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A High-Fat, High-Calorie Diet Can Reduce the Expression of Anxiety in Socially Housed Female Rhesus Macaques

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An abstract of
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Abstract

A High-Fat, High-Calorie Diet Can Reduce the Expression of Anxiety in Socially Housed Female Rhesus Macaques

By Quynh-Chau Ha

Socially housed rhesus macaques (Macaca mulatta) have a dominance hierarchy that is similar to the stratified society of humans and therefore can be used as an ethologically valid and translational model for the study of psychosocial stress, food intake, and emotionality. We used 2 groups of 5 socially housed female rhesus monkeys and observed their caloric-intake, using automated feeders, and behaviors under different diet conditions. Under the first diet condition, only a low-calorie (LCD) diet was available for 3 weeks, and under the second diet condition, the monkeys were given a choice between LCD and a high-calorie diet (HCD). Univariate analysis of variance (ANOVA) for repeated measures, and post hoc tests with Bonferroni correction were used to assess statistical significance. The data showed that all animals preferred HCD over LCD, and subordinate females consumed significantly more of both diets. Behavioral data from this study showed that the increased consumption of HCD was negatively correlated with the expression of agonistic and anxiety-like behaviors in subordinate females. Results of this study suggested that anxiety-like behaviors may be attenuated by the consumption of HCD and supported the notion that humans and animals under chronic stress increase the consumption of a preferred diet that is high in fat, calorie, and sugar as a coping strategy.

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Objective

Obesity is a worldwide concern that can be addressed by examining the complex relationship between genes, diet, psychological stress, and social and environmental factors (Adam 1989). The prevalence of overweight and obesity constitutes a global health epidemic (Popkin and Doak 1998; Wadden et al. 2002). The increased prevalence of obesity is seen across gender, age groups, socioeconomic status, and ethnicity (World Health Organization 2004). Although genetic influences predispose some individuals to obesity, it is the toxic environment that drives the alarming increase in its prevalence (Brown 1993; Van Dorsten and Lindley 2008; Wadden et al. 2002).

In recent years, research emphasis has been placed on designing behaviorally based weight-loss interventions because obesity is not only influenced by genetic and environmental factors, but also by psychosocial motivations to consume food in excess amounts (Van Dorsten and Lindley 2008; Wadden et al. 2002). This approach assumes that learned behaviors contributing to excessive food intake, poor dietary choices or habits, and sedentary activity habits can be modified and restructured to produce weight reduction (Jeffery et al. 2000). However, this behavioral approach should be coupled with an understanding of psychosocial stressors and their effects on emotional feeding, because it is difficult to modify physical activity and feeding behaviors without knowledge of the underlying motivation or the lack of motivation for these behaviors.

Chronic exposure to stressors has been shown to cause the activation and subsequent dysregulation of the limbic hypothalamic-pituitary-adrenal (LHPA) axis (Adam and Epel 2007; Dallman et al. 2005; Pecoraro et al. 2004). This is a precipitating factor in the emergence of a number of mood disorders that occur more often in women compared with men. Furthermore, stress-induced changes in emotion are often comorbid with other pathologies and can also induce significant changes in food intake (Dallman et al. 2005; la Fleur et al. 2005; Pecoraro et al. 2004).

Animal models have shown that decreased food intake and weight loss are the most reliable markers of stress severity (Armario 2006). However, when presented with palatable, calorically dense foods, such as lard or sugar, animals exposed to stressors increased their intake of the palatable food (Dallman et al. 2005; Pecoraro et al. 2004). The data suggest that stress-induced changes in appetite could reflect a coping strategy that provides relief from stressors; more specifically, the consumption of calorically dense foods could alleviate LHPA dysregulation, normalize mood, and provide emotional comfort (Wilson et al. 2008).

These data are consistent with observations in humans. The literature shows that most people increase their food intake during stress, especially in Westernized countries where palatable and calorically dense foods are available in abundance (Epel et al. 2004). In 2006, a survey conducted by the Practice Directorate of the American Psychological Association (APA) in partnership with the National Women's Health Resource Center and iVillage.com revealed that 47% of working

adults in the United States are concerned with the amount of stress in their life, and they often engage in unhealthy behaviors such as smoking as well as eating for relief (Stambor 2006).

Previous animal models have been informative and important from a heuristic point of view when studying the interactions between stress and eating behaviors. However, these animal models elicit behavioral and hormonal responses that are unique to a particular type of stress employed in a laboratory and do not fully reflect the biobehavioral effects of stressors that are likely to be shared by human populations (Wilson et al. 2008). The dominance hierarchy of socially housed female rhesus monkeys is similar to the stratified society of humans, wherein social subordination can lead to adverse consequences due to their social status. Therefore, rhesus monkeys can be used as an ethologically valid and translational model for the study of psychosocial stress, food intake, and emotionality (Wilson et al. 2008). An ethological animal model can help us better understand the interactions between these factors and shed light on the biobehavioral mechanisms that affect feeding behaviors of individuals who are engaging in unhealthy coping such as habitual consumption of high-fat and calorically-dense foods to alleviate feelings of discomfort.

Although psychosocial stress is only a small part in the etiology of obesity, it is an important factor to consider in the development of behavior-based obesity interventions and prevention. The focus of this thesis is to validate an ethological animal model that will increase our understanding of how diet composition

influences stress-induced changes in emotion and appetite. This experiment used socially housed female rhesus macaques to test the hypothesis that consumption of a high-calorie diet (HCD) can produce an anxiolytic effect to alleviate emotional discomforts caused by stress.

Background

A Definition of Obesity

Obesity is a complex metabolic disorder that is defined as an excess accumulation of fat in adipose tissue to an extent that can negatively affect a person's health (Garrow 1988). The most common method for classifying obesity is the Body Mass Index (BMI). BMI is defined as weight divided by the square of the height measured by kg/m^2. The World Health Organization (WHO) classified overweight as a BMI between 25 and 29.9 and obesity is a BMI of 30 or above (James et al. 2001; World Health Organization 2004).

Classifying obesity is complex. BMI is useful for measuring overweight and obesity in adults and for estimating their prevalence within a population, but it does not distinguish muscle weight from fatness and does not account for the wide variation in body fat distribution (James et al. 2001; World Health Organization 2004). While the BMI cut-off points for overweight and obesity in adults are commonly accepted worldwide, developing an internationally accepted standard of classification for childhood and adolescent obesity is much more difficult (World Health Organization 2004). Height and body composition are continuously changing during childhood and adolescence, but the rate of change is highly variable for different populations (World Health Organization 2004). Tim Cole and colleagues (2000) aimed to establish an international standard definition for childhood overweight and obesity by surveying six nationally representative cross-sectional growth studies to propose age and sex specific BMI cut-off points. The

survey included data from Brazil, Great Britain, Hong Kong, the Netherlands, Singapore, and the United States (Cole et al. 2000). This study linked BMI cut-off points for childhood overweight and obesity to the widely accepted adult BMI cut-off points of 25 and 30 kg/m^2 (Cole et al. 2000).

Health Risks Associated with Obesity

It is important to properly classify obesity in adults and children because it is more than just an aesthetic problem: It is a medical problem that has become a global epidemic (Kopelman 2000; Visscher and Seidell 2001). Data from life-insurance and epidemiologic studies show that increasing degrees of overweight and obesity are important indicators of decreased longevity (Lew 1985). Obesity is associated with many comorbidities such cardiovascular disease (CVD), type II diabetes mellitus (NIDDM), and certain cancers (Bray 2004; Burton et al. 1985; Centers for Disease Control and Prevention 2009). Obesity also increases the risk of stroke, hypertension, and dyslipidemia (Burton et al. 1985; Centers for Disease Control and Prevention 2009; Must et al. 1999). Studies have shown that obesity can influence the development of liver and gallbladder disease, sleep and respiratory patterns, and reproductive health (Centers for Disease Control and Prevention 2009; Clark et al. 1998; Dietl 2005; Kopelman 2000). Obesity has been considered an important risk factor for mortality and morbidity because it exacerbates the deleterious effects of other diseases. However, the exact mechanisms for these relationships are relatively unknown.

Studies have shown that an increase in body fat causes significant changes in physiological functions, and these changes vary depending on the regional distribution of adipose tissue (Kopelman 2000). Generalized obesity results in alternations in total blood volume and cardiac function because the increase in lean and fat mass causes an increase in total body oxygen consumption (Kopelman 2000). An increase in total circulating blood volume can lead to an increase in stroke volume and cardiac output, thus increasing the risk for hypertension and stroke (de la Maza et al. 1994).

Certain obese individuals have an increased risk for heart failure due to an increase in circulatory preload and afterload. This increase causes left ventricular dilation and can lead to systolic and diastolic dysfunction (He et al. 2001; Massie 2002). The Framingham Heart Study (1983) showed that the increase in body weight is directly related to the development of congestive heart failure. The study suggested that there is a positive correlation between obesity and the likelihood of developing CVD, and this correlation is independent of a person's age, history of smoking, cholesterol, and blood pressure problems (Hubert et al. 1983).

Type II diabetes is another health problem affected by obesity. Obesity is characterized by elevated fasting plasma insulin and exaggerated insulin response to glucose consumption (Kolterman et al. 1980). Kolterman et al. (1980) showed that there is a positive correlation between increasing upper body obesity and measures of insulin resistance. Cortisol and different fat deposits contribute to an exaggerated release of free fatty acids (FFAs) from abdominal adipocytes into the portal system

(Frayn et al. 1996). Elevation in plasma FFAs has a deleterious effect on insulin uptake by the liver and contributes to an increase in hepatic gluconeogenesis and hepatic glucose; this can lead to an inappropriate maintenance of glucose production and impaired glucose tolerance (Frayn et al. 1996).

Obesity can also exacerbate sleep-pattern abnormalities (Kopelman 2000). Increased amount of fat in the chest wall and abdomen is an important risk factor for obstructive sleep apnoea (OSA) (Schwartz et al. 2008; Vgontzas et al. 2003). The progressive increase in body weight compromises respiratory function, especially when obese individuals lie flat during sleep; this is characterized by a reduction in lung volume and obstruction of the larynx associated with the loss of muscle control in the tongue (Kopelman 1992). Incidence of upper airway collapse is also higher in obese compared to nonobese individuals (Sharp et al. 1964). The Swedish Obesity Subjects study have shown that loud snoring and excessive daytime sleepiness (hypersomnolence) are more prevalent in obese men and women (Grunstein et al. 1995; Stradling and Crosby 1991). The study examined 3,034 subjects with a BMI above 35 and found that over 50% of men and one-third of women reported snoring and apnoea (Grunstein et al. 1995). Daytime sleepiness is affected by alveolar hypoventilation and apnoea, accompanied by a fall in arterial oxygen saturation (hypoxia) and a rise in arterial carbon dioxide (hypercapnia) (Rochester and Eaton 1974). This pattern of hypoxia and hypercapnia can lead to pulmonary hypertension and respiratory failure (Kopelman 1992).

Epidemiology of Obesity

Obesity, as defined by a BMI above 30, is a condition that exists across the globe (Kopelman 2000). However, inconsistencies in the classification of obesity have made it difficult to compare epidemiologic data between different countries (World Health Organization 2004). Negative health consequences of obesity also differ according to sex, age, ethnicity, and socioeconomic conditions (Brown 1993; Flegal et al. 2002; Mokdad et al. 1999; Pickett et al. 2005).

Worldwide, obesity is more prevalent in women than in men (James et al. 2001; World Health Organization 2004). Women tend to have more fat tissue and a lower metabolic rate compared with men (Krotkiewski et al. 1983). There are also sex differences in the distribution of adipose tissues. In women, the distribution of adipose tissue is concentrated in the legs and hips (Krotkiewski et al. 1983). These sex differences are reflected in obesity data collected from different regions of the world.

The Middle East, Central and Eastern Europe, and North America are regions with the highest prevalence of obesity (Silventoinen et al. 2004). The WHO MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) Project derived data from 26 random populations throughout the world. This project included more than 42,000 men and women aged 35 to 64 years in the initial survey, and about 35,000 people during the final survey from 1989 to 1996 (Silventoinen et al. 2004; Tunstall-Pedoe 1988; WHO MONICA Project 1999; World Health Organization 2004). The study found that in Europe, more than half

the adult population between the age of 35 and 65 is either overweight or obese (WHO MONICA Project 1999). The US National Health Nutrition Examination Surveys also showed that more than half of the US population is overweight (BMI $\geq$25 kg/m^2) and around 20% is obese (BMI $\geq$30 kg/m^2) (Flegal et al. 1998). Other studies have shown that African-American women and other minority populations in the United States are more susceptible to becoming obese (Karter et al. 1996; Lovejoy et al. 1996).

Obesity is also prevalent in Latin America and has become a problem in the Caribbean (CIBA Foundation Symposium 1996). United Arab Emirates recognizes obesity as a major public-health problem associated with an increase in occurrences of chronic diseases such as CVD, type II diabetes, and hypertension by the United Arab Emirates (Miladi 1996). Data from the Regional Office for the Near East of the Food and Agricultural Organization of the United Nations have shown that food energy and protein availability have doubled and the level of fat in dietary consumption has increased three fold in Arab countries (Alwan 1997).

Southeast Asia and the Pacific islands have also seen a marked increase in the prevalence of obesity. Japan and China are observing increasing trends in childhood obesity fold the past two decades (Du et al. 2002; Matsushita et al. 2004). In Malaysia, obesity is more prevalent than undernutrition in both urban and rural communities (Ismail et al. 2002). The most alarming figures come from urban Samoa where the prevalence of obesity is greater than 75% in adult women and 60% in adult men (Hodge et al. 1995).

The increased prevalence of obesity varies significantly according to the stage of economic transition and industrialization of a country (Kopelman 2000). A shift from dietary deficit to one of dietary excess is often associated with a population-wide socioeconomic improvement (Monteiro et al. 2004). In China, a rapid increase in the consumption of animal-based foods and a reduction in the consumption of vegetables quickly followed the country's economy transformation (Du et al. 2002). Studies of socioeconomic status (SES) and obesity concluded that during the early phases of economic development, obesity is more prevalent among those of higher SES. However, during the later phases of economic development, obesity become more prevalent among populations with a lower SES compared with those with higher SES (Monteiro et al. 2004). There is also a striking increase in childhood and adolescent obesity in both industrialized and developing countries (Wang 2001). This increase in childhood and adolescent obesity can also lead to an increased prevalence of obesity-related disorders in later life (Kotani et al. 1997).

Etiology of Obesity

The wide variation in the prevalence of obesity and the associated health-risks reflect the complexity of its etiology. The cause of obesity is much more than just an imbalance of energy-intake and energy-expenditure. Instead, obesity is best understood as a heterogeneous group of conditions with multiple causes where body weight is determined by the interactions between genetic, environmental, and psychosocial factors (Kopelman 2000). Many theories and studies have examined

these different factors and the degree to which they contribute to the development of obesity.

In 1962, a geneticist named James Neel proposed the "thrifty genotype" hypothesis to explain the paradox observed in the high prevalence of diabetes, a disease that has a strong genetic basis, even though it had an adverse effect on reproduction (Neel 1962). The genotype is "thrifty" in the sense of being exceptionally efficient in the utilization of food (McDermott 1998). This hypothesis suggested that this thrifty genotype conferred survival advantages during frequent periods of food shortage that are common in hunter-gatherer and pre-industrial agricultural societies (Brown 1991; McDermott 1998). Individuals who possess the thrifty gene are better at storing fat during times of plenty and would not starve during times of famine. However, this genotype has become disadvantageous as rapid westernization, occurring in many countries since World War II, has changed the environment from one of relative scarcity of food to one of plenty and thus favoring the development of obesity and type II diabetes (McDermott 1998).

Studies of the Pima Indians of the Gila River Indian Community of central Arizona have being used to support Neel's "thrifty gene" hypothesis. The Pima Indians have the highest reported cases of obesity and type II diabetes in the United States; but this elevated prevalence is a historically recent phenomenon (Knowler et al. 1991). The ancestors of the present-day Pima Indians are the descendants of the Hohokam who moved into the Gila River valley from Mexico (Haury 1976). The Hohokam Pimas lived in the desert environment of what is now Arizona by

irrigation farming supplemented by hunting and gathering food; they are often exposed to dietary fluctuations between feast and famine (Haury 1976). However, the Pimas' agricultural lifestyle was replaced by a lack of physical activity and abundant access to calorie-dense diets that accompanied westernization in the late 19th century (Marchand 2002). As a result, the thrifty genotype that would have benefited the Pima Indians during waves of famine is now maladaptive and contributes to the high prevalence of obesity and associated chronic diseases. The thrifty gene hypothesis offers an evolutionary perspective on the link between the effects of natural selection on genes and the development of obesity, but it does not identify specific genes that can directly or indirectly influence the etiology of obesity.

Genetic influence on obesity can operate through susceptible genes. Susceptible genes increase the risk of developing a characteristic of obesity, but are not essential for its expression (Kopelman 2000). Findings from twin studies in which pairs of twins were exposed to periods of positive and negative energy balance support the susceptible-genes hypothesis (Bouchard et al. 1990; Stunkard et al. 1990). Bouchard et al. (1990) have found that differences in the rate of weight gain, the proportion of weight gained, and the site of fat deposition showed greater similarity within pairs than between pairs of monozygotic twins.

The search for candidate genes for obesity involves studies of animal models, human obesity syndromes, and a genomic-wide search using microsatellites covering the human genome (Kopelman 2000). The Human Obesity Gene Map

project publishes a yearly review in Obesity Research to discuss studies on genetic markers and mutations that have been found to be associated or linked to obesity phenotypes. These studies looked for candidate genes associated with human obesity or its metabolic complications that can effect body fat composition, anatomical distribution of fat, food intake, and energy expenditure. They include receptors that are important in mechanisms of thermogenesis as well as those involved in appetite regulation (Kopelman 2000). Many candidate genes have been identified as a result of rodent obesity models and the intense interest of the agricultural community in breeding livestock that will grow large on the smallest amount of feed possible (Comuzzie and Allison 1998).

Obesity has also been a consistent finding in many single-gene disorders such as Prader-Willi syndrome (PWS) and Bardet-Biedl syndrome. PWS is characterized by upper-body obesity, short stature, mental retardation, and hypogonadism; it is caused by a deletion of the paternal segment of chromosome 15 (Butler 1990). Barder-Biedl syndrome is a heterogenic condition that shares many characteristics with PWS; studies of affected families have identified mutations on different loci of chromosomes 16, 11, 3, and15 as the cause of the condition (Benzinou et al. 2006).

Genetic differences within a population may determine those who are most likely to become obese, but the influence of genotype on the etiology of obesity may be attenuated or exacerbated by nongenetic factors (Kopelman 2000).

The fetal origins hypothesis, also known as the Barker hypothesis, suggests that fetal under-nutrition *in utero* may determine the onset of obesity, type II diabetes, and hypertension independent of genetic inheritance (Barker 1995). The human fetus adapts by trading off the development of nonessential organs, such as kidney and pancreas, in favor of more essential organs such as the brain causing negative effects on the development of the cardiovascular system, physiology, and metabolism. These developmental adaptations are consequences of fetal programming that allow the fetus to target available nutrients while increasing the ability to store energy as fat in expectation of a nutrition-poor postnatal environment (Mahajan et al. 2004). However, these adaptations are detrimental when there is a constant supply of nutrition.

A study by Ravelli and colleagues (1976) compared cohorts born around the time of the Dutch famine during the winter of 1944-1945 to a control group not exposed to famine during pregnancy provided convincing evidence that both early and late gestation are critical periods for the development of obesity in later life. The data showed that prevalence of obesity was significantly higher in adults whose fetal exposure to famine coincided with the first two trimesters of pregnancy compared to those whose exposure to famine occurred in the third trimester of shorter after birth (Ravelli et al. 1976). Klebanoff et al. (1999) tested the Barker hypothesis by studying women who were born in Copenhagen, Denmark as members of the Danish Perinatal Cohort Study from 1959 to 1961, obtaining information on their pregnancies from 1974 to 1989 to predict the development of

hypertension. The data showed that hypertension developed in 11.3% of the pregnant women who were small for gestation age at birth compared with 7.2% of the pregnant women who were not small for gestation at birth, thus providing support for the Barker hypothesis.

The critical role of environmental factors in the development of obesity have been supported by studies of migration and "westernization" of diet and lifestyles in developing countries (Kopelman 2000; Popkin and Doak 1998). In Micronesia and Western Samoa, the increased prevalence of obesity accompanied the westernization of diet and lifestyle, characterized by an increased consumption of high-fat foods and a low level of physical activity (McGarvey 1991). Marked increase in BMI has been observed in migrant studies comparing populations with a common genetic heritage. On average, Pima Indians living in the United States are 25 kg heavier than Pima Indians living in Mexico (Ravelli et al. 1976). The mean BMI for Nigerian immigrants living in the United States is 27.1 for men and 30.8 for women compared to the mean BMI of 21.7 and 22.6 for men and women living in Nigeria (CIBA Foundation Symposium 1996).

Social environmental changes may have contributed to the weight gain among American adults (Zhang and Wang 2004). Technological advances and changes in food production, preparation, and consumption have lead to an increase in energy intake and decrease in energy expenditure (Cutler et al. 2003). In developed countries, the average time for food preparation and consumption have decreased; and these factors have contributed to the significant increase in food

consumption in the United States (Cutler et al. 2003). Agricultural policy in the United States subsidizes farmers, allowing them to produce grains and meats at low prices, and thus contributing to people's excessive intake of food and to the current obesity epidemic (Nestle 2007; Pollan 2003). The low prices of raw materials allowed many food industry companies to increase portion sizes without increasing the price per unit of food (Pollan 2003). Decreases in food preparation time, advertisements for energy-dense foods, and the low prices of unhealthy foods promote the overconsumption of energy while contributing to the high prevalence of obesity (Rolls 2003; Young and Nestle 2002). The availability of low-cost, high-fat, high-carbohydrate foods creates a toxic environment that can exacerbate the negative effects of poor dietary habits.

Technological developments such as automobiles, television, and personal computers have fostered more sedentary activities by reducing job- and leisure-related energy expenditure (Zhang and Wang 2004). In the United States, children who watch television for 5 hours or more each day have a relative risk of obesity that is 5 times greater compared with those who watch for less than 2 hours (Gortmaker et al. 1996). A longitudinal Finnish study found that a decline in physical activity at work and transportation is correlated with weight gain; Finnish individuals who reported little physical activity had twice the risk of gaining 5 kg or more (Rissanen et al. 1991). Overconsumption of food along with a sedentary lifestyle is a sure formula for weight gain.

Socioeconomic status (SES) is an important factor that influences an individual's access to resources, food choice, level of physical activity, and knowledge of nutrition and health (Sobal 1991). In developing countries, early studies have found that groups of high-SES are more likely to be obese (Du et al. 2002; Monteiro et al. 2004). However, there is an inverse relationship between SES and obesity in industrialized countries where individuals of low-SES are more likely to be obese than those of high-SES (Lynch et al. 1997; Silventoinen et al. 2004; Zhang and Wang 2004).

Data from England and Wales showed a strong correlation between social class and obesity, especially in women; in men and women, the prevalence of obesity was 10.7% for high-SES and 25% for low-SES (Bennett et al. 1995). Individuals of low-SES also reported significantly less physical activity and more time watching television compared to those of high-SES. The National Health and Nutrition Examination Surveys (NHANES) used education level as a measure of SES and have found that in the United States, there is a higher prevalence of overweight and obesity observed in those with a lower level of education and low income (Flegal et al. 2002). People of low-SES have also reported greater stress associated with economic uncertainties and have a higher prevalence of smoking, higher alcohol consumption, poorer diets, and sedentary lifestyles (Lynch et al. 1997).

Another important environmental factor that affects the development of obesity is stress. Psychosocial stressors, such as the increased level of stress

experienced by individuals of lower SES, may also influence the development of obesity via physiological mechanisms (Pickering 1999). Stress associated with low socioeconomic status has negative effects on mental health that can lead to depressive illness, post-traumatic stress disorder, and suicide attempts (Everson et al. 2002; Goodman 1999; Roy 2004). Regardless of SES, chronic exposure to stressful life events can lead to depression and anxiety. Depression and anxiety disorders are often present in individuals diagnosed with coronary heart disease, type II diabetes, hypertension, and cancer; also, these disorders are often cormorbid with eating disorders and the complications associated with metabolic syndrome (Dallman et al. 2005; Egede 2007; Epel et al. 2004; Sherbourne et al. 1996). The prevalence of depression, anxiety, and the associated psychopathologies are significantly higher in women than men (McElroy et al. 2004; Richardson et al. 2003). The risks of developing these psychopathologies can be reduced by social support networks and the ability to minimize or control the stressors (Dohrenwend 2000; Stambor 2006). Although it is possible that this relationship can be explained by the side effects of pharmacotherapy used to treat depression, epidemiological data have shown that the number of depressive episodes positively predicts the risk of developing obesity, suggesting that mood is the main driver of emotional eating (Bjorntorp 2001; Rosmond 2004; Scott et al. 2008).

Animal models have shown that chronic exposure to stressors lead to a reduction in the intake of standard, low-calorie diets (LCD), while indicating a strong preference for more palatable, high-calorie diets (HCD) (la Fleur et al. 2005;

Pecoraro et al. 2004; Wilson et al. 2008). These data support the notion that humans experiencing stress seek comfort by consuming palatable foods that are high in fat and sugar (Pecoraro et al. 2004). These types of foods are called "comfort food" because they have reinforcing properties of powerful and repetitive rewards that can alleviate feelings of anxiety and discomfort (Dallman et al. 2005). In a laboratory setting, caloric intake was positively correlated with negative mood following stress, and women with a greater cortisol response consumed more calories than those with lower cortisol level (Epel et al. 2001). Studies of young women who have been diagnosed with depression show the level of circadian adrenocorticotropin (ACTH) and cortisol concentration was slightly elevated (Young et al. 2001). Clinically depressed people have also reported that they look toward comfort food for consolation when feeling down and out (Dallman et al. 2003; Parker et al. 2002). The occasional use of comfort foods to relieve feelings of stress and anxiety may make a person feel better, but the habitual use of high-fat, high-calorie, and sugary food to alleviate emotional discomforts is likely to lead to chronic health problems such as diabetes, cardiovascular disease, and obesity (Dallman et al. 2003). A study in mice has shown that exposure to a highly preferred diet high in fat reduces stress sensitivity (Teegarden and Bale 2007). Teegarden and Bale (2007) found that mice significantly increased their total caloric intake when a high fat diet was available, but reduced their calories when returned to the standard chow. The data also indicate that consumption of preferred diets may lead to decreased anxiety-like behavior, but withdrawal from this high-fat diet

then led to states of arousal and emotionality that are similar to or greater than those observed in the control (Teegarden and Bale, 2007).

Comfort foods can lead to obesity because people tend to overconsume them (Bray et al. 2004). Foods that are high in fat and sugar stimulate excess energy intake through its high palatability and lack of satiating power (Astrup et al. 1997). It also appears that the ability to reduce food intake to compensate for the overconsumption of comfort food is impaired when a person has been exposed to habitual ingestion of foods that are high in fat and sugar (Sparti et al. 1997; Tremblay et al. 1991). These studies suggest that people who want to loose weight by reducing their caloric-intake are faced with a serious challenge. Weight regain has been the main problem for most dietary and behavioral interventions (Perri et al. 1984; Wadden et al. 2005). Patients typically regain about 30% to 35% of their lost weight in the year following behavioral treatments, such as dieting and increasing physical activity; and by 5 years, 50% or more of patients are likely to have returned to their baseline weight (Wadden et al.1989). The National Weight Control Registry (NWCR) have found that individuals who were able to maintain the weight loss practiced four key behaviors: regular exercise, consume a low-calorie diet, monitor weight regularly, and record food intake and physical activity (Klem et al. 1997; Wing et al. 2001). These studies also suggest that negative feelings and mood such as depression, anxiety, and helplessness vastly contribute to failed attempts at maintaining a weight loss regimen (Wing et al. 2001; Klem et al. 1997).

Unfortunately, there is little direct evidence to confirm the hypothesis that mood and obesity share a common neurobiological etiology (Wilson et al. 2008). It is possible that the increased consumption of HCD is simply due to a stress-induced change in the release of or sensitivity to peripheral anorexic or orexigenic signals; but it is more likely that this change could reflect a coping strategy that provides relief from stressors (Wilson et al. 2008). However, these questions are difficult to address in humans and rodent models of stress-induced obesity do not provide strong ethological evidence for the relationship between psychosocial stress and emotional feeding.

Introduction

An understanding of the neurobiological mechanisms that shift food preferences and increase appetite during periods of stress can help define suitable interventions that can alleviate the health burden imposed by comorbid factors of emotional eating and mood disorders in women (Wilson et al. 2008). However, this requires a direct investigation of the relationship between the consumption of a high-calorie diet and the expression of behaviors that can lead to the increase in emotional feeding.

Socially housed rhesus macaques were used to test the hypothesis that if the consumption of a palatable HCD does have a positive effect on emotional discomforts during periods of stress, then expressions of distress behaviors such as anxiety should be lower during periods of HCD consumption compared with periods of LCD consumption. The complex social structure of rhesus monkeys provides an ethologically valid model of psychosocial stress and can be used to explore the relationship between stress, emotion, and appetite.

In the wild, rhesus monkeys (*Macaca mulatta*) are mainly folivorous and frugivorous, but can become heavily reliant on agricultural crops and food from people in certain parts of their habitat range (Richard et al. 1989). Daily feeding and foraging time ranges between 2 and 6 hours (Thierry 2007). Rhesus macaques form strong dominance hierarchies recognizable by the differences in behaviors exhibited during interactions between dominant and subordinate individuals (Thierry 2007).

Dominant individuals have a steady walk and up-held tail while lower-ranking individuals promptly flee and avoid confrontations with the dominant animals (de Waal and Luttrell 1989). Subordinate individuals use a silent bared-teeth display (retracting the lips and exposing the teeth) to formally acknowledge their lower dominance status relative to higher-ranking individuals (de Waal and Luttrell 1985). Differences in dominance status depend on individual strength, personality, experience, as well as social power.

Social relationships between rhesus macaques also depend on formation of alliances. The social ranks of females are determined by inheritance through the matriline; mothers, daughters, and sisters maintain strong bonds and offer mutual support during conflicts (Datta 1992). The ranks of matrilines also depend on the number of kin able to support each other. Females often choose to support their youngest relatives in contests, and thus younger daughters often outrank their elder sisters (Chapais 1988). This social component of dominance relationships in rhesus monkeys have also been studied in groups of males. Bernstein and Gordon (1980) have experimentally shown that relative ranks were dependent on the social context. Random introductions of males to a new group show that the newest males always assume the lowest-rank despite their previous rank relationship in other groups (Bernstein and Gordon 1980). Whereas males simultaneously introduced into an enclosure during group formation will compete for rank, males who were introduced to an established group were attacked by residents and forced to accept the lowest ranking (Bernstein and Gordon 1974). This suggests that the dominance

hierarchy is influenced by social factors such as the number and positions of other animals that can come to an individual's aid during confrontations (Bernstein 1976; Bernstein and Gordon 1980).

In this context, social stress factors could be defined as challenging stimuli originating from conspecific interactions (Sgoifo et al. 2005). Agonistic behaviors with different degrees of aggression are involved in disputes of resources and have been observed in many animal species and in humans (Sgoifo et al. 2005). Social stress in humans includes competitive and hostile behaviors that can represent a severe challenge to physiological and psychological homeostasis (Sgoifo et al. 2005). Henry (1990) proposed that there are two innate response patterns related to dominance and subordinate behavior: acting coping and passive coping. Active coping response pattern is related to a dominant behavior, characterized by arousal, high levels of aggression, and territorial control (Henry and Grim 1990). It is associated with increased cardiac output and redistribution of blood flow to the brain and the skeletal muscles, mediated by a robust activation of the sympathetic-adrenomedullary (SAM) system (Henry and Grim 1990). On the other hand, passive coping response pattern is related to subordination, such as the feeling of defeat or the perception of a threat to or loss of control. It is characterized by a generalized behavioral inhibition and a stronger activation of the hypothalamo-pituitary-adrenocortical (HPA) axis (Henry and Grim 1990). Subordinate members of rhesus monkeys in captivity show dysregulation of the limbic-hypothalamo-pituitary-adrenal (LHPA) axis, reduced dopamine (DA) signaling, increased rates of anxiety-

and depressive-like behaviors, and a preference for calorically-dense diets compared with dominant animals (Wilson et al. 2008).

In humans, these patterns of active and passing coping have been defined as "type A" and "type B" personalities (Roseman and Friedman 1974). Individuals with type A pattern of behavior are often more hostile, competitive, and achievement oriented; and the absence of these characteristics defines type B behavioral pattern (Roseman and Friedman 1974). Studies have shown that individuals with type A behavioral pattern had significantly larger increments of systolic pressure, heart rate, and plasma adrenaline; and they are more likely to develop coronary heart disease compared with individuals of type B. These data support the notion that the style of behavioral coping can strongly influence patterns of physiological responsiveness (Herd 1991; Newton and Bane 2001; Roseman et al. 1975).

The stress appraisal process in humans and animals involves the activation of the hypothalamo-pituitary-adrenal (HPA) axis and the sympathethic-adrenomedullary (SAM) system (Adam and Epel 2007). Acute and chronic stressors activate the HPA axis located in the hypothalamus. In response to stress, corticotrophin-releasing hormones (CRH) stimulate the secretion of adrenocorticotropin (ACTH) from the anterior pituitary into the bloodstream. ACTH signals the adrenal glands, which sit atop the kidneys, to release a number of hormones that enable the body to response to threat; these hormones include epinephrine (adrenaline), norepinephrine (noradrenaline), and cortisol. Epinephrine

and norepinephrine increase blood pressure and heart rate to divert blood to the muscles and speed up reaction time. Cortisol, also known as glucocorticoid, releases sugar in form of glucose from the body reserves so that it can be used to power the muscles and brain. Cortisol also exerts a feedback effect to shut off further cortisol secretion by acting upon the hypothalamus, causing it to stop producing CRH after the threat has passed (Huizenga et al. 1998). CRH is also involved in the regulation of energy balance and food intake (Richard et al. 2002).

There are two types of stress responses associated with the activation of the activation of SAM and HPA axis. A high level of SAM activation occur when a stressor is perceived as a "challenge"—a demanding but controllable situation, or when a person has adequate resources to cope with the stressful situation. The SAM system prepares the body for the *fight-or-flight* response by stimulating the adrenal medulla to secrete catecholamines, epinephrine and norepinephrine, into the blood stream. This activation causes more blood to flow to the muscles and heart while reducing blood flow to the internal organs and the gastro-intestinal system. However, when a stressor is viewed as "threat"—a demanding situation that one does not have the resources to cope well with—there is a higher HPA axis activation. In humans, "threat stress" includes components of distress (feelings of fear or defeat), embarrassment, and learned-helplessness; this type of stress is a potent trigger of cortisol (glucocorticoid release) release (Dickerson et al. 2004; Folkman et al. 1986; Henry and Ely 1975; Minor and Saade 1997).

Acute and chronic stress has different effects on the release of glucocorticoid (GC). Glucocorticoid acts as a feedback inhibition of subsequent adrenocorticotropin (ACTH) secretion; GC-feedback inhibition occurs within the first 18 hr after an acute stress (Keller-Wood and Dallman 1984). However, exposure to persistent and chronic stressors significantly decreased the efficacy of GC-feedback inhibition of stimulated, but not basal, ACTH secretion. The chronic stress-response network in the brain becomes activated by GC after the first 24-hr of the onset of a stressor; this activation affects mechanisms associated with coping. The indirect effects of chronically elevated GCs inhibit the expression of the chronic stress-response network through signals of abdominal calorie storage (Sgoifo et al. 1999).

Many studies in mice have investigated the effects of chronic stress-response activity and its mechanisms. The numbers of c-Fos immunoreactive cells in naïve and chronically stressed rats were compared as a measure of the chronic-stress response. Lesions of the paraventricular nuclei (PVN) of the thalamus affect ACTH responses, only in chronically stressed rats (Bell et al. 2000). The PVN secretes glutamate which strengthens synaptic connections that are involved in the recruitment of the stress response network (Herman and Cullinan 1997). The amygdala is also involved in the chronic -stress response network; the basomedial, basolateral, and central nuclei of the amygdala have increased c-Fos cell numbers in acutely restrained rats who have been exposed to a prior condition of chronic stress (McGaugh 2002). The amygdala innervates cortical, subcortical, and brainstem

structures and it is important in memory consolidation (Gray 1993). Corticosterone (B) implanted in the central nuclei of the amygdala increased the expression of CRH and anxiety-like behavior in response to an acute stressor (Bell et al. 2002). In the absence of corticosteroid-induced increases in amygdalar CRH, the HPA component of the chronic stress-response network is not activated. Corticotropin-releasing factor cells in the amygdala also innervate monoaminergic neurons in the brainstem by increasing the basal firing rates of locus coeruleus (LC) neurons and norepinephrine secretion in the forebrain to increase arousal and attention. Chronically stressed rats have increased CRH tone in the LC, and the activity of serotoninergic neurons in the dorsal raphe is also affected by CRH and stress.

There are strong inverse relationships between the increase in corticosteroids and body weight and caloric efficiency (Pecoraro et al. 2004). GC concentrations during stress provide fuel for glucose synthesis in the liver by mobilizing peripheral amino acids from muscle, fatty acids, and glycerol from peripheral fat stores (Laugero et al. 2001). There is also a significant positive relationship between B and sucrose ingestion and mesenteric fat (Strack et al. 1995). This suggests that increasing B concentration during stress lead to a redistribution of stored energy toward an intra-abdominal distribution (Laugero et al. 2001; Strack et al. 1995). Evidence in rats suggest that chronic GCs secretion stimulate the proliferation of mesenteric energy stores in the periphery while providing feed forward stimulation in the brain.

GC also affects the central nervous system to increase the compulsive nature of some activities such as eating and drinking (Piazza and Le Moal 1997). Studies in rats have shown that increasing corticosterone in adrenalectomized rats led to an increase in the ingestion of saccharin, a pleasurable drink for rats (Laugero et al. 2002). These findings suggest that increased level of B during stress promote the consumption of comfort food that is highly palatable. Other experiments in rats have shown that central CRH expression after stress is decreased when the subjects were provided with comfort foods. Experimental rats exposed to inescapable tail shock 24 hr before a shuttle-box avoidance test performed more poorly than controls; but if these rats drank concentrated dextrose solutions during the night after inescapable tail shock and maintained their caloric intake and body weight, and then they were able to perform as well as control rats during the test. This suggests that corticosterone increases the salience of pleasurable stimuli, motivating the animal to engage in the rewarding activities associated with seeking, organize defensive responses, and consumption of nutrients such as sucrose and fat.

Increased food consumption during stress can become an addiction by stimulating the natural-reward pathways in the brain (Goeders 2003; Wise 2004). Naloxone, an opiate antagonist, will also inhibit feeding in mammals (Yeomans and Gray 2002). The endogenous opioid system appears to augment the intake of high-fat, high-sugar foods and underlie the rewarding properties of palatable foods (Zhang et al. 1998). Food intake can also activate the dopamine reward pathway in the brain (Volkow and Wise 2005). Pharmacological studies in rats have shown that

experimental damage to the forebrain dopamine systems attenuates free feeding and level-pressing for food reward (MacDonald et al. 2004). Obese Zucker rats, leptin-deficient *ob/ob* mice, and obesity-prone Sprague-Dawley rats have reduced dopamine activity in the tuberoinfundibular pathway that projects to the hypothamus; treatment with dopamine agonists reversed the obesity in these animals by activating dopamine D2 and D1 receptors (Fulton et al. 2000; MacDonald et al. 2004; Volkow and Wise 2005). Brain imaging studies show reductions in dopamine D2 receptors in the striatum of obese humans (Pijl 2003).

The neuroendocrine and behavioral links between the psychosocial stress responses of humans and animal models formed the basis for using animal models in stress-induced obesity studies. Even though many paradigms and animal models have been developed to examine the effects of chronic stress on the development of psychopathology, the hormonal and behavioral responses elicited were unique to the laboratory environment (Wilson et al. 2008). Although, it is important to understand the physiological connections between stress, behavior, and appetite; the investigation of the neuroendocrine mechanisms behind changes in behaviors and diet preferences are beyond the scope of this thesis.

To investigate the biobehavioral effects of food consumption on stress as it relates to the development of human obesity and adverse health events, the experiment should focus on those stressors that are likely to be shared by human populations. Socially housed female rhesus monkeys provide an ethologically valid and translational model in which to study these factors because, like humans, they

live in a stratified society wherein subordinate females experience adverse consequences due to their social status (Jeffery et al. 2000; Wilson et al. 2008). Their linear dominant hierarchy and group stability are maintained through continual harassment and threats of aggression (Bernstein 1976; Bernstein and Gordon 1974; Bernstein and Gordon 1980). Furthermore, the adverse effects of social subordinate are exacerbated when females live in groups with minimal social support. Social subordination in female macaques is widely used as a model to determine how psychosocial stress increases the risk of cardiovascular disease, addiction, reproductive dysfunction, and metabolic irregularities in women, but to date, has not been exploited to examine the mechanisms responsible for the comorbidity of emotional reactivity and appetite (Bastian et al. 2003; Bernstein and Gordon 1980; Hoffman et al. 2007; Jarrell et al. 2007; Kaplan et al. 1996; Morgan et al. 2002; Wilson et al. 2008).

Methods

Subjects and Housing

The subjects were 10 adult female rhesus monkeys (*Macaca mulatta*), previously ovariectomized to eliminate the effects of gonadal hormones. They were socially housed in indoor-outdoor enclosures at the Yerkes National Primate Research Center Field Station with access to their diets by means of automated feeders. The females were divided into two groups of five monkeys. Group 1 includes Yf1, Bf5, Jc6, De3, and Ct4; group 2 includes Zr2, Mu3, Vh5, Vg5, and Ee3. The social dominance hierarchy of each group has been established based on the outcome of dyadic agonistic interactions observed during formal and informal observation sessions prior to the beginning of this experiment (Bernstein and Gordon 1974; Wilson et al. 2008). Individual group hierarchy has been established 6 years prior to the start of this experiment. Social dominance stability is maintained through harassment and threat of aggression rather than through contact aggression (Bernstein and Gordon 1974). Females with ranks 1 and 2 were considered dominant (n=4) and females with ranks 3 through 5 were considered subordinate (n=6). No rank or status change occurred during the course of this study.

Feeding Apparatus and Data Collection

There were two automated food dispensers placed on each housing unit to allow ad-lib access to food (Figure 1). By use of the automated feeding devices, food intake data for each subject was recorded 24 hours per day, 7 days a week for a total of 6 weeks. The operation of the feeders has previously been described in detail (Wilson et al. 2008). The feeders are enclosed in a waterproof box and were hung outside of the enclosures. A plastic shield protected the boxes and connecting wires from the animal. AVID (American Veterinary Identification Devices, Norco, CA) microchips were implanted subcutaneously in both forearms of each animal. Each microchip contains a unique identification number that allows an AVID reader located in the opening of the pellet-dispenser to indentify each monkey. The monkeys must reach through this opening to obtain a food pellet. Detection by the AVID reader activates the dispenser and one pellet is delivered. An optical sensor built into the dispenser detects each pellet deposited into the food tray. A 4-second delay was built into the software program controlling the dispenser to require a new activation by the monkey for the next pellet to be dispensed.

The dispensers are hardwired to a computer that captures the ID of the animal and the time that each pellet was delivered to the nearest second. Research and animal care staff refilled the reservoir that holds the food pellets every 2 hours as needed, including at night. Previous experiments by Wilson et al. (2008) have validated this feeding system and showed that dominant females do not restrict access by subordinate females to the feeders. Furthermore, the data show that

dominant animals rarely (~1% of the time) take a pellet of food that the subordinate females obtained (Wilson et al. 2008). Thus, this feeding system allows the quantification and analysis of kilocalories (kcal) consumed by individual monkeys.

Experimental Design

The study was divided into two phases of 3 weeks each, during which animals had either no choice or a choice of diet. During the first phase, one group had access to both the high-calorie diet (HCD) and the low-calorie diet (LCD) while the other group only had access to the LCD. To control for the effects of order, the diets were exchanged in the following phase so that the group with the choice of diet was restricted to the LCD and vice versa.

The LCD diet is a Purina monkey diet (#5048) typically fed to animals in our facility. The diet contains 3.61 kcal/gm; the calorie distribution for this diet is 16% protein, 12% fat, and 72% carbohydrate, derived primarily from fiber. The HCD diet contains 4.47 kcal/gm and has a calorie distribution of 20% protein, 40% fat, and 40% carbohydrate, derived primarily from sucrose, glucose, and fructose. Compared to the LCD diet, the HCD contains significantly more fat (derived from lard and soy bean oil) and sugar. Both diets were prepared by Research Diets (New Brunswick, NJ) and were given a banana flavor. To maintain primate health, both diets contain the appropriate vitamins and minerals and have similar amounts of cholesterol. Animals had access to these diets through automated feeders that record the kilocalories consumed by individual monkeys 24 hours a day.

Behavior and Emotional Reactivity

Behavioral data were also collected for each phase of diet choice and no choice. Using a standard ethogram (Table 1), observation sessions were 30 minutes per group and were done in the morning, three times a week for each diet phase (thus nine 30-minute observations per phase). Behavioral observations were collected as a group scan to record affiliative (proximity, grooming), aggressive (open mouth threat, bite, slap/grab, chase), and submissive behaviors (grimace, squeal, avoid) (Altman 1974; Altman and Altman 1977; Wilson et al. 2008). However, the focus of this experiment is on the expression of anxiety-like behaviors (body shake, self-scratch, yawn, orally groom self, pacing).

All occurrences of actions and interactions were recorded for all individuals of the focal group during each sample period. Using a standard ethogram (Mook et al. 2005), behaviors were recorded by trained observers with an inter-observer reliability greater than 0.92. Each behavior was recorded using a data acquisition program on a Personal Digital Assistant (PDA) computer that sequentially records the initiator, behavior code, the recipient of the behavior, and the elapsed time. This program allowed observers to record both the behavioral frequency and duration. A record of social interactions was generated with each animal uniquely identified. Behaviors were summarized in categories of heuristic values consisting of several specific behavioral patterns. These categories include aggressive, affiliative, submissive, and anxiety-like behaviors.

Agonistic behaviors include attack (serious aggression including biting), chase (chasing around compound in aggressive interaction), display (shaking scaffold or bouncing display on ground), and threat to other animal (slap, grab, bark, lunge, or open mouth). Animals are considered subordinate when they express an unequivocal submissive gesture to another animal (Bernstein 1976; Bernstein and Gordon 1974; Wilson et al. 2008). Submissive and affiliative behaviors include squealing, lip smacking, grimacing (pulling back of lips to display teeth), withdraw (avoiding another animal or pulling away from another animal), proximity (coming in close within arm's length and include interactions with another animal), and grooming.

Anxiety-like behaviors include self-scratching, self-grooming, body shake, yawning, and pacing (animal continuously roams about the enclosure or strolls back-and-forth). These behaviors are called displacement activities and have been assessed in pharmacological studies of nonhuman primates to show that the frequency of their occurrence can be increased by anxiogenic compounds and reduced by anxiolytic drugs (Troisi 2001). Studies have shown that displacement activities occur in response to acute frightening stimuli as well as to subtle threats (Schino et al. 1991a; Troisi et al. 1991). Many pharmacological studies support the ethological hypothesis that displacement activities are the expressions of anxiety in nonhuman primates (Schino et al. 1996; Schino et al. 1991b; Troisi 2001; Troisi et al. 1991).

Data Analysis

The 24-hour feeding data were summarized by kcal consumed per hour and categorized into eight 3-hour time bins per week for each of the 3 weeks of both diet phases. Behavioral and feeding data were analyzed using univariate analysis of variance for repeated measures (ANOVA) with status as the between-subjects factor and time and diet phase as the repeated measures. This analysis was used to assess how diet choice affects different categories of behavior and how this interaction is affected by status. Individual behaviors were grouped and analyzed by their heuristic categories of agonistic, affiliative, submissive, and anxiety-like. Data were summarized by the mean and standard errors. All statistical tests with a $P<0.05$ were considered significant.

Results

Total calories consumed during LCD only and Choice (LCD + HCD)

The main effect of diet choice versus no choice on the total caloric intake per hour (kcal/hr) was significant ($p=0.02$). Food consumption primarily occurred during the daytime rather than at night ($p=0.00$). Subordinate females consumed significantly more kcal per hr compared with dominant animals during both conditions of diet (Figure 2: $p<0.01$). Compared to dominant animals, subordinate females consumed twice as much kcal per hr during the daytime and nighttime (Table 2: $p=0.04$).

Weight change data suggest that there was a significant status by diet interaction ($p=0.02$). Weight change was similar between the two statuses when diet was restricted to LCD only, but there was a substantial weight change in subordinate females when both LCD and HCD were available. During the control period when only LCD was available, both dominant and subordinate females lost weight. However, subordinate females gained weight while dominant females maintained weight loss when both diets were available (Table 3).

Diet intake of LCD in the absence and presence of HCD

The main effect of diet condition on the consumption of LCD is statistically significant ($p<0.01$). Females consumed less LCD when there was a choice between LCD and HCD (Figure 2). Compared with dominant females, subordinate females consumed 67% more kcal per hr of LCD during no choice (LCD only) and 57%

more LCD when there was a choice. However, there was no significant status by diet condition interaction ($p=0.38$). Food consumption occurred primarily during the day rather than at night, but daytime consumption of LCD was considerably less when HCD was also available (diet condition by diurnal interaction: $p<0.01$). Furthermore, the effect of diet by time of day varied across the 3 weeks, of each diet condition (diet condition by week by diurnal: $p<0.02$). Daytime consumption of LCD during the absence of HCD increased across the 3 weeks while changes in nighttime consumption of LCD were statistically insignificant (Table 4). However, when HCD was available, consumption of LCD decreased during the daytime while nighttime consumption increased during weeks 2 and 3.

Although the main effect of diet was not statistically significant ($p=0.28$), the data show that females consumed significantly more HCD than LCD when both diets were available. During diet choice condition, subordinate females consumed more than twice as much HCD than LCD compared with dominant females (Table 5).

Anxiety-like behavior and diet

There were no significant differences between dominant and subordinate females or a significant status by diet interaction for the frequency and duration of anxiety-like behaviors exhibited by females during both conditions of diet. However, the analysis of anxiety-like behaviors show that the interactions between diet by week by status are statistically significant ($p=0.01$). The frequency of

anxiety-like behaviors expressed by subordinate females increased across the weeks when only LCD was available, but significantly decreased from week 1 to week 3 of the diet choice condition (Figure 3b; post hoc, $p=0.01$). Furthermore, there were no significant differences in the frequency of anxiety-like behavior in dominant females over the weeks of either diet conditions (Figure 3a). During the diet choice condition, subordinate females consumed noticeably less HCD during week 1 compared with weeks 2 and 3 (Figure 4).

An analysis of anxiety-like behavior by individual rank shows that there were no significant interactions for the duration of the behavior. However, there were significant interactions of diet condition by week ($p=0.00$) and of diet by rank by week ($p=0.03$) for the frequency of anxiety-like behavior. Overall, the frequency of anxiety-like behavior decreased across the 3 weeks when both LCD and HCD were available, but increased across the weeks when only LCD was available. Females of rank 2 (Bf5 and Mu3) and rank 3 (Jc6 and Vh5) expressed remarkedly higher levels of anxiety than females of rank 1, 4, and 5 during both diet conditions (Figure 5a & 5b). When only LCD was available, the frequency of anxiety-like behavior increased from week 1 to week 3 in females of rank 1, 3, and 5 (Figure 5a). Although the frequency of anxiety-like behavior for females of rank 1, 3, 4, and 5 were higher during week 1 of the diet choice condition compared with week 1 of no choice, the frequency of anxiety-like behavior expressed by these females substantially decreased from week 1 to week 3 only when both LCD and HCD were available (Figure 5b).

Social behaviors and diet

Although the main effect of diet condition was not significant in the analysis of the frequency and duration of affiliative behaviors as well as the frequency of submissive behaviors exhibited by animals, it is barely significant in the analysis of agonistic behavior ($p=0.047$). Both dominant and subordinate females exhibited significantly less agonistic behaviors when both HCD and LCD were available compared with the LCD only condition (Figure 6; post hoc, $p<0.01$). There was no significant interaction for the frequency of submissive behaviors.

There was a significant interaction of diet condition by week by day in the frequency of affiliative behavior ($p=0.03$). Although the diet by week interaction for the frequency of affiliative behavior was not significant ($p=0.22$), it shows that females exhibited more affiliative behaviors during week 2 of the no diet choice condition compared with week 1 and 3 (Figure 7). However, when both LCD and HCD were available, the frequency of affiliative behaviors was much higher during week 1 and 2 than week 3 of the choice condition. It appears that the frequency of affiliative behavior was significantly less on day 1 and 3 of week 3 during the choice condition compared with all other days. The diet condition by week interaction for the duration of affiliative behavior was significant ($p<0.01$). The duration of affiliative behavior significantly decreased from week 1 and 2 to week 3 when both HCD and LCD were available (post hoc, $p=0.01$), but the duration of affiliation increased from week 1 through week 3 when only LCD was available (Figure 8).

Discussion

The results from this study provided valuable insights into the complex interaction between psychosocial stress, comfort food ingestion, and obesity. The notion that the consumption of a palatable, high-caloric diet (HCD) can alleviate the negative effects of psychosocial stress, and thus motivate subordinate females to consume more HCD was based on previous experiments by Wilson and colleagues (2008). The subordinate females used in the present study had physiologic and behavioral indications supporting the hypothesis that social subordination represents a psychosocial stressor in macaques (Kaplan and Manuck 2004; Wilson et al. 2008). In previous studies, these subordinate females expressed significantly higher frequency of anxiety-like behaviors and had elevated plasma cortisol following a dexamethasone suppression test, indicative of reduced glucocorticoid negative feedback (de Kloet et al. 2006; Heuser 1998; Kaplan and Manuck 2004; Wilson et al. 2008).

Before the effects of diet on behaviors were analyzed, we first looked at dietary consumption to see if status affects dietary preference and caloric-intake. In this experiment, all animals preferred HCD over LCD when they had a choice, but subordinate females consumed significantly more of both diets compared with dominant females during both feeding conditions. The data suggest that dominant animals calorically regulate by restricting their food intake when HCD was available, whereas subordinate females lack inhibition and consumed significantly more food regardless of the nutrient composition in each diet. Data from this study

are consistent with findings in other animal models where subordinate individuals consumed significantly more food after exposure to stress (Foster et al. 2006). Studies in Siberian and Syrian hamsters suggest that chronically defeated animals overconsume standard laboratory chow and developed obese phenotype, and that a consequence of subordination in social housing may lead to excess calorie consumption (Foster et al. 2006; Solomon et al. 2007).

Although weight change data suggest that subordinate females with access to HCD are more vulnerable to weight gain than dominant animals, due to the short duration of this study and the absence of information about the animals' level of physical activity, no significant conclusion can be drawn about the effects of dietary preference on the animals' weight.

Possible explanations for the observed differences in caloric intake between dominant and subordinate females suggest that it could be the result of metabolic or limbic hypothalamic-pituitary-adrenal (LHPA) dysregulation, or both. Metabolic signals, such as leptin, that would normally attenuate the rewarding value of food may become ineffective in the presence of chronic stress. Therefore, subordinate females exposed to chronic psychosocial stress would lack the appropriate signals for satiety, resulting in the overconsumption of food. The consumption of HCD may also be directly related to the reward pathway in the brain by reversing the hypodopaminergic tone created by the exposure to chronic stress. The increased preference for HCD by subordinate females could reflect the desire to stimulate the mesolimbic dopamine (DA) pathway, thus increasing Dopamine 2-like receptor

(D2R) binding density and activating the reward circuitry. Furthermore, consumption of HCD may provide an anxiolytic effect that normalizes mood and motivates subordinate females to consume more of the diet.

To test the hypothesis that HCD has an anxiolytic effect on subordinate females, we compared frequency and duration of anxiety-like behaviors between the LCD only and choice of diet condition for each social status. The frequency of anxiety-like behaviors expressed by dominant females remained high across the weeks for both diet conditions. However, for subordinate females, the increase in anxiety-like behaviors from week 1 through week 3 during LCD only compared with the decrease across the 3 weeks when both LCD and HCD were available correlated with the feeding data obtained from these animals. During the diet choice condition, subordinate females progressively consumed more kcal per hr of the HCD diet over the weeks, and the increase in caloric-intake is negatively correlated with the frequency of anxiety-like behavior. The relatively high frequency of anxiety-like behaviors expressed during week 1 of the diet choice condition could reflect the subordinate females' increased anxiety associated with wanting to consume as much of the preferred diet as possible.

An analysis by rank revealed that the females of rank 2 and 3 expressed the highest level of anxiety compared with other females during both diet conditions. It is possible that females of rank 2 (beta) and 3 (gamma) expressed more anxiety because of their position in the social hierarchy. Beta and gamma females face a higher threat of losing their social status to lower ranking females than the alpha

female. Rank differences in the expression of anxiety-like behaviors also negatively correlated with feeding data. Overall, the frequency of anxiety-like behaviors was lower for females of all ranks during the diet choice condition compared with the control diet. However, females of rank 1 and 2 did not show a significant decrease in anxiety-like behaviors across the 3 weeks compared with the decrease in females of rank 3, 4, and 5. This result may be because females of the dominant ranks (rank 1 and 2) consumed significantly less HCD than females of subordinate ranks. These findings suggest that the availability and subsequent consumption of HCD does provide an anxiolytic effect for the animals, and this effect is positively correlated with the amount of HCD ingested. The data also suggest that the anxiolytic effect of HCD is gradual, increasing over the weeks as the caloric-intake from HCD increased.

We also looked at social behaviors and determined how these behaviors were affected by the availability of HCD. Rates of social behavior were similar between dominant and subordinate females. The decrease in agonistic behaviors when HCD was available suggests that the diet has a mellowing effect on the animals. Although duration and frequency of affiliative behaviors were higher during weeks 1 and 3 of the diet choice condition, there was a significant decrease in affiliative behaviors during week 3. Although the animals were not spending a lot of time on affiliative interactions during week 3 of the diet choice condition, there were no significant increases in other behaviors to suggest that the animals were engaging a substantial amount of time in other social activities. Unfortunately,

we did not record self-isolating behaviors such as sit-alone and inactive behaviors such as sleep; therefore, we can only postulate what the monkeys may have been doing instead of engaging in affiliative behaviors and why they were doing so. The relatively small sample size and short duration of the study may be an important factor in explaining the observed changes in affiliative behaviors. It is also possible that the significant increase in HCD consumption during week 3 of diet choice condition led to an increase in periods of inactivity. However, without a systematic method for measuring physical activity, it is hard to come up with a definitive answer for why there was a low level of social behavior during week 3 of the diet choice condition compared to all other weeks.

Data from this study can be used as a model for future ethological and psychobiological experiments on obesity. Having an ethologically valid animal model to study the effects of psychosocial stress will allow scientists to manipulate a range of conditions that could not be achieved in human and rat studies. Non-human primates, especially rhesus macaques, express a range of behaviors that are similar to humans, and thus provide a more translatable link between the animal model of behavior and psychobiology with those of humans. The validity of this animal model can help scientists investigate a range of possible interventions and preventive measures to reduce the prevalence of obesity and its associated comorbidities.

Conclusion

In summary, the present study shows that anxiety-like behaviors may be attenuated by the consumption of palatable, high-fat, high-calorie food. The data collected support the validity of using socially housed female rhesus macaques as an ethological model that can provide insights into the complex interaction between psychosocial stress, ingestion of comfort food, and obesity. The data support the notion that humans and animals under chronic stress increase the consumption of a preferred diet as a coping strategy, and this habitual practice can lead to increased accumulation of adipose tissue and eventually obesity. This study also provides evidence for mood-motivated consumption of foods that are high in fat, calories, and sugar.

These findings are valuable because they suggest that the expression of certain behaviors can be indicators of stress and can lead to unhealthy habits of food consumption. Having a ethological animal model of psychosocial stress to study psycho-behavioral aspects of obesity will help epidemiologists identify and target unhealthy behaviors that can hinder a person's ability to maintain a healthy weight and can also suggest possible mechanisms that are affecting the etiology of obesity.

Figure 1: Automated feeder

Table 1: Rhesus monkey ethogram

SIO-5

Agonistic & Affiliative Codes

at	Attack (serious aggression including biting)
ch	Chase (chasing around compound in aggressive interaction)
di	Display (shaking jungle gym, bouncing display on ground, shaking fence)
gm	Groom (separation of hair)
gs	Groom solicit (should only be scored if recipient is obvious)
g-	Stop grooming (approximately 5 seconds)
gr	Grimace (pulling back of lips to display teeth)
tc	Threat with contact (slap or grab, not as severe as attack)
tn	Threat, noncontact (open mouth, barking, lunge)
wd	Withdraw (avoiding another animal or pulling away from another animal)
sm	Scream
ls	Lip smack

Distance Codes

px	Proximity (within arm's length; includes contact) Px bn (within arm's length of biscuit chow bin) Px fd (within arm's length of LFD or HFD feeder)
lb	Leave to beyond (ends prox)

Anxiety behavior (self-directed)

ss	scratch
ya	yawn
bs	body shakes
se	any form of self-inspection including sniffs self; self oral explore (mouths or licks feet or hands and biting nails); self-groom (groom self, pick at, rub or examine her toes, hands, tail or other body part)
e-	stop oral explore
pz	pace (start immediately when pacing is observed but wait approximately 5 sec when terminating behavior to see if female actually stops)
z-	end pace

Sexual Behavior

ht	Hiptouch (female touches hips of female with both hands)
hp	Hiptouch to present (male touches hips of female in response to female present)
mt	Mount
sl	Solicit (handslap, head bob, standup, threataway, crouch)
pr	Noncontact present (female raises hindquarters in direction of male)

Table 2: Social Status & Diurnal Feeding

Mean caloric intake (kcal/hr) of LCD and HCD during the daytime and nighttime

	Daytime		Nighttime	
Social Status	Mean	Std. Error	Mean	Std. Error
Dominant	291.495	59.85	48.42	20.835
Subordinate	547.815	48.87	98.475	17.01

Table 3: Weight Change Data

Mean body weight (kg) change of dominant and subordinate females during LCD only and when both LCD and HCD diets were available.

Social Status	**LCD Only**		**Choice (LCD + HCD)**	
	Mean	Std. Error	Mean	Std. Error
Dominant	-.130	.082	-.110	.030
Subordinate	-.142	0.067	0.157	0.025

Negative values indicate weight loss

Figure 2: Number of kcal/hr consumed during LCD only and LCD + HCD (Choice) for dominant (Dom) and subordinate (Sub) females.

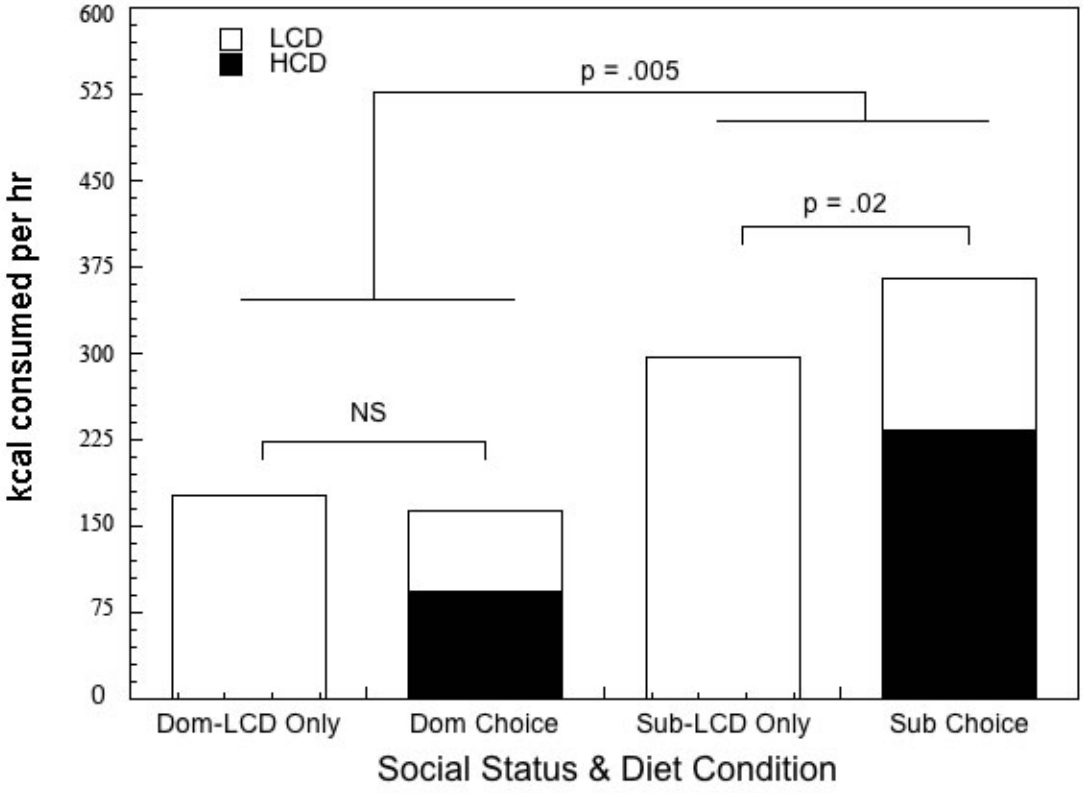
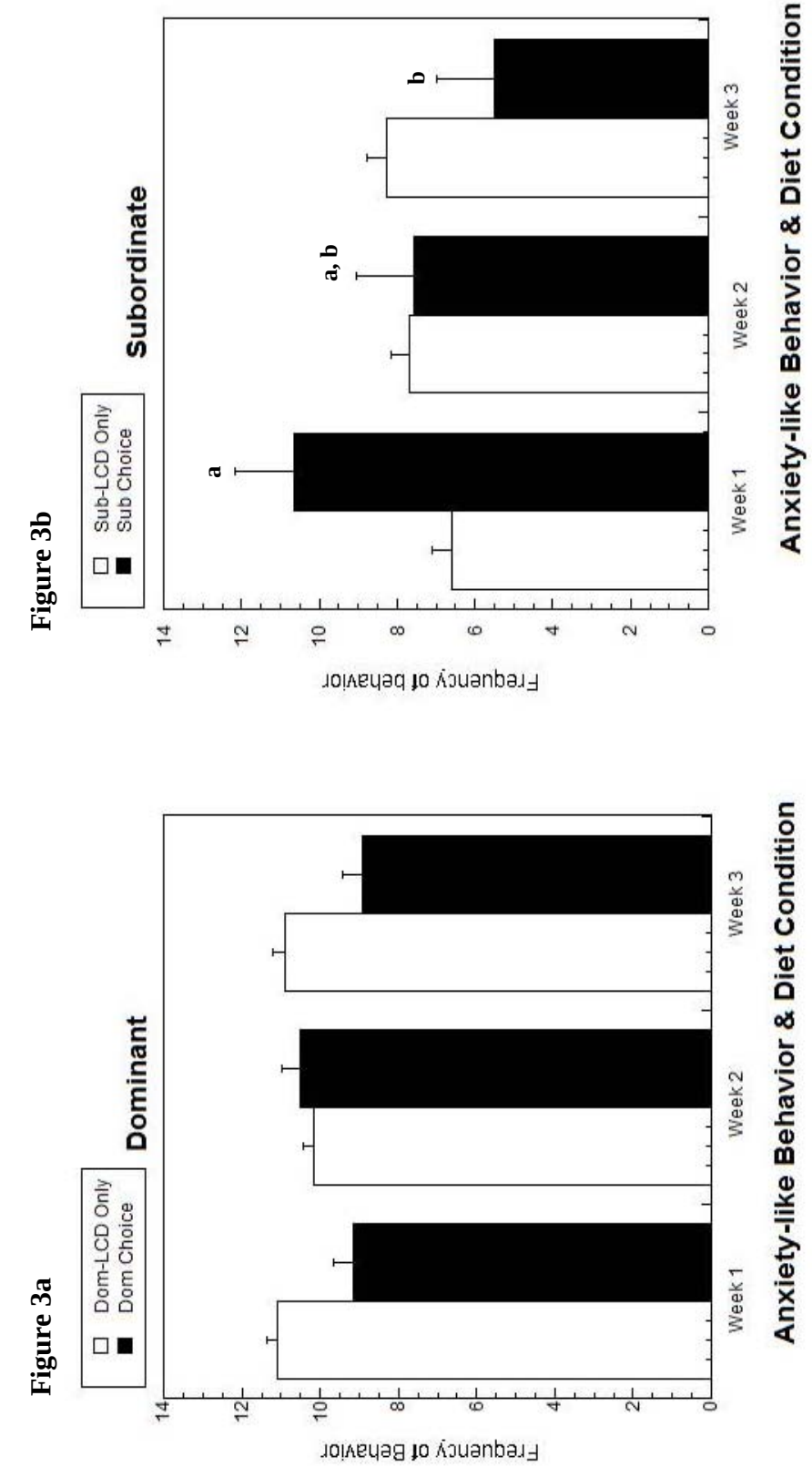


Table 4: Diurnal consumption of LCD (kcal/hr) during each diet condition

Diet Condition		Daytime		Nighttime	
	Week	Mean	Std. Error	Mean	Std. Error
LCD Only	1	326.475	42.45	65.955	17.565
	2	427.65	56.805	71.61	14.16
	3	465.675	64.11	63.93	11.13
Choice	1	173.97	26.1	40.245	6.225
	2	153.285	41.01	32.31	8.1
	3	131.115	36.165	70.29	33.015

Table 5: Diurnal consumption of LCD versus HCD during diet choice condition

		Daytime		Nighttime	
Social Status	**Diet**	Mean	Std. Error	Mean	Std. Error
Dominant	LCD	118.275	48.24	20.82	21.36
	HCD	171.885	86.88	14.52	11.85
Subordinate	LCD	187.305	39.39	74.415	17.445
	HCD	387.945	70.935	49.71	9.675



Bars with different letters indicate differences are statistically significant (post hoc test, $p<0.017$).

Figure 4: HCD consumption by status

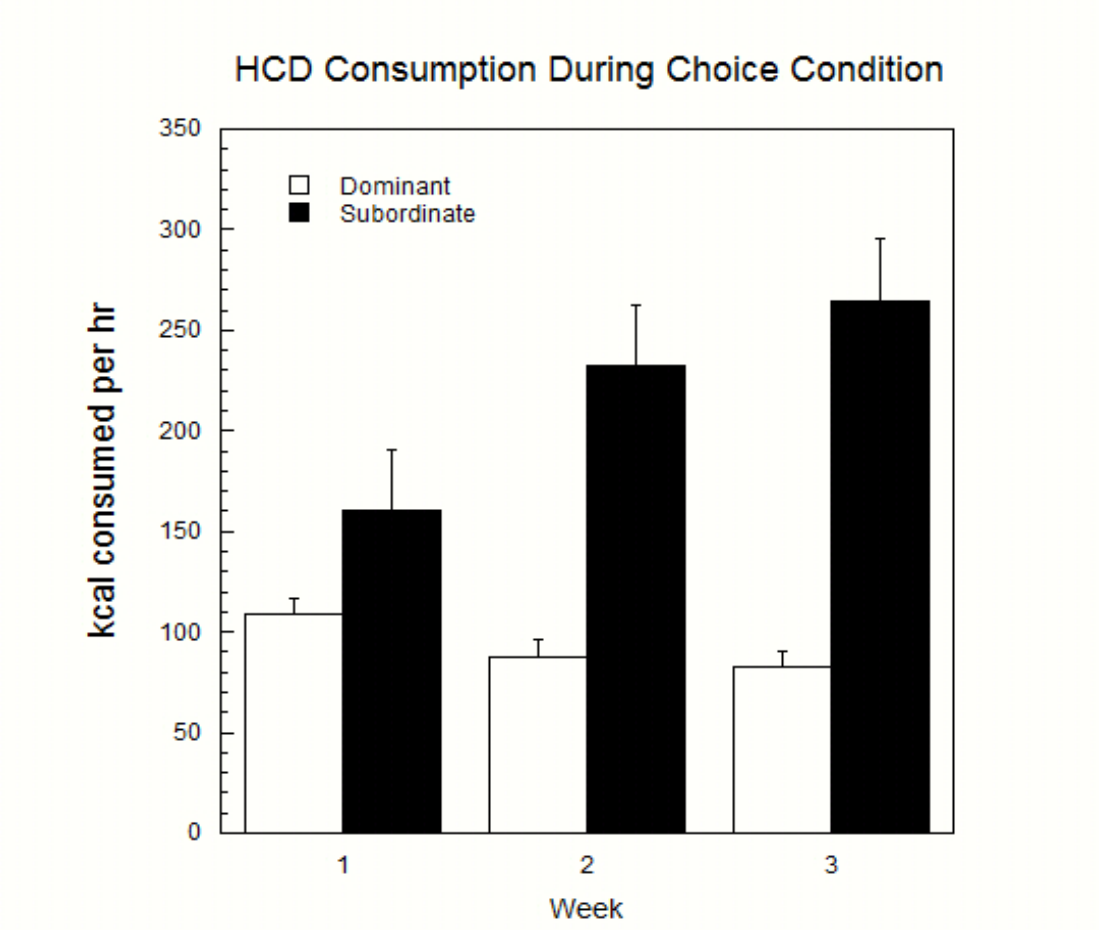


Figure 5b

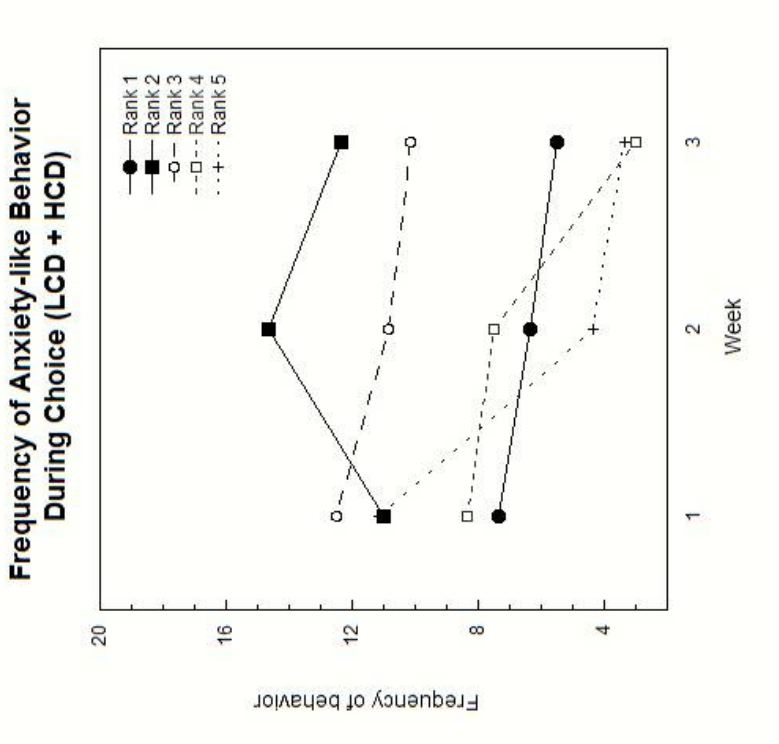


Figure 5a

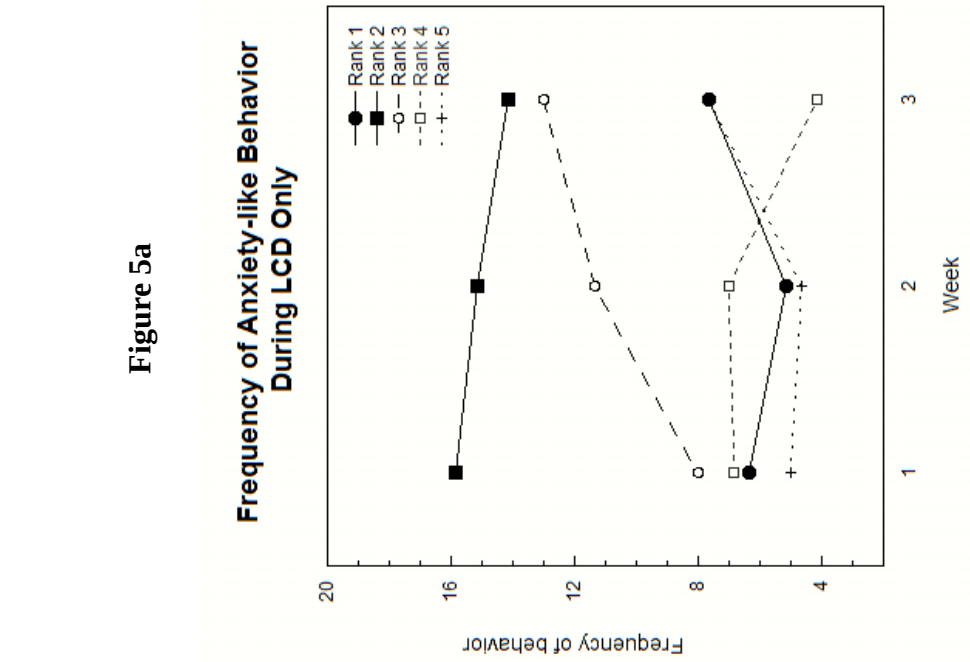
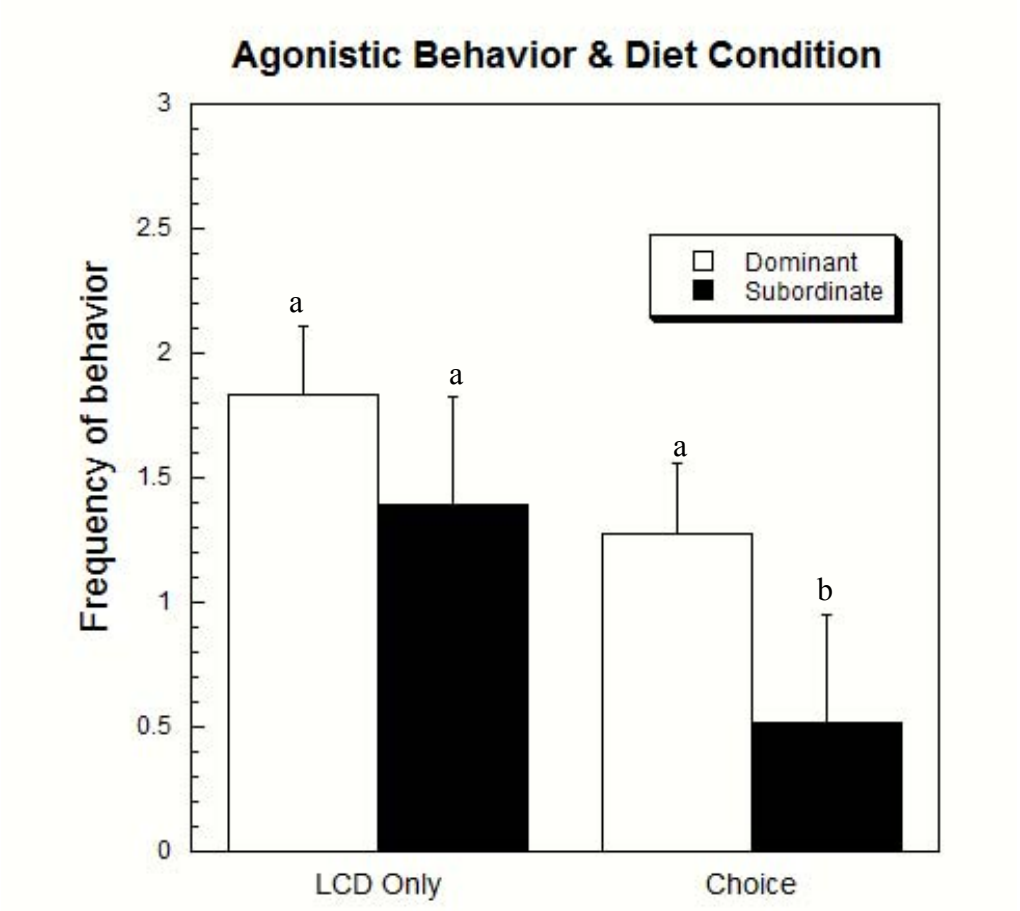


Figure 6



Bars with different letters indicate differences are statistically significant (post hoc test, $p<0.013$).

Figure 7

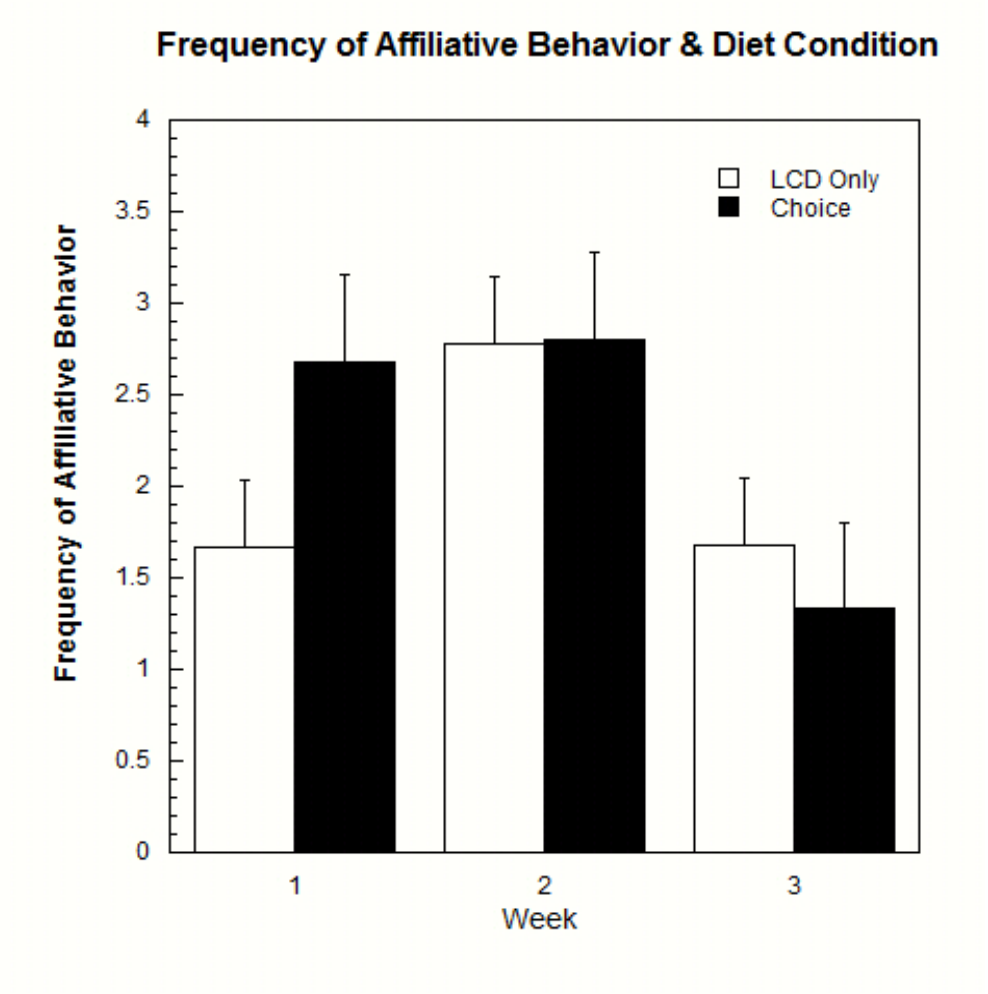
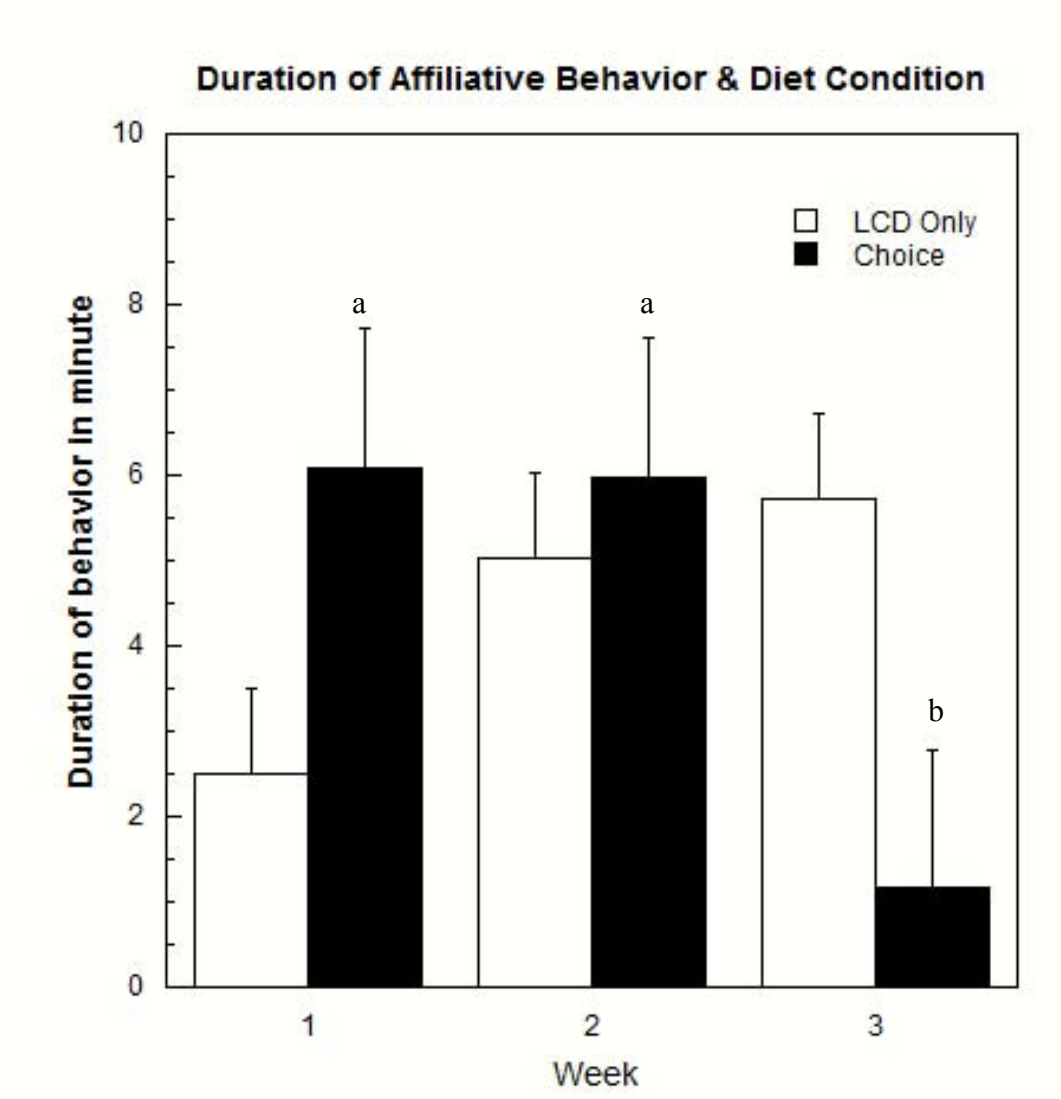

Frequency of Affiliative Behavior & Diet Condition
Frequency of Affiliative Behavior
LCD Only
Choice
4
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
Week

Figure 8



Bars with different letters indicate differences are statistically significant (post hoc test, $p<0.017$).

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