groups, compared to previously reported data. The time point used in this study was chosen to more closely mimic a binge period that would occur outside of a normal intake session as rats normally feed during their dark cycle. The current study was also extended for 30 days longer in order to further examine body weight and composition changes.

As mentioned above, limited 2-hour access to Crisco and HFD produced a binge compensate model in the Cris3D and HFD3D groups, but not the CrisED or HFDED. This pattern emerged in the first week and became more pronounced throughout the duration of the experiment, which is similar to previously reported work using a limited access protocol (8,9). Exposure to palatable foods or stress is capable of eliciting binge-intake behavior in rodents with a history of past caloric restriction when exposed to a nutritionally complete high fat diet similar to the one used in this study (2,3,4). In this study however, intermittent access to the nutritionally complete high fat diet alone was sufficient to induce binge type feeding in animals that had never been calorically restricted or stressed. Although stress and dieting are two known predictors of binge intake of high fat foods, these data suggest that they are not required to induce disordered eating. One implication of this is that feeding frequency and exposure could be equally strong determinants for binge-intake behavior. In addition, it is clear that the animals receiving daily access to Crisco or HFD, which are drastically different in energy content, are able to maintain stable caloric intake by limiting calories obtained from chow. One way this could be achieved is by peripheral or central caloric detection systems that monitor the overall amount of calories ingested during a particular feeding episode. Thus, the ability of both groups to consume the same amount of calories from two diets with differing caloric content could be regulated impart by such a system that can sense calories ingested in real time and then adjust future intake accordingly by regulating peripheral mechanisms. Although a role of this type of regulation during binge eating behavior has not yet been described, it is possible that the feeding paradigm used here could be useful in elucidating such mechanisms put in place to detect overall caloric load in addition to overall caloric volume.

In this study, however, intermittent access to Crisco or a diet high in fat did not lead to compensation in the amount of chow consumed prior to any binge session; in fact the compensation only occurred immediately after a binge session in both groups tested. Both groups receiving the test diet every third day under ate the day immediately following test diet exposure, and the group exposed to the high fat diet appeared to under eat more frequently across the sixty day feeding regimen than did the group receiving vegetable shortening. It is possible that this difference in the under eating observed between both three day groups after a particular binge session could be explained by micronutrient deficiency. In this way the animals' bingeing on the nutritionally complete test diet may have been more sated while the animals receiving the test diet comprised exclusively of fat (deficient in protein and carbohydrates, Table 1.) were not, perhaps due to a micronutrient imbalance experienced on the binge day itself. Nevertheless, the point of emphasis is that both a nutritionally complete diet as well as fat alone yields a binge phenotype accompanied by underrating as a consequence but not in preparation for a future binge episode, and this effect can be brought about by altering the frequency of dietary exposure.

Uncontrolled eating when energy reserves are met is a defining component of binge eating behavior (DSM-IV) and can be initiated by exposure to acute environmental cues that predict exposure to a 'forbidden' food source (9). However, our data suggest that animals receiving predictable, intermittent access to Crisco or HFD under-consume calories on non access days (days in which there is no access to the test diet) as a reaction to the increased caloric load rather than a predictor of it. This effect was present in both groups receiving the high fat test diet (Crisco or HFD), and supports the contention that compensation in anticipation of the

optional food source can be attributed to the frequency of exposure to the test diet rather than a reflection of the animals ability to predict a particular 'binge' session (8,9).

This study was also designed to examine feeding changes as well as overall body composition changes as a result of prolonged exposure to a limited access protocol of either a Crisco or high fat diet. As mentioned earlier, there were no differences in absolute bodyweight between groups; however extending the restricted access feeding regimen to 60 days did produce differences in body composition. In particular, there was an increase in overall fat composition which was consistent to both of the ED groups. Additionally, the group which received intermittent access to high fat diet also showed an overall increase in fat content at the conclusion of the study. This effect was absent in the group that received intermittent access to Crisco and could be attributed to the additional macronutrients contained in the high fat diet. Although both groups receiving the HFD meal feeding regimen displayed increases in body adiposity together with the group daily access Crisco group, only daily access to Crisco resulted in increased plasma leptin. Plasma samples were taken one day after the last binge session, a time that the HFD groups were under eating in comparison to control animals. Prior investigations from both rodents and humans have reported decreases in plasma leptin upon fasting (10,11,12). Thus, it is possible that the lowered plasma leptin in the HFD groups is a result of temporary caloric restriction or that the time point chosen here to examine plasma leptin in these groups was too early to detect a change using this restricted access feeding regimen.

In summary, these data suggest that a nutritionally complete high fat diet is capable of eliciting a binge/compensate pattern of feeding. Taken together these data support the idea that regulatory centers which control food intake and body composition can be dissociated in the binge/compensate model. This dissociation implicates the effects of disordered eating in other pathologies, such as metabolic syndrome, and is consistent with clinical data reporting that binge eating behavior can precede the onset of weight gain in young humans (18). Because previous reports suggest a direct relationship between obesity, metabolic syndrome, and visceral fat, (14,15,16) this model may afford the possibility to study endocrine alterations in isolation from overall weight gain. In addition, because humans often experience 'binge' type eating patterns for extended periods of time (17) the protocol employed here may be used to more closely mimic human binge eating behavior. An examination of the expression changes in neuropeptides which regulate meal initiation and termination, in addition to the effects of prolonged 'binge' type behavior on general motivation and rewarding processes are necessary to more completely understand the consequences of disordered eating and may perhaps elucidate potential mechanisms that aid in treatment of this disorder.

References

1. DSM-IV, Diagnostic and statistical manual of mental disorders. American Psychiatric Association; Washington, DC: 1994. p. 545-550.p. 729-731.
2. Hagan MM, Moss DE. Persistence of binge eating patterns after a history of restriction with intermittent bouts of refeeding on palatable foods in rats: implications for bulimia nervosa. *Int J Eat Disord* 1997;22(4):411–20. [PubMed: 9356889]
3. Hagan MM, Shuman ES, Oswald KD, Corcoran KJ, Proffitt JH, Blackburn K, Schwiebert MW, Chandler PC, Birbaum MC. Incidence of chaotic eating behaviors in binge-eating disorder: contributing factors. *Behavioral Medicine* 2002;28(3):99–105. [PubMed: 16463758]
4. Hagan MM, Wauford PK, Chandler PC, Jarret LA, Rybak RJ, Blackburn K. A new animal model of binge eating: key synergistic role of past caloric restriction and stress. *Physiology and Behavior* 2002;77(1):45–54. [PubMed: 12213501]
5. Corwin RL, et al. Limited access to a dietary fat option affects ingestive behavior but not body composition in male rats. *Physiol Behav* 1998;65(3):545–53. [PubMed: 9877422]

6. 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(4):436–45. [PubMed: 11054791]
7. Thomas MA, et al. Effects of aging on food intake and body composition in rats. *Physiol Behav* 2002;76(45):487–500. [PubMed: 12126984]
8. Corwin RL, Buda-Levin A. Behavioral models of binge-type eating. *Physiol Behav* 2004;82(1):123–30. [PubMed: 15234600]
9. Corwin RL. Binge-type eating induced by limited access in rats does not require energy restriction on the previous day. *Appetite* 2004;42(2):139–42. [PubMed: 15010177]
10. Wardle J. Conditioning processes and cue exposure in the modification of excessive eating. *Addictive Behavior* 1990;15(4):387–93.
11. Ahren B, Mansson S, Gingerich RL, Havel PJ. Regulation of plasma leptin in mice: influence of age, high-fat diet and fasting. *Am J Physiol* 1997;273:R113. [PubMed: 9249540]
12. Boden G, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* 1996;81:3419. [PubMed: 8784108]
13. Wisse BE, Campfield LA, Marliss EB, et al. Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentrations in obese women. *Am J Clin Nutr* 1999;70:321. [PubMed: 10479193]
14. Atzmon G, et al. Differential gene expression between visceral and subcutaneous fat depots. *Horm Metab Res* 2002;34(1112):622–8. [PubMed: 12660871]
15. Das M, Gabriely I, Barzilai N. Caloric restriction, body fat and ageing in experimental models. *Obes Rev* 2004;5(1):13–9. [PubMed: 14969503]
16. Gabriely I, et al. Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? *Diabetes* 2002;51(10):2951–8. [PubMed: 12351432]
17. Marcus, MD. Binge eating in obesity. In: Fairburn, CG.; Wilson, GT., editors. *Binge eating: nature, assessment, and treatment*. New York: The Guilford Press; 1993. p. 77-96.
18. Reas DL, Grilo CM. Timing and sequence of the onset of overweight, dieting, and binge eating in overweight patients with binge eating disorder. *Int J Eat Disord* 2007;40(2):165–70. [PubMed: 17089414]

Figure 1.
Mean caloric intake over 60 days in animals receiving daily or intermittent access to A) Crisco or B) high-fat diet, $p < 0.05$.

Cumulative Intake

Figure 2. Cumulative caloric intake from control as well as HFD or Crisco groups during the last restricted feeding regimen in animals receiving daily and intermittent access to Crisco, high-fat diet, or chow.

Figure 3.

Mean caloric intake during two-hour restricted feeding period on day 60 in animals receiving both daily and intermittent access to Crisco or high-fat diet. “*” = $p < 0.05$ relative to Crisco ED animals. “#” = $p < 0.05$ relative to control animals.

Figure 4.

Mean body weight from all groups after 61 days of the restricted access feeding regimen, $p < 0.05$ relative to control animals.

Figure 5.
% Adipose tissue from all groups after 60 days of the restricted access feeding regimen, “*” = $p < 0.05$ relative to control animals.

Figure 6. Mean plasma leptin levels from all groups 24 hours after the final day of the 8 week restricted access feeding regimen, $p < 0.05$.

Table 1Dietary composition of high fat butter diet, and *Crisco*.

Tso's High Fat Butter Diet #DO3082706		<i>Crisco</i>	
	gm	gm	
Protein	16.6	Total Fat	12
Carbohydrate	52	Saturated Fat	3
Fat	20	Trans Fat	0
Kcal/gm	4.54	Polyunsaturated Fat	6
		Monounsaturated Fat	2.5
Caesin 80 mesh	164	Cholesterol	0
L-cysteine	2.1	Sodium	0
		Total Carbohydrate	0
		Protein	0
Corn Starch	303.1		
Maldodextrin	115		
Sucrose	89.9		
Cellulose	58.6		
Soybean Oil	10		
Butter, Anhydrous	90		
Corn oil	0		
Olive oil	0		
Table 1.2. Dietary composition of Teklad standard rodent chow.			
	Teklad Standard Chow #7012		
	%		
Protein	19.92		
Carbohydrate			
Fat	5.67		
Kcal/gm	3.41		
AMINO ACIDS		Vitamins	
Arginine	1.33	Vitamin A	29.63 IU/g
Methionine	0.40	Vitamin D3	2.39 IU/g
Cysteine	0.34	Vitamein E	114.36 IU/KG
Histidine	0.48	Choline	2.23 mg/g
Isoleucine	1.01	Niacin	100.11 mg/KG
Leucine	1.80	Panthothenic Acid	86.66 mg/KG
Lysine	1.15	Pyridoxine	16.94 mg/KG
Phenylalanine	1.65	Riboflavin	13.68 mg/KG
Threonine	0.81	Thiamine	95.28 mg/KG
Tryptophan	0.25	Menadione	8.37 mg/KG
Valine	1.05	Folic Acid	6.70 mg/KG
Minerals		Biotin	0.77 mg/KG
Calcium	0.98	Vitamin B12	91.00 mcg/KG
Phosphorus	0.66	Vitamin C	-----
Sodium	0.32		
Choline	0.54		
Potassium	0.83		
Magnesium	0.25		
Iron	284.11 mg/KG		
Maganese	93.34 mg/KG		
Zince	63.59 mg/KG		
Copper	23.06 mg/KG		
Iodine	2.61 mg/KG		
Cobalt	0.72 mg/KG		
Selenium	0.16mg/KG		