

EFFECT OF FOOD AVAILABILITY
ON LONG- AND SHORT-TERM CONTROLS OF FOOD INTAKE

By

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This document is dedicated to my family and friends who have supported me in my educational endeavors ... and to those who haven't, for giving me the motivation to prove them wrong.

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Abstract of Thesis Presented to the Graduate School
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EFFECT OF FOOD AVAILABILITY ON LONG- AND SHORT-TERM CONTROLS
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In the wake of an epidemic of obesity, the factors that promote eating in excess of metabolic need must be explored and understood if effective prevention and treatment strategies are to be implemented. Food intake and body weight have most often been explored within the paradigm of homeostasis, the regulation of physiological parameters that maintain internal constancy. The literature suggests that there are oral and postingestive effects of food, such as taste, composition, and energy density, as well as feeding economics and learned mechanisms, which have been demonstrated to impact and overwhelm homeostatic mechanisms and promote overconsumption. The effect of food availability on caloric balance and compensation has not been thoroughly explored, yet has been suggested to play a roll in the development of obesity. In a series of six experiments using female rats, we studied the impact of different spatial, temporal, and qualitative availabilities of food on caloric intake and body weight gain. In the first experiment, we demonstrated that food intake and body weight gain did not increase as a

function of increased spatial availability of maintenance diet. In the second experiment, we demonstrated that uninterrupted temporal availability of a low calorie dessert promoted increased dessert consumption compared to intermittent availability, but did not disrupt caloric balance. In the third experiment, we demonstrated that the findings of the previous experiment were not a function of low protein availability, as the addition of protein to the low calorie dessert produced the same results. In the fourth experiment, we demonstrated that the availability of a single dessert with high energy density resulted in increased caloric intake and body weight gain, with some evidence of short- and long-term compensatory behaviors. In the fifth experiment, we demonstrated that previous experience with dessert increased the probability of consuming desserts in the future. In the sixth experiment, we attempted to produce an animal model of the human phenomenon of Night Eating Syndrome, but demonstrated that caloric intake and body weight gain did not increase as a function of diurnal access to a high calorie dessert. Together, these findings suggest that certain factors of availability impact food intake and caloric balance and demonstrate the need for further studies in this area.

CHAPTER 1 INTRODUCTION

The work described in this thesis originated in discussions concerning impulsive eating and the implications of overconsumption on theoretical models of body weight homeostasis. If the short-term and long-term limits of caloric compensation are tightly regulated, then the obesity epidemic that is sweeping most postindustrial societies would not exist. This introduction reviews the etiology of obesity, the mechanisms and limits of a model of homeostatic regulation of body weight, and the factors of food that mediate intake. The literature lacks examination of the effect of food availability and experimental protocols that explore situations of increased access to free food. We attempted to address this issue in a series of six experiments, which comprise this thesis.

Obesity and Public Health

The high incidence of obesity in the human population has become a significant threat to public health. Epidemiological studies in postindustrial countries show that food intake and body weight are rising at an alarming rate (Center for Disease Control 2004, Rudolf et al. 2004, Flegal et al. 2002, Gutierrez-Fisac et al. 2000). Obesity threatens health and productivity (Mokdad et al. 2003, Must et al. 1999) due to its strong positive correlations with diabetes (Chan et al. 1994), cardiovascular disease (Martins et al. 2003), and cancer (Bianchini et al. 2002).

Definition of Obesity

Obesity, ultimately the result of an imbalance between energy input and energy output, has been described as a multifactorial phenomenon resulting from genetic

predisposition either triggered or exacerbated by environmental pressures (Bray 1996). While consuming calories in excess of physiological need might have been evolutionarily advantageous in the unpredictable ancestral environment, the human genome has not adapted to the conditions in modern times in which food has become readily available. Today's trend toward obesity can be functionally viewed as a behavioral inability to compensate for this sudden change in the quantity and quality of food. The factors promoting eating in excess of physiological need must be understood if effective strategies are to be developed for the prevention and treatment of obesity.

Body Weight and Homeostasis

The genes, systems, and behaviors selected in times of lesser food availability have been studied extensively and most often have been organized within a homeostatic framework. Homeostasis is a term that was introduced by Cannon (1929) to describe the means by which the body achieves the relative constancy of physiological parameters. If body weight is a regulated variable controlled by a homeostatic mechanism, some factor(s) derived from food must be represented by the endogenous production of signals that can be detected by the brain and compared to some ideal or set point value dictated by genetics. One such factor is likely to be the amount of metabolizable energy stored in the body. The discrepancy between this hypothetical set point and energy influx and output would produce an error signal that would increase or decrease feeding behavior accordingly. For example, Cannon proposed that food intake is based primarily on peripheral cues, such as contractions by the empty stomach; however, feeding behavior usually occurs before the stomach empties fully and for this and other reasons, purely "peripheral" accounts are inadequate. Many correlates of the body's nutritive status have been identified and described in various theories.

The Glucostatic Hypothesis

Mayer (1954) hypothesized that short-term homeostatic regulation of eating was regulated through detection of changes in blood glucose levels. Glucose in the blood triggers the release of insulin, a pancreatic hormone that polymerizes glucose into glycogen and stores it in the liver and muscles. Insulin crosses the blood brain barrier at the circumventricular organs and centrally acts as a correlate of the mobilization of energy into cells, thus reducing food intake. Support of this action can be shown with exogenous administration, pharmacological manipulation, and genetic deficiencies (as reviewed in Benoit et al. 2004). The glucostatic hypothesis offers an explanation of the short-term control of glucose levels in blood, but Mayer argued that there must be means by which changes in whole-body nutritive status could be compared to overall body energy stores and thus, alter behavior over time. He supported a contemporaneous theory proposing the lipostatic control of body weight to round out glucostasis.

The Lipostatic Hypothesis

Kennedy's lipostatic hypothesis (1953) addressed energy balance by advocating fat stores as the regulated variable in the maintenance of body weight. Because adipose tissue is distributed in many depots and these do not have known neural connections to the brain, a circulating factor correlated with lipid stores is a necessary component for a lipostatic mechanism. After many years of searching, leptin was identified in the 1990's as a candidate mechanism. Leptin is secreted by adipocytes in amounts proportional to the amount of fat stored, has receptors in key brain regions, and peripheral or central administration of leptin has been shown to decrease food intake (reviewed in Benoit et al. 2004). The glucostatic and lipostatic hypotheses purport ways in which the peripheral

nutritive status of the body can be communicated to the brain, which uses the information to alter behavior by signaling states of hunger and satiety.

Central Integration

The findings related to the glucostatic and lipostatic theories added support to Stellar's (1954) proposal of a dual brain center located primarily in the hypothalamus that integrates multiple factors to control motivated behaviors of which feeding is a key example. Since then, most new findings in the field have been viewed in this construct and it has predictive value as a model. Numerous neural pathways and associated peptides have been discovered that support the concept of individual molecules or systems for hunger and satiety (Bagnasco et al. 1999).

The hypothalamus is often considered the brain area most directly responsible for integrating information about food stimuli and accommodating behavioral change. Separate areas are associated with certain physiological states and behavior patterns. The ventromedial nucleus of the hypothalamus (VMH) has been suggested to be a satiety center, since rats with lesions of this area engage in hyperphagia and gain a large amount of weight. The VMH contains receptors for a number of identified anorexigens, such as leptin and pro-opiomelanocortin (POMC). The lateral hypothalamus (LH) has been suggested to be a hunger center since its destruction results in aphagia and weight loss. The LH contains neurons that produce orexigens, such as neuropeptide Y (NPY) and agouti-related peptide (AgRP). It has been suggested that these areas interpret cues from food and endogenous correlates of nutritive status, integrate information concerning the need for nutrients, and change behavior accordingly. It has been suggested that lesions of these areas produce changes in body weight set point (Hoebel and Teitelbaum 1971). Friedman and Stricker (1976), however, argued against the perspective of hunger and

satiety controlled by the brain, and postulated basic biochemical processes of energy metabolism, especially liver function, as the foundation of food intake. This theory rejects the need for constructs such as body weight set point and explains changes in the food intake of VMH and LH lesioned animals as a result of disruption of lipogenesis.

None of these theories, however, address why intact animals with nutritional excesses might still eat, although this phenomenon has been extensively reported (Sclafani 1980, pp. 166-81). It is plausible that some variables in the environment may promote overconsumption and overwhelm these homeostatic mechanisms. The factors of food that influence consumption are discussed below.

Dietary Determinants of Energy Balance

Woods (1991) argued that since consuming food disturbs the internal milieu, the body must interpret cues inherent in or caused by food to emit appropriate behavior to maintain constancy. There are multiple aspects of food that can be detected and affect the body. Oral and postoral factors of food, such as taste, the relative hedonic evaluation of flavors (palatability), macronutrient composition, volume and moisture content, and the energy density of the food (Sclafani 2004), as well as the relative cost and learned feeding patterns affect intake. The consequences of these factors on physiological state would need to be assessed in order to determine energy balance and alter behavior to maintain relative constancy. Studies have been conducted addressing how these factors influence consumption.

Oral Cues

The taste and/or texture of a consumable are the first ways the body detects the influx of nutrients. By using experimental techniques that minimize postingestive effects, such as brief tests, licking, sham-feeding, taste reactivity, and operant methods,

we can ask animals questions about what behavior certain taste stimuli elicit and evoke (Spector 2000). Taste is often indicative of food composition and there seems to be innate preferences for sweet and avoidance of bitter tastes. Animals will work harder in the presence of a sweet taste that acts as a discriminative stimulus (“electronic esophagus” preparation, Sclafani 1990 as reviewed in 2004) or when the taste acts as a consequence of operant performance (progressive ratio breakpoints, Sclafani and Ackroff 2003). Taste independent of ingestion can cue feedback systems, such that insulin is released when a sweet flavor is detected (cephalic insulin responses, Steffens 1976 as in Woods 1991). Flavors can also be classically conditioned to reduce behavior, the most potent example being that of conditioned flavor avoidance (Garcia et al. 1956). Taste is linked to, but differs from palatability, the reward potential of an appetitive stimulus. Flavors are conditioned as palatable due to their postingestive effects, which demonstrates that consequences at the systemic level are able to influence behavior.

Postoral Cues

A food's taste is in part determined by its macronutrient composition, which relates to the innate preferences influencing intake that were described above. Warwick and Weingarten (1995) demonstrated that when contingent on a licking response requirement, rats that were infused intragastrically with nutrients proportionally high in calories from fat showed greater caloric intake and body weight gain than rats given nutrients high in carbohydrates. Woods et al. (2004, 2003) advocated the role of the postingestive consequences of the amount of fat in food. He developed a protocol in which rats were given either a high fat or low fat diet or fed the high fat diet matched daily to the caloric intake of the rats fed the low fat diet (since the two diets were not isocaloric). Rats fed ad libitum high fat diet gained more weight and developed hyperleptinemia and insulin

resistance compared to the rats fed low fat diet. The rats which were fed high fat diet matched to the caloric consumption of rats fed low fat diet had similar body weights to the rats fed low fat diet, but had much greater body adiposity, suggesting that the level of fat or the energy density of a maintenance diet affect fat deposition. Warwick et al. argued and demonstrated (2002, 1992) that increased caloric intake and body weight gain from high fat diets are a function of the fat content rather than the energy density. Rats were given isocaloric diets high in either fat or carbohydrate or those diets diluted to half the energy density. Rats given the diets high in fat, diluted or not, gained more weight than those on the high carbohydrate diets. Lucas et al. (1989) demonstrated fat selection and consumption as dependent on the type and form of fat and the availability of other macro- and micronutrients. He argued that the behavior change is determined primarily by postingestive factors and that the short-term controls of satiation and satiety are less affected by fat than other macronutrients due to weaker feedback signals.

The macronutrient composition of a food determines its caloric density, with fat contributing approximately 9 kcal of energy per gram (kcal/g). The water content also influences the amount of energy within a certain portion, resulting in a high satiating power of food with large amounts of bulk and water (Drewnowski 2004). Dry foods with high caloric density result in the reverse, having a small effect on satiation and promoting increased consumption. Rolls et al. (2005, 2000) demonstrated by altering the macronutrient status and palatability of foods that human satiety and satiation are influenced most directly by the energy density and portion size of food.

Behavioral Economics

The cost of food determines how readily and how much food is consumed. Since food in post-industrial societies has become easily available, this may also be

contributing to the rise in body weight. Ackroff and Sclafani (1999) have explored this by integrating the electronic esophagus paradigm with a foraging paradigm. In a protocol originally conceived by Collier (1985), food procurement cost is simulated by imposing a cost (usually a lever press) to gain access to a large amount of food. Collier found that in a wide range of conditions and species, as cost of procurement goes up, meal frequency goes down and mean meal size increases such that total intake is conserved except at the highest costs. Ackroff and Sclafani (1999) showed that increasing food cost blunts rats' responses to the changes in palatability. Collier and Johnson (2000) have also shown that the cost of sucrose correlates with its consumption and that the relative cost of nutrients plays a large role in food selection and intake.

Learned Feeding Patterns

The economics of food described above and the presence of circadian rhythms set a culture of appropriate behavior concerning food. In the last century, advances in food technology and distribution have led to an unprecedented range of food choices for most humans, often with the foods of higher energy density being the least expensive (Drewnowski 2004). Snacking has become a cultural norm and is prevalent in obese humans (Berteus Forslund et al. 2005). This behavior is maintained through schedules deemed appropriate by our verbal community, although aberrant behavior can be conditioned (e.g., Binge Eating Disorder and Night Eating Syndrome, Tanofsky-Kraff and Yanovski 2004).

Limits of Homeostasis

In a homeostatic system, the oral, postoral, economic, and learned cues about food must be integrated in a way to behaviorally control the stability of body weight. It has been demonstrated, however, that all these factors have the potential to override the

homeostatic controls of energy balance. This may be because in terms of “conscious awareness” (stimuli about which one can engage in verbal behavior), the only stimuli we utilize are those associated with the all-or-none behavioral assessment of, “eat this, don't eat that”. The impulsive choice of responding in the presence of consumables that have in the past resulted in pleasant oral and/or postingestive consequences will occur with a higher probability than the choice heeding the homeostatic push of the body to remain at a hypothetical set point. Body weight regulation seems to have evolved in a way which ensures defense against weight levels being lower than a certain level, but predisposes toward excess weight gain under comfortable conditions, since this, as stated previously, would have been a survival enhancing set of behaviors.

The homeostatic model, which advocates a body weight set point and behavioral maintenance of constancy, is incapable of addressing why humans are now in the midst of an obesity epidemic. Bolles (1980, pp. 63-76) argued against body weight regulation, attributing fluctuation to linear associations between environmental and physiological factors such as palatability, satiety, storage, and cost. All these factors have been shown to affect the body weight of even genetically homogeneous laboratory animals, the most well known being the phenomenon of diet-induced obesity and the effect of cafeteria diets (see Sclafani 1980 for review). New models of feeding and body weight address the impact of the environment and include both homeostatically 'compensated' and non-homeostatic 'uncompensated' factors (De Castro and Plunket 2002).

The Influence of Availability

The studies described in this introduction have addressed how qualitative aspects of food and their economics affect intake and body weight gain. Relatively few studies have explored the influence of food availability. Situations that promote increased access

to free food more closely model the modern postindustrial human environment, and so manipulations of food availability may provide information on which environmental factors disrupt caloric compensation and influence body weight. Availability has many facets, including spatial, temporal, and qualitative aspects, none of which are mutually exclusive. In a series of six experiments, we attempted to separate these parameters of food availability and we explored their effect on the balance of caloric intake and the compensatory mechanisms associated with body weight change in rats.

CHAPTER 2
EXPERIMENT 1–SPATIAL AVAILABILITY

Introduction and Rationale

Spatial availability can be defined as the amount of a commodity within a certain area at a specific time. It is plausible that the amount of food in the vicinity might influence how much is consumed at that time. Tordoff (2002) demonstrated that the amount and location of consumables affect preference and intake in rats. In his study, rats were provided with access to multiple fluid sources in their home cage, consisting of either water or a sucrose solution. Some rats were given more sucrose than water sources and some were given more water than sucrose sources. Rats provided with more sucrose locations than water consumed more sucrose than rats provided with more water locations than sucrose. He concluded that this shift in preference and intake was the result of increased spatial access to an otherwise free commodity. In the first experiment, we tested the hypothesis that increased intake as a function of increased spatial availability would generalize to increased consumption of maintenance diet in rats. Specifically, we hypothesized that rats presented with four jars of chow would eat more than those with one jar of chow.

Methods

Animals and Housing

The animals used were 30 female retired breeder Sprague Dawley rats (Harlan, Indianapolis, IN) weighing between 250 and 350 g at the start of the experiment. The animals were housed individually in polycarbonate tub cages (48x27x20 cm) containing

approximately 2 cm depth of Sani-Chips bedding (Teklad, Madison, WI) in a vivarium maintained at 22 ± 2 °C. The rats were kept on a reverse light cycle (lights on from 2200 to 1000 h and off from 1000 to 2000 h) such that all procedures were conducted in the dark, which is when rats are most active and consume the majority of their food. These and all the animal procedures presented in this thesis were conducted in accordance with good laboratory standards and were approved by the University of Florida Institutional Animal Care and Use Committee prior to implementation.

Food Conditions and Measures

All rats were provided with ad libitum access to tap water and standard powdered rat chow (Purina 5001, 3.34 kcal/g). Chow was presented in 50 ml glass jars suspended within the home cage using a custom-made metal stirrup. Daily intakes of chow were measured for a two week baseline phase by subtracting the remaining chow and hopper weight from that originally presented. The jars were refilled each day at the start of the dark cycle. Five groups of six rats were defined such that the average daily caloric intake during the baseline phase was equivalent between groups. Following the baseline period, the rats entered the experimental phase during which five different conditions of food availability were studied.

Table 2-1: Differences in the spatial availability of maintenance diet

Group	Type of maintenance diet	Number of jars	Where in cage
1	Standard chow	1	1 corner
2	Standard chow	4	4 corners
3	High fat diet	1	1 corner
4	High fat diet	4	4 corners
5	Both	2 of each	4 corners

Two groups of rats continued to receive the standard powdered chow, but with different spatial availability. Group 1 was maintained on baseline conditions with one jar

of chow in one corner of the cage and Group 2 was provided with a jar of chow hung in each corner of the home cage for a total of four jars of food. In order to assess if an effect would be different based on the quality (e.g., palatability, macronutrient composition) of the maintenance diet, a high fat diet was provided to the two other groups of rats. The high fat diet consisted of two parts powdered chow mixed with one part softened vegetable shortening (estimated to yield approximately 5.24 kcal/g), a combination known to be highly palatable to rats and to promote weight gain (Corbit and Stellar 1964). Group 3 was given one jar of high fat diet and Group 4 provided with four jars of high fat diet. The final group received two jars of standard chow and two jars of high fat diet (Group 5). The experimental phase lasted three weeks, during which food intake was measured daily. Data were excluded from analysis if upon visual inspection spillage appeared to be greater than 5 g.

Body Weight Measures

Body weight was measured twice weekly in the experimental phase. Cumulative body weight gain was calculated by subtracting the last body weight measurement taken during the baseline period from those taken throughout the experimental phase.

Data Analysis

Daily caloric intake and change in body weight of each rat were calculated. This was then averaged between rats within a single group over each week. Between group analyses were assessed using SPSS (SPSS, Chicago, IL) one-way ANOVAs and Tukey posthoc tests with a significance criteria of $p < 0.05$.

Results

Total Caloric Intake

Figure 2-1 shows the total caloric intake of the five groups by experimental week and compared to baseline consumption. Group differences in caloric intake were observed in experimental weeks 2 and 3. Rats in Groups 1 and 2, which were given either one or four jars of standard powdered chow, respectively, consumed more calories than those in Groups 3 and 4, which were given either one or four jars of high fat diet, respectively (Week 2: $F(4,29)=5.934$, $p<0.01$; Week 3: $F(4,29)=7.076$, $p<0.01$). Rats in Group 5, which were given two jars of each maintenance diet type consumed exclusively high fat diet and showed no difference in caloric intake from Groups 3 and 4. Total caloric intake did not vary as a function of the number of jars for either type of maintenance diet.

Body Weight

Figure 2-2 shows the average cumulative body weight gain from baseline of the five groups by experimental week. Those groups given high fat diet (Groups 3, 4, and 5) gained more weight than those given standard powdered chow (Groups 1 and 2) in each experimental week (Range of F's: $F(4,29)=9.405-3.931$, all $p's<0.01$). Body weight gain did not vary as a function of the number of jars for either type of maintenance diet.

Discussion

Based on results of Tordoff's study (2002), which showed that consumption of a sucrose solution was greater when it was presented in greater proportions, we hypothesized that the caloric intake of maintenance diet and body weight would increase as a function of increased spatial availability, such that rats given four jars of any diet type would consume more calories and gain more weight than those given one jar of that

diet. The results do not support the hypothesis since no effect of increasing the number of food jars was seen for either diet type.

There were some significant procedural problems that may have affected the results. One such factor was spillage of the maintenance diet by the rats. The food jars were not equipped with spill-proof lids and standard powdered chow is much easier to spill and harder to detect in bedding than the greasy high fat diet. The recorded intakes show that Groups 1 and 2 consumed more calories than Groups 3, 4, and 5 (Figure 2-1), but these rats did not gain more weight (Figure 2-2). This could be due either to a consistent inaccuracy of the food measurement as described above, or to a difference in feed efficiency (weight gained per kcal consumed) or both. These possibilities cannot be distinguished definitively from these results.

This failure to support the hypothesis that increased spatial opportunities to feed promote intake and weight gain is interesting since availability plays a large role in theories of foraging and patch selection and seems to impact overconsumption in humans as discussed in the introduction. Overconsumption in these arenas may be more attributable to availability of palatable foods and to the total time they are present, questions that will be explored in the subsequent experiments of this thesis.

Effect of 4 versus 1 jar of standard or high fat maintenance diet on average daily caloric intake

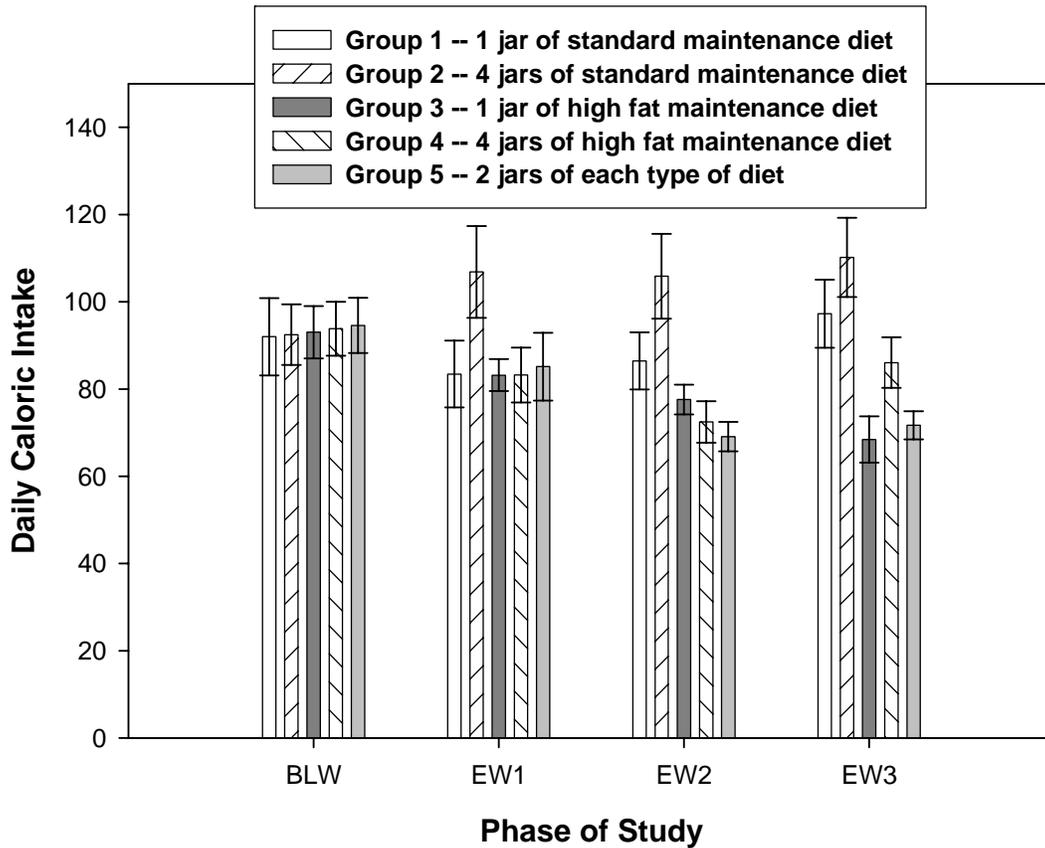


Figure 2-1: Average daily caloric intake in Experiment 1 (shown in bars and vertical lines represent SEM in this and all figures) of the five groups (shown in legend) by experimental week (EW) and compared to the average intake over two weeks of baseline conditions (BLW), in which all rats had ad libitum access to one jar of standard maintenance diet. In EW2&3, Group 1 and 2 consumed more calories than Groups 3, 4, or 5.

Effect of 4 versus 1 jar of standard or high fat maintenance diet on body weight change

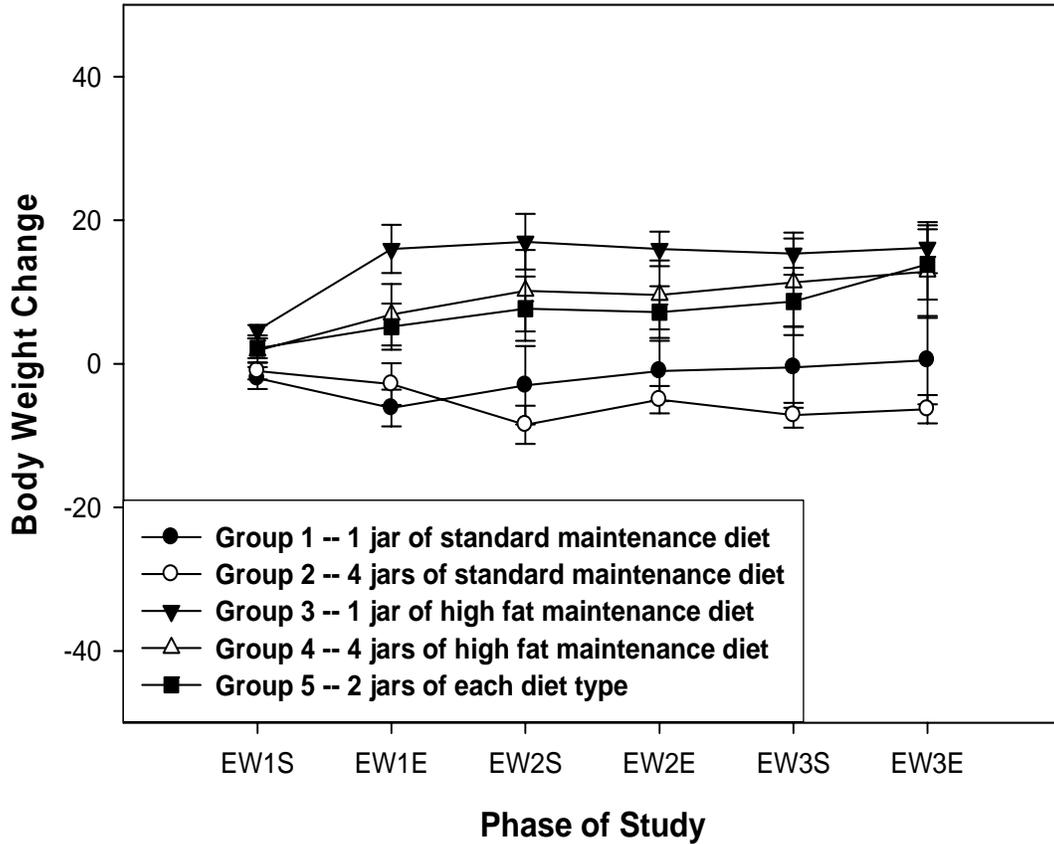


Figure 2-2: Average cumulative body weight change in Experiment 1 of the five groups (shown in legend) by experimental week (EW) calculated by subtracting the last body weight measurement of baseline conditions from body weight measurements during the three experimental weeks. Body weight was measured twice during each EW and each measurement is denoted as either S=start, beginning of the week or E=end, last day of the week. No significant differences were seen between groups.

CHAPTER 3
EXPERIMENT 2–TEMPORAL AVAILABILITY

Introduction and Rationale

In the previous experiment, we explored the role of spatial availability in food consumption. Another aspect of availability is that of temporal opportunity. Temporal availability can be defined as the number of times a commodity is available and the mean time it is present at each appearance. Temporal and spatial availability are not mutually exclusive, but based on the null findings of the previous experiment, when a commodity is present may have more impact than where or how much.

Snacking is positively related to energy intake and one of the most predictive correlates of obesity (Berteus Forslund et al. 2005). In a typical modern American home or workplace, people are constantly bombarded with opportunities to consume palatable food outside of normal meal taking. Opportunity to consume extra calorie sources may impact overconsumption more so than increased ingestion during meals. This can be explored in rats through the use of a dessert protocol in which rats are given free access to a palatable snack in addition to ad libitum maintenance diet. We hypothesized that giving rats a palatable extra calorie source would result in increased daily caloric intake and subsequent body weight gain.

The temporal dimensions of snack presentation may play a role in consumption of the snack and the meals taken after it. The phenomenon of sensory specific satiety is said to have occurred if intake and/or ratings of food that has been consumed recently are lower compared to new foods (Rolls et al. 1981). This has been explored extensively in

terms of food variety (Raynor and Epstein 2001), but very little in relation to food availability. If dessert access is uninterrupted, sensory specific satiety may occur quickly and intake may decrease and stop after an initial period of consumption. Intermittent or variable access may override this and the “novel” treat may be consumed equally at each presentation. Uninterrupted presentation of dessert may facilitate compensation of the extra calories, while intermittent access may impede this. We hypothesized that rats given intermittent access to dessert would consume more dessert compared to rats given uninterrupted access to dessert. We also hypothesized that the former group would not accurately compensate these extra calories by reducing intake of maintenance diet and thus display increased daily caloric intake and body weight gain.

Methods

Animals and Housing

The animals used in this study were 24 of the 30 rats from experiment 1 (the six rats that consistently spilled chow were excluded). The rats were maintained in the housing conditions previously described.

Food Conditions and Measures

All rats were provided with ad libitum access to one jar of standard powdered rat chow as in the baseline phase of experiment 1. Baseline intake was calculated for three days prior to study and these data were used to define four groups of six rats such that the average daily caloric intake was equivalent between groups.

In the two weeks of the experimental phase, rats in Groups 1 and 2 were maintained on one jar of standard powdered chow and Groups 3 and 4 on one jar of the high fat diet used in experiment 1. Food intake was measured daily and maintenance diet

was refilled daily at the start of the dark cycle. Data were excluded from analysis if upon visual inspection spillage appeared to be greater than 5 g.

Dessert Access

The extra calorie source in this experiment was a sugar gel made by dissolving 60 g of white sugar and 10 g gelatin in 1000 ml of tap water (estimated to yield approximately 0.31 kcal/g). The solution was poured into 50 ml glass jars, covered, and allowed to solidify in a refrigerator overnight. The jar of sugar gel was then attached to a metal stirrup and suspended in a corner of the home cage. The effect of evaporation of the sugar gel was measured prior to the experiment and since there was little effect, it was not considered in the analysis.

Table 3-1: Differences in the temporal availability of dessert

Group	Maintenance diet	Presentation of Sugar Gel across 8 h at Night
1	Standard chow	Uninterrupted
2	Standard chow	Intermittent
3	High fat diet	Uninterrupted
4	High fat diet	Intermittent

Groups 1 and 3 had uninterrupted nocturnal access to the sugar gel from 1000 h to 1800 h seven days a week during the experimental phase. Rats in Groups 2 and 4 were provided with sugar gel during the same 8 h time interval, but in a series of four 30-minute sessions every 2 h (sugar gel access from 1000 h – 1030 h, 1230 h – 1300 h, 1500 h – 1530 h, and 1730 h – 1800 h) for a total of 2 h of access. Daily intake of sugar gel was measured by subtracting the weight of the remaining gel and hopper from that originally presented. All jars of sugar gel were observed at the time of the third session and if greater than or equal to 40 g of gel had been consumed by that time, that rat received a new 50 ml jar of sugar gel.

Body Weight Measures

Body weight was measured twice weekly during the experimental phase. Cumulative body weight gain was calculated by subtracting the last body weight measurement taken during the baseline period from those measured during the experimental phase.

Data Analysis

Daily intakes of sugar gel, maintenance diet, and total calories, as well as change in body weight of each rat were calculated. These measures were then averaged between rats within a single group over a week. Between group analyses were assessed using SPSS (SPSS, Chicago, IL) one-way ANOVAs and Tukey posthoc tests with a significance criterion of $p < 0.05$. One rat from Group 1 was excluded from analysis due to extremely low gel consumption.

Results

Total Caloric Intake

Figure 3-1 shows the average daily total caloric intake of the four groups by experimental week and compared to baseline consumption. No differences in caloric intake were seen between groups during the experimental phase (Week 1: $F(3,22)=0.506$, $p > 0.05$; Week 2: $F(3,22)=0.655$, $p > 0.05$) and these intakes did not differ from baseline consumption. Caloric intake did not vary as a function of sugar gel presentation or differences in the temporal availability of sugar gel with either type of maintenance diet.

Caloric Intake from Maintenance Diet and Sugar Gel

Figure 3-2 shows the average daily caloric intake from maintenance diet and sugar gel of the four groups by experimental week and compared to baseline consumption. There were no differences in caloric intake from maintenance diet observed between

groups during either experimental week (Week 1: $F(3,22)=1.861$, $p>0.05$; Week 2: $F(3,22)=1.505$, $p>0.05$) and these intakes did not differ from baseline consumption. There were, however, differences seen in average caloric intake from sugar gel between groups during both experimental weeks (Week 1: $F(3,22)=9.066$, $p<0.01$, Week 2: $F(3,22)=7.163$, $p<0.01$). Tukey tests showed that Group 1, which had uninterrupted access to sugar gel in conjunction with standard maintenance diet, consumed more sugar gel than Groups 3 and 4, which were given sugar gel in conjunction with high fat maintenance diet.

Figure 3-3 shows the proportion of calories from maintenance diet and sugar gel averaged over the two experimental weeks and compared to baseline caloric intake from standard chow. In all groups, the extra calories consumed from sugar gel were accurately compensated through the slight reduction in the intake of maintenance diet.

Body Weight

Figure 3-4 shows the cumulative body weight change from baseline of the four groups by experimental week. There were no differences in body weight change from baseline or between groups (range of F's: $F(3,22)=0.925-0.706$, all p 's >0.05).

Discussion

Based on human data that suggest snacking to be predictive of obesity (Berteus Forslund et al. 2005), we hypothesized that providing rats with free access to a sugar gel dessert would increase total caloric intake and body weight. This dessert is palatable, evidenced by rats' high and immediate consumption of the preparation. The results do not support the hypothesis, but rather demonstrate the ability of rats to compensate for these extra calories by slightly reducing chow intake, thereby defending their baseline

body weight. Compensatory reduction in chow intake was seen independent of sugar gel access type or type of maintenance diet.

We also hypothesized that intermittent opportunities to snack would increase dessert consumption. The results do not support the hypothesis, as sugar gel consumption was greatest when it was provided uninterrupted for 8 h rather than intermittently for 2 h across the 8 h and in conjunction with standard maintenance diet. Thus, this type of intermittent presentation that emulates snacking does not promote increased intake of sugar gel or disrupt caloric compensation compared with uninterrupted access.

We cannot, however, state that the effects of snacking would not be important in other conditions. For example, our protocol may not have revealed an effect on total caloric intake due to the quality of the dessert used. Although the sugar gel is palatable, in that rats prefer it to chow when given the choice between them, it has a low caloric density so that a large amount in grams can be consumed without adding much caloric load. High water content (low caloric density) is thought to be a limiting factor on intake in humans (Drewnowski 2004). Also, from a nutritional perspective, sugar gel provides only carbohydrates, as protein in the form of gelatin is not readily used in mammalian digestive systems. The use of a higher calorie and/or more nutritive dessert may give different results, and this possibility will be explored in the next two experiments.

Effect of uninterrupted versus intermittent sugar gel access on average daily caloric intake

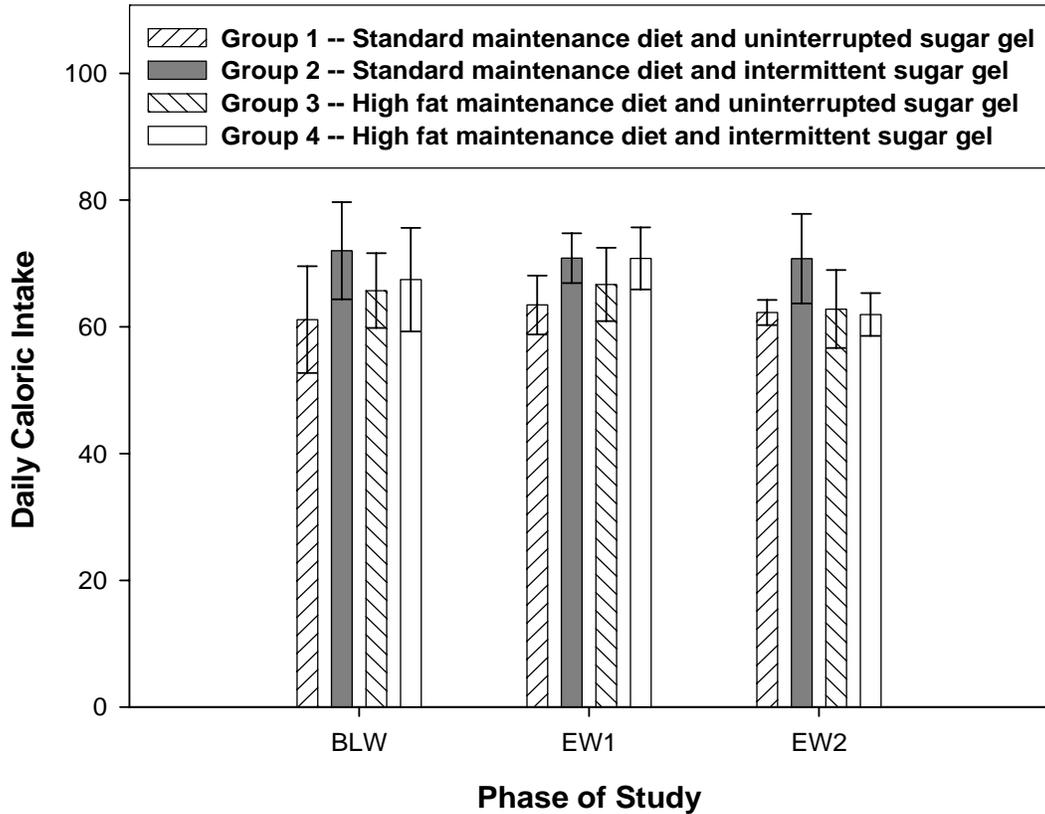


Figure 3-1: Average daily caloric intake in Experiment 2 of the four groups (shown in legend) by experimental week (EW) and compared to baseline consumption (BLW). No differences in caloric intake were seen between groups or from baseline.

Effect of uninterrupted versus intermittent access to sugar gel on caloric intake from maintenance diet and sugar gel

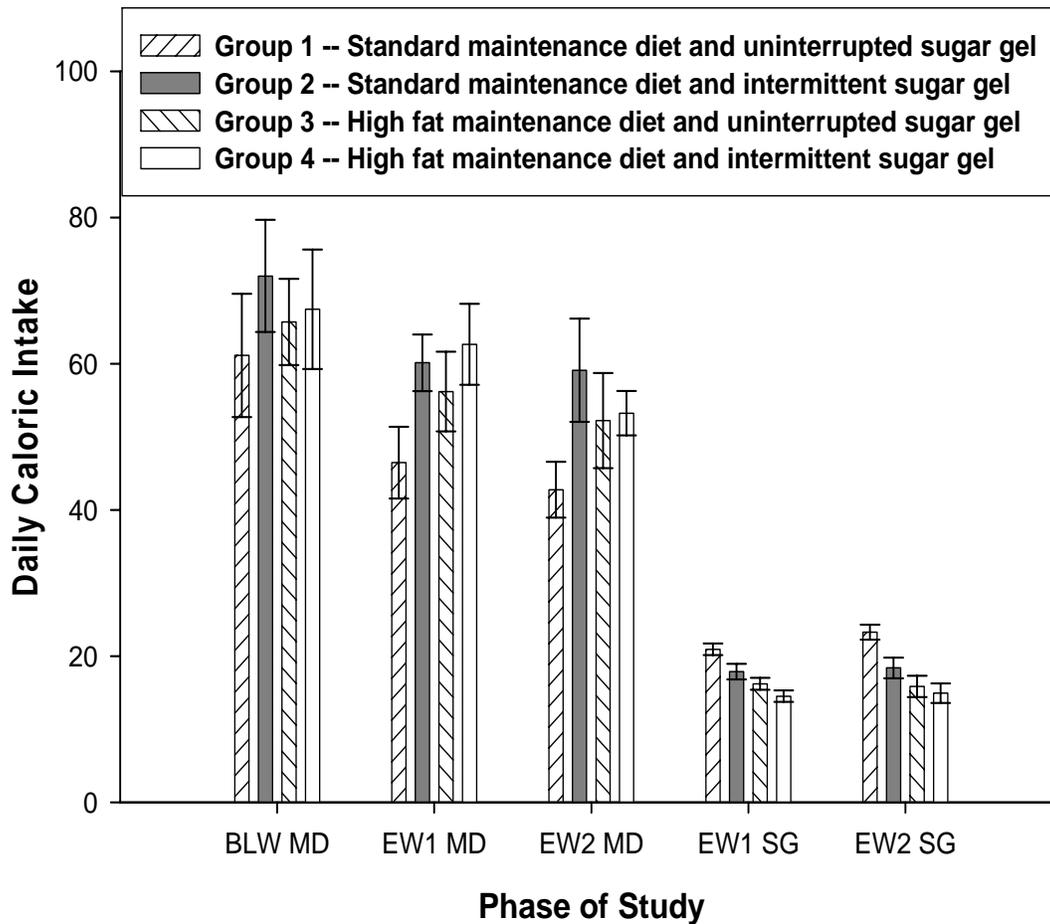


Figure 3-2: Average daily caloric intake from maintenance diet (MD) and sugar gel (SG) in Experiment 2 of the four groups (shown in legend) by experimental week (EW) and compared to baseline consumption (BLW). No differences in MD consumption were seen between groups. Group 1 consumed more SG than groups 3 and 4 in both experimental weeks.

Effect of uninterrupted versus intermittent sugar gel access on average caloric intake from maintenance diet and sugar gel

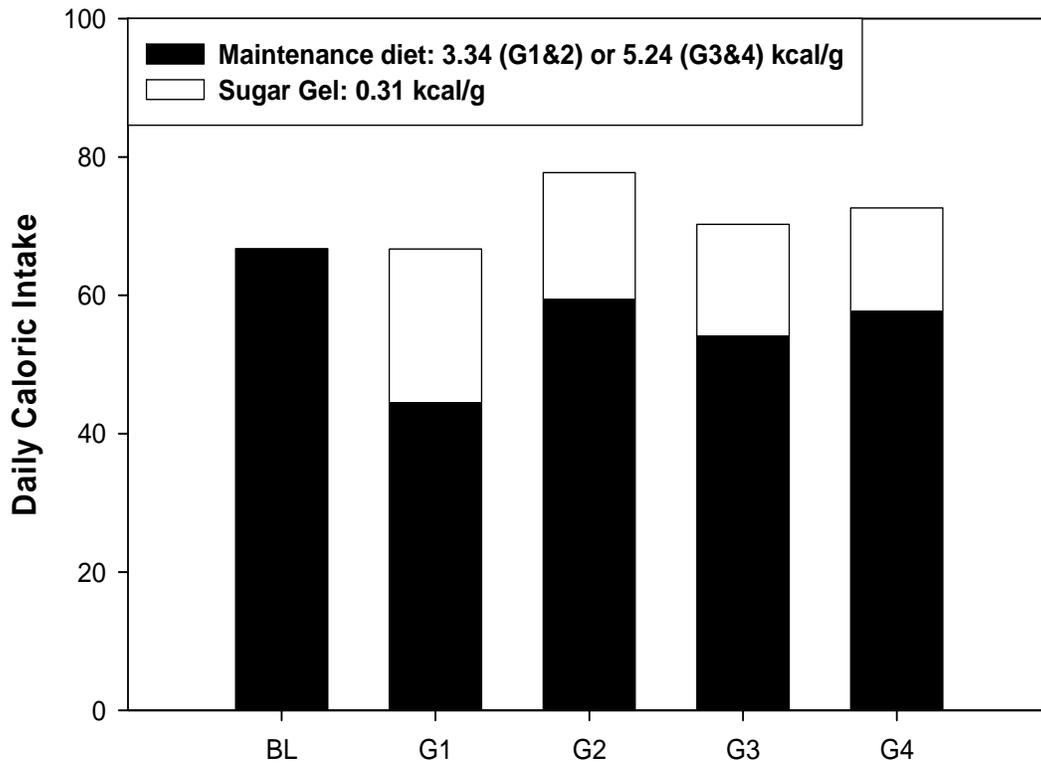


Figure 3-3: Proportion of caloric intake in Experiment 2 from maintenance diet and sugar gel by group (G, see legend of Figure 3-2 for description) averaged over the two experimental weeks and compared to the overall average baseline (BL) intake from standard maintenance diet.

Effect of uninterrupted versus intermittent sugar gel access on body weight change

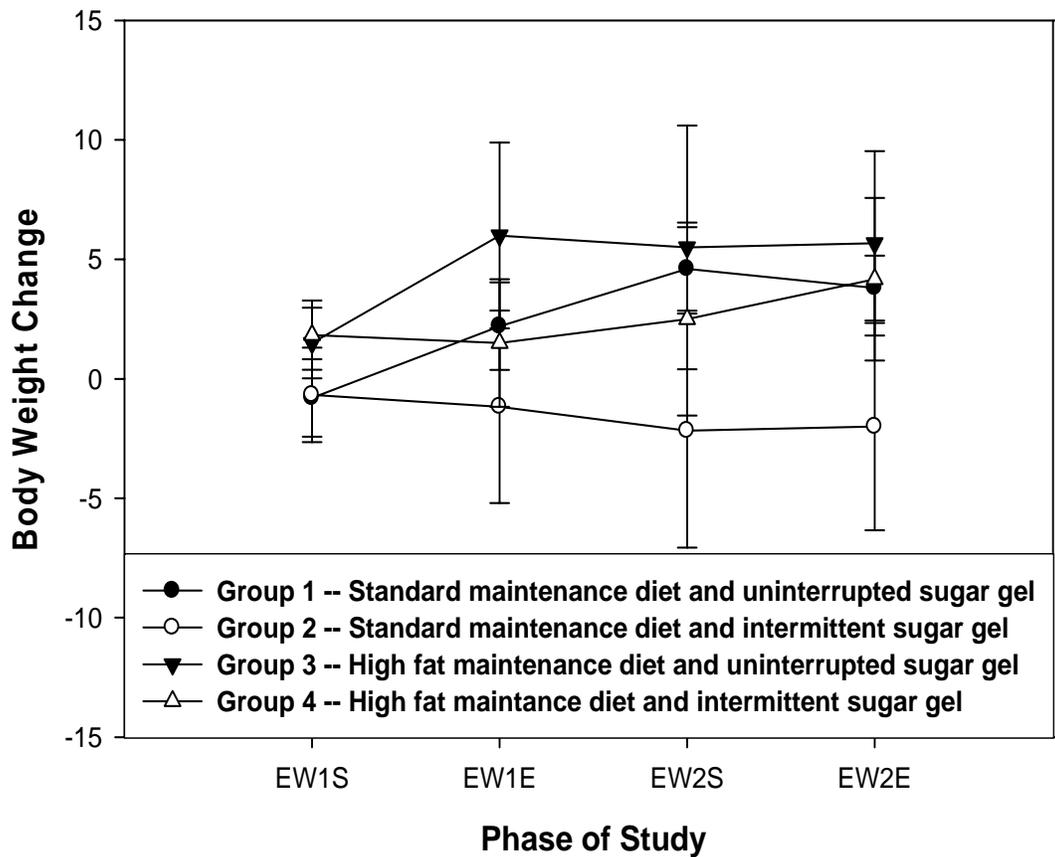


Figure 3-4: Average cumulative body weight change in Experiment 2 of the four groups (shown in legend) by experimental week (EW) calculated by subtracting the last body weight measurement of baseline conditions from body weight measurements during the two experimental weeks. Body weight was measured twice during each EW and each measurement is denoted as either S=start, beginning of the week or E=end, last day of the week. No significant differences were seen between groups.

CHAPTER 4
EXPERIMENT 3–MACRONUTRIENT QUALITY

Introduction and Rationale

We hypothesized in experiment 2 that caloric intake would increase as a function of access to a sugar gel dessert. This was not seen, possibly due to a ceiling effect resulting from the low protein to calorie ratio of the sugar gel. Protein to calorie ratio is the measure of the amount of calories consumed from protein compared to total caloric intake. This must be maintained at a stable level for survival. When rats are given the choice to eat from individual sources of different macronutrients, they select a protein to calorie ratio necessary for survival (Abraham et al. 1961, as in Pol and den Hartog 1966). Also, when the protein source is restricted, rats will consume separate sources of other macronutrients only such that approximately 8% of their calories are from protein (Pol and den Hartog 1966). Consumption of extra calorie sources may dilute the protein to calorie ratio of total consumption, posing a rate-limiting determinant of consumption of a dessert and may have been an influence on the results of experiment 2. Thus, the methods of experiment 2 were repeated with a dessert containing a protein content similar to that of chow, which has approximately 23% of its total calories in the form of protein. We hypothesized that the higher protein content of the gel would allow higher intakes of the dessert than were observed in experiment 2, and so might be more likely to reveal an increased overall caloric intake. This increased intake might show a greater consumption with intermittent compared with uninterrupted dessert access.

Methods

Animals, Housing, and Feeding Conditions

Experiment 3 began one week following experiment 2. All rats from experiment 2 were used in this study and housed as described in experiment 2. The rats were kept in the same groups and were fed the same type and amount of maintenance diet as in experiment 2. Since consumption and body weight did not change within experiment 2, no reassessment of baseline consumption was performed and all comparisons were made against the measures of baseline intake from experiment 2.

Dessert Access

In order to increase the protein to calorie ratio of the dessert, a sweetened milk gel was used instead of the sugar gel. The milk gel was made from 30 g white sugar, 30 g powdered milk, and 10 g gelatin dissolved in 1000 ml tap water. This composition is isocaloric to sugar gel (yielding approximately 0.31 kcal/g) but with a protein to calorie ratio similar to that of standard maintenance diet (approximately 20%). This solution was then poured into 50 ml glass jars and allowed to set overnight. The milk gel was presented to the groups of rats as described in experiment 2.

Body Weight Measures

Body weight was measured twice weekly during the experimental phase. Cumulative body weight gain was calculated by subtracting the last body weight measured during the baseline period from those taken during the experimental phase.

Data Analysis

Daily milk gel intake, maintenance diet intake, and total caloric intake, as well as change in body weight for each rat were calculated. These measures were then averaged between rats within a single group over each week. Between group analysis was assessed

using SPSS (SPSS, Chicago, IL) one-way ANOVAs and Tukey posthoc tests with a significance criterion of $p < 0.05$. One rat from Group 1 was excluded from analysis due to extremely low milk gel consumption and two rats from Group 2 were excluded due to excessive spillage of maintenance diet.

Results

Total Caloric Intake

Figure 4-1 shows the average daily caloric intake of the four groups by experimental week and compared to baseline consumption. No differences in caloric intake were seen between groups (Week 1: $F(3,20)=1.670$, $p > 0.05$; Week 2: $F(3,22)=0.160$, $p > 0.05$). Caloric intake did not vary as a function of milk gel presentation or differences in the temporal availability of milk gel with either type of maintenance diet.

Caloric Intake from Maintenance Diet and Milk Gel

Figure 4-2 shows the caloric intake from maintenance diet and milk gel of the four groups by experimental week and compared to baseline consumption of maintenance diet. No differences between groups were seen in caloric intake from maintenance diet during either experimental week (Week 1: $F(3,20)=2.892$, $p > 0.05$; Week 2: $F(3,20)=0.898$, $p > 0.05$). Differences were seen between groups in caloric intake from milk gel during both weeks of the experimental phase (Week 1: $F(3,20)=13.814$, $p < 0.01$, Week 2: $F(3,20)=16.456$, $p < 0.01$). Tukey tests showed that Group 1, which was given uninterrupted access to milk gel in conjunction with standard maintenance diet, consumed more milk gel than Groups 3 and 4, which had milk gel access in conjunction with the high fat maintenance diet.

Figure 4-3 shows the proportion of calories from maintenance diet and milk gel averaged over the two experimental weeks and compared to baseline consumption. In all groups the extra calories consumed from milk gel were accurately compensated through the slight reduction in the intake of maintenance diet.

Body Weight

Figure 4-4 shows cumulative body weight change from baseline of the four groups during the experimental weeks. There were no differences in body weight change from baseline or between groups (range of F's: $F(3,20)=0.612-0.070$, all $p's>0.05$).

Discussion

We hypothesized that access to milk gel would increase caloric intake and/or dessert intake compared to either baseline conditions or to the sugar gel access used in experiment 2, and that intake would vary as a function of the timing of access to the dessert. The results do not support the hypotheses because caloric intake in baseline and experimental phases were statistically equivalent and no differences were observed between groups. These data demonstrate that the results of experiment 2 generalize to isocaloric desserts that have higher protein content. These results may be due to the high protein to calorie ratio in standard maintenance diet and different results may be seen in protocols exploring protein deficiency. This low caloric density may be a limiting factor on consumption and diminish the probability of observing net overconsumption. Thus, in the next experiment, we examine the effect of a high caloric density dessert.

Effect of uninterrupted versus intermittent access to milk gel on average total caloric intake

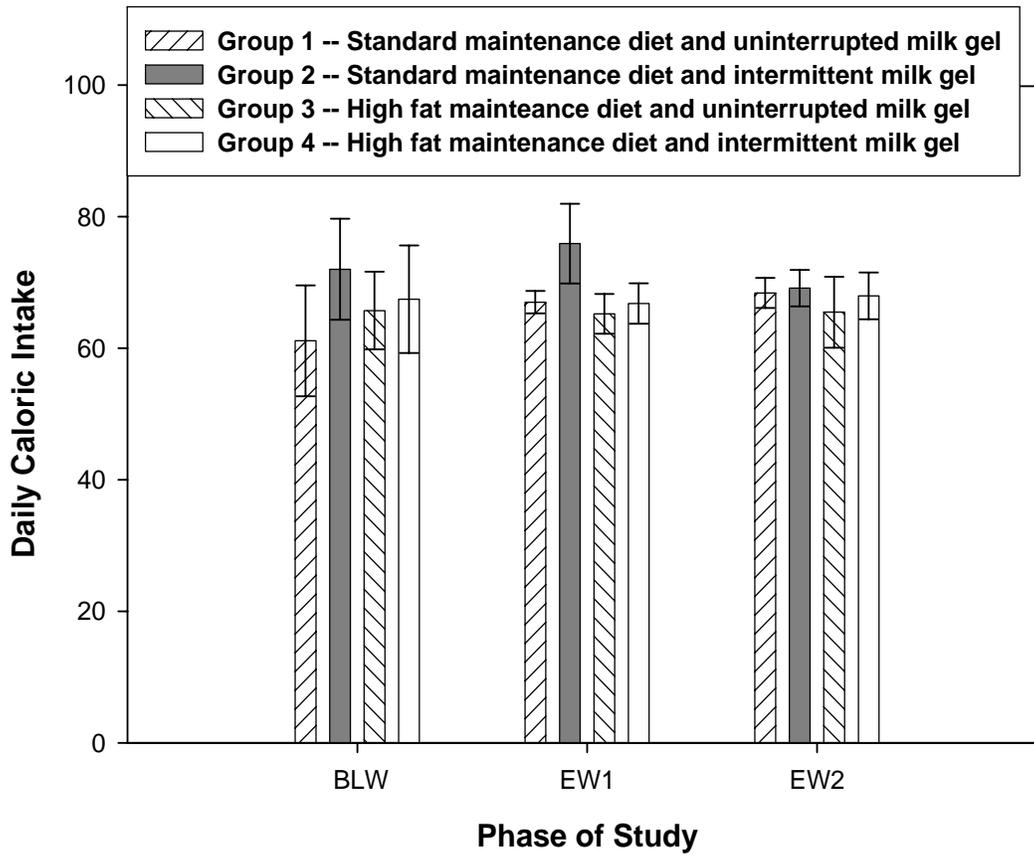


Figure 4-1: Average daily caloric intake in Experiment 3 of the four groups (shown in legend) by experimental week (EW) and compared to baseline conditions (BLW), during which all rats had access to standard maintenance diet only. No differences were seen between groups.

Effect of uninterrupted versus intermittent milk gel access on average caloric intake from maintenance diet and milk gel

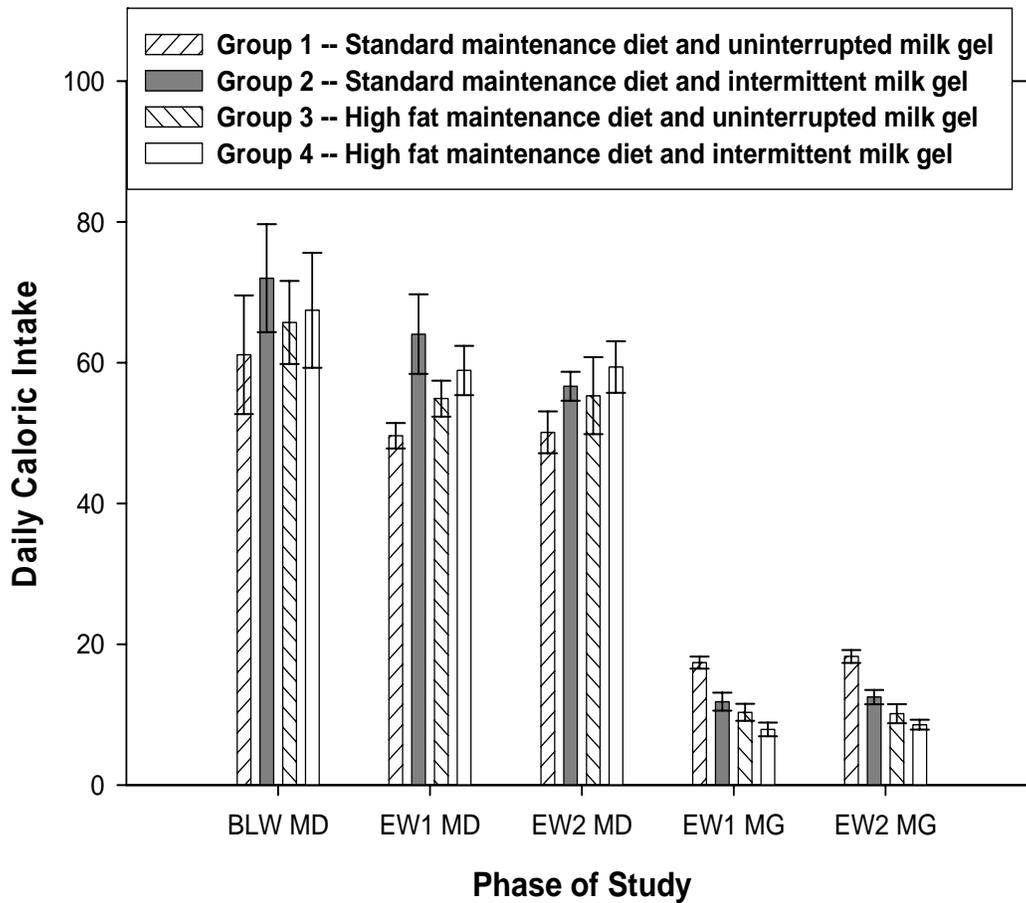


Figure 4-2: Average daily caloric intake from maintenance diet (MD) and milk gel (MG) in Experiment 3 of the four groups (shown in legend) by experimental week (EW) and compared to baseline conditions (BLW) in which rats had access to standard MD only. No differences in intake from MD were seen between groups. Group 1 ate more MG than Groups 3 and 4 in both experimental weeks.

Effect of uninterrupted versus intermittent milk gel access on average caloric intake from maintenance diet and milk gel

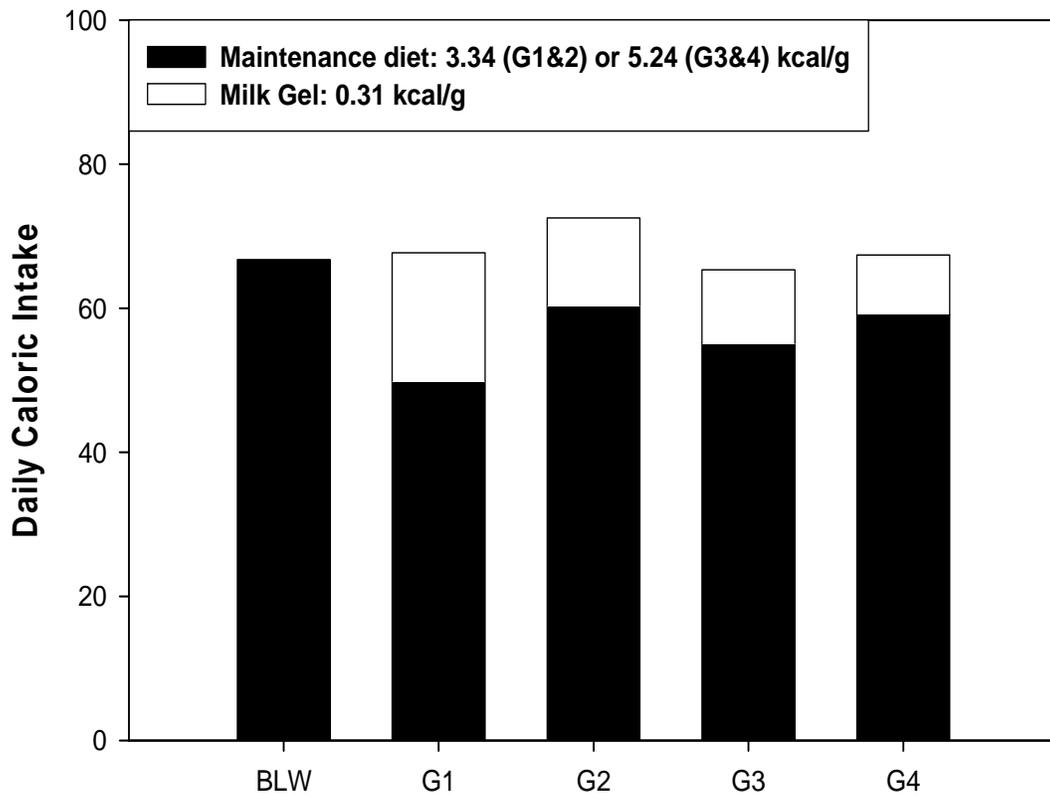


Figure 4-3: Proportion of caloric intake in Experiment 3 from maintenance diet and milk gel averaged over the two experimental weeks and compared to average baseline (BLW) consumption of standard maintenance diet.

Effect of uninterrupted versus intermittent milk gel access on body weight change

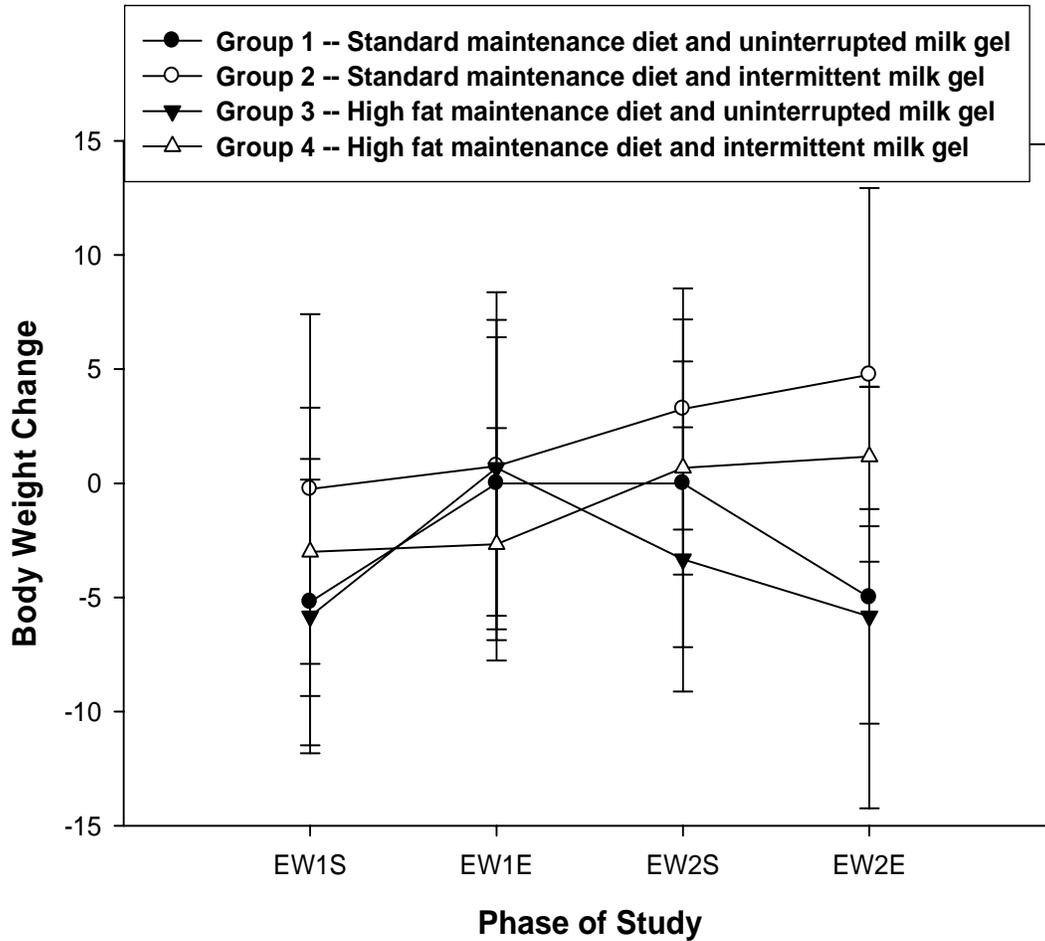


Figure 4-4: Average cumulative body weight change in Experiment 3 of the four groups (shown in legend) by experimental week (EW) calculated by subtracting the last body weight measurement of baseline conditions from body weight measurements during the two experimental weeks. Body weight was measured twice during each EW and each measurement is denoted as either S=start, beginning of the week or E=end, last day of the week. No significant differences were seen between groups.

CHAPTER 5 EXPERIMENT 4–CALORIC DENSITY

Introduction and Rationale

In the previous two experiments, we explored the effect of increased temporal availability of a palatable dessert on total caloric intake. Consumption of neither the sugar gel nor the milk gel promoted an increase in total caloric intake, but rather the extra calories were compensated by a slight reduction in intake of maintenance diet. Since the protein to calorie ratio of the dessert did not seem to influence the outcome, intake may have been regulated and caloric balance maintained as a function of the low energy density of the dessert (de Castro 2005). The snacks that humans prefer and report as palatable are typically high in caloric density, with most of the calories from fat (Berteus Forslund et al. 2005). Thus, a dessert protocol utilizing a high calorie snack may promote overconsumption of the snack and allow for the exploration of the parameters of compensation. We hypothesized that rats given a dessert of high caloric density would consume more total calories, with proportionally more calories from dessert and less from maintenance diet than rats given sugar gel or no dessert access and compared to baseline consumption of maintenance diet.

Methods

Animals and Housing

New animals were used in this study. The animals used were 24 female Sprague Dawley rats (Harlan, Indianapolis, IN) weighing between 250 and 350 g at the start of the experiment. The animals were housed in conditions described for the previous

experiments. The rats were maintained on a reverse light cycle with lights on from 2100 to 0900 h and off from 0900 to 2100 h, such that all procedures were conducted in the dark when rats are most active.

Food Conditions and Measures

All rats were provided with ad libitum access to tap water and a wet mash of equal parts Purina 5001 powdered rat chow and tap water (yielding approximately 1.67 kcal/g). This was presented in 50 ml glass jars equipped with spill-proof lids and suspended within the home cage on metal stirrups. Wet mash and lids were used to prevent spillage and associated measurement error, which may have occurred in the previous experiments. To establish baseline consumption, daily food intake was measured for one week by subtracting the remaining wet mash and hopper weight from that originally presented. Clean jars of fresh wet mash were presented each day at the start of the dark cycle. Three groups of eight rats were defined such that the average daily caloric intake was equivalent between groups. All rats continued to have ad libitum access to wet mash and food intake was measured daily during the three weeks of the experimental phase. Food intake was also measured for a week following the experimental phase.

Dessert Access

Table 5-1: Differences in the energy density of dessert

Group	Maintenance Diet	Dessert Presented Uninterrupted for 8 h at Night
1	Wet mash	none
2	Wet mash	Low calorie (sugar gel, 0.31 kcal/g)
3	Wet mash	High calorie (sugar fat whip, 7.35 kcal/g)

Group 1 was maintained on baseline conditions and had no access to any dessert during the experimental phase. Group 2 received uninterrupted 8 h nocturnal access

(0900 h to 1700 h) to the sugar gel used in experiment 2, which served as the low calorie snack, yielding approximately 0.31 kcal/g. Group 3 received the same uninterrupted 8 h nocturnal access to a “sugar fat whip”, which consisted of two parts of softened vegetable shortening blended with one part white sugar and served as the high calorie snack, yielding approximately 7.35 kcal/g. Both desserts were presented in 50 ml glass jars attached to a metal stirrup and suspended within the home cage. Uninterrupted dessert access was used since this had promoted the greatest consumption in experiments 2 and 3. Since most rats in Group 2 ate all 50 g of the sugar gel originally presented, a new jar of gel was put on at 1400 h. Conversely, since the most sugar fat whip consumed by any rat in Group 3 was 15 g, only 25 g of sugar fat whip was presented daily to prevent wastage. Intake of dessert was measured daily for the three weeks of the experimental phase.

Body Weight Measures

Body weight was measured twice weekly during the experimental phase and the week following the termination of the experimental phase. Cumulative body weight gain was calculated by subtracting the last body weight measurement taken during the baseline period from those taken during the experimental phase.

Data Analysis

Daily intakes of dessert, wet mash, and total calories, as well as change in body weight for each rat were calculated. These were then averaged between rats within a single group for each week. Between group analysis was assessed using SPSS (SPSS, Chicago, IL) one-way ANOVAs and Tukey posthoc tests with a significance criterion of $p < 0.05$.

Results

Total Caloric Intake

Figure 5-1 shows the total caloric intake of the three groups by experimental week and compared to total consumption in the one week of baseline and one week following the experimental phase, during both of which rats were given only wet mash. Differences between groups were seen in the first and second experimental weeks and in the week following the experimental phase (Week 1: $F(2,23)=35.149$, $p<0.01$; Week 2: $F(2,23)=5.297$, $p<0.05$; Reestablishment of baseline conditions: $F(2,23)=40.858$, $p<0.01$), but were not seen in the third experimental week ($F(2,23)=1.214$, $p>0.05$). Tukey tests showed that Group 3, which had sugar fat whip access, consumed significantly more total calories in the first and second experimental weeks compared to Groups 1 and 2, which had no dessert or sugar gel access, respectively. Groups 1 and 2 ate significantly more calories during the week following experimental conditions than Group 3.

Caloric Intake from Maintenance Diet and Dessert

Figure 5-2 shows the average caloric intake from wet mash by experimental week and compared to the wet mash consumption of the one week of baseline and the week following the experimental weeks. Differences in wet mash consumption between groups were seen in all the experimental weeks and during the week following the experimental conditions (Experimental week range of F's: $F(2,23)=33.494-196.844$, all p 's <0.001 ; Reestablishment of baseline conditions: $F(2,23)=40.858$, $p<0.01$). Tukey tests showed that Groups 1 and 2 consumed significantly more calories from wet mash than Group 3 during experimental week 1 and in the week following the experimental phase. Group 1 consumed more wet mash than Group 2, which consumed more than Group 3 in experimental weeks 2 and 3.

Figure 5-2 also shows the average caloric intake from dessert by experimental week. Differences in caloric intake from dessert were seen in all three experimental weeks between all three groups (range of F's: $F(2,23)=251.752-435.739$, all p 's <0.01). Group 3 ate significantly more calories from dessert than Group 2, which in turn consumed more calories than zero (Group 1). Figures 5-3, 5-4, and 5-5 show the proportions of caloric intake from wet mash and dessert for each of the three experimental weeks compared to the wet mash intake during the one week of baseline.

Body Weight

Figure 5-6 shows the average cumulative change in body weight of the groups from the last day of baseline by experimental week and the week following termination of the experimental phase. All points between groups were significantly different during the all three of the experimental weeks (range of F's: $F(2,23)=39.309-82.811$, all p 's <0.01) and the following week ($F(2,23)=6.979$, $p < 0.01$). Group 3 increased in body weight from baseline and gained more weight than and Groups 1 and 2, which did not deviate from baseline.

Discussion

Based on human studies that suggest energy density to be a key factor in increased caloric intake, we hypothesized that providing rats with a high calorie dessert would promote greater total caloric intake compared to rats given no dessert or access to the low calorie sugar gel used in experiment 2. The data support our hypothesis by showing that total caloric intake was greater in the group given high caloric density dessert compared to the low caloric density or the no dessert control group.

Although total caloric consumption increased with access to the dessert of high caloric density, some compensatory behavior was seen. Rats given access to sugar fat

whip ate fewer calories from wet mash than rats given sugar gel or no dessert. However, this reduction in wet mash intake did not offset the calories consumed from the sugar fat whip and so net caloric intake was substantially increased. This is similar to eating patterns seen in humans in which the consumption of high calorie snacks does not promote smaller meals (Berteus Forslund et al. 2005).

The caloric intake of rats given dessert of high caloric density declined over the experimental weeks, and in the following week of no dessert access, intake of wet mash was comparatively low. This is similar to the hypophagia and weight loss reported in rats following experimental overfeeding (Keesey 1989). A similar phenomenon may occur in humans, who show some compensation for snacks when measured over a two day span (de Castro 2005). This suggests that although short-term controls of body weight may be overwhelmed in conditions promoting overconsumption, long-term controls may facilitate a settling point of intake and body weight when the environment changes.

This preliminary study does not separate and address the differences in the palatability and macronutrient composition of the desserts, which may be partly responsible for the overconsumption and weight gain. These issues are difficult to separate in part because the maximum caloric density of a high carbohydrate snack is often only one-half that of a high fat snack. It would be interesting to develop diets for rats in which all the dietary components are equated and compared, as done by Rolls et al. (2005) in humans; however, this endeavor is beyond the scope of this thesis.

Effect of low calorie versus high calorie dessert access on average total caloric intake

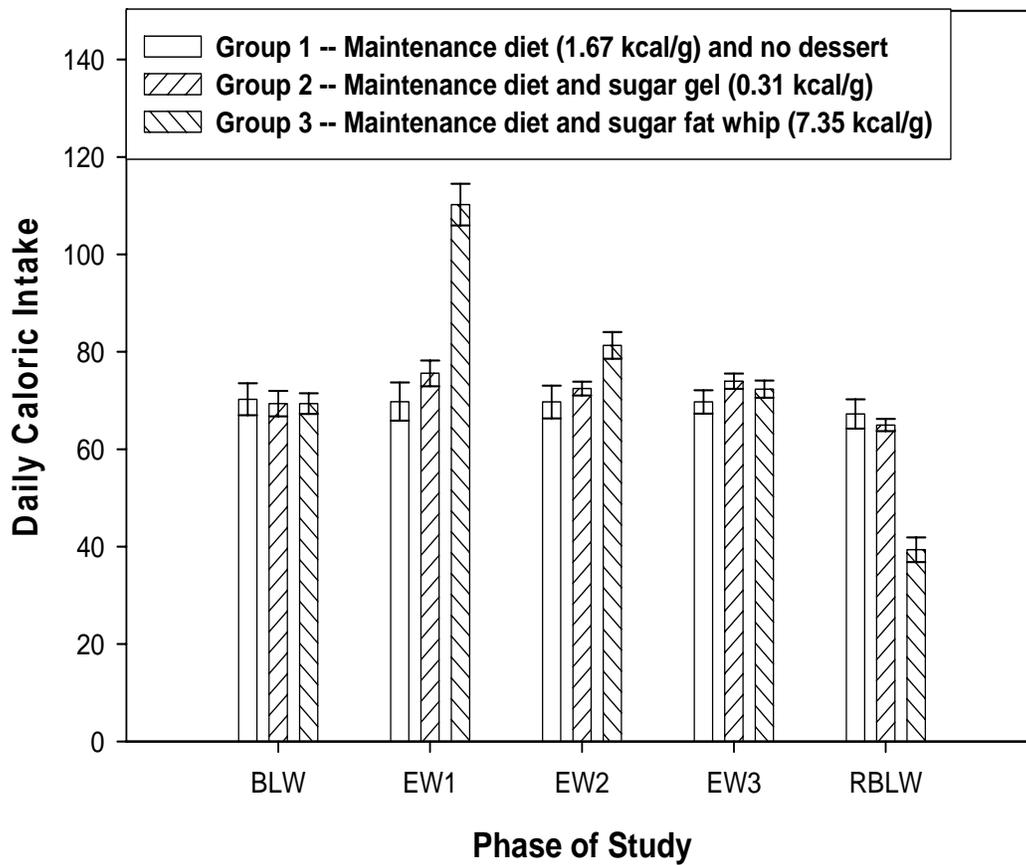


Figure 5-1: Average daily caloric intake in Experiment 4 of the three groups (shown in legend) by experimental week (EW) and compared to the week of baseline conditions (BLW) and one week following the experimental phase (RBLW), during both of which all rats had access to maintenance diet only. Group 3 consumed significantly more total calories in EW1&2 than Groups 1 and 2. Groups 1 and 2 ate significantly more calories than Group 3 during RBLW.

Effect of low calorie versus high calorie dessert access on average caloric intake from maintenance diet and dessert

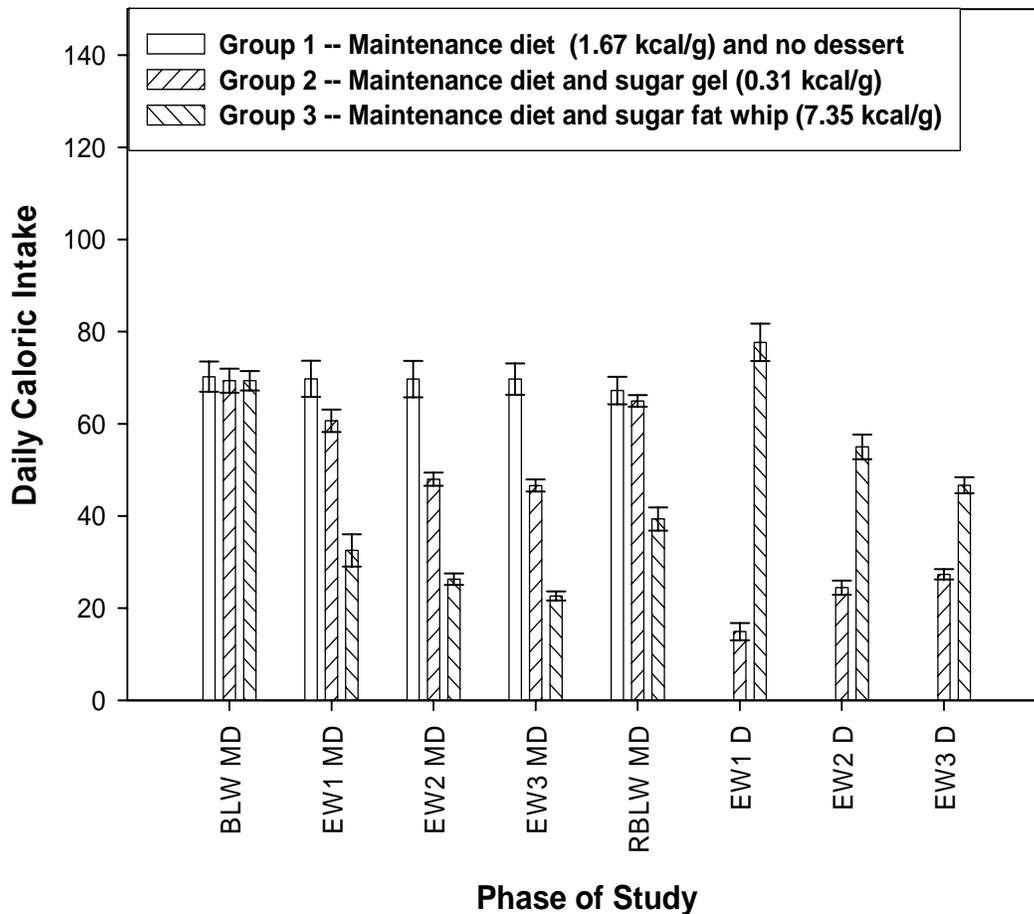


Figure 5-2: Average caloric intake from maintenance diet (MD) and dessert (D) in Experiment 4 of the three groups (shown in legend) by experimental week (EW) and compared to the week of baseline (BLW) and a week following the experimental phase (RBLW), during both of which all rats had access to MD only. Groups 1 and 2 consumed significantly more calories from MD than Group 3 during EW1 and RBLW and Group 1 consumed more MD than Group 2 during EW1 and RBLW and Group 1 consumed more MD than Group 2, which consumed more than Group 3 in EW2&3. Group 3 ate significantly more D than Group 2, which consumed more calories than Group 1 during all EW.

Effect of low calorie versus high calorie dessert access on distribution of caloric intake from maintenance diet and dessert -- week 1

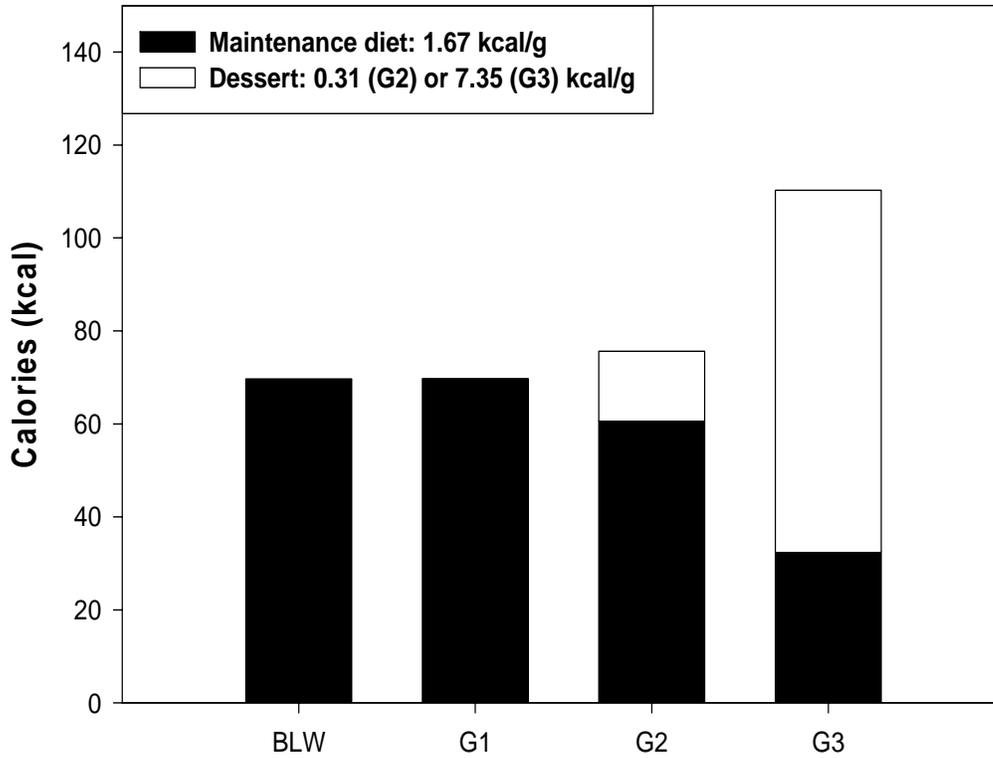


Figure 5-3: Proportion of caloric intake in week one of Experiment 4 from dessert and maintenance diet compared to baseline (BLW) measures.

**Effect of low calorie versus high calorie dessert access
on distribution of caloric intake from maintenance diet and dessert --
week 2**

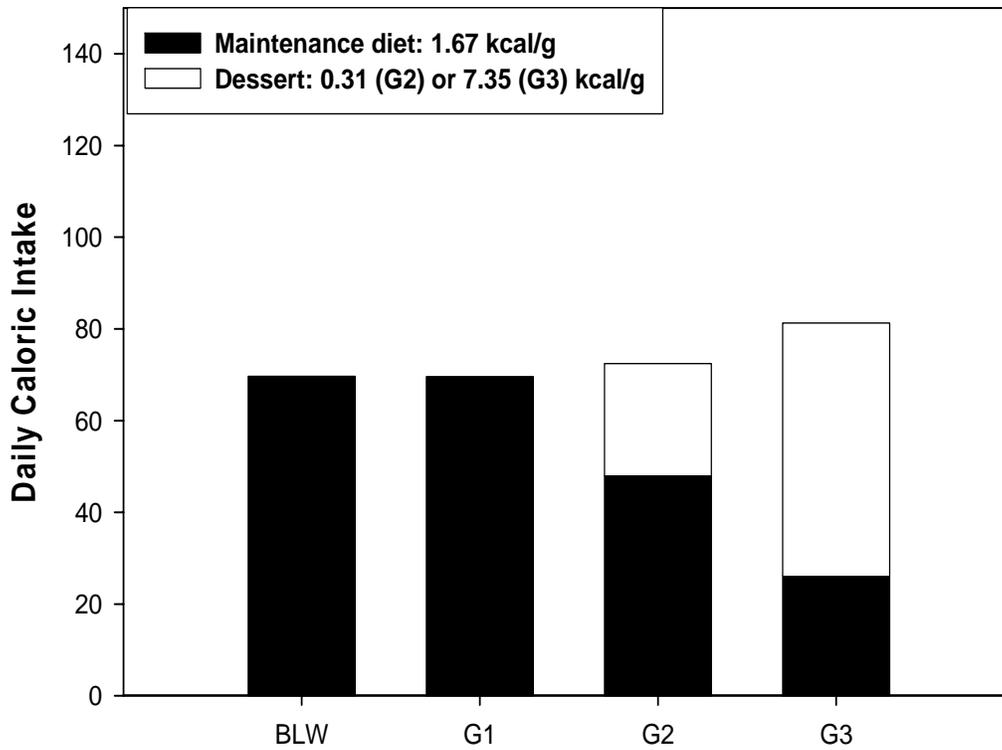


Figure 5-4: Proportion of caloric intake in week two of Experiment 4 from dessert and maintenance diet compared to baseline (BLW) measures.

**Effect of low calorie versus high calorie dessert access
on distribution of caloric intake from maintenance diet and dessert --
week 3**

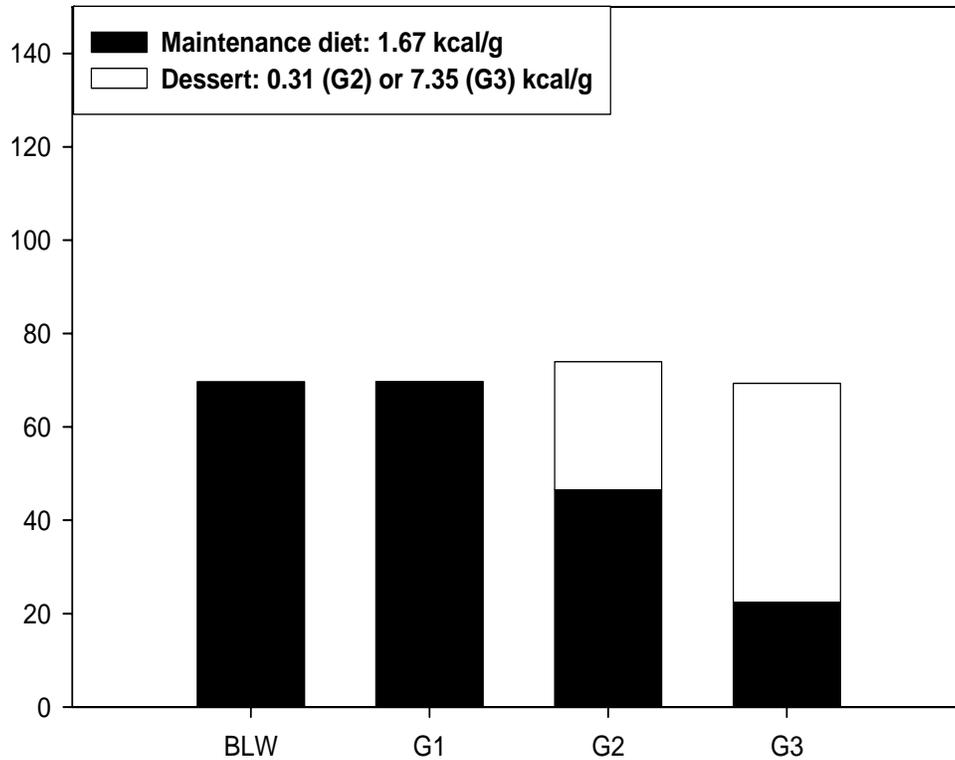


Figure 5-5: Proportion of caloric intake in week three of Experiment 4 from dessert and maintenance diet compared to baseline (BLW) measures.

Effect of low calorie versus high calorie dessert access on body weight change

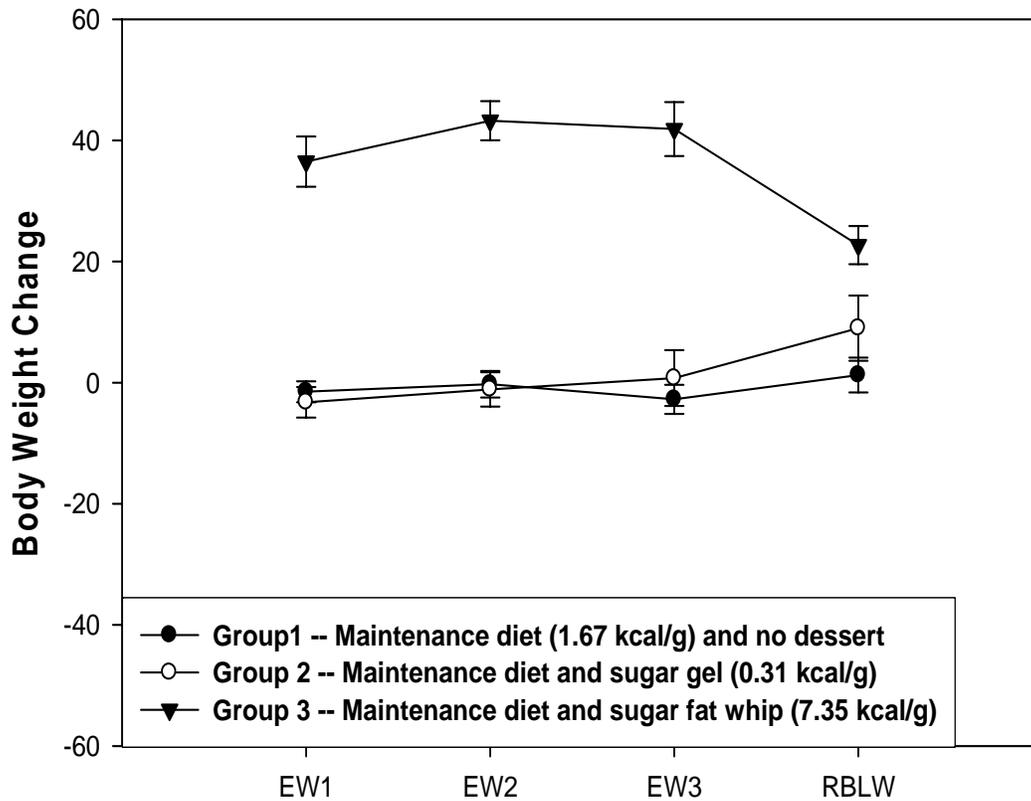


Figure 5-6: Average cumulative body weight change in Experiment 4 of the three groups (shown in legend) by experimental week (EW) and the week following the experimental phase (RBLW), during which all rats had access to maintenance diet only. Cumulative change is calculated by subtracting the last body weight measurement of baseline conditions from body weight measurements during the three EW. Group 3 increased in body weight more than Group 1 or 2, which did not deviate from baseline.

CHAPTER 6
EXPERIMENT 5–PREVIOUS DESSERT EXPERIENCE AND CHOICE

Introduction and Rationale

The behavioral strategy of selecting food based on previous experience with the positive and negative consequence of consuming it has been shown in a multitude of species (Darmaillacq et al. 2004, Leathwood and Ashley 1983). Organisms must have the ability to alter consummatory behavior in response to alterations in the environment in order to maximize feeding efficiency. Previous exposure to different desserts may affect selection and promote overconsumption when presented with access to different types of dessert. We hypothesized that rats previously exposed to any type of dessert would consume more of both types of dessert than those rats never having had dessert experience.

Methods

Animals, Housing, and Feeding Conditions

All 24 rats from experiment 4 were used in this study and maintained in the same housing conditions and groups. All rats had ad libitum access to tap water and wet mash as in experiment 4. Experiment 5 began immediately after the week of reestablishment of baseline conditions following the termination of the experimental phase of experiment 4 and so these consumption measures were used as the standard intakes for comparison. Daily food intake was measured for the two weeks of the experimental phase and for two weeks following the termination of the experimental phase, during which all rats were given wet mash only.

Dessert Access

Table 6-1: Differences in previous desert experience

Group	Previous Dessert Experience	Desserts Available Uninterrupted for 8 h at Night	Maintenance Diet
1	none	Sugar gel and sugar fat whip	Wet mash
2	Sugar gel	Sugar gel and sugar fat whip	Wet mash
3	Sugar fat whip	Sugar gel and sugar fat whip	Wet mash

All rats were given daily uninterrupted 8 h nocturnal access to both the sugar gel and sugar fat whip used in experiment 4. Both desserts were presented together in the same manner described in experiment 4. Intake of each dessert was measured daily for the two weeks of the experimental phase.

Body Weight Measures

Body weight was measured twice weekly during the experimental phase and during the week following termination of the experimental phase. Cumulative body weight gain was calculated by subtracting the last body weight measurement taken during the baseline period from those taken during the experimental phase and the following week.

Data Analysis

Daily intake of each of the desserts, maintenance diet, and the total calories, as well as change in body weight for each rat were calculated. This was then averaged between rats within a single group for each week. Between group analysis was assessed using SPSS (SPSS, Chicago, IL) one-way ANOVAs and Tukey posthoc tests with a significance criterion of $p < 0.05$. One rat from Group 4 died and so was excluded from analysis.

Results

Total Caloric Intake

Figure 6-1 shows the average daily total caloric intake of the three groups by experimental week and compared to baseline and the averaged two weeks of measures following termination of the experimental phase, during both of which all rats had access to wet mash only. Differences were seen between the groups in the week of baseline ($F(2,22)=40.858$, $p<0.01$), the first experimental week ($F(2,22)=7.944$, $p<0.01$), and second experimental week ($F(2,22)=4.356$, $p<0.05$). Tukey tests showed that in baseline and experimental week 1, Group 1 and 2, which had no dessert experience or experience with sugar gel, respectively, consumed significantly more calories than Group 3, which had experience with sugar fat whip. Group 2 consumed more calories than Group 3 in experimental week 2.

Calories from Maintenance Diet and Desserts

Figure 6-2 shows the average daily maintenance diet intake of the three groups by week and compared to baseline and the average of two weeks following the experimental weeks. Differences were seen between the groups in baseline only ($F(2,22)=40.858$, $p<0.01$), with Groups 1 and 2 consuming significantly more wet mash than Group 3.

Figure 6-3 shows the average daily intake of sugar gel and sugar fat whip of the three groups by experimental week. Differences in sugar fat whip consumption were seen between groups in the second experimental week ($F(2,22)=4.074$, $p<0.05$), with Group 2 consuming significantly more than Group 3 (this was also the trend in week 1, but $p=0.064$). Differences in sugar gel consumption were seen between the groups in both experimental weeks (Week 1: $F(2,22)=18.033$, $p<0.01$; Week 2: $F(2,22)=5.458$, $p=0.013$). In the first experimental week, Groups 2 and 3 consumed more calories from

sugar gel than Group 1. In the second experimental week, Group 2 consumed more sugar gel than Group 1. Figures 6-4 and 6-5 show the proportion of caloric intake from wet mash and each type of dessert during each of the experimental weeks and compared to the consumption of wet mash in the week of baseline and the week following the experimental phase.

Body Weight

Figure 6-6 shows the group average cumulative change in body weight from the last day of baseline by experimental week and for the two weeks following the termination of the experimental phase. All points between groups were significantly different (range of F's: $F(2,22)=8.224-22.187$, all p 's <0.01). During the first experimental week, Group 2 gained more weight than Group 3. In the second experimental week and the following week, both Groups 1 and 2 gained more weight than Group 3.

Discussion

Based on animal data that suggest food intake is affected by previous experience with it, we hypothesized that rats that had previously had access to either sugar gel or sugar fat whip would consume larger quantities of each dessert than those rats which had never experienced either dessert. The raw data did not support this hypothesis since rats previously given no dessert or sugar gel (Groups 1 and 2) tended to eat more total calories than those previously given sugar fat whip (Group 3). All three groups ate similar amounts of wet mash during the experimental weeks. Group 2 and 3 tended to consume more sugar gel than Group 1, but Group 2 tended to consume more sugar fat whip than Group 3. Groups 1 and 2 gained more weight than Group 3 during the experimental weeks, which corresponds with the data observed for total caloric intake.

Interpretation of these data is difficult since differences in intake were seen in the baseline conditions, making comparisons of the absolute data inadequate. When total calories are converted to percentages from baseline, Groups 2 and 3 consumed more calories from baseline (145% and 195%, respectively) than Group 1 (133%), supporting the hypothesis. During the experimental phase, Group 1 consumed approximately 67% of their calories from dessert, whereas Groups 2 and 3 consumed approximately 74% of their calories from dessert. This difference, while not significant, suggests that previous dessert experience may have a small effect on consumption. Also, during the two weeks following the experimental period, Group 1 and 2 consumed less chow than baseline (80%), similar to the compensatory behavior of Group 3 in experiment 4 (57%). During this time, Group 3 continued to consume 125% compared to baseline intake. This may suggest dysregulation of the long-term controls of feeding when repeatedly presented with desserts. Results may have been different if more time had been allowed between this experiment and the preceding one; however, extra time also could have interfered with the memory of the postingestive effects of the desserts.

**Effect of previous dessert experience
on choice and consumption of maintenance diet and multiple desserts --
average total caloric intake**

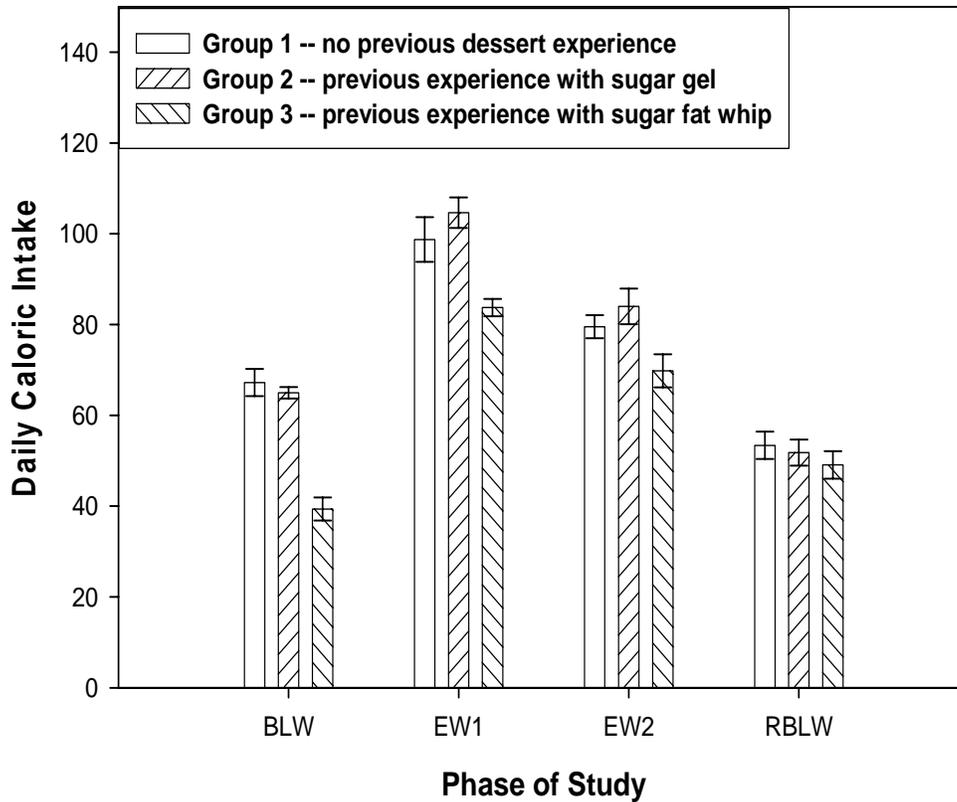


Figure 6-1: Average daily caloric intake in Experiment 5 of the three groups (shown in legend) by experimental week (EW) and compared to a week of baseline conditions (BLW) and the two weeks (averaged together) following the termination of the experimental phase (RBLW), during both of which all rats had access to maintenance diet only. Group 1 and 2 consumed more calories than Group 3 in BLW and EW1, and Group 2 consumed more calories than Group 3 in EW2.

**Effect of previous dessert experience
on choice and consumption of maintenance diet and multiple desserts --
average caloric intake from maintenance diet**

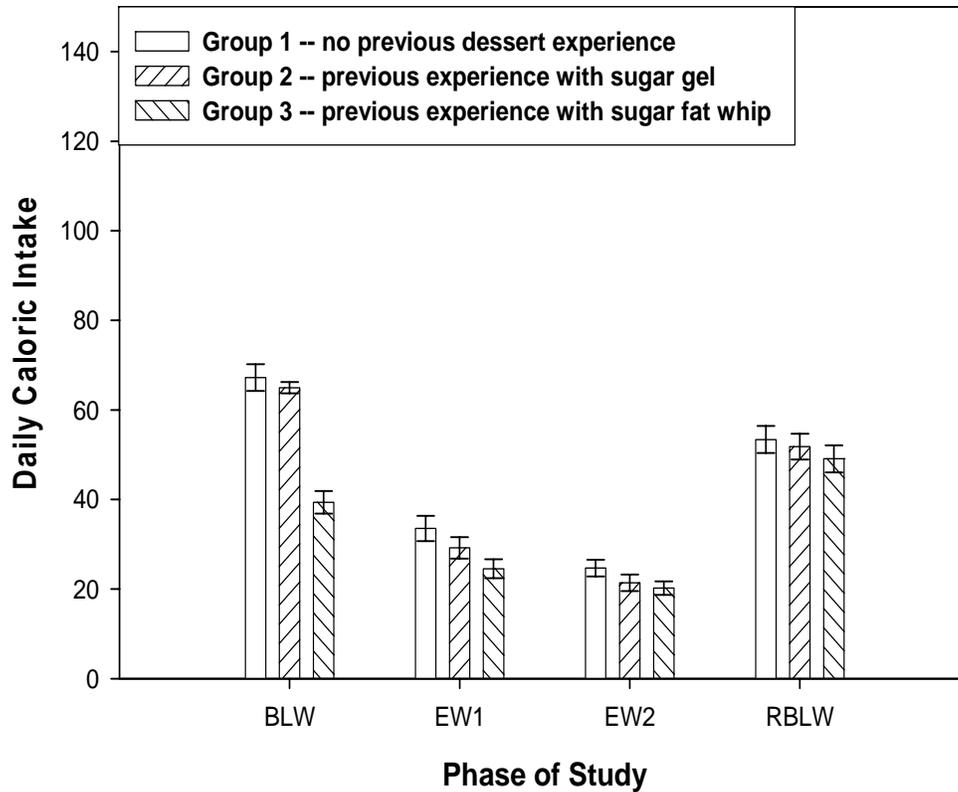


Figure 6-2: Average caloric intake of maintenance diet in Experiment 5 of the three groups (shown in legend) by experimental week (EW) and compared to a week of baseline (BLW) and two weeks (averaged together) following the termination of the experimental phase (RBLW), during both of which all rats had access to maintenance diet only. Groups 1 and 2 consumed more maintenance diet than Group 3 in BLW.

**Effect of previous dessert experience
on choice and consumption of maintenance diet and multiple desserts --
caloric intake of desserts**

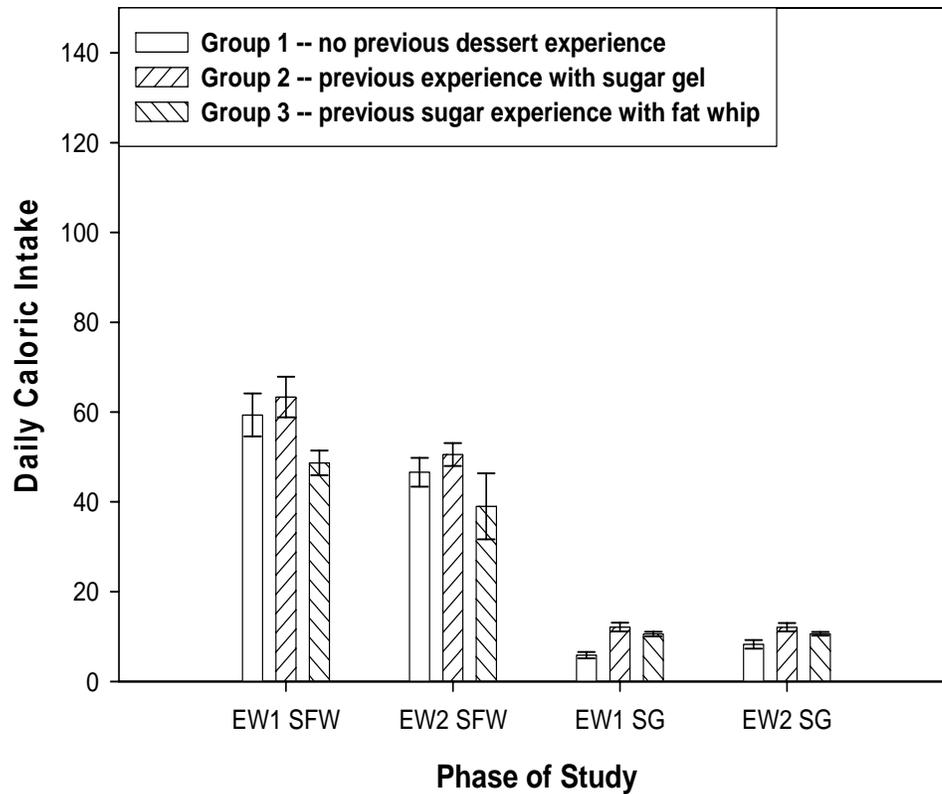


Figure 6-3: Average caloric intake of sugar fat whip (SFW) and sugar gel (SG) in Experiment 5 by the three groups (shown in legend) by experimental week (EW). In EW2, Group 2 consumed more SFW than Group 3. In EW1, Groups 2 and 3 consumed more SG than Group 1. In EW2, Group 2 consumed more SG than Group 1.

**Effect of previous dessert experience
on choice and consumption of maintenance diet and multiple desserts --
distribution of caloric intake from dessert and maintenance diet --
week 1**

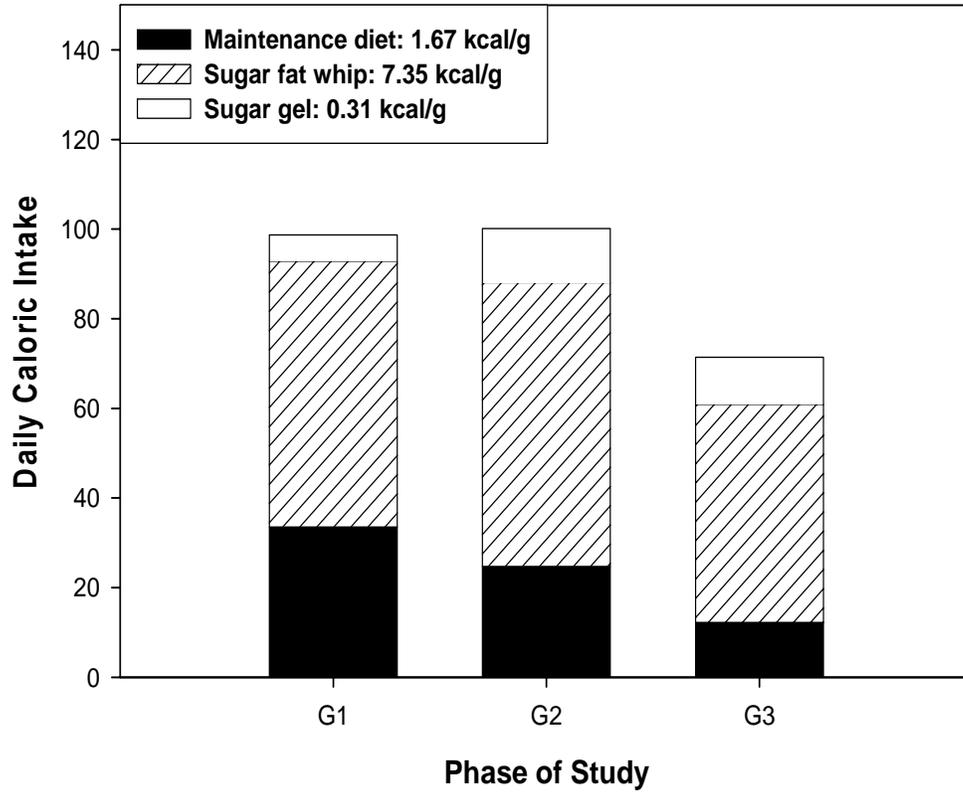


Figure 6-4: Proportion of caloric intake in week one of Experiment 5 from maintenance diet, sugar fat whip, and sugar gel of the three groups (G, see legend for Figure 6-3).

**Effect of previous dessert experience
on choice and consumption of maintenance diet and multiple desserts --
distribution of caloric intake from maintenance diet and dessert --
week 2**

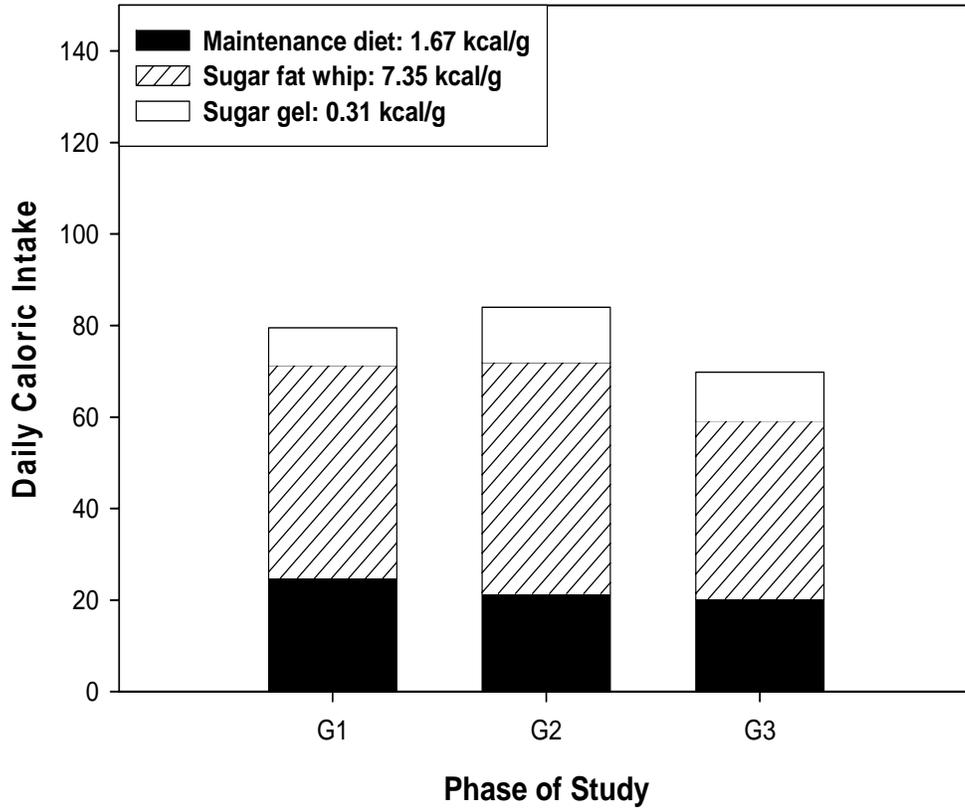


Figure 6-5: Proportion of caloric intake in week two of Experiment 5 from maintenance diet, sugar fat whip, and sugar gel of the three groups (G, see legend for Figure 6-3).

**Effect of previous dessert experience
on choice and consumption of maintenance diet and multiple desserts --
body weight change**

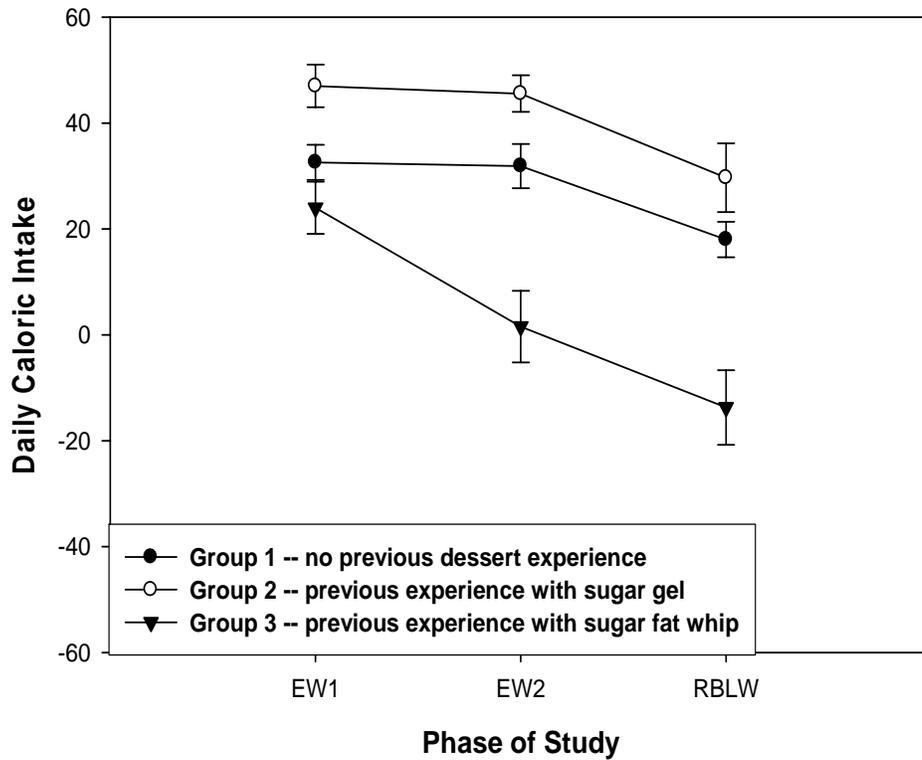


Figure 6-6: Average cumulative body weight change in Experiment 5 of the three groups (shown in legend) by experimental week (EW) and the average of the two weeks following termination of the experimental phase (RBLW), during which all rats had access to maintenance diet only. Cumulative change is calculated by subtracting the last body weight measurement of baseline conditions from body weight measurements during the three EW. Group 2 gained more weight than Group 3 in EW1 and Group 1 and 2 gained more weight than Group 3 in EW2 and RBLW.

CHAPTER 7
EXPERIMENT 6–CIRCADIAN RHYTHMS

Introduction and Rationale

Another aspect of availability concerns the circadian rhythms of appetitive processes. Night Eating Syndrome (NES) is a human clinical phenomenon that illustrates the effect of food eaten outside of normal consummatory rhythms (Stunkard et al. 2005). Individuals with NES show non-normative eating patterns and gain a considerable amount of weight by consuming large amounts of snack foods at night and early morning (Tanofsky-Kraff and Yanovski 2004). This suggests that a snack consumed during the normal period of food consumption may be more readily compensated than a snack eaten outside the normal period of food consumption. This phenomenon may generalize to rat behavior and represent some aspects of this clinical disorder through use of a protocol in which a palatable dessert is presented at the start of the light cycle. We hypothesized that rats given diurnal access to dessert (during the light cycle when rats are not very active; analogous to human nighttime) would consume more dessert and overall calories as well as gain more weight than rats given nocturnal dessert access (during the dark cycle when rats are active and feeding; analogous to human daytime).

Methods

Animals and Housing

The animals used were 11 female Sprague Dawley rats (Harlan, Indianapolis, IN) weighing between 250 and 325 g at the start of the experiment. All rats were caged as previously described. One group of five rats was housed in a vivarium with lights on

from 0800 h to 2000 h and the other group of six rats was housed in a vivarium with lights on from 2100 h to 0900 h. Both vivaria were maintained at 22 ± 2 °C.

Food Conditions and Measures

All rats were provided with ad libitum access to tap water and wet mash as previously described in experiment 4. Fresh wet mash in clean jars was presented daily at the start of the dark cycle. Food intake was calculated at 0, 12, and 24 h by subtracting the weight of the remaining wet mash from that originally presented. Baseline food intake was measured for one week prior to study and the rats were divided into two groups (Group 1: n=5 and Group 2: n=6) such that average daily caloric intake was equivalent between the two groups. Daily food intake was measured for two weeks during the experimental phase and for two weeks following termination of the experimental phase, during which rats had access to wet mash only.

Dessert Access

Table 7-1: Differences in the time of daily access to dessert

Group	Maintenance Diet	Dessert Available Uninterrupted for 4 h
1	Wet mash	Diurnally at lights on
2	Wet mash	Nocturnally at lights off

During the experimental phase, all rats were provided daily with 4 h access to 15 g of sugar fat whip, which was presented as in experiment 4. The start of dessert access depended on the light cycle of the vivarium that each group of rats was in. Rats in Group 1 had diurnal dessert access beginning at 0800 h at the onset of lights on and rats in Group 2 had nocturnal dessert access beginning at 0900 h at the onset of lights off. Dessert was removed 4 h later (1200 h for Group 1 and 1300 h for Group 2) and intake calculated by subtracting the weight of the remaining dessert from that originally presented. Daily intake was measured for two weeks.

Body Weight Measures

Body weight was measured three times weekly during the experimental phase and the two weeks following termination of the experimental phase. Cumulative body weight gain was calculated by subtracting the last body weight measurement taken during the baseline phase from those taken during the experimental phase and the week following.

Data Analysis

Daily intake of sugar fat whip, wet mash, and the total calories, as well as change in body weight for each rat were calculated. This was then averaged between rats within a single group for each week. Between group and condition analysis was assessed using SPSS (SPSS, Chicago, IL) one-way ANOVAs with a significance criterion of $p < 0.05$.

Results

Total Caloric Intake

Figure 7-1 shows the average daily total caloric intake of the two groups by experimental week and compared to the consumption of wet mash in baseline and the average of the two weeks following the termination of the experimental phase. Consumption was significantly different between baseline, experimental, and the reestablishment of baseline conditions ($F(3,40)=68.284$, $P < 0.01$), with rats consuming approximately 27% more calories in the first experimental week and approximately 15% more in the second compared to baseline consumption. Although access to dessert increased caloric intake, whether access to dessert was nocturnal or diurnal had no robust effect on total caloric intake (Week 1: $F(1,10)=1.589$, $p > 0.05$; Week 2: $F(1,10)=2.089$, $p > 0.05$).

Caloric Intake from Wet Mash and Dessert

Figure 7-2 shows calories from wet mash consumed by the two groups by experimental week and compared to the consumption of wet mash in the week of baseline and the average of the two weeks following termination of the experimental phase. In both groups, calories from wet mash fell to approximately 48% of baseline consumption in the first experimental week and approximately 54% in the second. This compensatory reduction in wet mash intake was insufficient to offset the large intakes from sugar fat whip and so intakes were elevated relative to consumption of wet mash during the week of baseline ($F(3,40)=91.000$, $p<0.01$). There were no significant differences between the diurnal and nocturnal access groups in the number of calories consumed from wet mash (Week 1: $F(1,10)=0.668$, Week 2: $F(1,10)=0.050$, $p>0.05$).

Figure 7-3 shows calories consumed from dessert by the groups by experimental week. On average, rats consumed calories from dessert in amounts equivalent to approximately 79% of total daily calories consumed in baseline. The amount of dessert eaten during the two weeks was different ($F(2,30)=116.847$, $p<0.01$), but no differences were seen between the diurnal and nocturnal access groups (Week 1: $F(1,10)=0.412$, Week 2: $F(1,10)=1.115$, $p>0.05$), with more eaten in the first experimental week.

Figure 7-4 shows the proportion of calories consumed from wet mash and dessert averaged over the experimental weeks and compared to consumption of wet mash in the week of baseline and the average of the two weeks following termination of the experimental phase.

Body Weight

Figure 7-5 shows cumulative change in body weight from the last day of baseline of the groups by experimental week and the two weeks following termination of the

experimental phase. By the end of the first experimental week, rats in both groups had gained an average of 26.2 g, and an average of 29.7 g by the end of the second experimental week. This is significantly different from baseline, but body weight changes were not robustly different between groups (only the first assessment of body weight change showed difference, $F(1,10)=11.025$, $p<0.01$; end of week 2: $F(1,10)=4.940$, $p>0.05$), and thus no reliable effect on body weight gain as a function of nocturnal and diurnal dessert access was observed.

Nocturnal and Diurnal Caloric Intake

Figures 7-6 and 7-7 show the average caloric intake of the two groups during the dark and light cycle, respectively, by experimental week and compared to the wet mash consumption in the week of baseline and the average of the two weeks following termination of the experimental phase. In baseline conditions, all rats consumed approximately 89% of their daily calories during the dark cycle; however, when provided with a dessert, feeding patterns shifted significantly (nocturnal intake: $F(3,40)=2.495$, $p=0.074$; diurnal intake: $F(3,40)=4.037$, $p=0.013$). Rats given diurnal dessert access (Group 1) consumed only an average of 35% of total calories nocturnally. Group 1 consumed their remaining calories during the light cycle and nearly all of these diurnal calories were from dessert. This was significantly different from the nocturnal intake of those rats given nocturnal dessert access (Group 2), which consumed approximately 96% of their calories nocturnally (Week 1: $F(1,10)=139.458$, Week 2: $F(1,10)=146.557$, $p<0.01$).

Discussion

Based on clinical reports of NES, in which calories in addition to meals are consumed in large amounts late at night or very early in the morning, resulting in

tremendous body weight gain in humans (Stunkard et al. 2005), we hypothesized that rats with diurnal access to a dessert, shown previously to result in weight gain in rats, would consume more calories and/or gain more weight compared to rats given nocturnal dessert access. This hypothesis was not supported by the data. Although access to sugar fat whip resulted in an increase in caloric intake compared to baseline, rats given either nocturnal or diurnal access to this dessert consumed similar amounts and gained similar amounts of weight. This suggests that, in rats, circadian periodicity of caloric intake does not affect body weight as it has been suggested to affect the body weight of humans with NES.

There are, of course, many differences between this rat model of NES and human behavior. Although NES is not currently classified as an eating disorder by the DSM-IV-TR, it is similar to Binge Eating Disorder, which is associated with anxiety caused by the recurring dysfunctional eating behaviors (Tanofsky-Kraff and Yanovski 2004). The distress associated with nighttime eating may facilitate the perpetuation of this behavior and be a large component of the weight gain seen in these individuals. This was not assessed in the rats and presumably is not a factor affecting rats' consumption. Also, the rats had ad libitum access to maintenance diet whereas individuals with NES tend to engage in non-normative meal taking as well as night eating. Arranging rat meals and snack access in bouts analogous to human patterns and adding a type of chronic processive stressor may produce different results that would more closely model NES.

Effect of nocturnal versus diurnal access to a high fat dessert on average daily total caloric intake

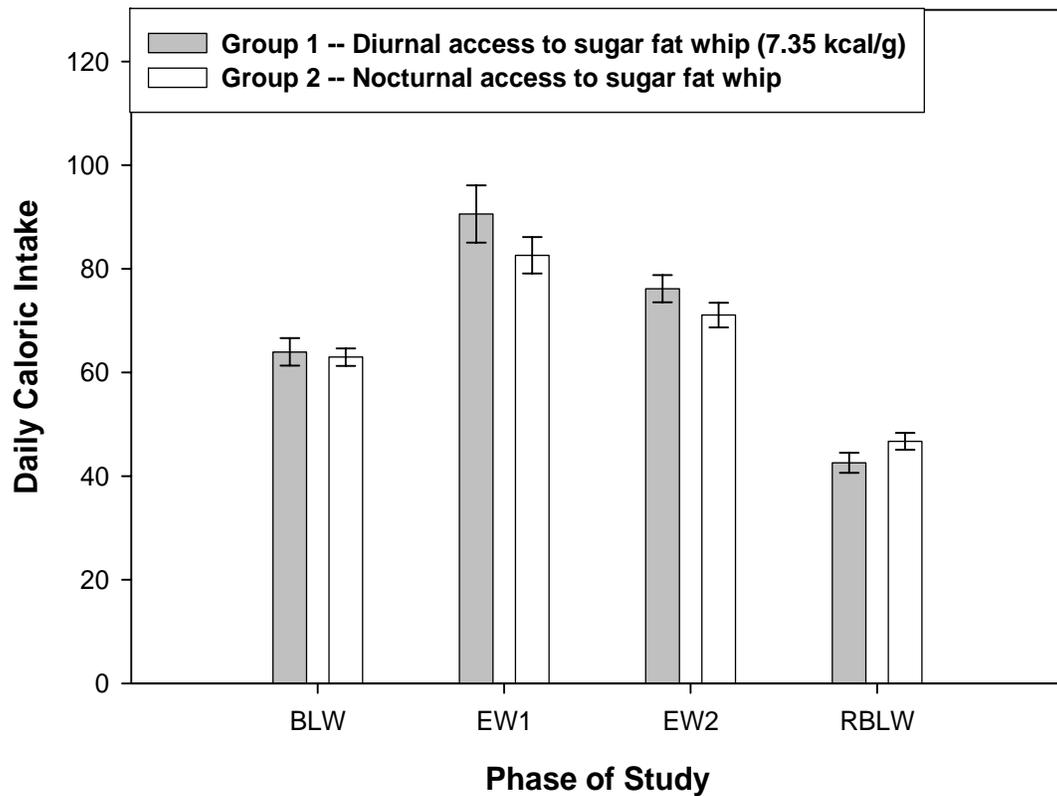


Figure 7-1: Average daily caloric intake in Experiment 6 of the two groups (shown in legend) by experimental week (EW) and compared to a week of baseline (BLW) and the two weeks (averaged together) following the termination of the experimental phase (RBLW), during both of which all rats had access to maintenance diet only. There were no differences seen between groups.

Effect of nocturnal versus diurnal dessert presentation on average caloric intake from maintenance diet

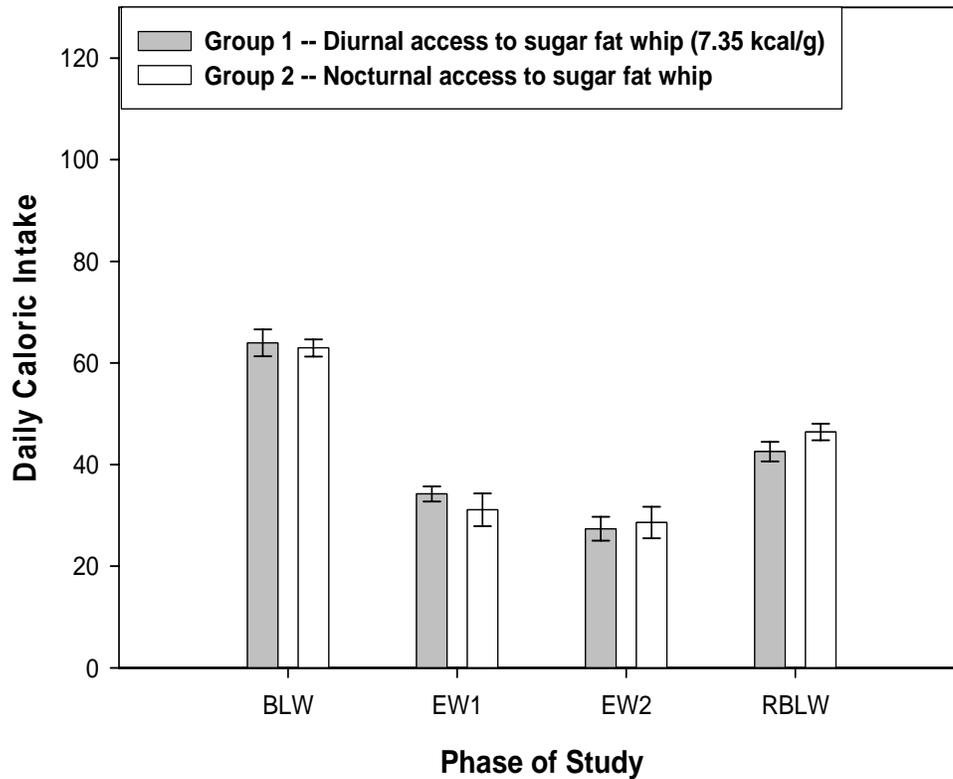


Figure 7-2: Average caloric intake from maintenance diet in Experiment 6 of the two groups (shown in legend) by experimental week (EW) and compared to a week of baseline (BLW) and the two weeks (averaged together) following the termination of the experimental phase (RBLW), during both of which all rats had access to maintenance diet only. There were no differences seen between groups.

Effect of nocturnal versus diurnal access to dessert on average caloric intake from dessert

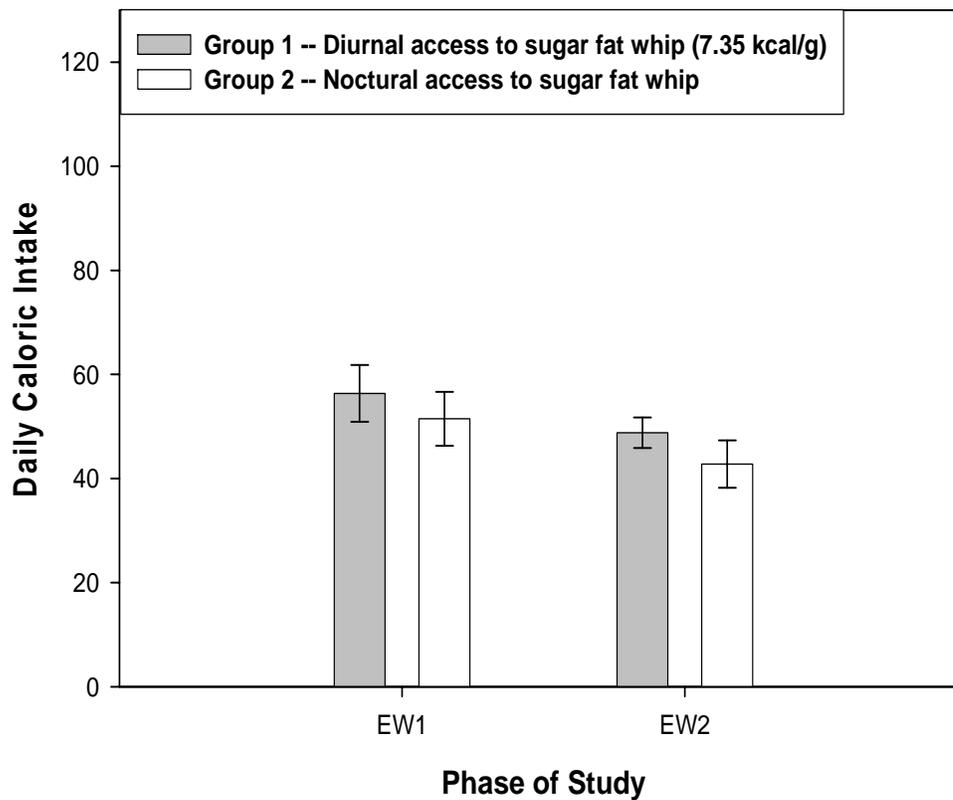


Figure 7-3: Average caloric intake from sugar fat whip in Experiment 6 of the two groups (shown in legend) by experimental week (EW). There were no differences seen between groups.

**Effect of nocturnal versus diurnal access to dessert
on average distribution of caloric intake from maintenance diet and dessert**

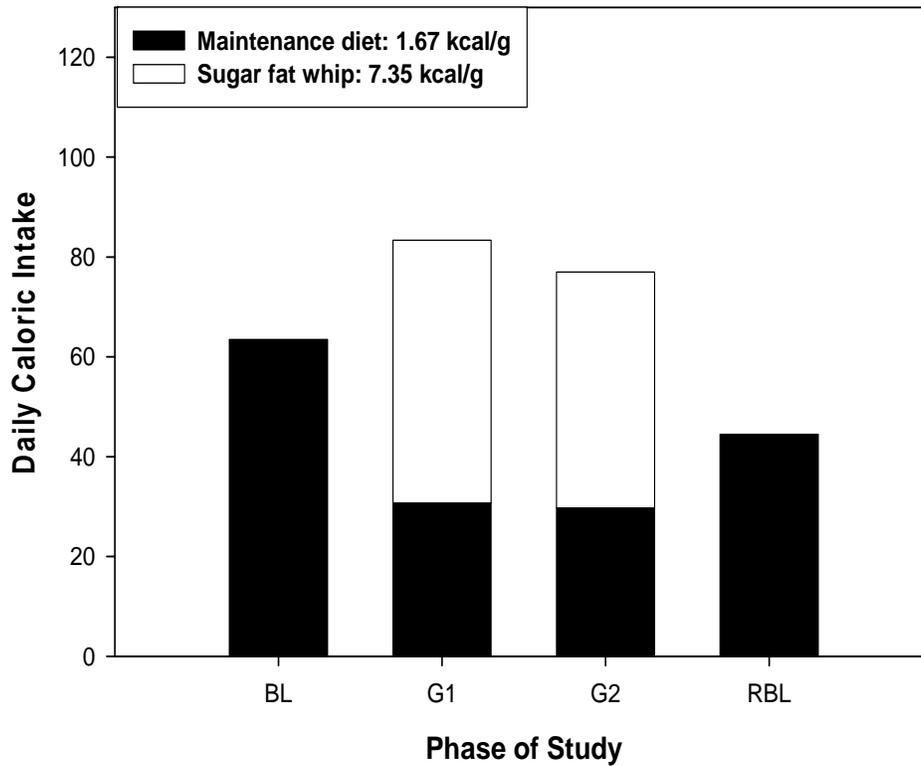


Figure 7-4: Proportion of caloric intake in Experiment 6 from maintenance diet and sugar fat whip of the two groups (G, see legend for Figure 6-3) averaged over both experimental weeks and compared to maintenance diet consumption in the week of baseline conditions (BL) and the two weeks (averaged together) following the termination of the experimental phase (RBL).

Effect of nocturnal versus diurnal dessert presentation on cumulative body weight change

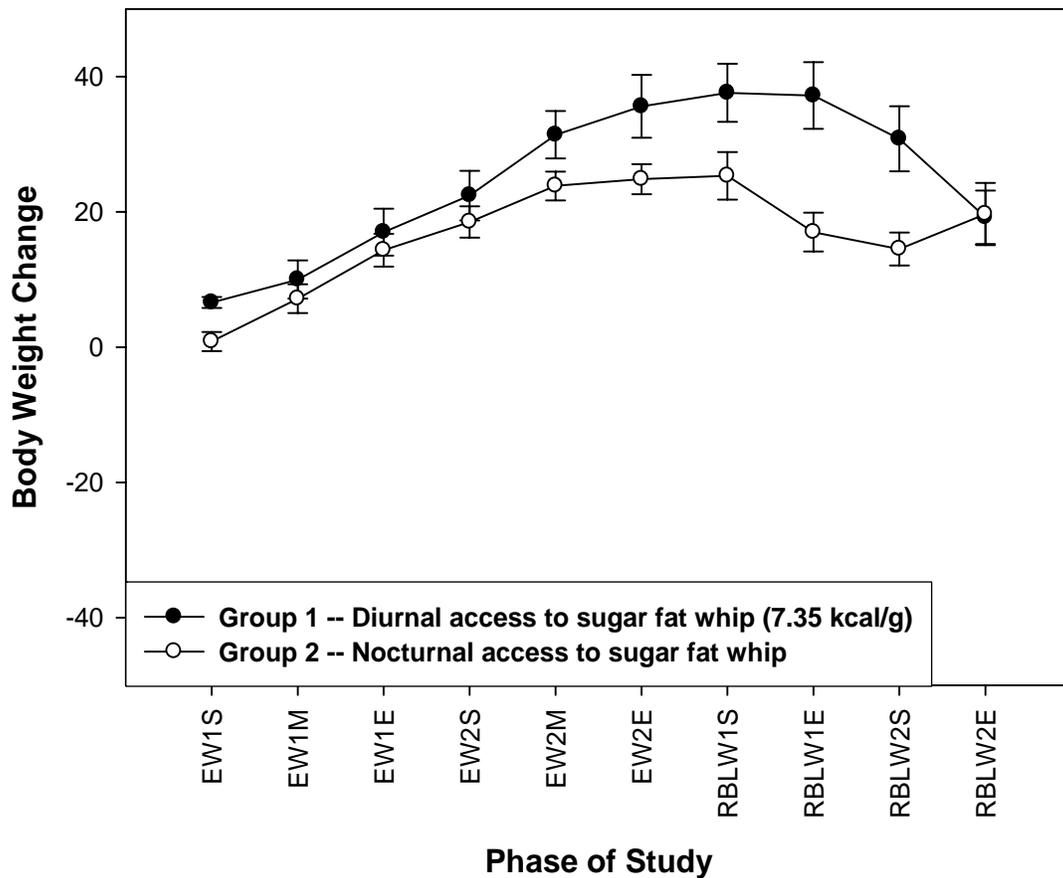


Figure 7-5: Average cumulative body weight change in Experiment 6 of the two groups (shown in legend) by experimental week (EW) and the two weeks following termination of the experimental phase (RBLW). Cumulative body weight change is calculated by subtracting the last body weight measurement of baseline conditions from body weight measurements during the three EW. Body weight was measured three times during each EW and RBLW and each measurement is denoted as either S=start (at beginning of the week), M=middle (in the middle of the week), or E=end (last day of the week). No significant differences were seen between groups in the EW, but there were differences in RBLW between groups.

Effect of nocturnal versus diurnal access to dessert on average nocturnal total caloric intake

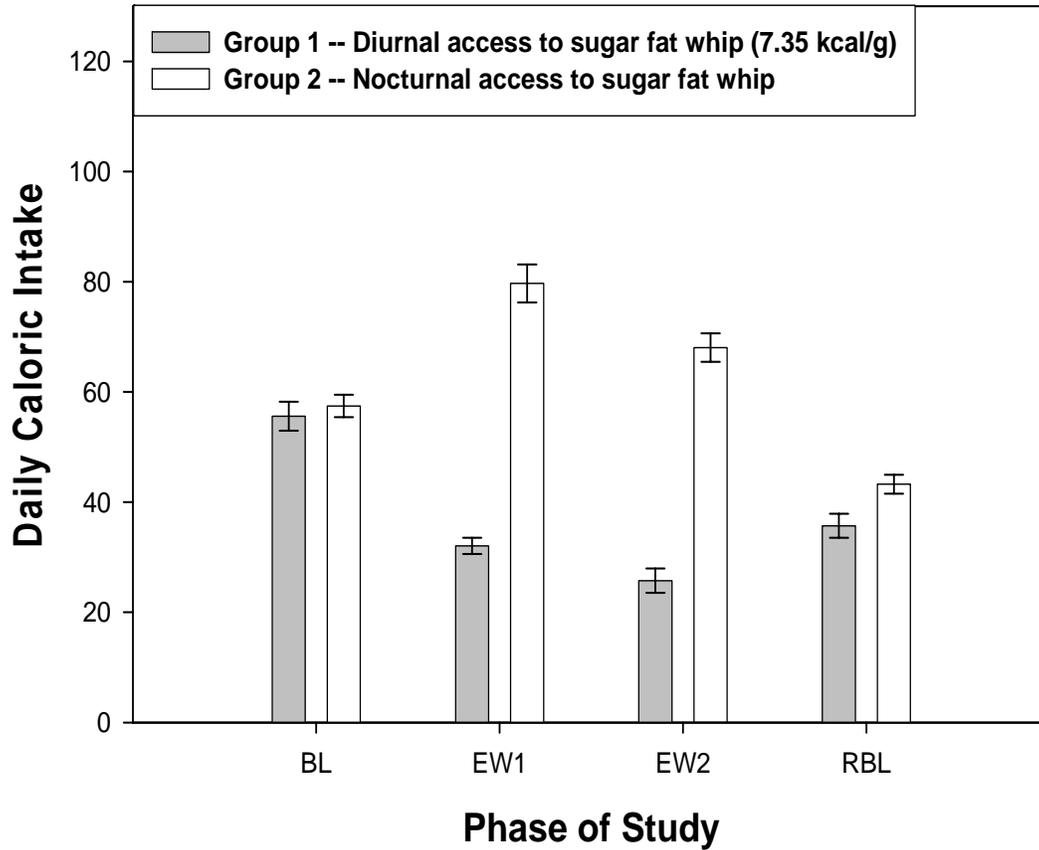


Figure 7-6: Average nocturnal caloric intake in Experiment 6 of the two groups (shown in legend) by experimental week (EW) and compared to a week of baseline (BLW) and the two weeks (averaged together) following the termination of the experimental phase (RBLW), during both of which all rats had access to maintenance diet only. Group 2 consumed more calories in the dark cycle than Group 1.

Effect of nocturnal versus diurnal access to dessert on average diurnal total caloric intake

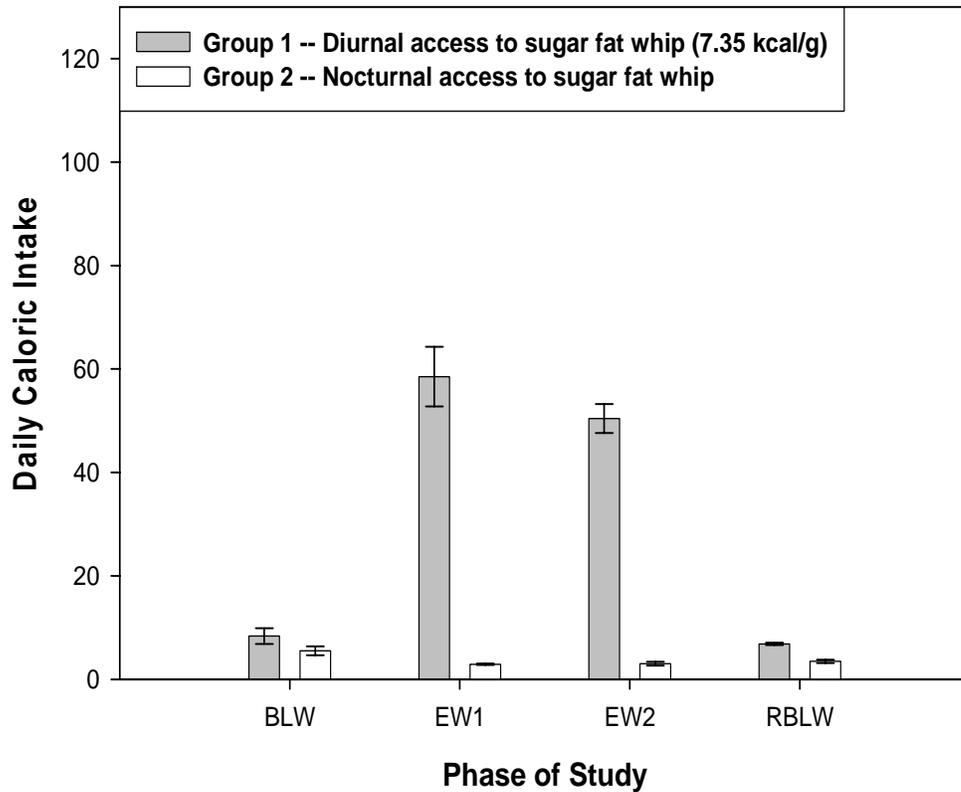


Figure 7-7: Average diurnal caloric intake in Experiment 6 of the two groups (shown in legend) by experimental week (EW) and compared to a week of baseline (BLW) and the two weeks (averaged together) following the termination of the experimental phase (RBLW), during both of which all rats had access to maintenance diet only. Group 1 consumed more calories in the light cycle than Group 2.

CHAPTER 8 DISCUSSION

The global aim of this thesis was to discuss which factors in the environment promote eating in excess of physiological need and how this is compensated within a homeostatic system. The literature pertaining to homeostatic models of body weight and its limits and studies demonstrating the effect of food quality on intake were reviewed. The effect of availability has not yet been thoroughly explored although increased access to palatable food has been touted as the culprit of the obesity epidemic. This series of six experiments attempted to address the effects of increased spatial, temporal, and qualitative availability on the compensatory nature of caloric intake and body weight gain in female rats.

Experiment 1–Spatial Availability

In our first experiment, we attempted to extend Tordoff's (2002) findings that the amount and location of sucrose access compared to water access affects preference and intake to the situation of increased spatial availability of maintenance diet. We observed no effect on caloric intake or body weight as a function of increasing the amount of jars of maintenance diet provided to rats, and this was independent of the quality of the maintenance diet provided. This does not refute Tordoff's findings, but suggests that the effect he demonstrated either doesn't generalize to maintenance diet or that this protocol (and the problems encountered in it) is not capable of showing an effect of the increased spatial availability of maintenance diet.

This failure to support the hypothesis is in conflict with a review by Wansink (2004), which argues that highly visible food promotes hunger and that portion sizes and amount of food available set the norms of how much to eat. This could be the result of verbal behavior in humans, in which ratings of hunger is set contingent on the amount of food presented rather than from internal cues. This obviously can not be assessed in a rat model. Wansink also suggests that the salience of a constantly available consumable may decrease with time and that differences in temporal, rather than spatial, availability may have a more robust effect on food intake and body weight.

Rolls et al. (2005) argued that external cues of availability such as portion size do influence intake, but that this is only important in the context of energy density. If foods with a high energy density are consumed in excess due to increased availability, body weight will increase. Snacking on palatable foods has been demonstrated to be the best predictor of obesity (Berteus Forslund et al. 2005) and thus, protocols representing the increased availability of extra calorie sources may more completely demonstrate the effect of availability on caloric intake body weight gain in rats. These issues were addressed in the subsequent experiments.

Experiment 2–Temporal Availability

Through the use of a dessert protocol in which a sugar gel was presented for either an uninterrupted period or intermittently over that period, we explored the effect of the facets of temporal availability of a palatable calorie source in addition to maintenance diet on caloric intake and body weight gain in rats. We observed that the intake of a sugar gel was greater when available for an uninterrupted 8 h period in conjunction with a standard maintenance diet than when sugar gel was available intermittently for a total of 2 h across the 8 h session or presented in conjunction with a more palatable, high fat

maintenance diet. Regardless of the amount of dessert consumed, the calories from sugar gel were compensated by a reduction in the intake of maintenance diet across all the groups such that all the rats continued to consume the amount of calories they had when given maintenance diet only in baseline conditions.

The data failed to support the hypothesis that intermittent access to a dessert would increase its reinforcing saliency and diminish sensory specific satiety compared to uninterrupted access, an argument suggested by Rolls et al. (1981) and Wansink (2004). This suggests that the sugar gel may have a long-term acceptability (Vickers 1999) and thus, not be prone to the effects of sensory specific satiety, or that with a single consumable, availability does not affect the intake parameters concerning reinforcing saliency or sensory specific satiety.

These data are interesting in relation to Corwin and Buda-Levin's (2004) demonstration of binge-eating patterns, increased caloric intake, and body weight gain in rats presented with vegetable shortening every other day compared to those given daily access. This suggests that temporal availability does influence intake, but perhaps only across days and with a high calorie snack. A different effect may have been observed with the use of a dessert of higher caloric density in this protocol, an issue discussed below.

The failure to support the hypothesis of an increase in caloric intake as a function of dessert access is surprising since snacks are touted as driving the obesity epidemic (Berteus Forslund et al. 2005); however, the snacks most often consumed by humans are usually much higher in fat and energy density than the sugar gel used in this protocol. The rats ate a large amount of the sugar gel, but at a caloric density of approximately 0.31

kcal/g (compared to maintenance diets of 3.34 or 5.24 kcal/g), large intakes of this diluted diet wouldn't have much impact on total daily calories. As mentioned above, Rolls et al. (2005) argue that energy density of foods is the most potent influence of body weight gain and Drewnowski (2004) supports this by proposing that the water content of food is indicative of its satiating power.

Intake of the sugar gel could also have been limited by its low protein to calorie ratio relative to maintenance diet. Pol and den Hartog (1966) reported that even when food deprived, rats will self-manage the selection of different macronutrient sources such that approximately 8% of their calories are from protein. This exclusively carbohydrate sugar gel may have set a ceiling of consumption that along with the low energy density was unable to demonstrate the effect of dessert consumption on calorie balance. The effect of dessert protein to calorie ratio was addressed in the next experiment, and dessert energy density was explored in experiment 4.

Experiment 3–Temporal Availability and Protein to Calorie Ratio

The same pattern of dessert and maintenance diet consumption as in the sugar gel protocol of experiment 2 was seen with uninterrupted or intermittent milk gel access. The addition of protein to the low calorie dessert did not seem to have any effect on gel consumption or maintenance diet compensation, but rather shows that the results of experiment 2 generalize somewhat to isocaloric desserts of different macronutrient quality. The protein to calorie ratio of food consumed may not be as influential a determinant of food choice in situations of increased access to free food as it is in those of deprivation. However, the low caloric density of the dessert is still an unresolved factor since the 0.31 kcal/g gels seem to be incapable of increasing caloric intake from baseline values. This prompted us to use a high calorie dessert, and the next experiment

addressed the effect of access to a high fat dessert of high caloric density compared to sugar gel or no dessert on intake, compensation, and body weight.

Experiment 4–Energy Density

In this experiment, we observed that access to a dessert of high caloric density, sugar fat whip, resulted in higher total caloric intake, dessert intake, and body weight gain than rats given access to the lower density sugar gel or no dessert. Although intake of maintenance diet was lower in rats given sugar fat whip than rats given sugar gel, this compensation did not allow for maintenance of caloric balance. These findings are in line with the arguments of Rolls et al. (2005) presented above and with those of de Castro (2005), who demonstrated in humans that the greater the energy density of food consumed, the greater the short-term caloric intake.

This study is an initial demonstration that caloric intake and body weight can be influenced by access to a single optional calorie source. It is difficult, however, to separate the effect of energy density from that of macronutrient content, especially that of fat. The influence of fat and palatability are discussed in the introduction, and it has been suggested that fat is not as satiating as carbohydrates or protein. The combinations of macronutrients that make up a food also influence its palatability, which has also been demonstrated to have a positive relationship with intake (as discussed in the introduction). It would be interesting to implement comparisons of different desserts representing the spectrum of combinations of energy density, macronutrient composition, and palatability for long-term studies in rats as Rolls has done in short-term human studies, but that task is beyond the scope of these studies.

These data also imply some means of long-term compensatory behavior and regulation of body weight. The rats who gained weight on sugar fat whip showed lower

maintenance diet intake throughout the periods of dessert access and this continued throughout the weeks following during which only maintenance diet was available. Sugar fat whip intake decreased through the three experimental weeks, leveling by the third week to the caloric intake seen in the rats given sugar gel. In the weeks following termination of the experimental phase, the rats previously given sugar fat whip lost weight and approached the body weights of the other groups of rats. This may be indicative of increased leptin levels in response to increase in body adiposity, which would then decrease subsequent food intake while maintaining a new body weight.

Since eating foods of a higher caloric density increases feeding efficiency, it is possible that information regarding energy density can be defined and used to aid future food selection and consumption. Since we had rats with different histories of dessert access, it seemed ideal to assess the effect of prior experience with desserts of different densities or no dessert at all on calorie selection from maintenance diet and both forms of dessert.

Experiment 5–Previous Experience and Choice

In this study, we assessed the effect of previous experience with neither or either of one of two desserts of different caloric densities on choice and consumption of the two desserts when presented together and on subsequent compensation of maintenance diet intake. Since baseline intakes were not equivalent between groups at the start of the experiment, the results of this study are somewhat difficult to interpret. The differences in baseline intake between groups was the result of decreased consumption of maintenance diet of the rats previously given sugar fat whip, which may be indicative of differences in production and effect of long-term correlates of body fat. When the results are examined in terms of change in caloric intake from baseline, the rats that had

experience with sugar fat whip consumed more total calories than those with sugar gel experience, which consumed more than the rats with no previous dessert experience. The rats that had experience with either of the desserts also tended to eat more of both desserts (significant for sugar gel but not sugar fat whip).

This is in line with Ramirez's (1991) study in rats, which demonstrated that previous experience with bitter tastes reduces rejection of maintenance diet containing sucrose octaacetate (a substance reported by humans to be bitter), and Darmaillacq et al.'s (2004) study in cuttlefish, which suggested that prey preferences were determined by early feeding experience. Leathwood and Ashley (1983) argue that learning from previous food selection is a key component of both the metabolic and behavioral mechanisms that promote adaptive eating in the prevailing environment. It would be evolutionarily advantageous for animals to consume large quantities of familiar foods that had previously been associated with positive oral and postingestive consequences when they were readily available. The modern postindustrial food environment of humans promotes experience with an abundance of different foods, possibly priming future overconsumption.

Previous experience with food may also have implications for the acquisition and maintenance of eating disorders, although the literature is not clear. Neudeck et al. (2001) demonstrated that when presented with a high calorie 'forbidden food' two days in a row, bulimic women rated urges to binge at a lower level on the second day compared to the first. Jansen (1998) argues that the uncontrolled eating in binge eating disorder (BED) is under the stimulus control of familiar cues, similar to drug addiction, and that exposure to previously learned cues causes an increase in binge eating behavior. As

previously mentioned, Corwin and Buda-Levin (2004) has demonstrated the effect of availability on binge eating in rats. Binge eating and perhaps overconsumption in general seem to be influenced by multiple factors, including temporal opportunities and previous experience. It was this that prompted our next experiment which explored the impact of availability on the circadian metabolic rhythms of feeding in an attempt to delineate a model of an eating disorder similar to binge eating -- night eating syndrome (NES), in which humans gain a considerable amount of weight by consuming excess calories at times of typical inactivity and sleep (Stunkard et al. 2005).

Experiment 6–Circadian Rhythms

In this study, rats were given access to sugar fat whip either at the onset of the dark cycle, during which rats are active and consume the majority of their daily intake, or at the onset of the light cycle, during which rats are inactive and consume very little food, in an attempt to model the increased caloric intake and body weight gain seen in NES. Although all the rats consumed more calories and gained weight when provided access to sugar fat whip compared to baseline (as seen in experiment 4), there were no differences as a function of the periodicity of dessert access. Rats given sugar fat whip during the light cycle consumed a large amount of dessert and the majority of their daily calories during their “nighttime”, but showed compensatory decrease of maintenance diet intake such that total daily intake and body weight did not differ from rats given sugar fat whip during the dark cycle.

These results are interesting in light of a study by Walls et al. (1991), which suggests that compensatory decreases in oral food intake in the presence of intravenous administration of nutrients are more accurate when the nutrients are presented nocturnally than when presented diurnally. Walls makes the argument that this is due to the

association of gut-related signals that are present when rats normally feed (during the night) and that are absent during the light cycle when the rats are undergoing lypolysis. Although experiment 6 presents supplemental calories, it does so in the form of a food that is eaten orally, thus allowing for gut-related signals. These aspects of the protocol may have undermined the effect of circadian rhythms on compensation of feeding.

These results imply that some factor other than availability may be promoting increased caloric intake in this model of NES, as opposed to Corwin's model of BED, in which temporal availability seems to be key. This may imply that considering NES and BED as components of a single recurring dysfunctional eating pattern (Tanofsky-Kraff and Yanovski 2004) may not be functional, or at least a component to be considered in construction and interpretation of rat models. Human behavioral antecedents, patterns, and consequences of food intake (e.g., anxiety, social facilitation, meal structure and frequency, and fear of weight gain) are not available in this rat model and thus, need to be addressed before conclusions can be made.

Concluding Remarks

These studies explore the facets of availability and their effect on food intake, caloric balance, and body weight gain. The results suggest that some aspects of availability, especially the caloric density of supplemental calorie sources (snacks) and previous experience with foods impact these measures. The effect of spatial and temporal availability need to be further explored. It is possible that increased spatial availability of a dessert or intermittent presentation of a dessert of high energy density could elucidate the impact of these factors. These also need to be explored and animal models constructed in the context of eating disorders, such as NES and BED. Food availability seems to have the power to overwhelm homeostatic controls of body weight

and must be further explored and understood if effective prevention and treatment strategies for obesity are to be developed.

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BIOGRAPHICAL SKETCH

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