Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyrights of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

_____ _____

Carla Jennings Moore Date

Effects of Psychosocial Stress on
Food Preference, Caloric Intake, and Obesity Risk

By

Carla Jennings Moore

Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences

Nutrition and Health Sciences

_____	_____
Mark E. Wilson, Ph.D. Advisor	Charles F. Gillespie, M.D., PhD. Committee Member
_____	_____
Zachary P. Johnson, Ph.D. Committee Member	Dean P. Jones, Ph.D. Committee Member
_____	_____
K.M. Venkat Narayan, M.D., M.Sc. Committee Member	Thaddeus W. W. Pace, Ph.D. Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____
Date

Effects of Psychosocial Stress on Food Preference, Caloric Intake, and Obesity Risk

By

Carla Jennings Moore

B.S., Clemson University, 2005

M.P.H., University of North Carolina, 2006

Advisor: Mark E. Wilson, Ph.D.

An abstract of

A dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Graduate Division of Biological and Biomedical Sciences

Nutrition and Health Sciences

2014

Abstract

Effects of Psychosocial Stress on
Food Preference, Caloric Intake, and Obesity Risk

By Carla Jennings Moore

In recent decades, the prevalence of obesity has increased steadily worldwide leading to a concomitant surge in the incidence of related metabolic complications and chronic diseases. While obesity can be explained in biological terms as the consequence of prolonged positive energy imbalance (i.e., energy intake exceeding energy expenditure), a number of complex psychological, social, and environmental factors affect both sides of this equation. Stress-induced eating has received substantial attention in both human and animal model research. Yet, the stress-eating-obesity relationship has not been fully elucidated, and the role of the food environment in the stress-eating relationship has only recently gained interest. Previous studies using social subordination as a model of chronic stress among group housed female rhesus monkeys have shown that subordinate females consume fewer kilocalories than dominant animals when a typical laboratory chow diet is available but become hyperphagic in a rich dietary environment providing access to chow and a more palatable diet, high in fat and sugar. The present investigations expanded upon this work using long-established and recently formed groups of female rhesus monkeys. Significant findings included: 1) Pharmacological antagonism of the physiological stress response system attenuated caloric intake in a rich dietary environment among socially subordinate female rhesus monkeys within long-term stable social groups. 2) Formation of new social groups led to marked weight loss among all subjects within a standard laboratory chow dietary environment, and all animals within recently formed groups, regardless of status, increased caloric intake and gained weight when access to a high-fat, sugary diet was provided. 3) Exposure to an acute, psychological stressor among subjects in recently formed groups markedly reduced caloric intake among high and middle ranking subjects regardless of diet availability while the lowest ranking females, who consumed fewer calories than conspecifics during control conditions, did not further reduce their caloric intake in response to the acute stressor. These findings support a role of stressor exposure in promoting excess caloric intake from palatable diets but also highlight the potential importance of stressor intensity, duration, and intermittency in shaping the bidirectional effects of stressor exposure on dietary patterns.

Effects of Psychosocial Stress on Food Preference, Caloric Intake, and Obesity Risk

By

Carla Jennings Moore

B.S., Clemson University, 2005

M.P.H., University of North Carolina, 2006

Advisor: Mark E. Wilson, Ph.D..

A dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Graduate Division of Biological and Biomedical Sciences

Nutrition and Health Sciences

2014

ACKNOWLEDGEMENTS

When I decided to pursue a doctoral degree, I was unaware of how challenging, humbling, and enlightening the experience would be. I was also unaware that the most rewarding outcome of this endeavor would not be the degree itself but the people I would meet and the relationships that would be strengthened throughout its pursuit. There are many individuals to whom I owe a tremendous amount of gratitude for the opportunities that were provided through their efforts. There are others whose presence in my life simply enriched the experience and made the journey more worthwhile.

First and foremost, I must thank my advisor Dr. Mark Wilson for undertaking the challenging task of directing my graduate training and finding a way to integrate my interests as a nutrition and health sciences student with the ongoing activities of his neuroscience laboratory. The excellent training I received as a result of joining his laboratory could not have been obtained elsewhere, and I am certain that the skills that I acquired through this experience will serve me well in whatever career I ultimately choose. Additionally, I am tremendously grateful to the efforts of my committee members. Dr. Zachary Johnson provided the infrastructure for several experiments that were critical to my dissertation and offered thoughtful feedback and constructive criticism on all resulting publications. Dr. Charles (Rickey) Gillespie took a personal interest in my professional development and coordinated an invaluable clinical experience with the Grady Trauma Project. Dr. K.M. Venkat Narayan was a consistent source of encouragement and a critical liaison with the Molecules to Mankind program. Dr. Thaddeus Pace expressed enthusiasm and provided insight regarding the practical

relevance of investigations whenever I reached out to him for assistance, and Dr. Dean Jones always found a way to make time in his schedule to ensure my progress was on track and congruent with departmental requirements.

Further, I am forever indebted to the laboratory technicians who are the linchpins in the conduct of quality research. Although data collection processes seem effortless and routine in published manuscripts, the day-to-day execution of these procedures requires an enormous degree of planning, patience, strength, and ingenuity. I thank Jennifer Whitley for coordinating the acquisition of all necessary data despite limited resources and time constraints imposed by concurrent investigations. I thank Angela Tripp for taking a significant leadership role in the collection and management of all biological samples and behavioral data, for being dependable and responsive to any request for information or assistance, and for approaching every task with enthusiasm and the attitude that no job is beneath her. I thank Jonathan Lowe for his assistance with the logistics involved in all feeding studies as well as his outstanding technical support and engineering of the automated feeders. I thank Natalie Brutto and Casie Pricinsky for their demonstration of impeccable clinical skills and general awesomeness, and I thank Shannon Moss for her patience, nurturing spirit, and frequent serenades while I was initially assimilating to life in the laboratory. Additionally, I thank former and fellow graduate students Dr. Vasiliki Michopoulos, Amanda Mummert, Jodi Godfrey, and Jordan Kohn for informational support and occasional assistance with data collection. I feel fortunate to have gotten to know each of these individuals throughout this experience and consider them not only invaluable colleagues but also friends.

In closing, I must acknowledge the ever-present and unwavering support I have received from my closest friends and family. I thank Jennie Ellis Haithcock, Alex de Voux, Lucien de Voux, Adonis Bovell, Kathy Reding, Adam Wall, and Deborah Salvo for helping me maintain a healthy perspective, for embracing my quirks, for quelling my self-doubt, and for enriching my life beyond measure. I thank my brother Jason Moore for fully supporting me while keeping me grounded with his offensive humor, and I must acknowledge my parents Paul and Sheree Moore, who have exemplified hard work, discipline, and integrity in their every undertaking. I am extremely grateful for the sacrifices they have made throughout every phase of my life, and to them, I say, thank you for tolerating my rebellious spirit and encouraging my independence, for always supporting me emotionally and occasionally financially, and for being a constant source of unconditional love and practical advice.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

In recent decades, prevalence of obesity, defined as excess adiposity and indicated by a body mass index (BMI) greater than 30 kg/m^2, has increased significantly (1, 2). Though once viewed as an inert storage vat of energy, adipose tissue is a metabolically active secretory organ, and its endocrine and proinflammatory roles are becoming increasingly apparent as explanatory biological mechanisms that link the condition of excess adiposity with comorbid diseases including type 2 diabetes, cardiovascular disease, and certain cancers (3).

The forces that drive the emergence of an obese phenotype are vast and complex in terms of their interactions and relative contributions at the level of the individual. Nonetheless, factors that promote eating in the absence of hunger have garnered substantial attention in both the scientific community as well as the mainstream media, and the phenomenon colloquially termed "stress-eating" has been investigated as a possible contributor to the current obesity epidemic (4). This interest is driven by anecdotal evidence of a widely embraced practice of turning to "comfort food" during stressful periods as well as significant clinical evidence illustrating that excess levels of cortisol, a primary effector of activation of the physiological stress response, promotes consumption of excess calories and subsequent weight gain (5). Although investigations into the directionality and underlying mechanism for stress-induced alterations in food intake have been carried out for decades, a clear consensus has not been established (6), and the current literature is limited by practical and ethical constraints conducting human research (7). Existing animal research is also limited because many of the stressors utilized in these studies are

not ethologically relevant to the psychosocial stressors that people experience, and only a subset of investigations has provided access to palatable food (8). Further, despite higher prevalence of obesity (2), emotional eating (9-12), anxiety and depression (8), and perceived psychosocial stress (13) among women relative to men, the vast majority of animal studies investigating the effects of stressor exposure on food intake and body composition have been conducted in male subjects (8).

Female rhesus monkeys provide an ethologically relevant model of psychosocial stress, and recent investigations using this model have highlighted significant effects of psychosocial stress on food intake within the context of a rich dietary environment. Studies have shown that chronically stressed, subordinate animals become hyperphagic in a rich dietary environment while more dominant animals appear to regulate caloric intake regardless of whether they were maintained on a palatable diet or a standard laboratory chow diet (14, 15). These data support a plausible causal link between stressor exposure and obesity within a rich dietary environment, similar to that of humans. However, previous studies were very brief in duration. While changes in body weight tracked status differences in caloric intake, these changes did not reach statistical significance.

Expanding on previous studies, three critical investigations were conducted using this same model to advance our understanding of the relationships between stressor exposure, appetite, and obesity risk. The first study explored whether pharmacological alleviation of the physiological stress response would attenuate excess caloric intake among subordinate animals in a rich dietary environment. The second investigation assessed caloric intake and body weight among newly formed social groups of female rhesus monkeys over a prolonged period of time in the presence and absence of a palatable diet

to determine whether subordinate animals would become hyperphagic in a rich dietary environment leading to meaningful changes in adiposity. The final experiment evaluated the effects of acute stressor exposure on short-term caloric intake within a standard laboratory chow environment as well as a rich dietary environment. The justification for these studies is provided through the discussion of homeostatic and hedonic appetite regulation, the physiological stress response and its effects on food intake, and the utility of using socially housed female rhesus monkeys to investigate questions related to stressor exposure and obesity risk. The chapter concludes with a more detailed outline of the three research questions and the corresponding hypotheses for each investigation.

Homeostatic and Hedonic Regulation of Food Intake

Food and caloric beverages provide the necessary energy and molecular substrates for the survival and growth of living organisms. Energy is acquired from the environment through the ingestion of macronutrients – protein, fat, carbohydrate – and alcohol (16). Utilization and storage of acquired food energy requires vitamins and minerals, collectively termed micronutrients, which function as cofactors and coenzymes in the enzymatic reactions that are necessary to provide cellular energy to sustain life (16). For the majority of evolutionary history, food intake was motivated predominantly by a drive to maintain energy homeostasis and avoid starvation (17). However, in modern times, food consumption is motivated predominantly by factors other than acute energy deprivation (17). Thus, discrete episodes of food intake and emergent dietary patterns are shaped by the complex interplay of peripheral physiological hunger and satiety signals and related central hedonic neural processes as well as environmental, social, and psychological factors.

The roles of homeostatic hunger and satiety signals in appetite regulation have been reviewed extensively (18-21), and an overview of findings is summarized as follows. Homeostatic control of appetite is driven by the biological need to maintain the body's energy stores (20), and stimulation and inhibition of food intake are coordinated via episodic (short-term) and tonic (long-term) signals of peripheral and central origin (20). During short-term energy deficits, hunger signals provide motivation to seek and consume food (20). Ghrelin is a peripheral hunger-stimulating factor while central appetite stimulants include neuropeptide Y, agouti-related peptide, melanin concentrating hormone, and the orexins (20).

Ghrelin is an orexigenic (i.e., appetite stimulating) peptide hormone that is secreted primarily from the gastric mucosa (18); however, neurons within the hypothalamus also synthesize and release ghrelin to some degree (20). Ghrelin concentrations peak just before meal initiation and fall following intake to impart episodic regulation of appetite (20). Circulating ghrelin crosses the blood brain barrier to exert effects via two potent orexigenic peptides in the hypothalamus – neuropeptide Y and agouti-related peptide (21). *Neuropeptide Y* (NPY), synthesized primarily in the arcuate nucleus of the hypothalamus, is one of the most potent orexigenic substances that has been identified, and central administration has been shown to promote sustained hyperphagia and weight gain (21). *Agouti-related protein* (AgRP) is co-synthesized in the NPY neurons of the arcuate nucleus of the hypothalamus (20), and this peptide stimulates food intake and decreases energy expenditure by functioning as an endogenous antagonist at two receptors of the melanocortin system (MCR-3 and MCR-4), discussed later (21). Similar

to NPY, central administration of AgRP results in marked and prolonged hyperphagia (20).

Melanin concentrating hormone (MCH) is an orexigenic neuropeptide expressed in discrete populations of neurons in the lateral hypothalamus, often coexpressed with *cocaine and amphetamine-regulated transcript*, a satiety factor discussed later. Though less is known about this neuropeptide relative to other orexigenic signals, elevations in MCH mRNA and peptide levels have been observed in fasted animals, and central administration produces rapid dose-dependent increase in food intake in sated rats (20). The *orexins* are another class of neuropeptides secreted by neurons in the hypothalamus that play a role in appetite stimulation. Orexin secretion is stimulated in response to hypoglycemia; however, these signals are promptly inhibited by gastric distention and elevations in portal glucose levels (20).

When food is ingested, negative feedback signals are generated leading to a state of satiety in which the hunger drive is inhibited and eating eventually ceases (20). Initially, mechano-receptors in the stomach detect gastric distention, and this information is transmitted indirectly to the hypothalamus via the solitary tract of the brain stem as a consequence of vagal signaling (20). Chemo-receptors in the gastrointestinal tract also detect the chemical composition of ingested food and direct secretion of appropriate digestive enzymes and additional satiety signals including cholecystokinin, glucagon-like peptide 1, and peptide YY.

Cholecystokinin (CCK) is a peptide hormone secreted by the I-cells in the proximal intestinal tract (i.e., duodenum) in the presence of long-chain (>12C) fatty acids and

protein (19, 20). The role of CCK as an episodic satiety factor has been well established in rodents and monkeys as direct peripheral infusion dose-dependently decreases food intake in these animals. Likewise, human studies consistently show reduced meal size and shorter duration of eating episodes following the peripheral administration of CCK (20). CCK functions peripherally to delay gastric emptying (19). CCK also exerts its satiety-inducing effects through the vagus nerve, which relays the signal to the solitary tract of the brain stem and ultimately directs signaling at the level of the hypothalamus (19). CCK concentrations peak at 25 minutes following meal initiation, suggesting a role in meal termination and early phase satiety, and begin to fall around 3 hours following meal cessation, suggesting role in between meal satiety (20). Vagal signaling between CCK and brainstem melanocortin system, discussed next, plays a role in termination of food intake.

Melanocortin neurons produce neuropeptides that are derived from the precursor proopiomelanocortin (POMC), and of these POMC-derived neuropeptides, α- and β-*melanocyte stimulating hormone* (*MSH*) inhibit feeding (20). The melanocortin system has five known receptor subtypes, two of which (MC3-R and MC4-R) are expressed within the hypothalamic nuclei and are involved in energy balance. Both receptors appear to mediate the hypophagic effects of the POMC neuropeptides, though MC4-R is thought to play a more prominent role (20). Although the precise role of *cocaine and amphetamine-regulated transcript* CART in feeding behavior has not been fully determined, CART mRNA is expressed in the paraventricular nucleus of the hypothalamus, and this neuropeptide system is proposed as a supplementary avenue through which CCK exerts its effects (20). Additionally, *serotonin* has been implicated in

the within-meal processes of satiation and post-meal satiety of CCK, and pharmacological treatment with selective serotonin reuptake inhibitors reduces food intake among free-feeding rats (22).

Glucagon-like peptide 1 (GLP-1) is synthesized and released L-cells in distal small intestine (i.e., ileum) in response to carbohydrate ingestion and, to a lesser degree, dietary fat (19). GLP-1 induces satiety by stimulating insulin release, inhibiting glucagon production, and delaying gastric emptying (20). GLP-1 levels rise in anticipation of food intake and during the postprandial period and fall in the fasted state. GLP-1 receptors are found throughout central nervous system (CNS) and peripheral tissues, though not all signaling is associated with appetite regulation, and whether GLP-1 crosses the blood brain barrier to exert effects directly has been a topic of debate (20).

Peptide YY (PYY) is co-released with GLP-1 from the L-cells of the distal intestine (i.e., ileum) in the presence of fatty acids, fiber, and bile acid, and release appears to be confined to the period immediately preceding or immediately following meal cessation resulting in delayed gastric emptying (20). PYY shares similar structural elements with neuropeptide Y (NPY), a potent orexigenic peptide in the CNS, and PYY appears to cross exert its effects by directly antagonizing NPY Y2 receptors, though some effects may be dependent upon vagal afferents from periphery to the solitary tract of the brain stem (20). Other gut-derived peptides that may reduce meal size and induce satiety include *bombesin*, *somatostatin*, and *enterostatin* though the potential mechanisms of action of these signals have not been thoroughly explored (16).

While CCK, GLP-1, and PYY and the associated neuropeptide satiety factors, coordinate ingestive behavior in response to acute metabolic fluctuations, several peptide hormones are secreted from the peripheral organs to provide a direct indicator of long-term energy reserves (i.e., adiposity). Most notably, *leptin* is a protein secreted from adipose tissue in proportion to total body adiposity. Leptin enters the CNS in proportion to plasma concentration, and exerts hypophagic (i.e. appetite suppressing) effects via actions at neuropeptide systems located in the hypothalamus, medulla, and other sites that express its receptor (OB-R). Within the hypothalamus, leptin exerts its satiety effects via the melanocortin system and functions synergistically with CCK (20). Leptin also suppresses expression of NPY in neurons in the arcuate nucleus of the hypothalamus, reducing the appetite stimulating effects of this system (3).

Insulin and *amylin* are released from pancreatic beta cells in response to elevations in circulating glucose. However, these two peptide hormones also circulate in proportion to body fat. Thus, insulin and amylin levels reflect the interaction of ongoing metabolic processes as well as body adiposity (16). Insulin gains access to the brain through areas with a reduced blood-brain barrier and by a receptor-mediated transport system (16). Insulin binds to receptors expressed in the arcuate nuclei of the hypothalamus, and central infusion of insulin in rodents has demonstrated potent hypophagia and subsequent weight loss (16). Amylin appears to reduce food intake via central mechanisms in area postrema of the brainstem as well as through peripheral mechanisms that delay gastric emptying (20). Peripheral administration of amylin in rodents reduces food intake and body weight, and notably, pramlintide, a human amylin analogue, has been shown to lower subjective

feelings of hunger, increase satiety, and decrease food intake in lean and obese humans, suggesting this agent may hold promise as weight loss aid in humans (20).

Given the elaborate systems that have evolved to inhibit food ingestion in the absence of an energy deficit, the current obesity epidemic seems to be at odds with these biological processes (23). Indeed, many investigations have explored altered homeostatic signaling among obese individuals. However, evidence suggests that a very small percentage – less than 5% – of all obesity is a direct consequence of genetic mutations involving the homeostatic mechanisms that govern appetite (24). Instead, it appears the vast majority of individuals who develop excess adiposity do so as a consequence of the asymmetrical nature of the homeostatic energy balance system coupled with external factors that can influence appetitive behaviors (25). Indeed, this evidence suggests that the system is more sensitive to under-consumption rather than over-consumption of energy. Thus, given the rich dietary environment in which people now exist, the sensory properties of food as well as environmental and psychosocial cues can easily override satiety signals to promote consumption of excess calories and accumulation of body fat.

Palatability and pleasure are powerful motivators of food intake, and the rewarding value of food appears capable of overriding homeostatic control to promote ingestion of food in the absence of energy deficits (20). An increasing proportion of food consumption appears to be driven by pleasure, and an emerging body of work is elucidating the underlying mechanisms that likely involve the endogenous *opioid system*, the *cannabinoid system*, and the *dopamine circuitry* of the brain. These cortico-limbic systems are collectively termed hedonic systems of appetite regulation due to their roles in promoting eating as a function of sensory pleasure rather than biological need (20),

and activation of these systems by highly palatable food influences the cognitive, motivational, and emotional aspects of food intake.

Although hedonic mechanisms are dissociable from homeostatic mechanisms, the two systems demonstrate substantial cross-communication (17). There is considerable evidence that ghrelin enhances the hedonic value of rewarding substances, including highly palatable food (26). Functional magnetic resonance imaging studies among humans have shown increased neural response to food images within several brain regions implicated in hedonic feeding with peripheral administration of ghrelin versus without (27) as well as in the fasted versus fed state when ghrelin concentrations are elevated (28). Additionally, multiple rodent studies have demonstrated that exogenous ghrelin administration induces dopamine release from the ventral tegmental area (VTA) neurons that project to the nucleus accumbens, critical regions of neural reward circuitry (26). Rodent studies have also demonstrated increases in opioid messenger RNA expression within the VTA in response to ghrelin administration, and although the mechanisms have not been fully explored, ghrelin may also mediate the hedonic actions of endocannabinoid signaling (26). Conversely, central leptin and insulin infusions in rodents reduce operant responding for palatable foods, likely through the modulation of dopaminergic and opioidergic pathways (29). Thus, the general conclusions seem to suggest that homeostatic satiety may slightly diminish the perceived pleasantness of foods while homeostatic hunger signals may actually heighten the pleasure attained from the consumption of highly palatable foods.

In summary, although food provides energy and nutrients that are necessary for survival, eating is a multidimensional process. The CNS integrates numerous signals from the

gastrointestinal tract, pancreas, and adipose tissue to monitor acute and long-term energy status. These signals are further modified, and in some cases overridden, by the sensory experience of eating. Additionally, a multitude of environmental and psychosocial cues can direct appetitive behavior. Absence or presence of food obviously dictates whether there is an opportunity to eat and, if so, what types of foods are consumed. However, more subtle environmental cues such as the size of packaging or served portions can also influence the amount of food eaten on a single occasion (30). Because eating is often a social activity, the types and amounts of foods consumed within any given eating episode are also vulnerable to social influences and the desire to convey a socially acceptable image (31). Additionally, due to the adulation of thinness in Western cultures, many individuals attempt to exert psychological control over homeostatic, hedonic, environmental, and social cues that influence food consumption in an effort to maintain a desired body weight, a practice known as dietary restraint or "dieting" (32). Thus, the factors that drive the emergence of an obese phenotype are vast and complex in terms of their interactions, and attempts to unravel the underlying mechanisms must consider the relative influence of physiological, environmental, and psychosocial factors on long-term energy balance.

Stress-induced alterations in appetite and associated emotional eating have attracted substantial interest in the obesity research. Qualitative surveys generally report that a majority of respondents and study participants self-identify as stress eaters, indicating that they consume highly palatable – usually sweet, high-fat – food in excess of usual intake in response to emotional distress (33-35). While it is important to note that not all individuals self-identify as emotional eaters, not all emotional eaters are overweight or

obese, and not all obese individuals are emotional eaters, this behavior is significantly more prevalent among obese and overweight individuals relative to lean individuals (36). Thus, this observation suggests that emotional eating, particularly stress-induced eating, is a potential contributor to the current obesity epidemic.

Some epidemiological investigations into the relationship between perceived stress and subsequent eating behaviors have indeed demonstrated increased caloric intake during periods of heightened stress relative to less stressful periods (37, 38). However, other, similar investigations report no change in caloric intake in response to stressors (39, 40). Still, other investigations have demonstrated that subjects shift preferences to high-fat, sweet, snack-like foods and forego traditional meals during stressful intervals, which may or may not significantly alter total caloric intake (41). These investigations are complicated by the reliance on naturalistic stressors which encompass a range of occurrences from daily hassles to major life events to trauma or abuse (42). Further, while dietary assessment methods are intended to quantify usual caloric intake, recall methods are limited by dependence on memory and cooperation of the subject as well as communication skills of the interviewer, and food diaries can induce changes in dietary patterns through the actual process of recording food intake (43).

Laboratory studies involving humans are similarly inconsistent, reporting reduced (12), increased (44), and similar (39, 45, 46) food intake in response to acute, lab-induced stressors relative to control conditions. These investigations typically employ mild, acute stressors such as cognitive tasks (i.e., mental arithmetic, mirror tracing, stroop word test) or challenging interpersonal/audience performance tasks (i.e., Trier Social Stress Test) and provide short-term access to an array of palatable foods (47). While these studies are

informative in that they permit controlled testing environments, the few food options that are available may not be typical of the subjects' dietary environments, and follow-up assessments of potential compensatory dietary behaviors are generally not conducted. Additionally, the stressors employed in the laboratory may not be relevant to the types of stressors that individuals encounter outside of the laboratory, and in some cases, there is no measure of whether the stressor was perceived as stressful or elicited a hormonal stress response.

Given the lack of consistency in human epidemiological and laboratory investigations regarding the effects of stressor exposure on caloric intake, some may be inclined to dismiss this concept of stress-induced, emotional eating as purely anecdotal. However, as discussed, these human investigations are rife with limitations and rely on subjective measures and self-report. Clinical observations as well as animal studies employing more objective measures of the physiological stress response have demonstrated consistent associations between circulating levels of stress hormones and subsequent consumption of palatable food, supporting a role of stressor exposure on food intake and obesity risk. While these investigations are not without limitations and the biological mechanisms underlying consistent findings have not been fully elucidated, they provide the bulk of evidence regarding stress-induced alterations in food intake and are subsequently discussed in detail.

The Physiological Stress Response and Food Intake

From a physiological standpoint, stressors are any physical or psychological factors that disrupt or threaten to disrupt homeostasis (i.e., the optimal level for physiological endpoints) (48). Any biochemical or physiological response to a stressor is termed stress

or the stress response (49), which involves the acute activation of two physiological pathways: the sympathetic adrenal medullary (SAM) axis and the limbic hypothalamic pituitary adrenal (LHPA) axis (47). Activation of the SAM axis results in the immediate release of norepineprhine from sympathetic nerve terminals in peripheral tissues and the release of epinephrine and norepinephrine from the adrenal medulla into the systemic circulation. This is a rapid response, with plasma concentrations of epinephrine and norepineprhine increasing in seconds and reaching their peak within minutes (50). This response, known as the fight-or-flight response, and increased levels of circulating epinephrine and norepinephrine heighten vigilance and arousal to escape the threat (51). The second pathway involved in the stress-response, the limbic hypothalamic pituitary adrenal (LHPA) axis, comes into operation over minutes and hours rather than milliseconds (52). LHPA axis activation results in a hormonal cascade initiated by the release of corticotropin releasing factor (CRF) from limbic structures and the paraventricular nucleus of the hypothalamus, stimulating adrenocorticotropic hormone (ACTH) secretion from the pituitary, and the synthesis and release of glucocorticoids from the adrenal glands (53). The primary glucocorticoid in both humans and non-human primates is cortisol (50). Plasma cortisol levels peak within 20-60 minutes depending upon the nature of the encountered stressor, and increased levels of circulating cortisol mobilize energy reserves by promoting lipolysis within adipose tissue and glycogenolysis within the liver (50).

Although stress is generally considered a negative experience, the stress response is critical to survival and functions to help an organism adapt and overcome challenges (7). In healthy individuals, the stress response is short-lived. Activation of the SNS is rapidly

counterbalanced by the parasympathetic branch of the autonomic nervous system (7), and LHPA axis activation is terminated through an intricate negative feedback network in which elevated glucocorticoids in the circulation inhibit further secretion of CRF and subsequent release of glucocorticoids by acting at the level of the hypothalamus, hippocampus, medial prefrontal cortex, and pituitary gland (54). However, while the stress response is necessary for survival and adaptation, pathology can arise when continued exposure to adverse experience is prolonged (7).

The psychophysiological investigation of chronic stress and stress-related diseases has spanned many decades of research efforts (47). A substantial body of literature supports the notion that chronic life stress contributes to poor mental health, chronic disease risk, and decreased longevity (55). Additionally, evidence suggests that chronic, psychosocial stress may promote excess caloric intake from palatable foods, contributing to the high prevalence of obesity (7).

The clinical observation that patients with Cushing's syndrome, a condition resulting in excess production of cortisol secondary to tumor growth on the pituitary gland, demonstrate increases in appetite and central adiposity supports a potential role for stressor-induced elevations glucocorticoids in the development of obesity (6). Additionally, exogenous administration of glucocorticoids among human patients consistently induces increased food intake and, if prolonged, subsequent weight gain (56, 57). Likewise, in normal rats, central infusion of the synthetic glucocorticoid dexamethasone has produced sustained increases in food intake and body weight (58). In adrenalectomized rats, glucocorticoid replacement normalizes food intake and appears to dose-dependently increase intake of lard, sucrose, and saccharine (59).

While the relationship between exogenously administered glucocorticoids and food intake appears to be direct and consistent, discrepancies arise when animal studies attempt to induce stress via experimental manipulations. However, these inconsistencies are likely a function of the types of stressors that are utilized as well as the absence or presence of a palatable diet. Early rodent studies investigating the effects of acute stressor exposure on food intake consistently reported reduced food intake in response to acute, physical stressors including tail pinch, electric shock, injection, restraint, and immobilization in a standard laboratory environment (60). However, recent investigations have demonstrated binge-like consumption of a palatable diet in response to acute, electric shock among female rats (61).

Rodent studies of chronic stress and food intake have also produced varying results. Investigations using the social defeat model, in which hamsters experience brief, 7-minute exposures to a more aggressive cage mate for 15 repeated trials across 34 days, have induced hyperphagia of a standard chow diet following stressor exposure (62, 63). Conversely, male rats housed within a visible burrow system demonstrate reduced chow intake and reduced body weight in response to chronic social subordination that is enforced by unrelenting physical aggression from more dominant housing mates (8, 64, 65). Additionally, the chronic mild stress paradigm exposes rats to 6 weeks of repeated mild stressors, including food or water deprivation, light flashes, paired housing, cage tilting, and soiled bedding applied for 10 to 14 hours at a time. Although formal dietary assessment studies have not been conducted using this model, a consistent finding is that sucrose preference is significantly diminished in male and female rats exposed to these conditions (66).

Explanations as to why certain foods may be preferred during or following stressful experiences are based on the theory that high-fat, high-sugar foods act centrally on neuropeptide systems that influence the physiological stress response (67). Availability of lard and a sucrose solution has been associated with diminished ACTH and corticosterone concentrations after restraint stress in male rats (68), and CRF mRNA expression in the paraventricular nucleus was lower among male rats fed a high-fat diet (40% kcal from fat) following exposure to saline injection as a mild stressor compared to chow-fed controls (69). These findings are not consistent, however, as other male rats fed a high-fat diet (20% kcal from fat) demonstrated elevated basal corticosterone levels compared to low-fat diet (4% kcal from fat) fed controls (70). In addition, these animals also demonstrated increased ACTH release in response to restraint stress and impaired recovery of corticosterone concentration during the following stressor exposure compared to animals maintained on the low-fat diet (4% kcal from fat) (70). Conflicting results of these studies may be a result of variations in diet composition. Additionally, most of these mechanistic studies have been short-term in nature, and some findings suggest that the effects of diet composition on the physiological stress response are transient, disappearing within weeks (71, 72).

An alternative and possibly complementary theory is that signals from the LHPA axis target dopamine (DA) neurons in the reward pathways of the brain (73-75) producing a dysregulation of DA neurotransmission (76). Mounting evidence suggests a functional consequence of chronic stress is a "reward deficiency syndrome," characterized by reduced DA activity (77). Because pleasure is a powerful motivator of food intake (20), functional changes in DA signaling provide the rationale for stress-induced anhedonia

and accompanying reductions in food intake. However, a provocative alternative hypothesis asserts that down regulation of dopamine D2 receptors in these critical pathways may actually drive some individuals to seek out and over consume palatable, rewarding foods in an effort to compensate for a hypofunctional reward system (8). However, more work is needed in this area to fully understand the neurobiological underpinnings of this purported phenomenon.

A Nonhuman Primate Model of Psychosocial Stress

For many decades, rodent models have provided the foundation for understanding the mechanistic underpinnings of many human biological processes (78). Indeed, rodent studies are invaluable in that the minimal resources required for their execution permit carefully controlled longitudinal studies, resulting in a vast literature explaining these animals' physiology and behavior. Additionally, investigators can utilize these rodent models to manipulate genetic expression via knockout and transgenic technology (8). However, rodents possess notable differences from primates in terms of their lifespan, neuroanatomy, neurophysiology, and reproductive cycles, which may explain why many therapeutic interventions in rodents fail to exhibit the same properties in clinical trials with humans (78). Further, despite the disproportionate prevalence of emotional eating, obesity, and anxiety/depressive disorders among women, very few rodent models of psychosocial stress are applicable to females (8). Social dominance hierarchies do not emerge when female rodents are group-housed, and female rodents are not generally responsive to the resident-intruder model of social stress that is commonly used with male rodents (8). An element of social stress appears to arise among female rodents only when pregnant females are housed together; however, studies employing this method are

highly confounded because the endocrine changes associated with pregnancy and lactation may have profound effects on the stress response (8).

Nonhuman primates, specifically rhesus monkeys, circumvent many of the limitations of rodent studies, and provide a model in which to study psychosocial stress and obesity that is more ethologically relevant to humans, given that social subordination in rhesus macaques is a well established model to study the adverse effects of psychosocial stress on cardiovascular disease (79), addictive behaviors (80), reproductive dysfunction (81), and immune compromise (82, 83). Regardless of size, groups of rhesus monkeys are organized by a dominance hierarchy that functions to maintain group stability (84, 85). Lower ranking animals receive more aggression from higher-ranking group mates and terminate these interactions by emitting submissive behavior, a defining feature of subordination (84-87). Subordinates have less control over their environment (88), and a consequence of continual harassment is LHPA dysregulation, evidenced by reduced GC negative feedback and hypercortisolemia (83, 89-93). In addition, macaques exhibit a specific set of behaviors in stress-eliciting situations that are considered anxiety-like (94-98) and these occur more often in subordinates (89, 99).

Like humans, rhesus monkeys share cortical brain regions (e.g. the prefrontal cortex, anterior cingulate, and subgenual cingulate) that are not present or are underdeveloped in rodents (100). Rhesus monkeys share 93% of their DNA with humans (101). Nonhuman primates are also highly dependent on social interaction, and since animals are group-housed for extended periods of time, the social stress that is modeled is more ethologically relevant to humans than the intermittent social stress models that are utilized in rodent studies (8).

Further, rhesus monkeys demonstrate age-related changes in body composition similar to those observed in humans, and the development of spontaneous obesity has been documented in feral and captive populations (102). Though different definitions of obesity in rhesus macaques have been employed, (e.g., >22% bodyfat , > 2 standard deviations above the population mean, > 15 kg body weight), excess adiposity in macaques is associated with increased risk for spontaneously developing type 2 diabetes, and there appears to be a consistent linear relationship between adiposity and adiponectin, leptin, serum triglycerides, and fasting insulin (103). Of note, rodents do not typically develop spontaneous diabetes and are generally resistant to the development of diet-induced obesity-mediated type 2 diabetes. Instead, rodent diabetes research typically relies on chemical induction of diabetes with streptozotocin, which models type 1 diabetes, or genetic knockout models of type 2 diabetes (104). Finally, macaques share with women a true menstrual cycle that is more similar to in length, during which the endometrium is shed periodically, rather than absorbed as in the estrous cycles of rodents (78), and a substantial literature documents the behavior of many species of non-human primates in the wild and in captivity, which provides an established framework for interpreting results from emerging investigations (8).

The chapters that follow provide detailed descriptions of three investigations that were conducted to fill gaps in the existing literature using a rhesus monkey model of psychosocial stress to addresses stressor-induced alterations in feeding behavior. The first investigation explored the effects of pharmacological antagonism of the stress hormone response on food intake among socially housed rhesus monkeys in a rich dietary environment. As discussed, dysregulation of the LHPA axis among subordinate females

in stable social groups has been well documented, and previous findings using socially housed female rhesus monkeys have demonstrated that subordinate females became hyperphagic in a rich dietary environment while dominant animals appeared to regulate caloric intake. Given these findings, we hypothesized that pharmacological treatment with a potent brain penetrable CRF_1 receptor antagonist would attenuate emotional feeding among subordinate animals in a rich dietary environment but would be without effect in dominant females when both a low caloric, chow diet and a high caloric, palatable diet were available *ad libitum*.

The previous finding that subordinate animals became hyperphagic when access was granted to a highly palatable diet supports a plausible causal link between stressor exposure and obesity within a rich dietary environment. However, previous studies were very short in duration, and while changes in body weight tracked status differences in caloric intake, these changes were not statistically significant. The second experiment monitored caloric intake and weight status among newly formed social groups and built upon the previous findings by replicating these studies over an extended period of time. Because the time interval following initial group formation is typically characterized by high rates of aggression, assessing caloric intake and body weight following formation of new groups provided an unprecedented opportunity to determine if a greater dose of stressor exposure predicts greater consumption of palatable food and an associated metabolic phenotype. Thus, we hypothesized that the consequences of social subordination would be exacerbated among subordinate animals in the newly formed groups leading to excess caloric intake from a palatable diet and significant weight gain among the most subordinate animals.

The final study was designed to test the overriding hypothesis that caloric intake following exposure to an acute stressor would be influenced by the dietary environment and a female's chronic stress history. We hypothesized that animals would be hypophagic or unaffected by stressor exposure in the absence of a palatable diet but that animals would be significantly hyperphagic following stressor exposure in a rich dietary environment that included access to highly palatable food in addition to laboratory chow. We further hypothesized that hyperphagia would be significantly more pronounced among the most subordinate animals in a rich dietary environment, reasoning that experiencing greater ongoing aggression from group mates would exacerbate the consequences of acute stressor exposure. Finally, we hypothesized that greater cortisol reactivity, independent of social status, would be predictive of greater consumption of palatable food when animals were given a choice between a standard chow diet and a high-fat, high sugar diet.

The closing chapter provides a summary of all study findings and revisits the strengths and limitations of each investigation. The significance of these results are framed within the context of the existing literature and current public health strategies to promote health and wellness, and we conclude with recommendations for future investigations that will answer the questions that remain in the field of stress-induced alterations in appetite and food intake.

CHAPTER 2

Abstract

Social subordination in macaque females is a known chronic stressor and previous studies have shown that socially subordinate female rhesus monkeys consume fewer kilocalories than dominant animals when a typical laboratory chow diet is available. However, in a rich dietary environment that provides access to chow in combination with a more palatable diet – high in fat and refined sugar – subordinate animals consume significantly more daily kilocalories than dominant conspecifics. A substantial literature supports a role of products of the neuroendocrine stress response in shaping dietary preferences and promoting consumption of palatable, energy dense foods. The present investigation was conducted using stable groups of adult female rhesus monkeys to test the hypothesis that pharmacological treatment with a potent, brain penetrable corticotropin releasing factor type 1 (CRF_1) receptor antagonist would attenuate stress-induced consumption of a palatable diet among subordinate animals in a rich dietary environment but would be without effect in dominant females. Results showed that administration of the CRF_1 receptor antagonist significantly reduced daily caloric intake of both available diets among subordinate females compared to dominant females. However, effects were not uniform, as a subgroup of animals was unresponsive to Antalarmin. Together, findings support the involvement of activation of CRF type 1 receptors in stress-induced consumption of excess calories in a rich dietary environment and support a growing literature on the importance of CRF for sustaining emotional feeding.

Introduction

Collective evidence from animal and human studies indicates that stressor exposure affects a number of appetitive behaviors and may induce either increases or decreases in food intake (6). This bidirectional relationship is multifactorial, likely arising from differences in food availability (67, 105), individual physiology (4), and stressor severity and duration (106). In animal models, stress-induced decreases in food intake have been consistently documented in the presence of a standard laboratory chow diet (60). However, stress-induced increases in caloric intake have been observed when highly palatable food is available, particularly among subjects with enhanced glucocorticoid reactivity to acute stressors (4, 107) and among subjects enduring chronic stressors (5, 108). The notion of stress-induced consumption of "comfort foods" is widely accepted, and eating in response to negative emotional states has been associated with increased risk of obesity and its associated comorbidities (6). However, while this phenomenon has garnered substantial attention in the interest of public health, a more thorough understanding of the neurobiology that underlies this observed trend is necessary for the development of potential strategies to circumvent undesirable behavioral and metabolic consequences of stressor exposure.

Exposure to threatening stimuli elicits a highly coordinated physiological response engaging both the sympathetic nervous system and the limbic-hypothalamic-pituitary-adrenal (LHPA) axis. Activation of corticotropin-releasing factor (CRF) neurons in the amygdala, bed nucleus of the stria terminalis, and hypothalamus, coordinates the central and HPA response to a stressor (109-111). CRF triggers the release of ACTH from the anterior pituitary, which stimulates biosynthesis and secretion of glucocorticoids from the

adrenal glands (112). The actions of CRF are mediated by at least two distinct receptor subtypes (CRF_1 and CRF_2) that exhibit specific pharmacological and anatomical characteristics (113). Evidence suggests that the CRF_1 receptor plays a primary role in this pituitary-adrenal response to stress (113) and mediates central CRF action on neural circuits coordinating behavioral and physiological responses to stressors (111). The activity of the LHPA cascade is normally tightly regulated by negative feedback circuits that restore homeostasis when the threat is no longer present (114, 115). However, unrelenting exposure to stressors can overwhelm these regulatory circuits, resulting in elevated central CRF activity (59, 116, 117) and increased risk for a number of stress-dependent disorders (118).

Socially housed female rhesus monkeys (*Macaca mulatta*) permit assessment of the role of CRF signaling in the consumption of highly palatable food in response to a daily, unrelenting psychosocial stressor. Regardless of group size, female rhesus monkey societies organize themselves in a clear, linear dominance hierarchy (84, 85). Social subordination is enforced with both contact and noncontact aggression from more dominant animals, requiring subordinates to emit submissive behaviors to terminate these interactions (84-87). In stable hierarchies, subordinate animals consistently show dysregulation of the LHPA axis characterized by reduced glucocorticoid negative feedback, elevated basal cortisol, and/or delayed recovery following exposure to an acute stressor (83, 90-93, 119). Thus, social subordination in female macaques is a well-established model to study the adverse effects of psychosocial stress on a number of phenotypes (79-83).

In previous studies, socially subordinate female rhesus monkeys sustained on a standard laboratory chow diet weighed less and had less total body fat than dominant females (120), and this profile was associated with mild inappetence (121, 122). In contrast, subordinate females consumed significantly more daily kilocalories relative to dominant monkeys when a more palatable diet – high in fat and refined sugar – was presented in combination with the standard chow diet (14, 122). While dominant monkeys preferred the more palatable diet to the chow, their daily caloric intake did not increase during the dietary choice condition compared to the chow-only condition. (122). Together, these data suggest that the consequences of subordination in stable social groups of female monkeys increases vulnerability to consumption of excess calories when highly palatable food is available.

The mechanisms underlying stress-induced changes in appetite are complex and not fully understood, but evidence supports direct involvement of both CRF and glucocorticoids in shaping dietary intake and preferences (6). The present investigation built upon the aforementioned findings by testing the hypothesis that antagonism of CRF_1 receptors would reduce caloric intake among subordinate female rhesus monkeys in a rich dietary environment when both a standard chow diet and an energy dense, palatable diet were available *ad libitum*. Because dominant females did not significantly increase caloric intake in this rich dietary environment relative to chow-only conditions during previous trials (122), we predicted antagonism of CRF_1 receptors would be without effect in dominant females.

Materials and Methods

The Emory University Institutional Animal Care and Use Committee approved all procedures in accordance with the Animal Welfare Act and the US Department of Health and Human Services "Guide for the Care and Use of Laboratory Animals."

Subjects and the Dietary Environment

Subjects were ovariectomized, adult female rhesus monkeys (n = 23) that were members of five separate social groups at the Yerkes National Primate Research Center Field Station. Groups consisted of five or six animals (4 to 5 females and 1 male). Selected demographic information is shown for each subject in Table 2.1. Each group was housed in adjacent indoor-outdoor enclosures measuring 3.8 m by 3.8 m by 3.8 m. Indoor light cycles were maintained on a 12h: 12h schedule; however, access to outdoor caging allowed the natural photoperiod to prevail. Social groups were established approximately six years prior to the initiation of the current study using previously described methods, and the outcome of dyadic interactions between females obtained from formal, repeated group observations were used to establish group dominance ranks (91). The groups used in the study had been stable, with no changes in dominance rank for a minimum of 2 years. There were no differences between dominant and subordinate females in terms of age during the study, years in the group, years from ovariectomy, and body weight (Table 1). These animals formerly served as subjects in NIH-funded studies to determine the effects of psychosocial stress, induced by social subordination, on a number of behavioral, metabolic, and reproductive outcomes (91, 120, 122-126).

During the study, animals were provided with *ad libitum* access to both a standard, laboratory chow diet (LCD; 3.6 kcal/g, 12% fat, 18% protein, 4.1% simple carbohydrate,

and 65.9% complex carbohydrate; Purina #5038, re-pelleted by Research Diets) and a more calorically dense diet (CDD; Research Diets, D07091204; 4.74 kcal/gram, 40% fat, 20% protein, 25.3% simple carbohydrate, and 14.7% complex carbohydrate). Each diet was presented to the animals through two separate, automated feeders attached to each housing enclosure as described previously (119). Prior to the study, each animal's wrists were subcutaneously implanted with unique RFID microchips (DATAMARS). When an animal placed its hand in a feeder, a reader detected the microchip, relayed a signal to a remote computer that identified the study subject, and triggered the delivery of a single food pellet. This system allowed for continual quantification of caloric intake among individual monkeys embedded in social groups (119). Because the diets were available *ad libitum*, allowing animals to free feed, food competition did not occur.

Experimental Design

The study tested the hypothesis that the administration of a CRF_1 receptor antagonist would attenuate caloric intake among subordinate females (n = 13) but not dominant females (n = 10) in a rich dietary environment. Each subject served as her own control across study phases (treatment vs. placebo). During each trial, animals received daily injections at 0800 hr on two consecutive days with either Antalarmin (1 mg/kg/day, IV) or the vehicle (0.3 mL/day, IV). This dose of Antalarmin was chosen as it had been shown to normalize patterns of gonadotropin secretion following exposure to a psychosocial stressor in female macaques (127, 128). Animals were not subjected to any additional experimental manipulation, and food intake was quantified in the 24 hours following each injection using the previously described automated feeders. Order of treatment phase was counterbalanced across groups, and the diet dispensed by each

feeder was alternated at the midpoint of each trial to eliminate the potential confound of feeder-specific preference based on feeder locations within each housing unit. A three-week washout period separated the placebo and drug treatment trials. During the washout period, animals were maintained on LCD and food intake was not quantified.

Antalarmin (Sigma-Aldrich) was the pharmacological agent of choice, as this drug can be administered peripherally to diminish the central activity of CRF mediated through its Type 1 receptor (129). Antalarmin solutions were prepared on the day of each experimental manipulation following previously described procedures (127, 128). Placebo injections for both experiments were prepared in the same manner without the incorporation of Antalarmin.

Statistical Analyses

Following convention (124), females ranked 1 and 2 were classified as dominant while females ranked 3 through 5 were considered subordinate. Our *a priori* hypothesis predicted subordinates would consume more calories during the placebo condition compared to dominant females but that a status by treatment interaction would be present, with Antalarmin attenuating caloric intake, particularly of the CDD, in subordinate females but not dominant females. Social status differences in daily caloric intake were assessed as a function of experimental condition (placebo versus Antalarmin) using repeated measures ANOVA performed in SPSS. Group membership was included as an additional between-subject factor to determine whether the consequences of social status were consistent across the five social groups. Treatment day for caloric intake was included as an additional within-group factor. Post-hoc pairwise comparisons were generated to assess simple main effects from significant interactions. Results are

presented as the mean ± SEM for main effects and interactions. P-values < 0.05 were considered significant. Finally, effect sizes (Cohen's *d*) for selected main effects were also reported. Following accepted nomenclature, effects sizes greater than 0.50 were considered moderate and those greater than 0.80 were considered large.

Results

Caloric Intake

As illustrated in Figure 2.1, the effect of Antalarmin on daily caloric intake varied significantly by social status ($p = 0.012$), with Antalarmin reducing caloric intake in subordinate ($p = 0.001$) but not dominant monkeys ($p = 0.81$). During the placebo condition, subordinate females consumed significantly more calories relative to dominant females ($p = 0.002$, Cohen's $d = 1.63$), but this difference was no longer present during the Antalarmin treatment phase ($p = 0.24$, Cohen's $d = 0.52$). While all animals consistently preferred the CDD (722 ± 71 kcal) over the LCD (348 ± 44 kcal, $p = 0.005$, Cohen's $d = 1.36$), dietary preference did not vary by status ($p = 0.45$) nor treatment by status interaction ($p = 0.23$). Importantly, membership in a specific social group contributed significantly to explaining variance in caloric intake, as significant treatment by social group ($p < 0.001$) and treatment by status by social group ($p = 0.048$) interactions emerged.

Because the consequences of Antalarmin administration differed significantly between dominant and subordinate females, separate analyses were conducted for each status category. The significant attenuating effect of Antalarmin on caloric intake in subordinate females ($p = 0.001$, Cohen's $d = 2.48$) was not influenced by day of treatment ($p = 0.76$) nor was it due to a greater reduction in a specific diet. Although the

reduction in consumption of the CDD following Antalarmin was greater than the reduction in LCD intake (Figure 2.2A), the difference was not significant (treatment by diet interaction: $p = 0.60$). As illustrated in Figure 2.3A, however, this main effect of Antalarmin treatment varied significantly by social group membership ($p = 0.001$). Given the small sample size per group within the subordinate status category ($n = 2$ to 3 per group), parametric analyses of these group differences were not possible. However, qualitative observations of total caloric intake in response to Antalarmin administration among subordinate animals in each group revealed that 4 groups decreased (groups 1, 2, 3, & 5) caloric intake while one group actually increased caloric intake (group 4). Furthermore, in some groups, not all subordinates responded similarly (groups 1, 3 and 5). Finally, this effect of social group membership on the response to Antalarmin was not influenced by day of treatment ($p = 0.27$). In total, 62% (8/13) of subordinate females responded to Antalarmin with a decrease in total caloric intake.

As described above, Antalarmin did not have a significant effect on total caloric intake in dominant females ($p = 0.81$, Cohen's $d = 0.14$). In addition, consumption of each specific diet was also unaffected by treatment condition in dominant females (Figure 2.2B; $p = 0.77$). Unlike the effect observed in subordinate females, the response to Antalarmin in these dominant animals was not affected by group membership ($p = 0.07$). However, inspection of the data (Figure 2.3B) shows that the response to Antalarmin between the groups was variable, albeit non-significant. The most notable differences in caloric intake among dominant females in response to Antalarmin treatment were documented in Group 1, in which dominant animals increased caloric intake, and Group 2, in which dominant animals decreased caloric intake. While the dominant females in

Groups 3 to 5 showed a consistent decrease in caloric intake in response to treatment, this represented an average of 69 kcal per day. Finally, the effect of social group membership on the response to Antalarmin was not influenced by day of treatment ($p = 0.49$). In total, 70% (7/10) of dominant females responded to Antalarmin with a decrease in total caloric intake.

To further examine the status-dependent effects of Antalarmin on caloric intake, the analysis was then rerun using only animals that responded to Antalarmin treatment with a decrease in caloric intake (n= 7 dominant and n= 8 subordinates). In this restricted analysis, Antalarmin decreased caloric intake relative to placebo conditions (730 ± 46 vs. 366 ± 19; $p = 0.001$). Importantly, this main effect of treatment was significantly modified by status ($p = 0.03$). Because subordinate females were consuming significantly more calories during the placebo condition than dominant females ($p = 0.02$; Cohen's $d = 1.38$), the decrease among subordinate females during Antalarmin treatment compared with control (888 ± 68 vs 368 ± 27, Cohen's $d = 3.88$) was greater than that of dominant females (573 ± 71 vs. 364 ± 28, Cohen's $d = 1.60$). Finally, restricted analyses examining those females that showed an increase in caloric intake during Antalarmin treatment showed no main effect of treatment ($p = 0.06$), and no status by treatment interaction ($p = 0.19$).

Discussion

Findings from this study support previous work demonstrating that socially subordinate female rhesus monkeys consume more daily kilocalories relative to dominant animals in a rich dietary environment. Furthermore, the data support a role for the activation of CRF_1 receptors in this phenomenon because administration of Antalarmin significantly

reduced daily caloric intake of both available diets among these subordinate females. Dominant females, whose daily caloric intake was lower than that of subordinate group mates during the placebo condition, did not significantly reduce caloric intake during the Antalarmin treatment. However, these effects of Antalarmin were not uniform across all subjects within each social status category. While the attenuation of caloric intake by Antalarmin was sufficient across subordinate females to reach statistical significance with a large effect size, some subordinate animals did not respond to the treatment (5 out of 13). Indeed, dominant females responded similarly, with most decreasing and others (3 out of 10) increasing caloric intake during the Antalarmin condition. Limiting the analysis to those females among whom treatment with Antalarmin attenuated caloric consumption during the Antalarmin condition showed that the effect was still significantly greater in subordinate compared with dominant females. Nonetheless, the data highlight that some females, both dominant and subordinate, are insensitive to the appetite suppressing effects of CRF_1 receptor antagonist in this rich dietary environment.

Several studies have been published to date that directly assess the effects of CRF_1 antagonist administration on food intake in diet-cycled rats. Diet cycling or intermittent dietary restriction of a palatable diet is a mild stressor that elevates CRF positive cells in the central nucleus of the amygdala (130) (130) compared with control rats (131, 132), similar to the effects of withdrawal from drug administration in rodent models of addiction (133, 134). A recent report (131) using this model of intermittent access to chow and a palatable diet reported findings similar to the results in the present study. Specifically, microinfusion of Antalarmin into the CeA of diet cycled male rats fully blocked hyperphagia of a palatable food with no effect on standard chow intake (131).

Infusions into the bed nucleus of the stria terminalis or basolateral nucleus of the amygdala were without effect. The use of another brain penetrable CRF_1 receptor antagonist, R121919, administered peripherally in rats has produced mixed results, attenuating palatable food intake in diet cycled males (132) but failing to reduce binge-like intake of an intermittently available high fat, high sugar diet in females (135). However, consistent with observations from the present study, peripheral administration of Antalarmin reduced palatable food consumption induced by yohimbine, an α-adrenergic antagonist employed as a pharmacological stressor (136). For the present study, all animals preferred the CDD, yet the effects of Antalarmin were not limited to the reduction of this diet alone. There was no interaction of treatment and diet, suggesting antagonism of the CRF_1 receptor effectively reduced total caloric intake from both diets.

The present results warrant a discussion of the significance of the dietary environment as well as the potential roles of CRF_1 and CRF_2 receptor subtypes in the bidirectional relationship between stress and appetitive behaviors. While stimulation of downstream glucocorticoids via CRF_1 activation in the pituitary appear to increase preference and intake of palatable, energy dense diets (67, 105), CRF itself is a potent anorexigenic peptide (137), perhaps predominantly operating via CRF_2 receptors (138). However, studies show that microinfusion of CRF into the nucleus accumbens (95) potentiates cue-induced responding for sucrose (139), supporting the notion that the effects of CRF on food intake may be modified by the neuroadaptation that results from the dietary environment where palatable food is available (105). Indeed, our observations of significant attenuation in palatable food consumption by subordinate but not dominant

females following Antalarmin administration is reminiscent of the effect of CRF_1 receptor antagonism on attenuating drug reinstatement in drug dependent but not nondependent rats (see (133, 134, 140) for review).

CRF_1, and to a lesser degree CRF_2, receptors are distributed throughout rhesus monkey cortico-limbic and striatal regions (141), providing neuroanatomical evidence for a possible role of CRF in the response to appetitive and aversive stimuli in this species. Evidence from rodent models clearly implicates a role for CRF from the extended amygdala and specifically CRF_1 receptor activation in the NAc and or ventral tegmental area (VTA) in producing deficits in brain reward salience and in sustaining drug dependence (134). In these models, administration of CRF_1 receptor antagonists reduces drug intake (see (140) for review). Signals from the stress axis target dopamine (DA) neurons in mesolimbic regions (73-75) producing a dysregulation of DA neurotransmission (76) that increases the expression of anhedonia and the risk for developing an addictive phenotype (133, 134, 142). The particular pattern of stress-induced DA dysfunction in reward pathways may vary by species and stressor, but the functional consequences of chronic stress is a "reward deficiency syndrome", characterized by reduced DA activity (77) that is both predictive of an addictive phenotype (143, 144) and observed in obesity (145, 146). Thus, the implication is that palatable food intake diminishes the hyperactivation and the adverse effects of these central stress response systems (105). Furthermore, like drugs of abuse, abstinence from palatable food intake that occurs spontaneously between meals results in reactivation of the CRF system and binge-like feeding behavior (140) which may be exacerbated in the context of chronic stress. Previous observations that subordinate females consume larger

but not more frequent meals when both chow and a palatable diet are available supports this hypothesis (147). Taken together, the results of Antalarmin administration on attenuating daily caloric intake in subordinate but not dominant females supports the hypothesis that CRF_1 receptor activation is important for sustaining emotional feeding.

An important, yet unexpected, observation from this study was that the response to Antalarmin was not uniform. Despite the variability, a significant status effect was seen regardless of whether the entire sample was used or analyses were restricted to only those females – both dominant and subordinate – who reduced caloric consumption following Antalarmin administration. We do not know what accounted for the lack of sensitivity to Antalarmin among some animals. While individual variability would be expected, of particular note is that three of the subordinate females who failed to decrease food intake in response to Antalarmin were from the same social group and two of the dominant females who showed an increase in caloric intake during Antalarmin administration were from another social group. Although social behaviors were not measured in this study, it is possible that group dynamics varied and thus accounted for differences in responsivity. On the individual level, the impact of relative social position within a group can be variable for macaques (124, 148, 149) and non-primate animals (65), which could be due to experiential or genetic factors.

Peripheral effects of Antalarmin on serum cortisol were not measured in this study. However, a previous study using female cynomolgus monkeys showed that a similar dose and route of administration of Antalarmin had no effect on baseline and stressor-induced increases in ACTH or cortisol (150, 151). Nonetheless, administration of Antalarmin reversed the adverse effects of the stressor on reproductive function and luteinizing

hormone secretion, suggesting the activation of central CRF_1 receptors and not changes in peripheral cortisol release are important for this stressor-induced effect (150, 151). A similar reasoning could be used to interpret the effects of Antalarmin on daily caloric intake in subordinate females in the present study.

Lastly, a critical consideration for interpreting the results of the present study is that animals were ovariectomized and were untreated during the feeding assessments. Both estradiol and progesterone are known to affect appetite and meal size (152-154). Thus, the generalizability may be considered somewhat limited. However, previous work with this model has shown that estradiol treatment of ovariectomized female rhesus monkeys resulted in reduced total caloric intake and significant reductions in meal size when only a chow diet was available (155), consistent with well-established appetite suppressing effects of estradiol (156). In contrast, during a choice dietary condition when both palatable and chow options were available, estradiol treatment had no observable, attenuating effect on caloric intake (155), suggesting that the hedonic value of palatable food may override homeostatic mechanisms that typically reduce appetite and caloric intake. Thus, it is not clear how antagonism of CRF_1 receptors would have interacted with social subordination to affect daily caloric intake in this rich dietary environment in the presence of estradiol.

Conclusion

In summary, the observation that emotional feeding was attenuated among subordinate animals in a rich dietary environment following administration of a CRF_1 antagonist supports the involvement of activation of central CRF_1 receptors for sustaining this stress-induced phenotype, which substantiates numerous epidemiologic studies that have

linked chronic social stress with excess consumption of high caloric diets, obesity, and metabolic disorders (157-161). However, the response to Antalarmin was somewhat variable, even for subordinate animals, as a sub-group of monkeys did not reduce caloric intake during treatment. Consequently, the results of the present study warrant replication, assessing the consequences of central CRF_1 antagonism on caloric intake in a rich dietary environment for a longer duration and measuring peripheral effects of Antalarmin on circulating cortisol levels to identify factors that account for variability in responsiveness.

Table 2.1
Demographic information on each subject including social group membership; dominance rank within their social group; age at the time of the study; years in their group; years from ovariectomy (OVX); body weight; and the change in total kcals from the Antalarmin to placebo condition. Mean ± sem are shown for dominant monkeys (ranks 1 and 2) and subordinate monkeys (ranks 3 – 5). P-values are from t-tests. *See text for status differences in calorie consumption during the placebo and Antalarmin conditions.

Animal ID	Social group	Rank	Age (yr)	Years in Group	Years OVX	Body Weight (kg)	Kcal Change
RRa7	1	1	12.77	5.82	6.36	9.98	1450
RTv6	1	2	12.82	5.84	6.55	7.68	354
ROb6	1	3	14.72	5.84	6.48	9.03	139
RGs6	1	4	13.65	5.81	6.64	9.18	(260)
RZp6	2	1	13.70	5.84	6.34	10.86	(452)
RYn5	2	2	15.78	5.84	6.58	7.84	(650)
RIz6	2	3	12.79	5.82	6.50	9.13	(1018)
RRu6	2	4	12.89	5.84	6.41	6.78	(1734)
RZd7	2	5	13.03	5.82	6.36	7.46	(1221)
ROy4	3	1	17.56	5.84	6.66	10.21	10
RWb7	3	2	12.75	5.82	6.37	9.00	(139)
RYh4	3	3	18.57	5.84	6.45	8.29	(256)
RFp8	3	4	10.74	2.22	9.96	11.05	(17)
RIp7	3	5	11.79	2.22	11.15	8.97	212
RBe5	4	1	16.70	5.90	6.41	10.28	(101)
RHc4	4	2	18.70	5.88	6.64	10.53	84
RMg3	4	3	20.59	2.92	11.06	8.83	124
RRb7	4	4	12.75	5.90	6.66	8.75	107
RZt5	4	5	15.62	5.87	6.51	9.23	52
RNf6	5	1	14.62	5.84	6.35	11.27	(5)
RZk6	5	2	13.70	5.84	5.93	10.62	(125)
RQq4	5	3	17.70	5.82	6.62	9.33	(274)
RFc6	5	4	14.71	5.82	6.50	9.76	(442)
		Dominant	14.91	5.85	6.42	9.83	28.51
			0.68	0.01	0.07	0.39	179.41
		p-value	0.88	0.11	0.09	0.07	*
		Subordinate	14.58	5.06	7.49	8.91	-352.98
			0.79	0.41	0.52	0.29	168.25

Figure 2.1
Mean kilocalories ± SEM consumed averaged across the two-day placebo and Antalarmin conditions for dominant and subordinate females. White sections reflect intake of the laboratory chow diet (LCD) and black sections reflect intake of the calorically dense diet. The p-value reflects the significant treatment by status interaction.

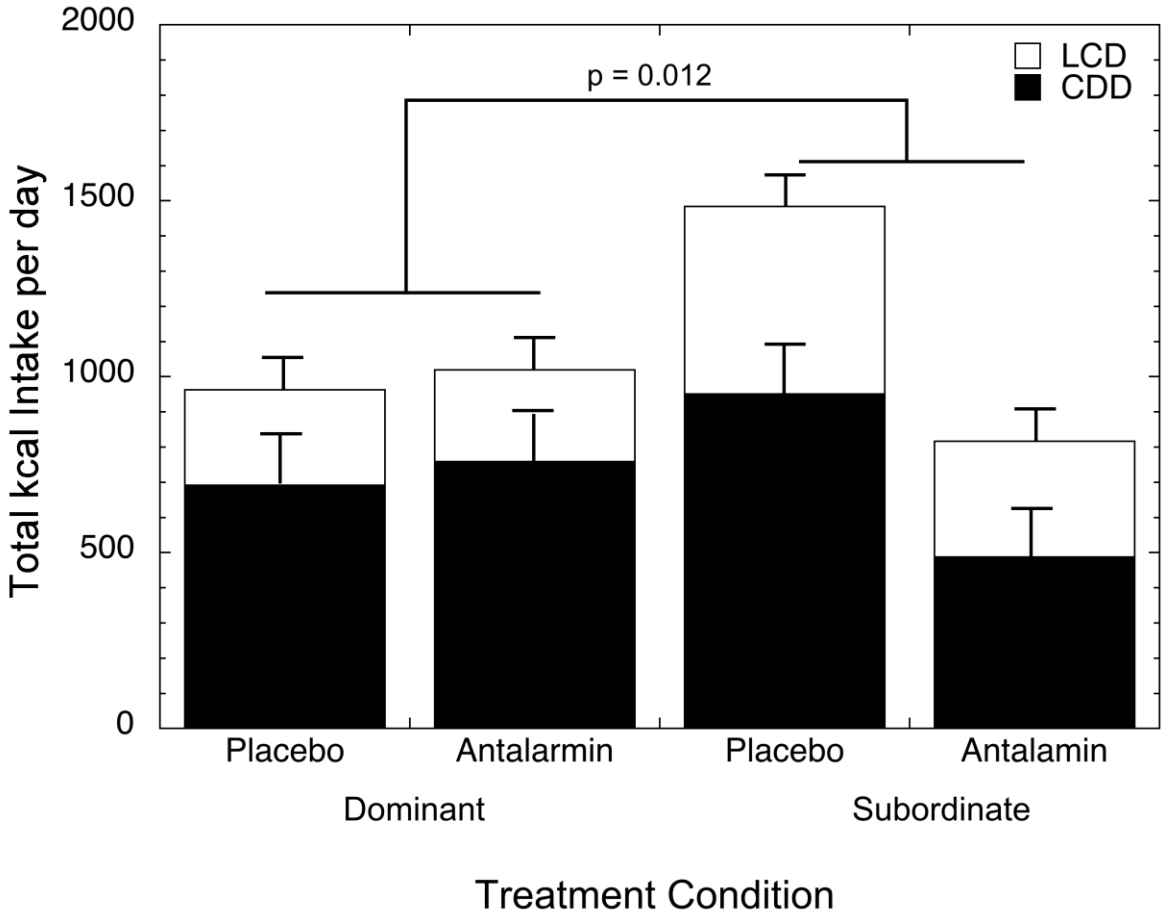


Figure 2.2
Mean kilocalories ± SEM consumed from each diet averaged across the two-day placebo and Antalarmin conditions for subordinate (Panel A) and dominant females (Panel B). Asterisks indicate significant ($p < 0.05$) treatment effects within a social status group.

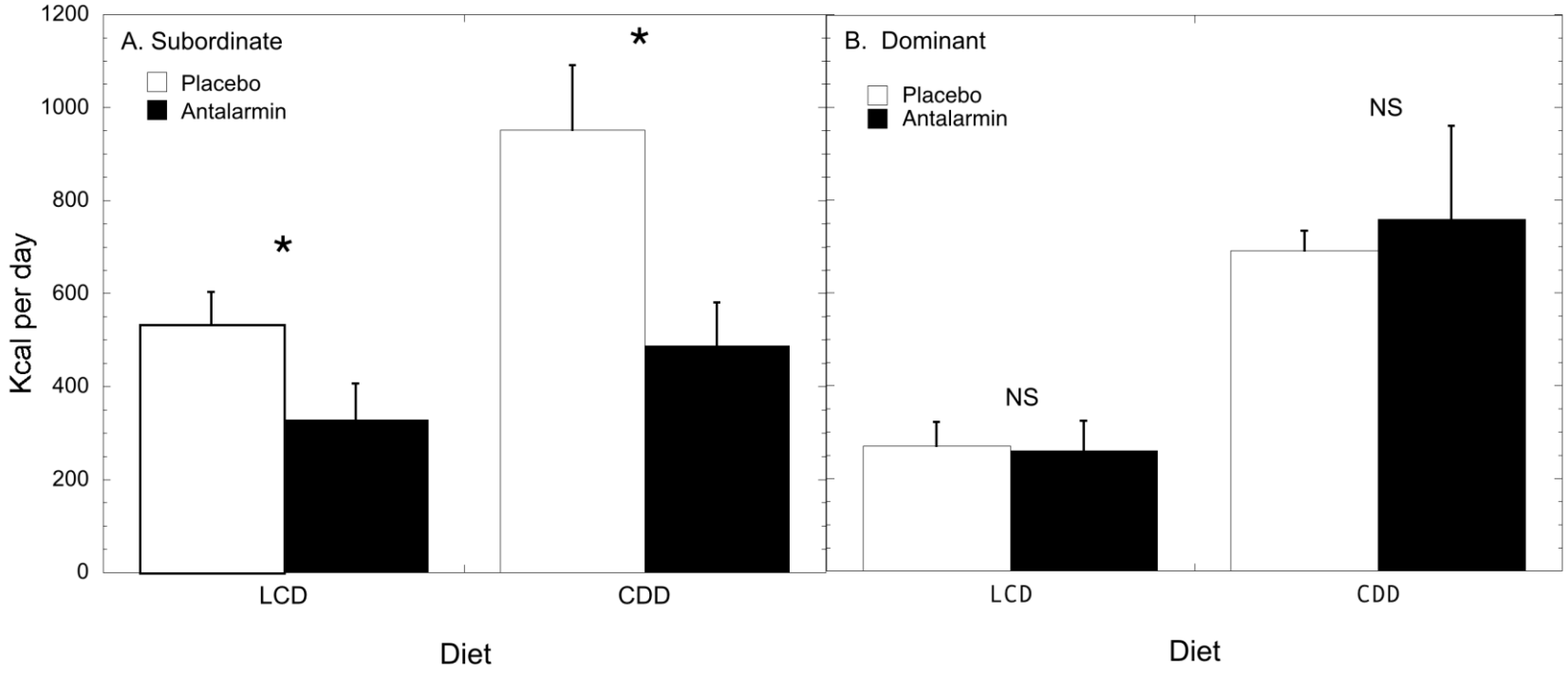
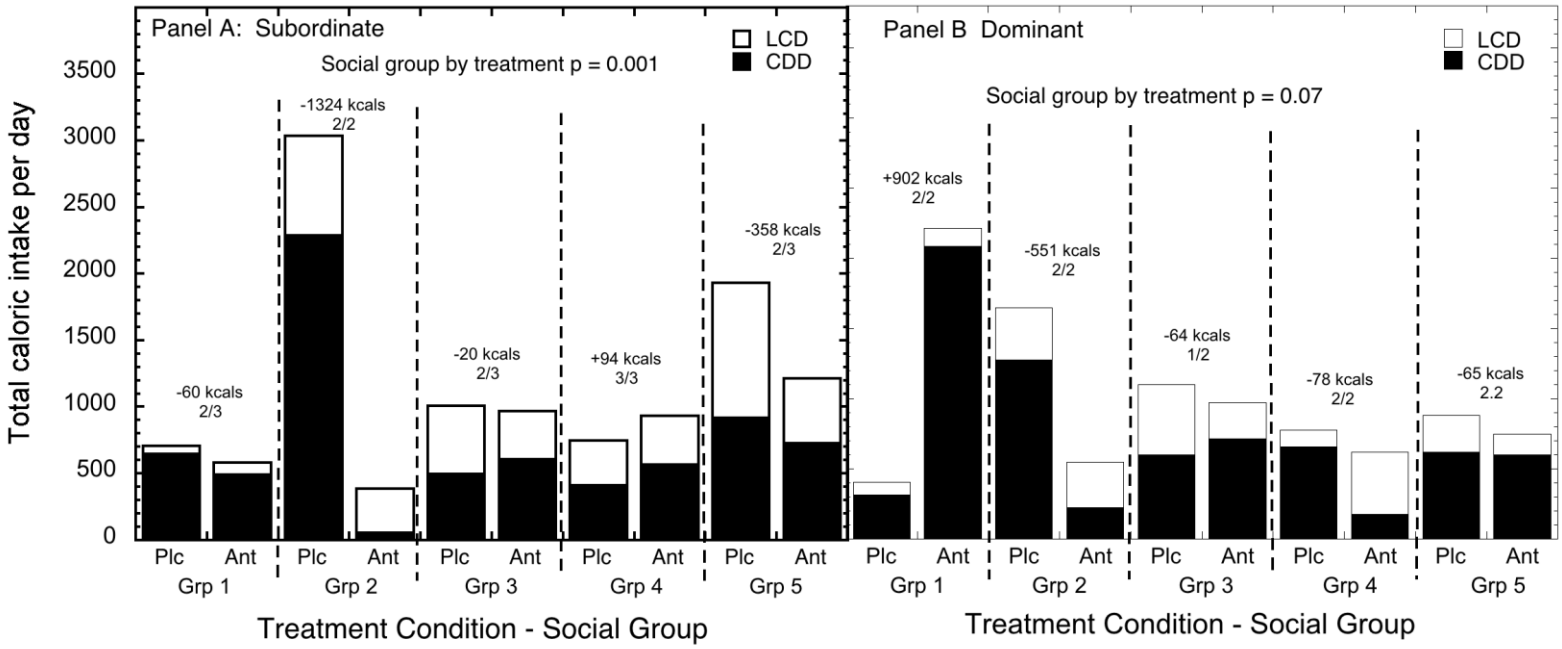


Figure 2.3
Mean kilocalories ± SEM consumed from each diet averaged across the two-day placebo and Antalarmin conditions for subordinate in each of the five social groups (Panel A) and dominant females in each of the five social groups (Panel B). There was a significant social group by treatment interaction for subordinate but not dominant females. Shown for each group is the kcal change from placebo to Antalarmin conditions as well as the number of animals from the status category that contributed to the decrease or increase in caloric intake.



CHAPTER 3

Abstract

Previous studies have shown that socially subordinate female rhesus monkeys in stable social groups consume significantly more kilocalories than dominant conspecifics when a palatable diet, high in fat and sugar, is provided, supporting a plausible causal relationship between chronic stress and obesity risk within a rich dietary environment. However, investigations using this model were too brief to induce significant changes in body weight. The present experiment quantified caloric intake and body weight among female rhesus monkeys within newly formed social groups across a 24-week study period that was divided into three, 8-week dietary phases (chow only, chow and a palatable diet, and chow only) to test the hypothesis that the most subordinate females within newly formed social groups would consume excess kilocalories and gain significantly more weight relative to higher ranking conspecifics in the presence of a palatable diet. Contrary to our hypothesis, results showed all animals, regardless of emergent social status, demonstrated significant increases in caloric intake following introduction of the palatable diet, which led to significant increases in body weight. Additionally, the lowest ranking females in each group weighed significantly less and had less total body fat than mid ranking and high ranking females at the end of each of the three dietary phases. Findings suggest that reorganization of social groups can function as a significant stressor for even the most dominant subjects within a social hierarchy. Future investigations are necessary to determine the point at which status-related differences in stress axis function and neural reward circuitry emerge among socially-housed female rhesus monkeys.

Introduction

In recent decades, populations worldwide have experienced an increase in the prevalence of obesity, defined as excess adiposity and indicated by a body mass index (BMI) greater than 30 kg/m^2. Although the most recent data from the United States indicate that national obesity prevalence is stabilizing, affecting approximately 32% of men and 36% of women (162), this substantial proportion of the population suffers decreased life expectancy, endures reduced quality of life, and imposes a financial burden on the remainder of the population (163). Given the detrimental effects of obesity at the individual and societal levels, the development of effective treatment and prevention strategies is imperative. However, the complex, multifactorial etiology of obesity complicates this task, and many prevailing treatment and prevention strategies often focus on individual behavior change without addressing underlying environmental and psychosocial factors that sustain this phenotype.

Consistent with the first law of thermodynamics, obesity results when energy intake exceeds energy expenditure over a prolonged period of time (164). However, both sides of this equation are influenced by intricate interactions between genetic, environmental, and psychosocial factors (165). Persistent exposure to psychosocial and environmental stressors has been implicated as one potential contributor to the observed increase in obesity prevalence within Westernized countries (64). Indeed, evidence suggests that exposure to stressors and/or manipulation of circulating glucocorticoids, a product of the neuroendocrine stress response, can alter dietary preference, food intake, weight gain, and fat distribution (7). Yet, the directionality of these relationships is not fully

understood as evidence has supported roles of stressor exposure in increasing and diminishing appetite and food intake (6).

From a physiological stand point, the stress response involves the activation of two biological pathways: the sympathetic adrenal medullary (SAM) axis and the limbic hypothalamic pituitary adrenal (LHPA) axis (47). Activation of the SAM axis results in the immediate release of norepinephrine from sympathetic nerve terminals in peripheral tissues and the release of epinephrine and norepinephrine from the adrenal medulla into the systemic circulation (51). Products of this pathway heighten vigilance and arousal promoting what is commonly dubbed the "fight-or-flight" response (7). Activation of the LHPA axis involves a hormonal cascade initiated by the release of corticotropin releasing factor (CRF) from limbic structures and the paraventricular nucleus (PVN) of the hypothalamus, which stimulates adrenocorticotropic hormone secretion from the pituitary and the synthesis and release of glucocorticoids (GC) from the adrenal glands (53). The primary glucocorticoid in primates, including humans, is cortisol, and increased levels of circulating cortisol mobilize energy reserves by promoting lipolysis within adipose tissue and glycogenolysis within the liver (50). In the face of a real challenge, the stress response is highly adaptive, and the body readily reestablishes homeostasis when the threat is no longer eminent. Activation of the SNS is rapidly counterbalanced by the parasympathetic branch of the autonomic nervous system, and the LHPA response is terminated via negative feedback loops (i.e., GC act on receptors at the level of the limbic structures, PVN, and pituitary to inhibit further production and release of CRF and ACTH) (7). However, prolonged activation of the stress response can lead to impairment of this negative feedback loop, and excessive production of glucocorticoids may

eventually lead to a number of adverse health outcomes (48).

The consequences of chronic activation of the stress response are especially concerning among humans and other species that can mobilize this response in anticipation of adverse events, even in the absence of a true threat to physiological homeostasis (48). Many epidemiological studies have linked indices of psychosocial stress with obesity and metabolic disorders (7), and increased consumption of calorically-dense diets in response to stressor exposure is proposed as the critical behavioral mediator of the purported stress and obesity association (33). Consistent with this hypothesis, laboratory studies have highlighted a tendency for people to prefer and consume highly palatable "comfort foods" during or following stressful episodes (4), and some epidemiological studies suggest that individuals under chronic stress shift preferences to energy-dense foods (157-161). Nonetheless, while it appears that stressor exposure may lead to consumption of less nutritionally balanced foods, at least among some vulnerable groups, the degree to which diet mediates a relationship between stress and obesity cannot be determined based on qualitative rather than quantitative assessments of dietary intake. Similarly, because obesity results from a prolonged period of positive energy imbalance, even quantitative assessment of dietary behaviors at a single point in time makes inferences related to diet and obesity difficult (166).

Given these limitations, animal studies provide the majority of data regarding the mechanisms by which stress influences physiology, ingestive behaviors, and weight status (7). Furthermore, animal models that utilize a species in a social environment confer greater construct validity with regard to human conditions (7). While many rodent studies have employed social defeat to model the effects of chronic stress on food intake

and body weight (8), quantification of dietary intake is not always included in the design of these studies, and the dietary environment typically consists solely of a standard laboratory chow. Further, despite the growing evidence highlighting significant gender differences in the stress-eating-obesity relationship (9-12), much of the stress-eating research has been conducted exclusively in male rodents (8).

Social subordination in female rhesus macaques provides an animal model of chronic psychosocial stress that is ethologically relevant to the animals and to humans. Macaque groups, regardless of size, are organized by a dominance hierarchy that functions to maintain group stability (84, 85). Lower ranking animals receive more aggression from higher-ranking group mates and terminate these interactions by emitting submissive behavior, a defining feature of subordination (84-87). Subordinates have less control over their environment (88), and a consequence of continual harassment is dysregulation of the limbic hypothalamic pituitary adrenal axis, evidenced by reduced glucocorticoid negative feedback and hypercortisolemia (83, 89-93). Social subordination in female macaques results in a number of phenotypes consistent with chronic stressor exposure (120) and is a well established model to study the adverse effects of psychosocial stress on cardiovascular disease (79), addiction (80), reproductive dysfunction (81), and immune compromise (82, 83).

Recent findings with this model support its utility in investigating and elucidating causal mechanisms underlying stress-induced consumption of palatable foods (122, 167). Specifically, when animals were fed a standard laboratory chow diet, caloric intake was similar between dominant and subordinate females. However, when animals were given a choice between a palatable diet, high in fat and sugar, and a typical chow diet, caloric

intake varied significantly by social status (122, 167). While dominant females preferred the palatable diet, they did not significantly increase caloric intake relative to chow-only conditions. Conversely, subordinate females consumed significantly more calories relative to dominant females during the dietary choice condition and relative to their own baseline levels of intake in the chow-only condition (122, 167).

These data support a plausible causal link between stressor exposure and obesity within a rich dietary environment, similar to that of humans. However, previous studies were very short in duration, and while changes in body weight tracked status differences in caloric intake, these changes were not significant. What is unclear is whether the observed hyperphagia among subordinate animals would persist with prolonged exposure to the palatable diet to promote meaningful increases in body weight and adiposity. Thus, the current investigation built upon the previous findings by replicating these studies over an extended period of time using newly formed social groups of female rhesus monkeys. Because the time interval following initial group formation is typically characterized by high rates of aggression (91), assessing caloric intake and body composition following formation of new groups provided an unprecedented opportunity to determine if a greater dose of stressor exposure predicts greater consumption of palatable food and an associated metabolic phenotype. We hypothesized that the consequences of social subordination would be exacerbated among subordinate animals in the newly formed groups leading to excess caloric intake from a palatable diet and significant weight gain among the most subordinate animals.

Materials and Methods

Subjects were 42 adult female rhesus monkeys (*M. mulatta*) between 5 and 14 years of age (10.68 ± 0.29 yr) that were initially members of three breeding groups located at the Yerkes National Primate Research Center Field Station, Emory University. The breeding groups were predominantly comprised of adult females along with 2-3 adult males and juvenile offspring. Groups ranging in size from 30 to 50 animals were housed in outdoor compounds with attached indoor quarters as described previously (91).

Females were removed from their natal groups to form seven, six-member groups that were housed in indoor-outdoor enclosures measuring approximately 3.8 m^3. Because females within each social group were formerly housed within the same natal group, all animals were introduced simultaneously within the new housing conditions. Animals were first housed in two adjacent enclosures with the connecting door removed. Animals were monitored continuously during the first week of introduction by veterinary and animal care staff. As rates of physical aggression declined, animals were condensed to a single housing unit, initially for 8 hours per day and ultimately continuously. The time required for the completion of this process varied from group to group and was confirmed when a linear dominance hierarchy was evident. Groups were established for 12.04 ± 0.69 weeks (range 6.71 to 18.43 weeks) prior to the study onset, and clear dominance ranks within each group emerged during this time interval. The Emory University Institutional Animal Care and Use Committee approved all procedures in accordance with the Animal Welfare Act and the US Department of Health and Human Services "Guide for Care and Use of Laboratory Animals."

Experimental Design and Diet Intervention

A 24-week study period was subdivided into three, 8-week dietary phases. During the first dietary phase (Phase 1, weeks 1-8), a standard laboratory chow diet was provided (no choice). The second dietary phase (Phase 2, weeks 9-16) permitted a dietary choice between a more palatable diet and the standard chow (choice). The final dietary phase (Phase 3, weeks 17-24) eliminated the dietary choice and a chow-only environment was reestablished (no choice).

The two diets utilized in the study were selected to resemble the Prudent and Western profiles used in human epidemiological studies (168). The chow diet contains 3.60 kcal/gram (12% fat, 72% carbohydrate, and 16% protein; Of the total 2.59 kcal/gram of carbohydrates, 2.44 are derived from fiber and 0.15 from sugar; Purina #5038, re-pelleted by Research Diets). The palatable diet contains 4.74 kcal/gram (Research Diets, D07091204; 40% fat, 44% carbohydrate, and 16% protein; Of the total 2.08 kcal/gram of carbohydrates, 0.6 are derived from fiber and 2.02 from sugar). Both diets contained similar and appropriate vitamin and mineral fortification.

Food intake was quantified continuously throughout the 24-week study. Behavioral outcomes, anthropometric measures, serum cortisol values, and physical activity levels were also assessed at regular, specific intervals described as follows.

Behavioral Outcomes

Individual ranks (1 through 6) within each of the seven groups were determined by the outcome of unequivocal dyadic agonistic interactions that were documented during formal group observations following an established ethogram (91). Observations were conducted in half-hour sampling intervals during weeks 1-7 of each dietary phase. During

each behavioral sampling interval, all occurrences of dyadic interaction involving agonistic behavior and affiliative behavior were recorded in the format of actor-behavior-recipient using a netbook computer. Aggressive behavior was defined as threats, slaps, bites, and chases. Grimaces, withdrawals, and screams were categorized as submissive behavior. Affiliation was defined as grooming and sitting in proximity with other animals. Solitary anxiety-like behavior was defined as body shakes, yawns, self-grooming, scratching, and pacing. Behaviors were recorded by two previously trained observers who maintained an inter-observer reliability of >92%.

LHPA Reactivity

Because previous studies have demonstrated that social subordination results in dysregulation of the LHPA axis, indicated by impaired glucocorticoid negative feedback, animals in the present study were subjected to an acute, psychosocial stressor during each dietary phase to assess dysregulation of the LHPA axis as a function of rank within the new group. The acute stressor involved temporary removal of each animal from her group followed by exposure to the Human Intruder (HI) task, a standardized 30-minute behavioral paradigm used to assess the emotional response of a monkey to threatening stimuli (169). Between 0900 and 1030 hour on the day of the Human Intruder tests, animals were accessed for blood collection (baseline, 3 mL) from the saphenous vein. All animals used in this study were trained for conscious venipuncture following procedures previously described (170). Animals were then transferred to an adjacent behavioral testing room and exposed to the acute stressor task. A second blood sample was collected (3 mL) immediately following completion of the task (30 min), and animals were promptly returned to their social groups. Animals were accessed for additional blood

samples (3 mL) at 1-hour and 4-hour post-stressor intervals to assess recovery of serum cortisol, an indicator of the sensitivity of the negative feedback regulatory mechanism of the LHPA axis. Repeated exposures to the HI task were separated by 8 weeks, and the time of testing was held constant for each animal across repeated trials. Blood samples were centrifuged at 4°C for 15 minutes and the serum layer was pipetted into Cryovials (Fisher Scientific, Atlanta, GA) and stored at -20°C until assay. Serum levels of cortisol were determined by liquid chromatography-mass spectrometry as previously described (171).

Anthropometric Measurements

Crown-heel length of each animal was obtained at the time of the group formation while the animals were anesthetized (Telazol, 3 mg/kg, IM). Crown-heel length was measured to the nearest millimeter with the animal in supine position on a calibrated ruler with a fixed head rest. Measures were collected in duplicate by two independent laboratory technicians, and the average of the two values was used in analyses. Body weights were measured to the nearest gram using an electronic scale at the time of group formation, at the start of the study (week 1), and at the end of each dietary phase (weeks 8, 16, and 24). Whole body scans were performed using dual x-ray absorptiometry (DEXA, Norland Eclipse) to obtain measures of fat mass at the time of group formation and at the end of each dietary phase (weeks 8, 16, and 24).

Food Intake

Prior to the start of the study, unique radiofrequency identification (RFID) microchips (DATAMARS) were subcutaneously implanted in each animal's wrists while animals were anesthetized. This procedure permitted animals to use automated feeders that were

attached to each housing enclosure. When an animal placed either hand in a feeder, a reader detected the microchip, relayed a signal to a remote computer that identified the study subject, and triggered the delivery of a single food pellet. This system allowed for continual quantification of caloric intake of individual monkeys embedded in social groups.

Two separate, automated feeders were attached to each housing enclosure to permit *ad libitum* access to experimental diets. Validation studies (89) show that dominant females do not restrict subordinate animals' access to the feeders and rarely (≈1% of the time) take food that subordinate females obtained. Two weeks prior to the study onset, automated feeders were activated and filled with pelleted chow. Animals were free to feed from the feeders or consume traditional chow biscuits *ad libitum* during this provisional period. Use of the automated feeders was monitored during this time to ensure that all animal codes registered prior to the study onset.

When the study commenced, animals were fed exclusively with the automated feeders. Food intake was quantified over the course of 24 weeks that were subdivided into three, 8-week dietary phases. During the first 8 weeks of the experiment (Phase 1), both feeders dispensed pelleted chow (no choice). During weeks 9-16 (Phase 2), one feeder dispensed a standard chow diet while the other dispensed the more palatable diet resembling a typical American diet (choice). During this phase, the diets dispensed by each feeder were alternated every two-weeks to eliminate the potential confound of feeder-specific preferences based on feeder locations within the housing unit. During weeks 17-24 (Phase 3), the palatable diet was no longer available, and animals were returned to a chow-only dietary environment (no choice). Over the course of the 24-week study period,

food intake was quantified continuously. Because anthropometric and physiologic assessments corresponding to the end of each dietary phase began on the first day of the eighth week of each dietary phase, food intake data from the first 7 weeks of each dietary phase were used in statistical analyses.

Physical Activity

Because physical activity is an important component of the energy balance equation, Actical accelerometers (MiniMiter, Bend, OR) were used to provide an unbiased indicator of physical activity during each dietary phase (172). Once during each dietary phase, each animal was fitted with a collar (Primate Products) to which an Actical accelerometer was attached. The accelerometer recorded activity in 30-second epochs for 5 days during each assessment. Raw data were extracted using Actical version 3.1 software (Mini Mitter, Bend, OR), and data were reported in metabolic equivalents (METS) - a physiological measure expressing the energy cost of physical activities- per epoch.

Statistical Analyses

Stepwise multiple regression analyses were performed to identify baseline factors that predicted emergent rank (1 through 6) within each newly established six-member group. Differences in rates of aggression and submission across individual ranks and dietary phases were assessed using repeated measures ANOVA (RM-ANOVA). Based on distinct behavioral profiles, described in the results section, females were further grouped into high (ranks 1 and 2), mid (ranks 3 and 4), and low (ranks 5 and 6) rank categories to improve statistical power of the subsequent planned statistical analyses involving social status.

Using the defined rank categories as a between-subjects variable, differences in baseline demographic characteristics were assessed with multivariate ANOVA, and differences in social behavior (hourly rates), body weight (kg), body fat (kg), body fat percentage, serum cortisol reactivity and recovery (micrograms/dL), food intake (kcal), and physical activity (METS) were assessed across dietary phases using RM-ANOVA. For analyses related to body composition, baseline weight, body fat, or body fat percentage and baseline age were included as covariates within each RM-ANOVA. Regression analyses were applied to assess the predictive value of behavioral indices of psychosocial stress exposure on caloric intake within each dietary phase. Regression analyses were also performed to assess combined effects of caloric intake and physical activity as predictors of change in body weight within each dietary phase. Statistical values with $p \leq 0.05$ were considered significant.

Results

Determination of Emergent Social Rank and Designated Rank Categories

Stepwise multiple regression analyses revealed that pre-study rank within the larger natal groups and age explained 47% of the variance in emergent rank within the newly formed, six-member groups ($R^2 = 0.47$, $F_{2,39} = 19.158$, $p<0.001$). Higher pre-study rank within the natal group ($\beta = 0.605$, $p<0.001$) and older age ($\beta = - 0.310$, $p=0.010$) significantly predicted higher rank within the new group. Of note, baseline body weight ($p=0.620$), body fat ($p=0.793$), and body fat percentage ($p=0.813$) were not predictive of emergent rank. Throughout the entire study period, individual rank (1 through 6) within each newly established social group was associated with significant differences in aggression received ($F_{5,36} = 12.336$, $p=0.007$, Figure 3.1). The lowest ranking animals in each group,

rank 6, received significantly more aggression than all other ranks (1 through 5, $p<0.001$). Additionally, animals that assumed rank 5 received significantly more aggression than animals ranked 1 ($p=0.016$) and 2 ($p=0.016$). Thus, for subsequent analyses, females were further grouped into three rank categories – high rank (ranks 1 and 2), mid rank (ranks 3 and 4), and low rank (ranks 5 and 6) – to improve statistical power of analyses involving social status.

Baseline Descriptives

Multivariate ANOVA showed no significant differences in baseline demographic characteristics between subjects stratified by emergent social status, Table 3.1. Specifically, no significant differences in age ($p=0.064$), body weight ($p=0.077$), body fat ($p=0.213$), body fat percentage (0.214), or crown-heel length ($p=0.354$) were apparent across the three emergent rank categories at the time of group formation.

Behavioral Outcomes

There was a significant main effect of dietary phase on rates of aggression ($F_{2,78} = 10.520$, $p<0.001$), submission ($F_{2,78} = 5.904$, $p=0.004$), affiliation ($F_{2,78} = 8.669$, $p<0.001$), and anxiety ($F_{2,78} = 3.635$, $p=0.031$) as shown in Figure 3.2 . Animals demonstrated significantly higher rates of aggressive behaviors toward other animals during Phase 1 (no choice) relative to Phase 2 (choice, $p<0.001$) and Phase 3 (no choice, $p<0.005$). Similarly, rates of submission emitted decreased throughout the study interval with higher rates during Phase 1 (no choice) relative to Phase 2 (choice, $p=0.011$) and Phase 3 (no choice, $p=0.003$). Conversely, rates of affiliation received increased throughout the study with higher rates in Phase 2 ($p<0.001$) and Phase 3 ($p=0.001$)

relative to Phase 1. Rates of anxiety displayed were also higher in Phase 2 ($p=0.028$) and Phase 3 ($p=0.027$) relative to Phase 1.

In addition to differing across study phases, agonistic and anxiety behaviors differed as a function of social status. Rates of aggressive behavior directed toward others differed significantly by rank category ($F_{2,39} = 7.941$, $p=0.001$) with high ranking and mid ranking animals displaying significantly higher ($p<0.001$ and $p=0.047$, respectively) rates of aggressive behavior than low ranking animals independent of dietary phase (Figure 3.3). A significant interaction between social status and dietary phase also emerged with regard to aggressive behavior ($F_{4,78} = 5.988$, $p<0.001$). High ranking animals displayed significantly higher rates of aggression than both mid ranking ($p=0.017$) and low ranking ($p<0.001$) animals in Phase 1, and high ranking animals continued to display significantly greater aggressive behaviors than low ranking animals ($p=0.004$) in Phase 2. However, differences in rates of aggression did not differ significantly by social status in Phase 3 ($p>0.06$).

Rates of submissive behavior also differed significantly by social status ($F_{2,39} = 29.759$, $p<0.001$, Figure 3.4). Low ranking animals displayed significantly higher rates of submissive behaviors than high ranking ($p<0.001$) and mid ranking ($p<0.001$) animals. Mid ranking animals also displayed significantly higher rates ($p=0.021$) of submissive behaviors than high ranking animals. These effects were consistent across dietary phases as no significant interaction effect emerged ($p=0.354$).

Anxiety behavior differed significantly by social status ($F_{2,78} = 3.635$, $p=0.031$, Figure 3.5), and a significant rank by dietary phase interaction effect emerged ($F_{4,78} = 3.288$,

p=0.015). Post hoc analyses revealed that high ranking animals displayed significantly fewer anxiety behaviors than mid ranking (p=0.033) and low ranking (p=0.018) animals. Additionally, although anxiety among high ranking and mid ranking animals did not significantly differ across dietary phases (p>0.231), low ranking animals displayed increasing anxiety as the study progressed from phase 1 to phase 2 (p<0.001) and phase 2 to phase 3 (p=0.001). Despite an overall increase in affiliative behavior received from group mates from Phase 1 to Phases 2 and 3, there was no significant effect of social status on affiliative behavior (p=0.099) nor was there significant rank category by dietary phase interaction (p=0.245; data not shown).

LHPA Reactivity

Exposure to the acute stressor significantly elevated serum cortisol in each dietary phase (Figure 3.6A, $F_{3,111} = 368.320$, $p < 0.001$). Post-stressor cortisol, sampled at the immediate conclusion of exposure to the 30 minute stressor paradigm, was significantly elevated relative to baseline levels (p<0.001). One hour following exposure to the acute stressor, cortisol levels significantly decreased (31.946 ± 0.944, p<0.001) from levels measured immediately post-stressor but remained significantly elevated relative to baseline. At 4 hours post-stressor, cortisol levels were significantly lower (p<0.001) than levels measured at all previous time points. Cortisol levels also differed across dietary phases ($F_{2,74} = 32.827$, $p < 0.001$, Figure 3.6A), and a significant diet phase by time interaction emerged ($F_{6,222} = 4.605$, $p < 0.001$). Post-hoc pairwise comparisons revealed that cortisol levels were significantly lower at all four time points in phase 3 relative to the first two phases (p<0.001). However, this effect of dietary phase on cortisol responsivity was not affected by social status ($p = 0.861$). Thus, Figure 3.6B shows the

response in cortisol for each social status category prior to and following the acute stressor collapsed across dietary phases. As illustrated, serum cortisol did not differ by social status ($p=0.337$) nor was there significant interaction between rank category and time of cortisol assessment ($p=0.919$).

Changes in Body Weight and Anthropometrics

All animals lost weight during the interval between formation of the new social groups and the start of the study. However, social status did not significantly affect weight loss between group formation and study start (Figure 3.7, $F_{2,39} = 0.174$, $p = 0.841$). Additionally, linear regression analyses showed time between group formation and study onset was not predictive of initial weight loss ($p=0.951$)

Across rank categories, body weights differed significantly at weeks 1, 8, 16, and 24 ($F_{3,111} = 2.785$, $p=0.044$), the intervals that correspond with the study onset and the end of each dietary phase (Figure 3.8). Post hoc comparisons revealed significant differences between weights at the end of Phase 1 (8.524 ± 0.151 kg, $p<0.001$), the end of Phase 2 (9.334 ± 0.173 kg, $p<0.001$), and the end of Phase 3 (8.826 ± 0.170 kg, $p<0.001$). Although weights at study onset (8.596 ± 0.118 kg) differed significantly from weights at the end of the dietary choice condition of Phase 2 ($p<0.001$), they did not differ from measures at the end of Phase 1 ($p=0.513$) or Phase 3 ($p=0.177$), the two chow-only dietary phases. Despite no effect of social status on weight change from group formation to the start of the study, a significant main effect of rank category emerged with regard to body weight during the study ($F_{2,37} = 5.88$, $p=0.006$), and post hoc comparisons revealed that this effect was due to low ranking animals weighing less than both high and mid ranking animals at all four time points of assessment ($p<0.006$, Figure 3.8).

In addition to these differences in absolute body weights, a significant interaction between social status and dietary phase emerged regarding change in body weight ($F_{4,74}$ = 2.700, p=0.037, Figure 3.9). Post hoc analyses revealed that low ranking animals lost a significant amount of weight (-0.507 ± 0.189 kg) in Phase 1 relative to high ranking (0.083 ± 0.183 kg, p=0.037) and mid ranking animals (0.084 ± 0.180 kg, p=0.033), and low ranking animals gained significantly less weight (0.506 ± 0.154 kg) than high ranking animals (1.088 ± 0.149 kg, p=0.013) during Phase 2. Weight gain among mid ranking animals (0.835 ± 0.146 kg) did not differ significantly from low ranking (p=0.138) or high ranking (p=0.228) animals during Phase 2 (Figure 3.9). Change in body weight did not differ as a function of social status in Phase 3 (p>0.156).

As with total body weight, a significant main effect of social status emerged for total body fat ($F_{2,37}$ = 5.785, p=0.007) and body fat percentage ($F_{2,37}$ = 5.379, p=0.009, Table 3.2). Post hoc comparisons revealed that this effect was due to low ranking animals having less body fat (p<0.008) and a lower body fat percentage (p<0.018) than both high and mid ranking animals at the end of all dietary phases (Table 3.2). Changes in fat mass were highly and significantly correlated with changes in weight from baseline to the end of Phase 1 (r=0.873, p<0.001), during Phase 2 (r=0.838, p<0.001), and during Phase 3 (r=0.731, p<0.001).

Food Intake

A significant main effect of dietary phase emerged with regard to average daily caloric intake ($F_{2,78}$ = 85.346, p<0.001) as shown in Figure 3.10. Post hoc tests revealed that animals consumed significantly more daily kilocalories when the highly palatable diet and standard chow were available as a choice (850.873 ± 28.641) relative to the initial

chow-only condition (756.851 ± 35.030, p=0.003) and the final condition when the animals were returned to a chow-only environment (477.884 ± 21.693, p<0.001). Animals also consumed significantly fewer kilocalories during the final chow-only, no choice condition relative to the initial chow-only, no choice condition (p<0.001). There was no significant main effect of social status on average daily caloric intake ($F_{2,39}$ = 1.533, p=0.229, Figure 3.10). However, compared to the dominant and middle ranking females, subordinate females consumed 13% and 16% fewer calories in Phase 1 and 2, respectively. In contrast, caloric intake by subordinates was only 2% less than dominant and middle ranking females in Phase 3.

With respect to time within each phase, there was a significant main effect of week ($F_{6,234}$ = 11.838, p<0.001, Figures 3.11A – 3.11C) and significant week by phase interaction ($F_{12,468}$ = 3.326, p<0.001). During Phase 1 (no choice), animals' average daily caloric intake differed significantly across weeks, with the animals decreasing intake during the last three weeks of the dietary phase relative to the first four weeks (Figure 3.11A). During phase 2 (choice), average daily caloric intake differed significantly across weeks, with the most notable difference being that animals consumed significantly more calories during the first week when the palatable diet was initially introduced (Figure 3.11B). During phase 3 (no choice), significant differences between weeks emerged, with the general observation that animals decreased average daily caloric intake in the final weeks of the dietary phase relative to the initial weeks (Figure 3.11C). Finally, rank category did not modify the week by phase interaction (p = 0.850).

When data were analyzed from the choice diet condition to assess the relative contributions of each diet to daily caloric intake, a significant main effect of diet was

observed in that animals consumed significantly more calories from the palatable diet relative to the chow diet ($F_{1,37} = 37.819$, $p<0.001$, Figure 3.12). Social status did not modify caloric intake from either diet ($p=0.969$). However, a significant week by diet interaction emerged ($F_{6,37} = 21.690$, $p<0.001$), with animals decreasing caloric intake of the palatable diet across the study phase and increasing intake of the chow diet across the study phase (Figure 3.13). This effect of time on diet intake was not modified by rank category ($p = 0.056$).

At the individual level, regression analyses revealed significant behavioral correlates with regard to average daily caloric intake within each dietary phase (Table 3). Specifically, higher rates of anxiety behavior were predictive of greater average daily caloric intake during Phase 1 (no choice, $r=0.370$, $p=0.016$) but not Phase 2 (choice, $p=0.326$) or Phase 3 (no choice, $p=0.494$). More frequent aggression received was predictive of lower average daily caloric intake during Phase 1 (no choice, $r = - 0.332$, $p=0.031$) but not Phase 2 (choice, $p=0.143$) or Phase 3 (no choice, $p=0.822$). Higher rates of submissive behavior were predictive of lower average daily caloric intake during Phase 1 (no choice, $r = - 0.372$, $p=0.015$) and Phase 2 (choice, $r = - 0.318$, $p=0.040$) but not Phase 3 (no choice, $p=0.948$). Of note, rates of anxiety behavior, aggression received, and submission displayed were not associated with baseline cortisol values or cortisol reactivity during any dietary phase ($p>0.085$).

Physical Activity

No main effect of dietary phase emerged with regard to physical activity (METS) per epoch ($F_{2,74} = 1.860$, $p=0.163$, Figure 3.14), However, physical activity levels differed significantly as a function of rank category ($F_{2,37,} = 3.529$, $p=0.040$) with mid ranking

animals demonstrating greater activity than high ranking animals (p=0.012). METS values for low ranking animals were statistically intermediate between high and middle ranking females (Figure 3.14).

Collective Predictors of Weight Change

Stepwise multiple regression analyses revealed average daily caloric intake alone explained 38% of the variance in weight change during Phase 1 ($R^2 = 0.383$, $F_{1,40} = 26.492$, $p<0.001$), with greater intake predicting increases in weight ($\beta = 0.631$). During Phase 2 average daily caloric intake combined with physical activity (METS per epoch) accounted for 33% of the variance in weight change ($R^2 = 0.333$, $F_{2,39} = 11.252$, $p<0.001$), with greater caloric intake ($\beta = 0.541$) and reduced physical activity ($\beta = -0.346$) predicting more weight gain ($p<0.010$). In Phase 3, average daily caloric intake again directly and significantly predicted weight change and explained 19% of the variance ($R^2 = 0.193$, $F_{1,39} = 10.352$, $\beta = 0.463$, $p=0.003$). Physical activity was not predictive of weight change in Phase 1 (p=0.076) or Phase 3 (p=0.953).

Discussion

Data from the present investigation did not support our hypothesis that the most subordinate females within newly formed social groups would consume excess kilocalories and gain significantly more weight relative to higher ranking conspecifics in the presence of a palatable diet. Instead, all animals, regardless of emergent social status, demonstrated significant increases in caloric intake following introduction of the palatable diet, which led to significant increases in body weight and body fat. Additionally, all animals reduced caloric intake upon the withdrawal of the palatable diet, resulting in significant weight loss. Social status differences in body weight and body fat

did emerge as the study progressed; however, these differences contradicted our expectations in that the highest ranking animals demonstrated significantly greater increases in body weight and body fat relative to the lowest ranking animals during the dietary choice condition. Further, the lowest ranking females weighed significantly less and had less total body fat than mid ranking and high ranking females in the newly formed social groups at the end of each of the three dietary phases. Despite a lack of significant differences in average daily caloric intake between rank categories, caloric intake emerged as a significant predictor of change in body weight during each of the dietary phases while estimates of energy expenditure only accounted for a significant portion of the variance in body weight during the dietary choice condition.

The failure to see pronounced hyperphagia among socially subordinate animals with access to the palatable diet was surprising given the results of previous investigations. However, an important consideration regarding these data is the social history of the animals used. Generally, in species in which subordination is enforced by more dominant animals in the group, socially subordinate animals are subject to a disproportionate share of physical and psychological stressors leading to physiological indices of chronic stress (48). In each of the previous studies (122, 167), females had been members of their social groups for a minimum of three years and were thus very stable, producing distinct differences in stress-related phenotypes (120) and LHPA axis activity (124) between dominant and subordinate females. In unstable hierarchies, however, the advantages of dominance as indicated by stress-related physiology are often absent (48). In the present investigation ranks were clearly defined among subjects, but the recency of group formation likely induced a lack of predictability and control that

functioned as a stressor among all animals, independent of social status (88). Indeed, weight loss among all animals following group formation is consistent with previous data indicating that new social organization is initially a stressor for all animals involved, whether it be among female macaques (91) or male rats housed the visible burrow system (65). Additionally, there were no status-related differences in cortisol responsivity or recovery in response to an acute stressor at any point throughout the course of the study.

Comparing the behavioral data from the present investigation to previously published results from observations of stable social groups lends further support to the notion that all animals in the newly formed groups were experiencing considerable psychosocial stress. Although a consistent finding within both study populations was that anxiety behaviors, which are indicative of chronic stress exposure, were elevated among more subordinate animals relative to dominant counterparts, reported rates appear to be quite different between the two groups. Earlier studies among females living in stable, social groups reported average rates of anxiety behavior in the range of 4-6 occurrences per hour among dominant subjects (ranks 1 and 2) and 7-10 incidents per hour among subordinate subjects (ranks 3 through 5) (119). In the present investigation, average rates of anxiety behaviors among high ranking animals (ranks 1 and 2) ranged from 11-13 behaviors per hour; mid ranking animals (ranks 3 and 4) displayed 14-17 behaviors per hour; and low ranking subjects (ranks 5 and 6) demonstrated 11-19 behaviors per hour. Both investigations utilized the same behavioral ethogram in scoring behavior. Thus, rates of anxiety behavior among the most dominant animals in the newly formed groups appear to be analogous to rates of anxiety behaviors among the subordinate animals in previous investigations. Evaluation of agonistic behavior and affiliative behavior across

the study interval also provide critical insight into group dynamics. Decreases in aggression and submission coupled with increases in affiliation suggest that groups were stabilizing and animals were forming alliances within their new social settings as the investigation progressed. Additionally, progressive increases in anxiety behavior among the most subordinate subjects may be indicative of the emergence of the previously documented subordinate phenotype. However, lack of prolonged follow-up observations limits this notion to speculation.

Investigations into the psychophysiological effects of stressor exposure on appetite have spanned decades of research (47), and collective evidence from animal and human studies indicates that stress affects ingestive behavior in a bidirectional manner, inducing either increases or decreases in food intake (6). While the exact mechanisms underlying this phenomenon are not fully understood, the biological pathways that have been most thoroughly investigated with regard to mixed findings are the LHPA axis and the mesolimbic pleasure-reward pathways of the brain.

Studies in animals and humans have consistently shown that administration of GCs increases caloric intake (56, 173). However, experiments designed to activate the LHPA axis via exposure to external stressors have produced inconsistent results. These conflicting results may be due in part to opposing effects of two hormones within the LHPA cascade with regard to homeostatic feeding mechanisms. CRF, the neuropeptide that initiates the stress response, is considered a potent anorexigenic peptide while GC, the steroid end products of the LHPA axis cascade, appear to be orexigenic, promoting preferential consumption of highly palatable “comfort foods” (6).

Explanations as to why certain foods may be preferred during or following stressful experiences are based on the theory that sweet and/or high-fat "comfort" foods act centrally on neuropeptide systems that influence the physiological stress response and anxiety. Indeed, some data indicate that the opportunity to consume a highly palatable diet reduces LHPA axis activation and adverse behavioral effects of an acute stressor (173-175). Conversely, other work contradicts this assertion and actually suggests that consuming energy dense, palatable diets may increase basal and stress-induced LHPA activity (11, 14, 72). Of note, no differences in baseline cortisol or cortisol reactivity were detected in the present study when animals were exposed to the rich, dietary choice environment.

An alternative and possibly complementary theory is that signals from the LHPA axis target dopamine (DA) neurons in the reward pathways of the brain (73-75) producing a dysregulation of DA neurotransmission (76). Specifically, PET imaging in macaques has demonstrated a reduction in D2R binding potential (93, 176-178) and estimates of central DA release (93, 179) among subordinate animals relative to dominant conspecifics in stable social groups. These results suggest the functional consequence of chronic stress is a "reward deficiency syndrome," characterized by reduced DA activity (77). Because pleasure is arguably the most powerful motivator of food intake (20), functional changes in DA signaling provide the rationale for stress-induced anhedonia and accompanying reductions in food intake. However, a provocative alternative hypothesis asserts exposure to intermittent social stress may lead to neuroadaptations within critical reward pathways that drive some individuals to seek out and over consume palatable, rewarding foods (8).

Although the long-term effects of social stressors on food intake have not been well explored, particularly within the context of a rich dietary environment, a series of elegant studies in rats and mice has demonstrated the significance of the intermittency and duration of social stressors in shaping other appetitive behaviors including self-administration of cocaine and consumption of ethanol (180-182). Results have consistently shown that continuous, unrelenting social subordination typically leads to anhedonia-like behavioral profiles while brief, intermittent episodes of defeat stress are associated with increased appetite for and consumption of pleasurable substances (180-182). Further, the data show clear divergent effects of different social stressors on dopaminergic pathways (180, 181) and expression of brain derived neurotrophic factor (181) as potential explanatory mechanisms underlying the intensification of binge-like consumption of rewarding substances in response to episodic social stressors versus the anhedonia-like deterioration of reward processes during unrelenting social subordination stress.

Results of these rodent studies provide a potential explanation for the inconsistencies in results from the present investigation relative to previous findings from stable social groups. It is plausible that social conditions within newly formed social groups resemble the chronic, unrelenting social subordination stress demonstrated in these rodent studies while conditions within stable groups that produced hyperphagia of a palatable diet among socially subordinate animals may be more analogous to episodic social defeat stressors. While a dominance hierarchy was clearly established in all groups at the start of Phase 1 in the present study, with the lowest ranking females receiving significantly more aggression, this process of establishing and maintaining this social structure may be a stressor for all

females. Thus, the increase in caloric intake among all animals during the dietary choice condition when the palatable diet was available may have been a result of stress-induced over-indulgence in "comfort foods". On the other hand, the greater energy density of the preferred diet may have simply compensated for the reduction in appetite characteristic of stress-induced anhedonia. Indeed, increasing the energy density and palatability of available foods is a clinical strategy that is used to preserve body mass among humans experiencing reduced appetite or anorexia as a function of aging or illness (183). Possibly with additional study time, rates of aggression towards the lowest ranking females would lessen and become more periodic, perhaps resembling the episodic pattern of social defeat shown in the abovementioned rodent studies (180-182). We would predict that once this social structure was established, the neurobiology of this social experience would change, shifting the lowest ranking females from an anhedonic phenotype to one in which a palatable diet would be over consumed.

When a chow-only dietary environment was again imposed following the dietary choice condition, all subjects reduced caloric intake relative to the previous diet conditions. This observation is supported by numerous studies showing that withdrawal of highly preferred food results in reduced consumption of less preferred, but otherwise acceptable, food (184) and suggests that the reinforcing value of a previously acceptable food decreases among animals with a history of access to a more preferred alternative. However, this finding was again contrary to previous findings in which subordinate females living in long-established social groups continued to consume excess calories when the palatable diet was removed and animals were returned to a chow-only condition (15).

At the individual level, analyses were conducted to determine whether behavioral indices of chronic psychosocial stress were predictive of caloric intake. During Phase 1, the initial no choice, chow only condition, more frequent anxiety behavior was associated with greater caloric intake while greater aggression received and submission displayed were associated with lower caloric intake. During Phase 2, when animals had a choice between the palatable diet and standard chow, the association between greater submissive behavior and lower caloric intake persisted but the significance of the other behavioral correlates diminished. There were no behavioral predictors of caloric intake in Phase 3, when the animals were returned to a chow-only environment. These results suggest that stressor exposure was associated with reduced caloric intake in the initial chow-only environment and the choice dietary environment; however, behavioral predictors were no longer significant in the final dietary phase as groups were stabilizing. Thus, these results provide further evidence that the directionality of the stress-eating relationship may be contingent upon the severity and/or frequency with which stressors are encountered.

Final analyses were conducted to assess combined effects of caloric intake and physical activity as predictors of change in body weight within each dietary phase. In all three phases, caloric intake explained a significant portion of the variance in weight change. Physical activity significantly attenuated weight gain in the choice dietary phase but was not a significant predictor of changes in weight during either of the chow-only dietary phases. The direct association between caloric intake and changes in body weight is well established, and physical activity, the most modifiable component of energy expenditure, is a behavioral strategy for weight loss and weight maintenance among humans embedded in rich dietary environments. Though not unexpected, these results help

illustrate an important point with regard to obesity given that the most significant predictor of changes in body weight in the present investigation was caloric intake. Although there was no statistically significant difference in caloric intake between rank categories, body weight did differ significantly across study phases as a function of social status. Thus, these findings demonstrate how small differences in average daily caloric intake can produce significant changes in body composition over time.

Conclusion

Findings from the present study suggest that social rank may not always be an appropriate indicator of social stress. Instead, reorganization of social groups can function as a significant stressor for even the most dominant subjects within a social hierarchy. In the present investigation, all animals lost weight following new group formation when a standard laboratory chow diet was available. All animals gained weight when given access to an energy dense palatable diet in addition to the chow, and all animals lost weight when the palatable diet was removed and they were returned to a standard chow diet. Because our study design assessed the response to a new group formation and imposition of new ranks different than those from their natal group is was not possible to determine independent and synergistic effects of stressor exposure and the dietary environment on food intake among these subjects. Thus, each successive dietary phase was confounded with time from the formation of the new groups. We would anticipate that with continued assessment of these animals, indices of stress may decline in more dominant animals yet increase in more subordinate animals as imposition of their subordinate status by dominant cage mates would be more periodic and random.

Critical factors that have yet to be determined are the point at which status-related differences in LHPA axis function and neural reward circuitry emerge among socially-housed female rhesus monkeys and whether identifiable individual differences predispose some individuals to becoming anhedonic while others become hyperphagic in response to social stress. Nonetheless, results of the present investigation provide valuable insight into group dynamics during the early stages of group formation and provide the rationale for future studies that must explore long-term effects of social subordination on limbic hypothalamic pituitary adrenal functioning, dopaminergic function, ingestive behaviors, and weight status. Investigations that utilize neuroimaging techniques can elucidate mechanisms involving reward circuitry of the brain as an explanation for the paradoxical anhedonia vs. excess consumption of palatable food among chronically stressed individuals.

Table 3.1
Descriptive characteristics of subjects at baseline stratified by eventual rank category. F-value and p-value pertain to between group differences. Results are presented as mean ± SEM.

Descriptive	High Rank (n=14)	Mid Rank (n=14)	Low Rank (n=14)	$F_{2,39}$	p-value	Combined Mean
Age (yr)	11.35 ± 0.45	10.96 ± 0.49	9.75 ± 0.51	2.96	0.064	10.69 ± 0.29
Weight (kg)	10.23 ± 0.44	10.09 ± 0.31	8.93 ± 0.0.51	2.74	0.077	9.75 ± 0.26
Body Fat (kg)	2.90 ± 0.34	2.65 ± 0.29	2.04 ± 0.39	1.61	0.213	2.53 ± 0.20
% Body Fat	27.34 ± 2.22	25.76 ± 2.31	20.78 ± 3.40	1.61	0.214	24.62 ± 1.58
Crown-Heel (cm)	84.16 ± 0.96	83.65 ± 0.64	82.36 ± 1.05	1.06	0.354	83.39 ± 0.52

Table 3.2
Body fat and body fat percentage by dietary phase and social status. Results are presented as mean (± SEM). A single asterisks (*) indicates statistical significance at the $p<0.05$ level relative to other values within a column.

	Phase 1 (Choice) Body Fat		**Phase 2 (No Choice) Body Fat**		**Phase 3 (Choice) Body Fat**	
	kg	**%**	**kg**	**%**	**kg**	**%**
High Rank (n=14)	1.340 (0.230)	13.844 (2.179)	2.725 (0.309)	31.397 (3.939)	2.467 (0.310)	24.712 (2.720)
Mid Rank (n=14)	1.343 (0.225)	13.096 (2.135)	2.514 (0.302)	26.511 (3.860)	2.193 (0.303)	21.508 (2.666)
Low Rank (n=14)	0.602* (0.235)	6.579* (2.231)	1.187* (0.316)	14.421* (4.033)	1.048* (0.317)	11.258* (2.785)

Table 3.3
Regression coefficients for behavioral predictors of caloric intake during each dietary phase. A single asterisks (*) indicates statistical significance at the p<0.05 level while double asterisks (**) indicate statistical significance at the p<0.01 level

	Average Daily Caloric Intake (kcal)		
Predictor	**Phase 1 (No Choice)**	**Phase 2 (Choice)**	**Phase 3 (No Choice)**
Anxiety Behavior (per hour)	**0.370***	0.155	0.108
Aggression Received (per hour)	**-0.332***	-0.230	0.036
Submission Displayed (per hour)	**-0.372***	**-0.318***	-0.010

Figure 3.1
Rates of aggression received per hour across the full study interval by individual within-group rank (1 through 6). Different typographical symbols indicate statistically significant differences ($p<0.05$) between individual ranks (mean ± SEM).

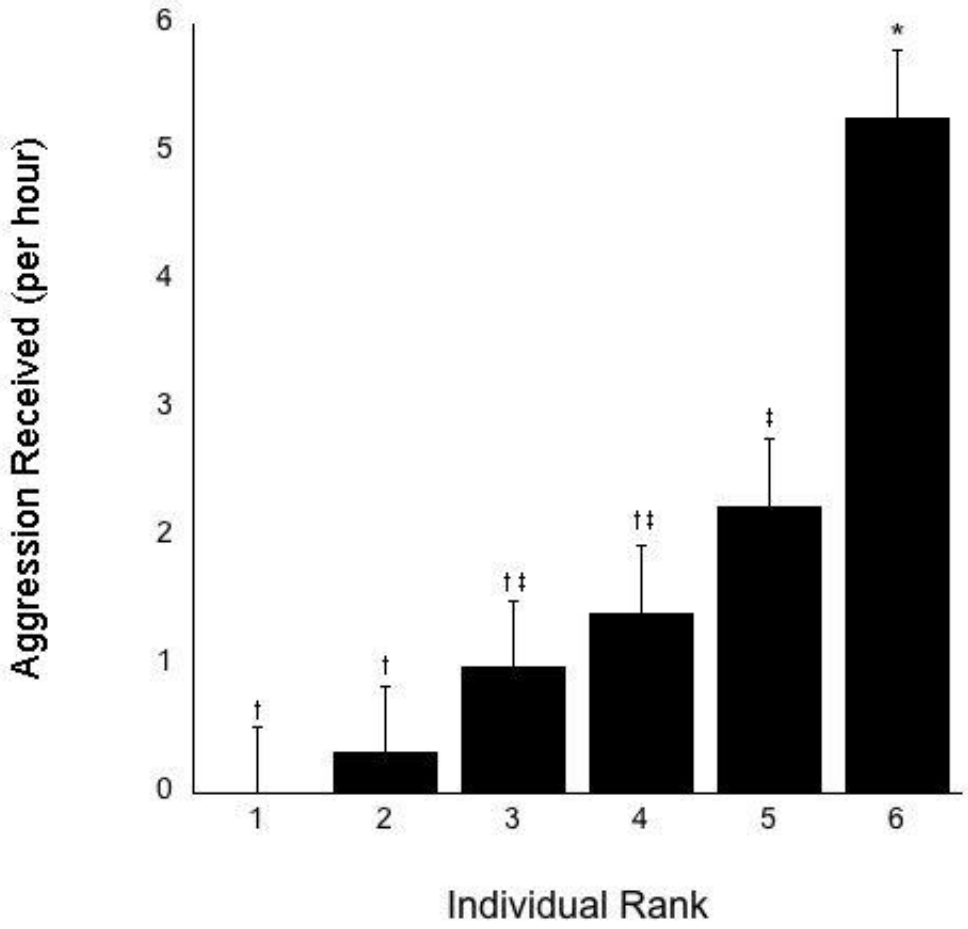


Figure 3.2
Rates of aggression directed, submission emitted, affiliation received, and anxiety displayed collapsed for rank across the three dietary phases. Results are presented as the mean ± SEM. An asterisk (*) indicates the rate of the given behavior was significantly ($p<0.05$) different in the dietary phase that is noted relative to the unmarked phases.

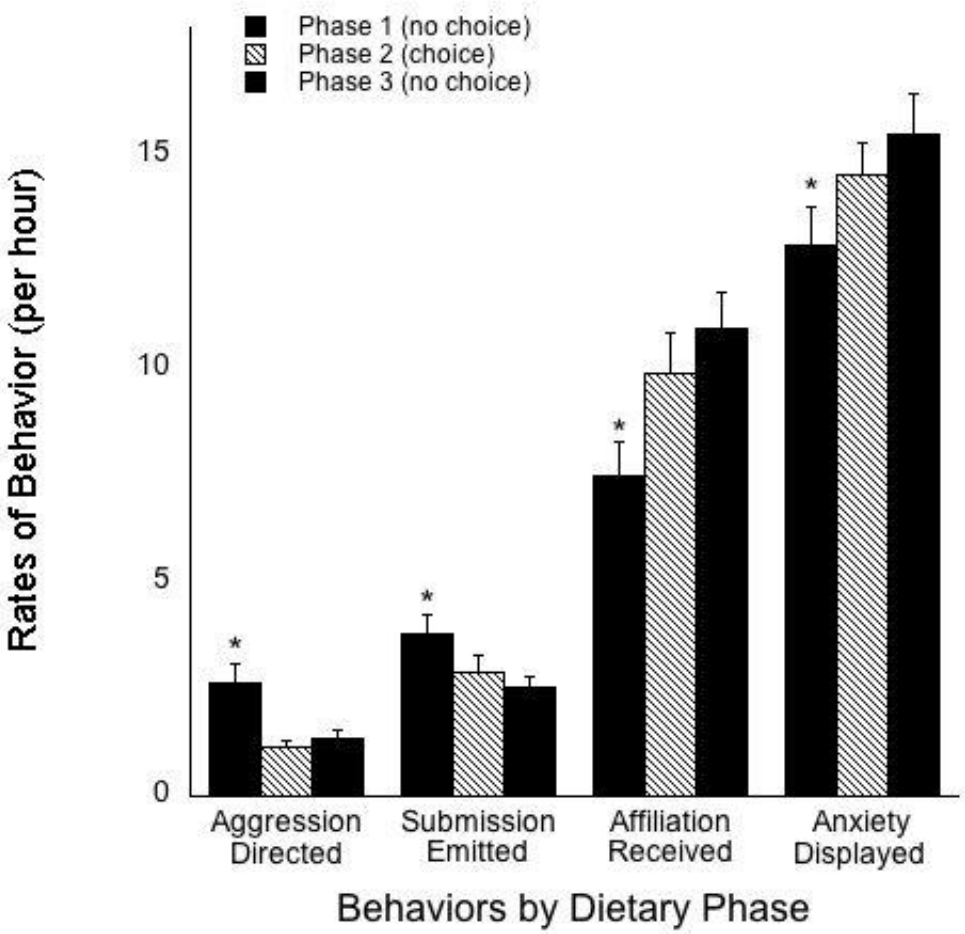


Figure 3.3
Rates of aggression emitted per hour by rank category (high – ranks 1 and 2, mid – ranks 3 and 4, low – ranks 5 and 6). Results are presented as the mean ± SEM. Different alphanumeric characters indicate statistically significant differences ($p<0.05$) between rank categories. An asterisk (*) indicates a significant difference by phase within rank category.

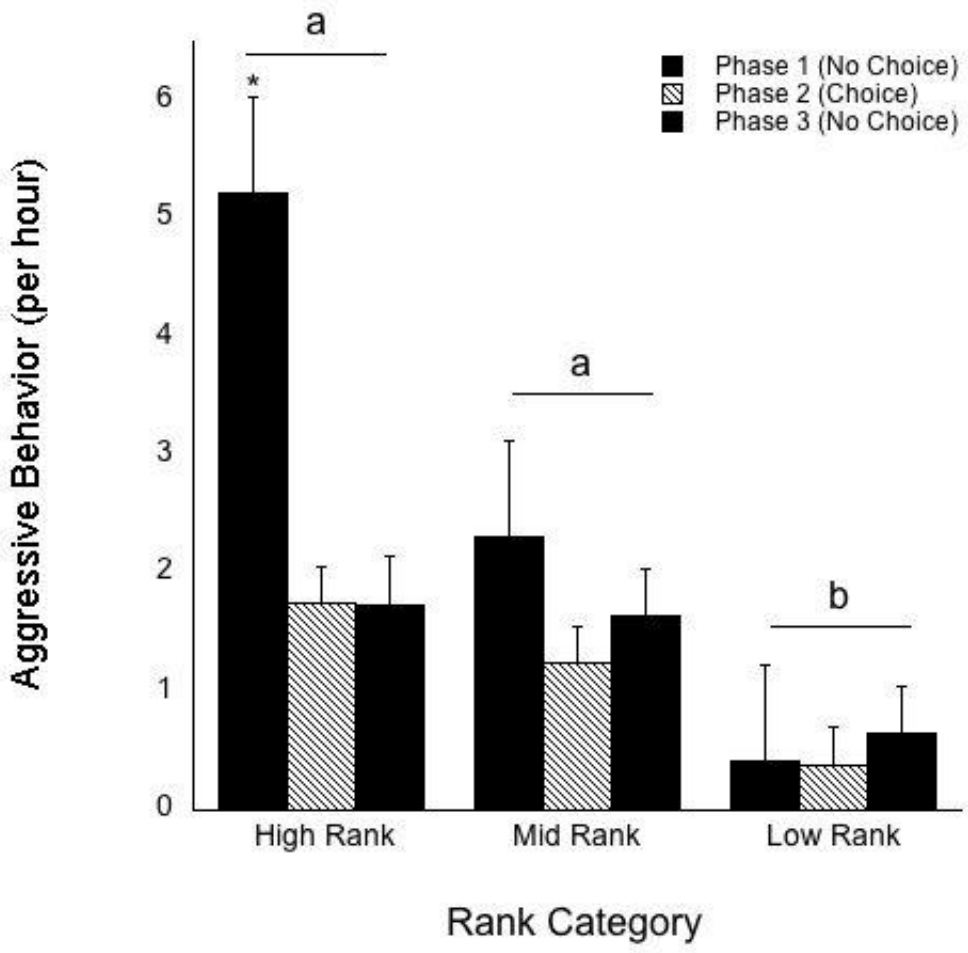


Figure 3.4
Rates of submission displayed per hour by rank category. Results are presented as the mean ± SEM. Different alphanumeric characters indicate statistically significant differences ($p<0.05$) between rank categories.

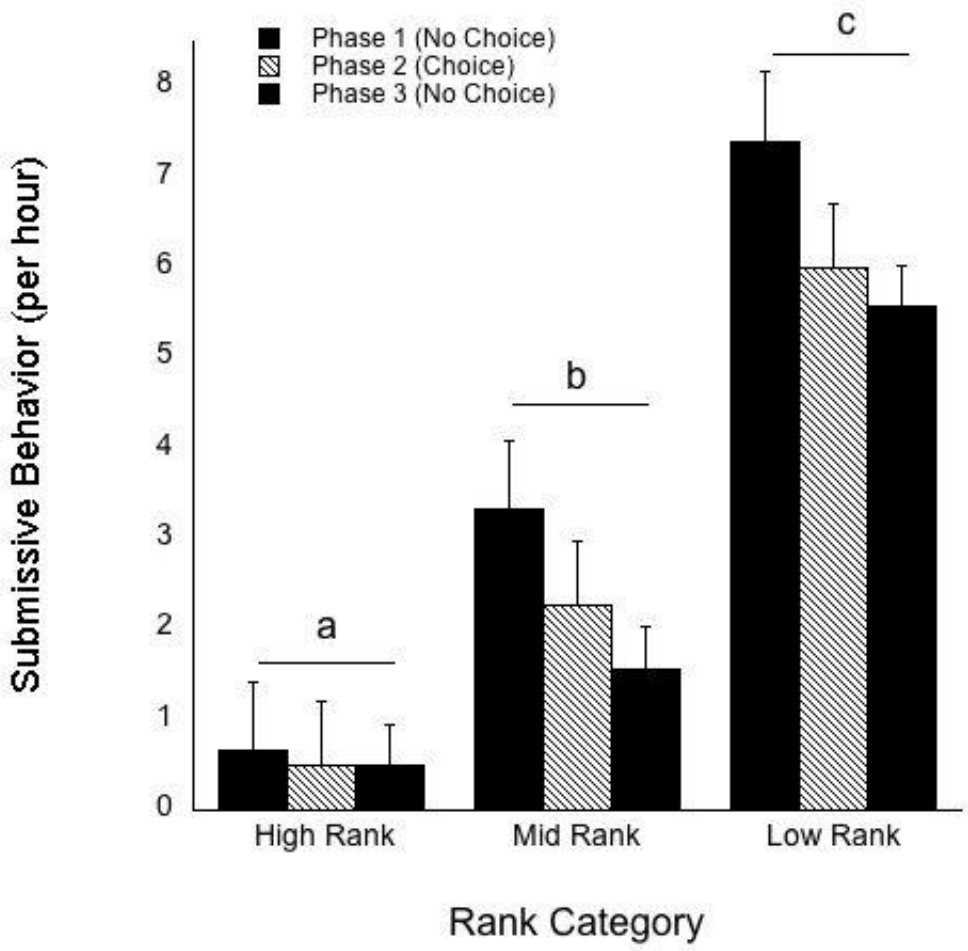


Figure 3.5
Rates of anxiety-like behavior per hour by rank category. Results are presented as the mean ± SEM. Different alphanumeric characters indicate statistically significant differences ($p<0.05$) between rank categories. An asterisk (*) indicates a significant difference by phase within rank category.

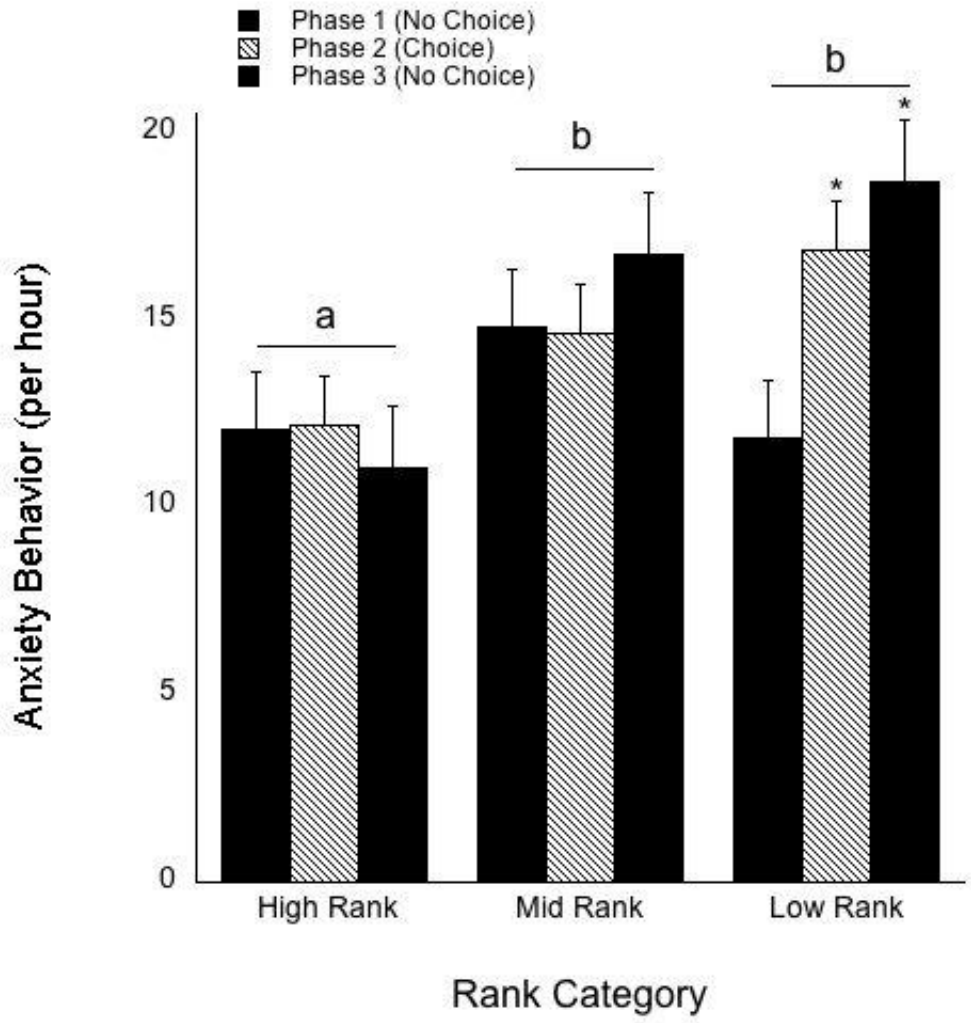


Figure 3.6A
Serum cortisol levels (μg/dL) pre-stressor (baseline), immediate post-stressor (30 min), and 1-hour and 4-hours post-stressor stratified by dietary phase and collapsed for rank category. Results are presented as the mean ± SEM. Different alphanumeric characters indicate statistically significant differences ($p<0.05$) between dietary phases. Different typographical symbols indicate statistically significant differences ($p<0.05$) between sampling intervals.

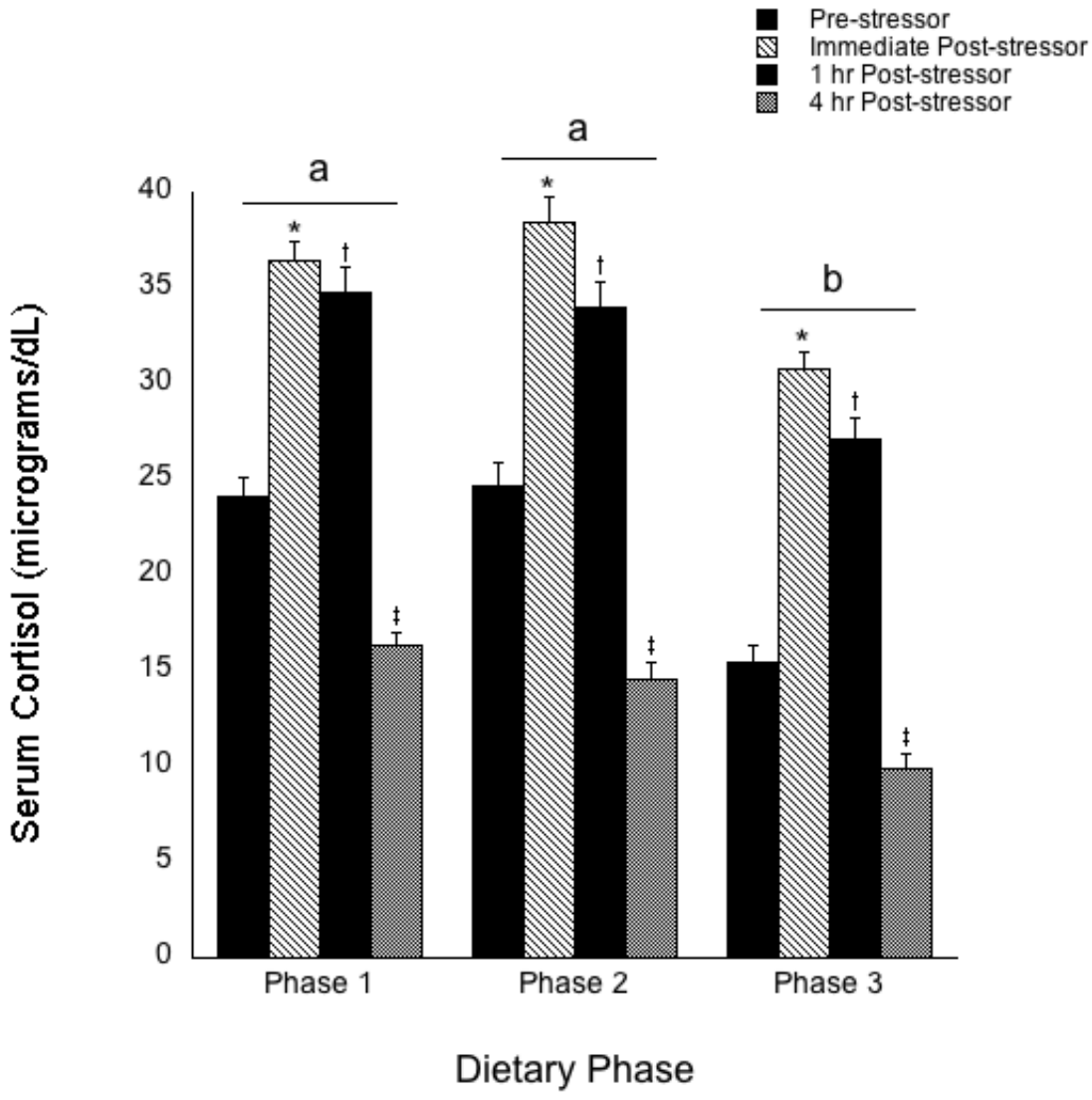


Figure 3.6B
Serum cortisol levels (μg/dL) stratified by sampling interval and rank category and collapsed for dietary phase. Results are presented as the mean ± SEM.

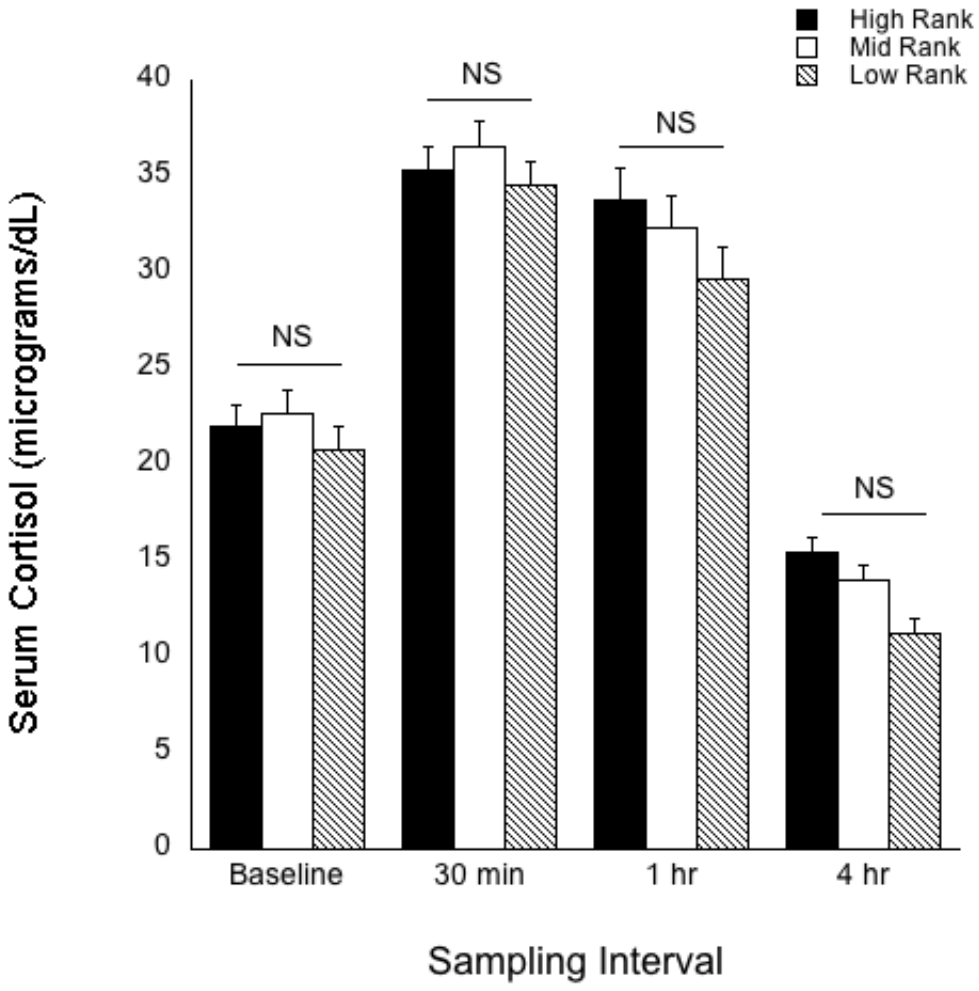


Figure 3.7
Change in body weight (kg) from the time of group formation to study start stratified by rank category. Results are presented as the mean ± SEM.

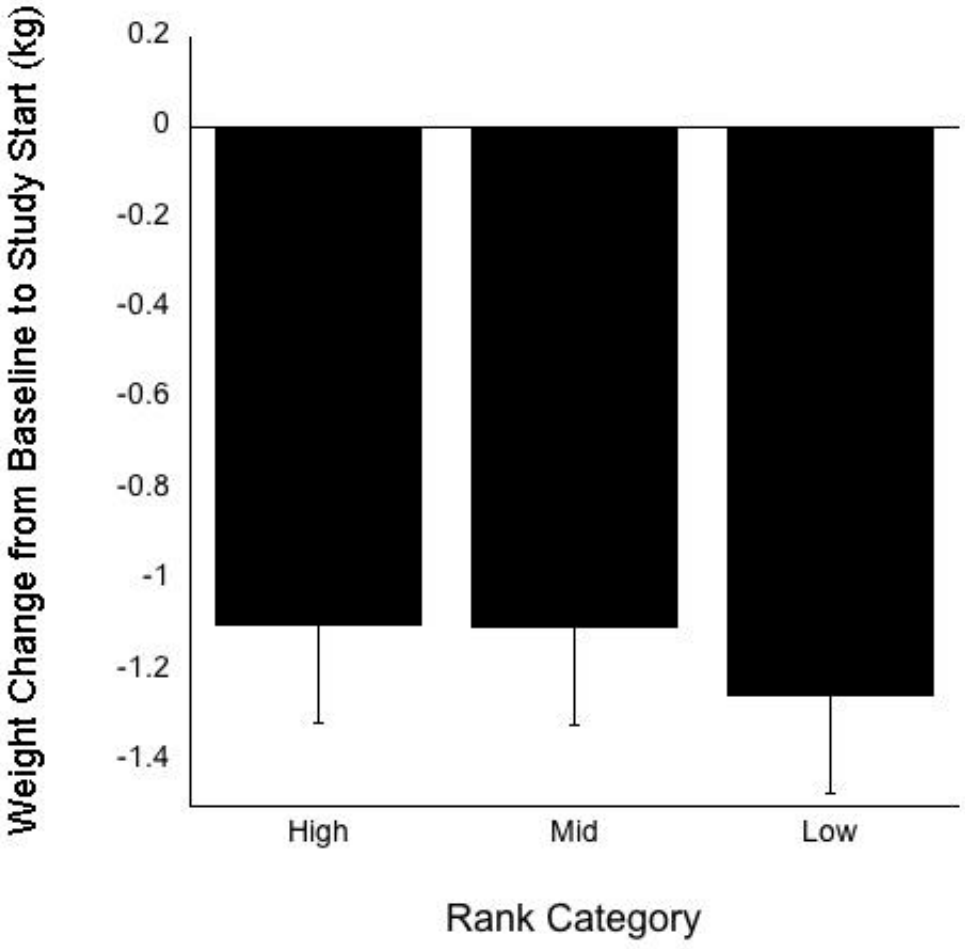


Figure 3.8
Body weights (kg) at the beginning of the study (week 1) and the end of phase 1 (week 8), phase 2 (week 16), and phase 3 (week 24). Results are presented as the mean ± SEM. Different alphanumeric characters indicate statistically significant ($p<0.05$) differences between rank categories. Different typographical symbols indicate statistically significant differences between measurement intervals.

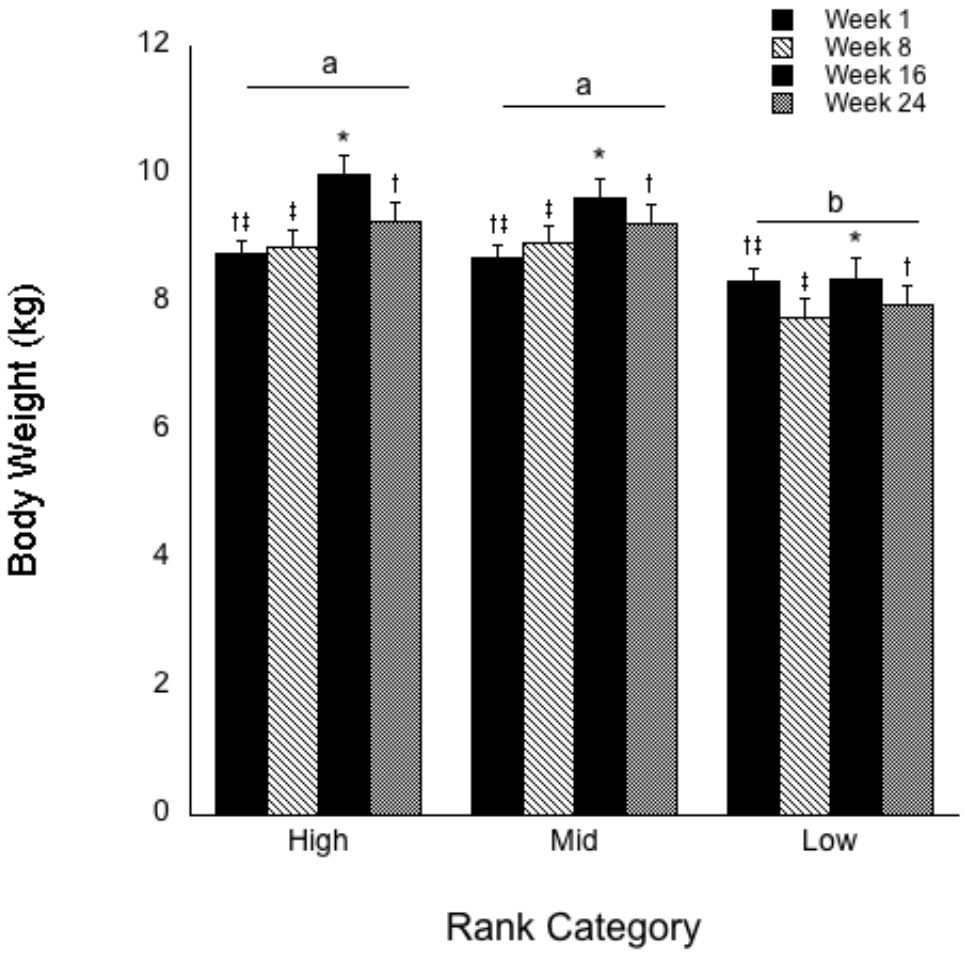


Figure 3.9
Change in body weights (kg) during each dietary phase by rank category. Results are presented as mean ± SEM. Different typographical symbols indicate statistically significant differences ($p<0.05$) between rank categories.

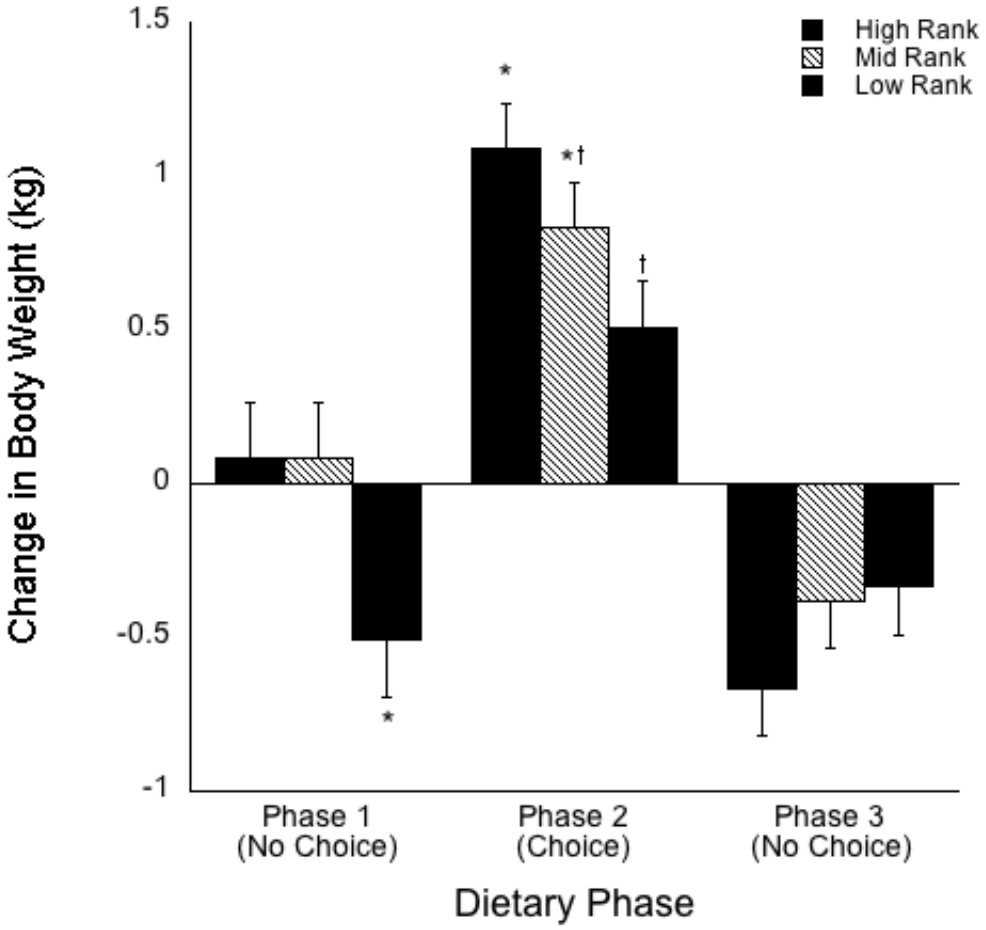


Figure 3.10
Average daily caloric intake (kcals) consumed during each dietary phase by rank category. Results are presented as mean ± SEM. Different alphanumeric symbols indicate statistically significant differences ($p<0.05$) between dietary phases.

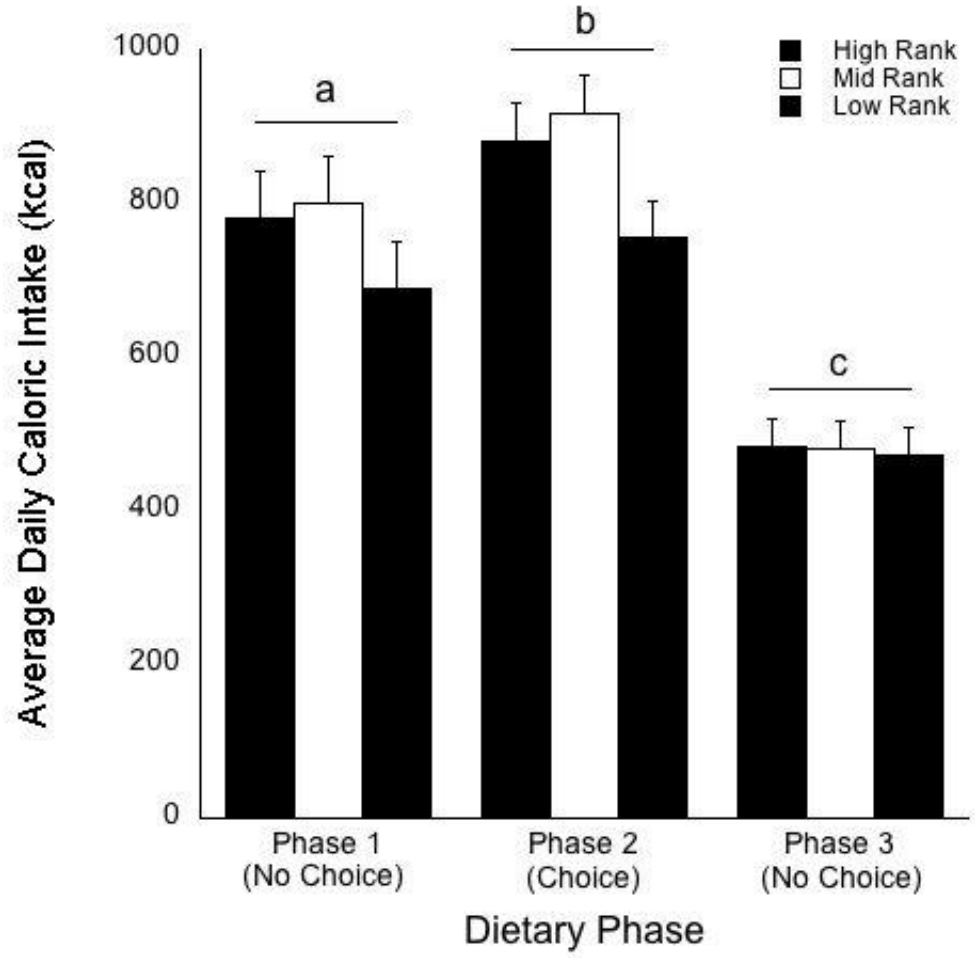


Figure 3.11A
Average daily caloric intake (kcal) by week during Phase 1, the first no-choice dietary phase. Results are presented as mean ± SEM. Different typographical symbols indicate statistically significant differences ($p<0.05$) between individual weeks.

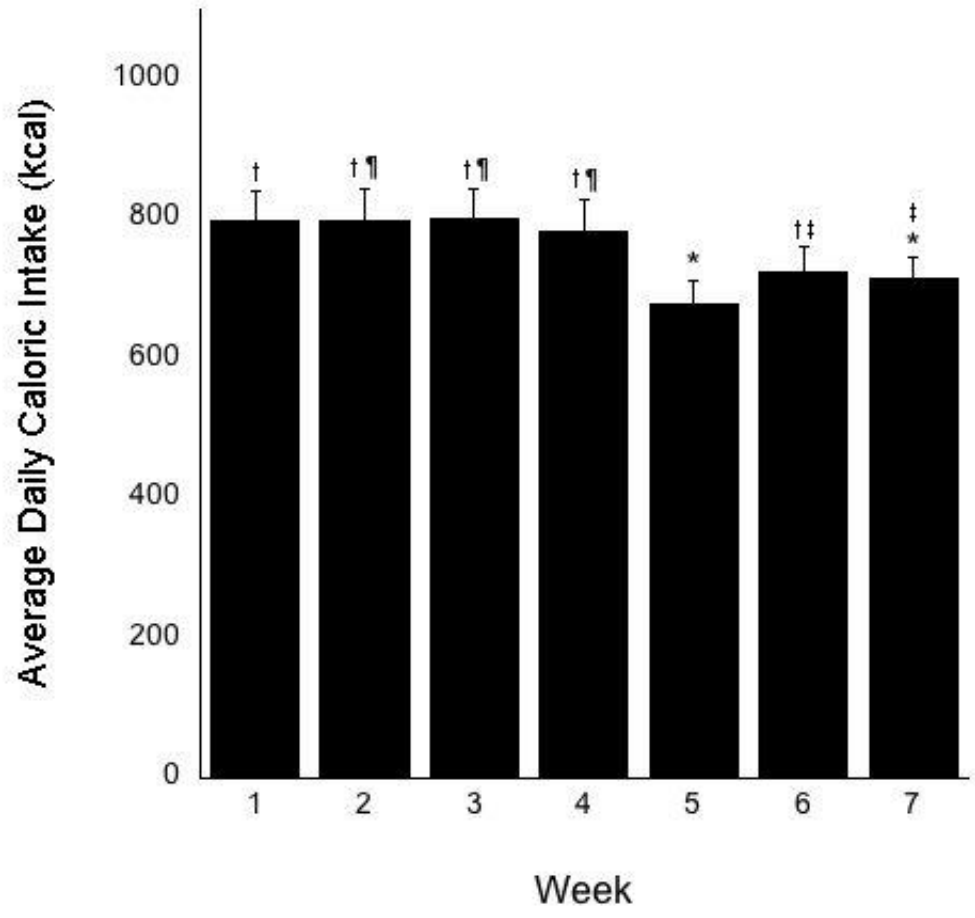


Figure 3.11B
Average daily caloric intake (kcal) by week during Phase 2, the dietary choice phase. Results are presented as mean ± SEM. Different typographical symbols indicate statistically significant differences ($p<0.05$) between individual weeks.

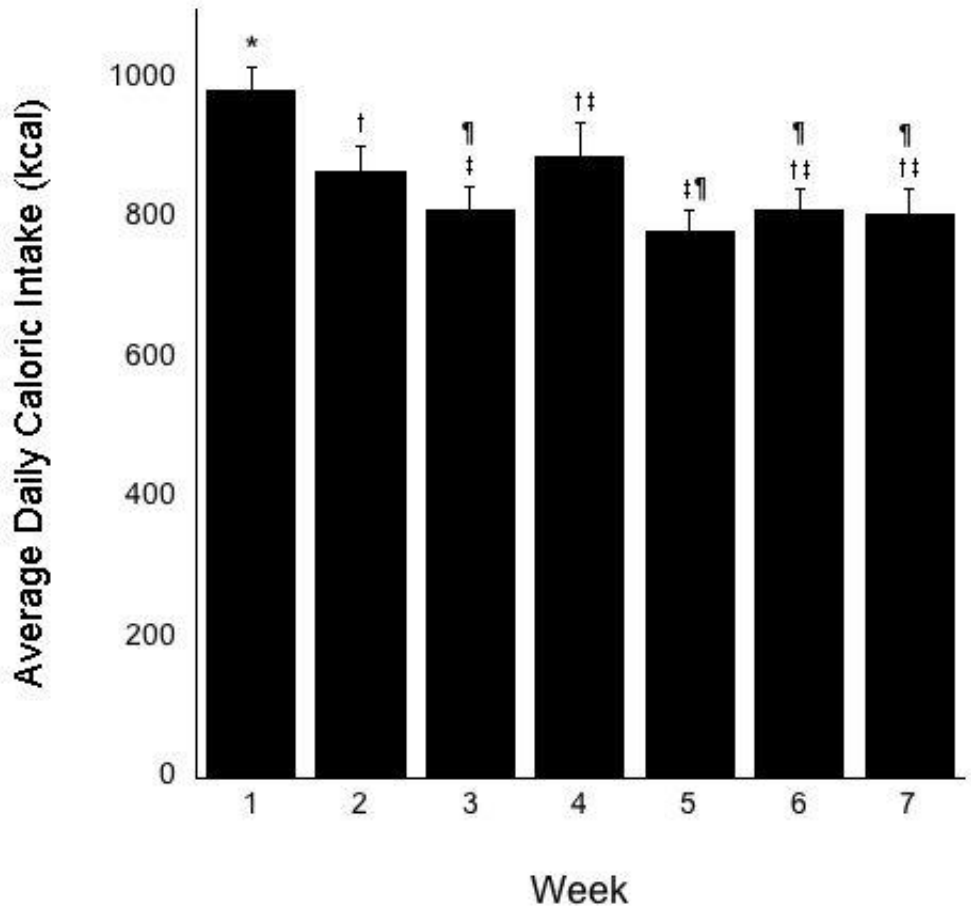


Figure 3.11C
Average daily caloric intake (kcal) by week during Phase 3, the final no-choice dietary phase. Results are presented as mean ± SEM. Different typographical symbols indicate statistically significant differences ($p<0.05$) between individual weeks.

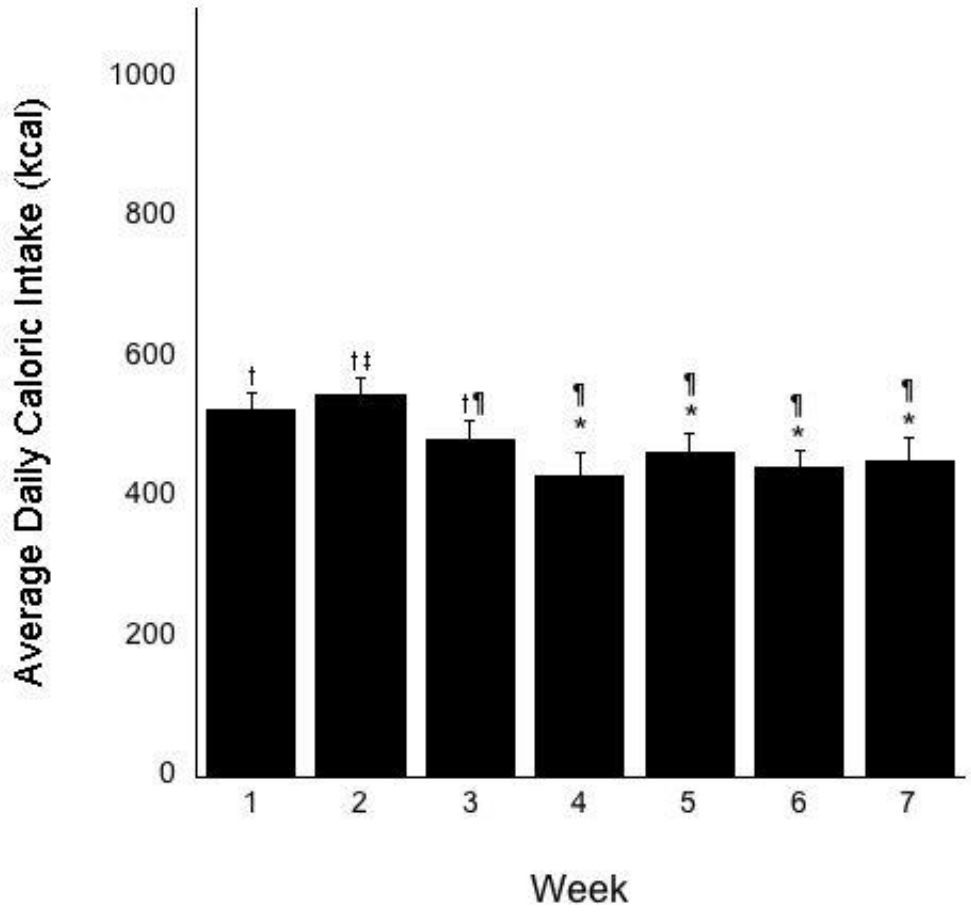


Figure 3.12
Average caloric intake (kcal) during the choice dietary condition stratified by rank category and diet. An asterisk (*) indicates a significant difference ($p<0.05$) in calories consumed from each diet.

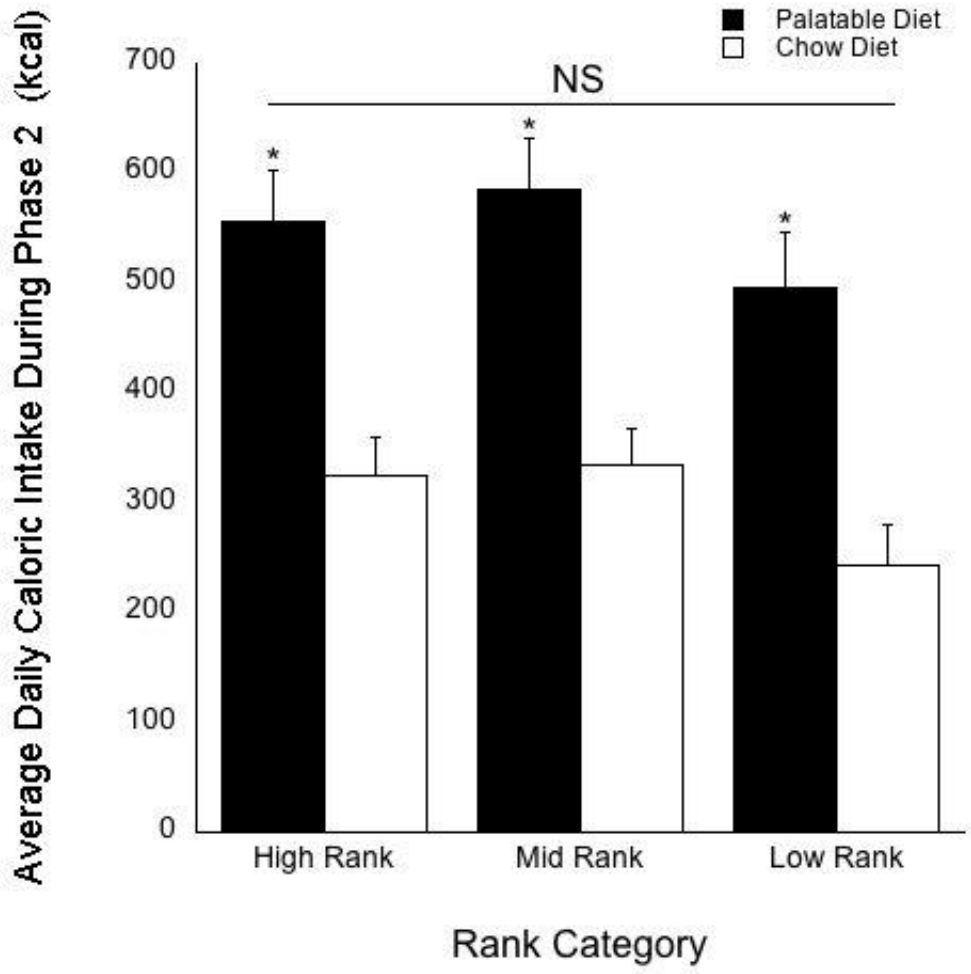


Figure 3.13
Average daily caloric intake (kcal) by week during Phase 2, the dietary choice phase, stratified by diet. Results are presented as mean ± SEM. Different typographical symbols indicate statistically significant differences ($p<0.05$) in caloric intake for each diet between individual weeks.

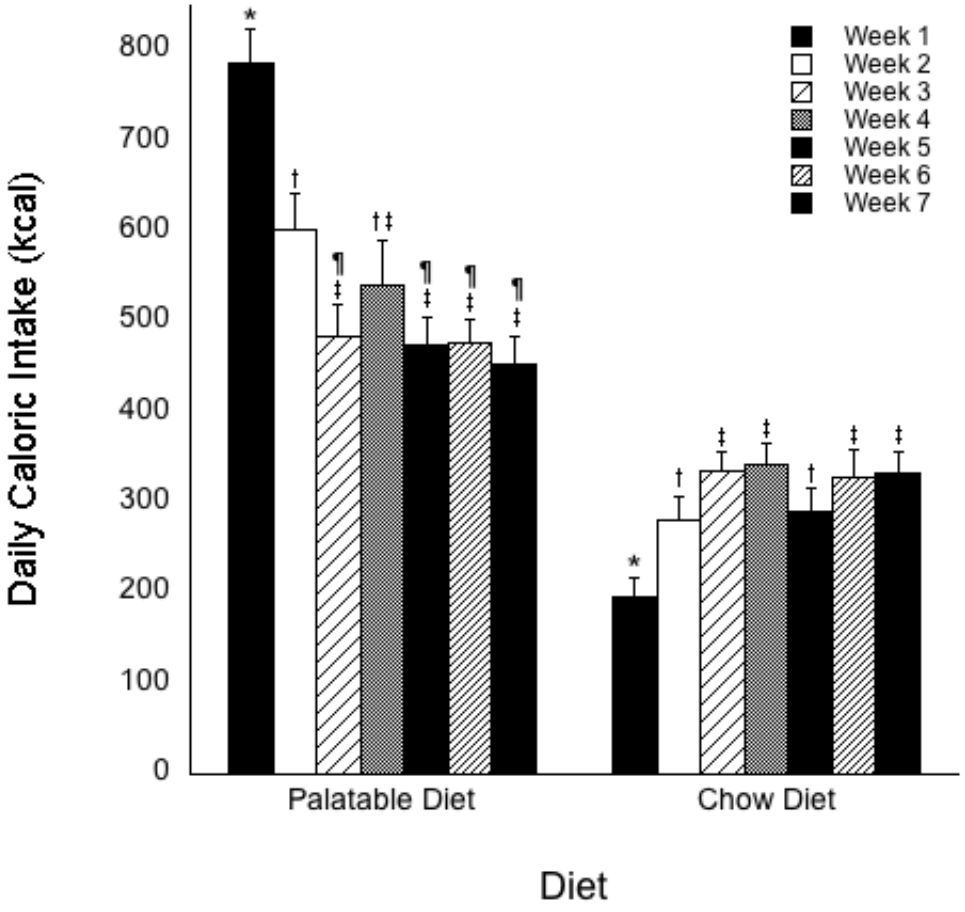
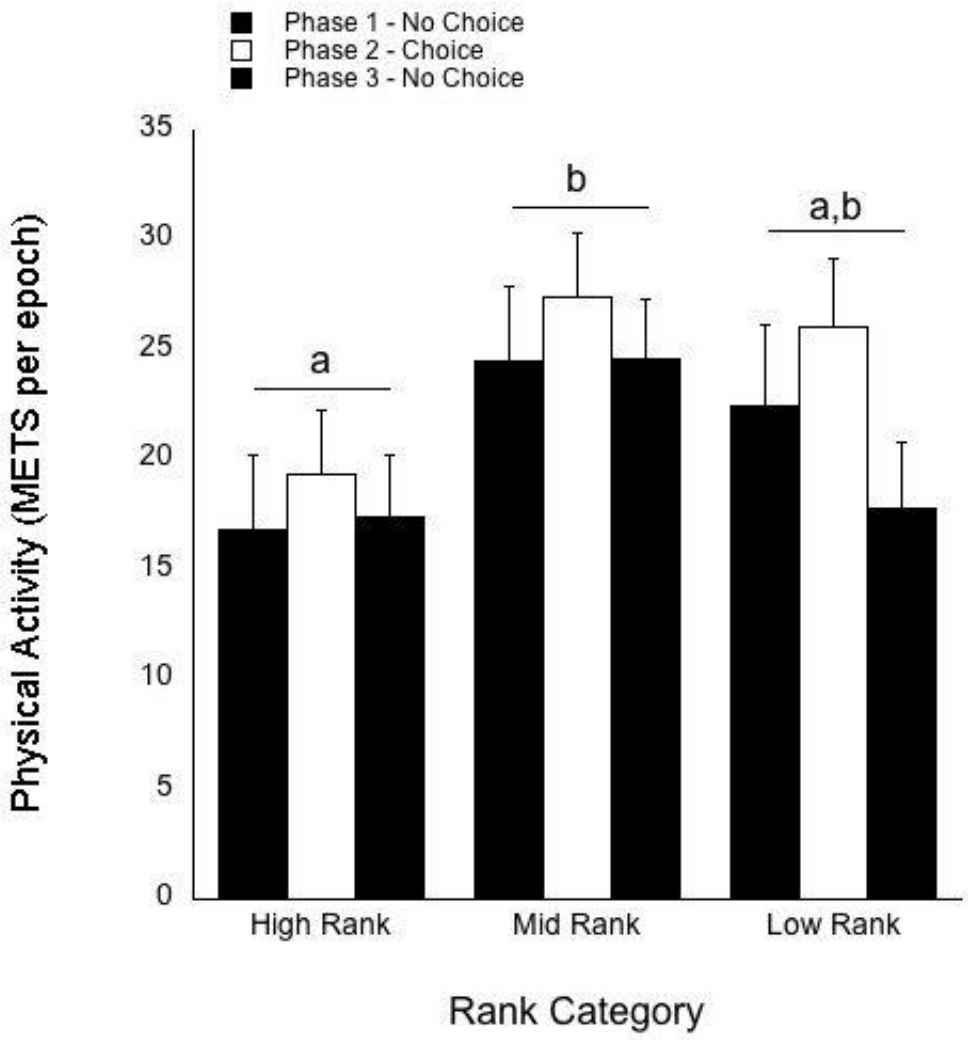


Figure 3.14
Average rates of physical activity (METS per epoch) during each dietary phase by rank category. Results are presented as mean ± SEM. Different alphanumeric symbols indicate statistically significant differences ($p<0.05$) between rank categories.



CHAPTER 4

Abstract

Appetitive behaviors, including food intake, appear to be particularly susceptible to stress-induced alterations and are likely a primary avenue through which stressor exposure manifests as adverse health outcomes. The present investigation tested the overriding hypothesis that female rhesus monkeys, particularly the most subordinate females, would be hyperphagic following exposure to an acute stressor in an environment that included access to highly palatable food but that animals would be hypophagic or unaffected by stressor exposure when only a standard laboratory chow diet was provided. Results showed exposure to an acute, psychological stressor markedly reduced caloric intake among high and middle ranking subjects regardless of diet availability while the most subordinate females, who consumed fewer calories than conspecifics during control conditions, did not further reduce their caloric intake in response to the acute stressor. Despite the fact that animals did not increase caloric intake in response to the stressor, basal cortisol levels prior to the acute stressor were predictive of caloric intake from the palatable diet in the 24-hour interval following exposure to an acute stressor. Findings from the present study suggest that stressor intensity and duration may be critical factors in the bidirectional relationship between stressor exposure and food intake and support the notion that stressor exposure promotes a shift in preference to high fat, high sugar comfort foods, which may or may not alter total caloric intake relative to usual dietary patterns.

Introduction

Exposure to – or in some cases, anticipation of – a threatening stimulus results in a highly coordinated physiological response engaging both the sympathetic nervous system (SNS) and the limbic-hypothalamic-pituitary-adrenal (LHPA) axis (48). LHPA axis activation results in a hormonal cascade initiated by the release of corticotropin releasing factor (CRF) from limbic structures and the paraventricular nucleus of the hypothalamus, stimulating adrenocorticotropic hormone (ACTH) secretion from the pituitary, and the synthesis and release of glucocorticoids (primarily corticosterone in rodents or cortisol in Cercopithecine primates, including humans) from the adrenal glands. This response is highly adaptive for managing infrequent stressors, and when the threat is no longer eminent, a sequence of neuronal and hormonal events is coordinated through negative feedback to quickly restore homeostasis (53). However, over time and as a result of repeated and/or continuous stressor exposure, the negative feedback loop of the LHPA axis can become dysregulated, giving rise to glucocorticoid resistance and a number of adverse health outcomes, including metabolic abnormalities (48).

Appetitive behaviors, including food intake, appear to be particularly susceptible to stress-induced alterations and are likely a primary avenue through which stressor exposure manifests as adverse health outcomes (41). Although a sizeable literature has investigated the relationship between stressor exposure and dietary patterns, no consistent effect has emerged. Instead, studies have produced bidirectional results, with some individuals becoming hyperphagic in response to stressor exposure and others becoming hypophagic (6). Inconsistencies in findings have been attributed to difficulty obtaining valid and reliable information on food intake and stressor exposure outside of the

laboratory (33). Additionally, mixed results could be a function of differential severity of stressor exposure and/or stable individual differences in stress responsivity and coping strategies (33).

Evidence suggests that chronic stress, defined as prolonged or repeated stressor exposure over a extended period of time (6), is likely of greatest importance with regard to stress-related pathologies (7). Nonetheless, investigations utilizing acute stressors are frequently employed to assess the effects of stressor exposure on food intake. Laboratory studies involving humans report reduced (12), increased (44), and similar (39, 45, 46) food intake in response to acute stressors relative to control conditions. These investigations typically employ challenging cognitive tasks (e.g., mental arithmetic, mirror tracing, Stroop word test) and/or challenging interpersonal performance tasks (e.g., Trier Social Stress Test) and provide short-term access to an array of palatable foods (47). While these studies are informative in that they permit controlled testing environments, the few food options that are available may not be typical of the subjects' dietary environments, and follow-up assessments of potential compensatory appetitive behaviors are generally not conducted (35). Additionally, the stressors employed in the laboratory may not be relevant to the types of stressors that individuals encounter in their usual environments (35), and in some cases, there is no measure of whether the stressor was perceived as stressful or elicited a hormonal stress response (185).

Animal studies have likewise assessed the effects of acute stressor exposure on short-term dietary intake, and these investigations have also produced mixed results. Early studies with rodents consistently demonstrated hypophagia in response to physical stressors such as tail pinch, electric shock, and restraint within the context of a standard

laboratory dietary environment (186). However, evidence from more recent investigations using similar physical stressors indicates that access to a palatable diet may be a critical factor in the emergence of stress-induced hyperphagia (61). Additionally, experiments that have employed social defeat, a psychosocial stressor that is deemed more ethologically relevant to human conditions than physical stressors (8), have induced hyperphagia of a standard chow diet following stressor exposure (62, 63), suggesting that the type of stressor encountered may be an important determinant of post-stressor ingestive behaviors. In short, existing rodent studies are informative, but they are limited by the use of either stressors or dietary environments that are not analogous to human conditions. Further, much of the animal research addressing stress-induced alterations in food intake has been conducted exclusively in male rodents (8) despite the growing evidence highlighting significant gender differences in susceptibility to emotional eating (9-12). The need for more studies in female subjects is evident as women are not only disproportionately affected by emotional eating (187) but also demonstrate higher prevalence of obesity (163) and anxiety and depressive disorders (8) relative to men.

The social organization of female rhesus monkey (*Macaca mulatta*) societies is based on a linear dominance hierarchy (84, 85). Regardless of group size, each animal within this social structure has a clearly defined rank. Lower ranking animals receive more aggression from higher-ranking group mates and terminate these interactions by emitting submissive behaviors, a defining feature of subordination (84-87). A consequence of prolonged social subordination is dysregulation of the LHPA axis (124) and the emergence of a number of stress related phenotypes (120). Within newly formed social groups, dominance ranks become evident very quickly (91); however, it is unclear at

what point the chronic stress-induced phenotypes begin to emerge. Nonetheless, group-housed female rhesus monkeys provide a model for studying the consequences of chronic psychosocial stressors that is highly relevant to human populations (8), and this social structure permits the evaluation of how behavior following exposure to an isolated, acute, psychogenic stressor is modified by an individual's stressor history.

The mechanisms through which stressor exposure affects ingestive behaviors are not fully understood, and investigations are complicated by the observation that CRF, the neuropeptide that initiates the stress response, suppresses food intake while glucocorticoids, the end product of the LHPA axis cascade, appear to be orexigenic, promoting preferential consumption of highly palatable "comfort foods" (6). Data from some rodent studies indicate that exposure to a palatable diet directly reduces acute LHPA axis activation (173-175, 188). Conversely, studies of rhesus monkeys show that the serum cortisol is increased when these animals consume a palatable diet (14), corroborating other rodent data showing ingestion of a high-fat, high-sugar diet increases both basal and stress-induced LHPA activity (11, 72).

The present investigation was designed to test the overriding hypothesis that caloric intake following exposure to an acute stressor would be influenced by the dietary environment and a female's stress history, thereby augmenting the existing evidence regarding the effects of stressor exposure on food intake. In addition to utilizing an ethologically relevant model of acute and chronic psychosocial stressors among females, the study allowed quantification of food intake under different dietary conditions within the context of animals' usual social groups during the 24-hours immediately following exposure the acute stressor and permitted evaluation of potential modification of the

effects of acute stressor exposure by recently acquired social position. Because evidence suggests that a palatable diet may be essential to elicit stress-induced hyperphagia (61), we hypothesized that animals would be hypophagic or unaffected by stressor exposure in the absence of a palatable diet but that animals would be significantly hyperphagic following stressor exposure in a rich dietary environment that included access to highly palatable food in addition to laboratory chow. We further hypothesized that hyperphagia would be significantly more pronounced among the most subordinate animals in a rich dietary environment, reasoning that experiencing greater ongoing aggression from group mates would exacerbate the consequences of acute stressor exposure. Finally, we hypothesized that greater cortisol reactivity, independent of social status, would be predictive of greater consumption of palatable food when animals were given a choice between a standard chow diet and a high-fat, high sugar diet. However, based on the previously referenced investigations with rhesus monkeys (14), we did not expect the provision of the palatable diet to directly attenuate activation of the LHPA axis.

Materials and Methods

Subjects

Subjects were 42 adult, female rhesus monkeys (*M. mulatta*) ages 5 to 14 years (10.68 ± 0.29). Animals were members of seven recently formed six-member social groups that were housed in indoor-outdoor enclosures at the Yerkes National Primate Research Center Field Station, Emory University. The formation of these seven groups followed procedures for simultaneous introduction as described previously, and groups were formed 12.04 ± 0.68 weeks prior to study onset (range 6.71 to 18.43 weeks). The Emory University Institutional Animal Care and Use Committee approved all procedures in

accordance with the Animal Welfare Act and the US Department of Health and Human Services 'Guide for Care and Use of Laboratory Animals.'

Experimental Design

The present investigation was conducted within the context of a larger study in which caloric intake was continuously quantified among socially housed female rhesus monkeys throughout a 24-week study period that was subdivided into three, 8-week dietary phases. During the first dietary phase (Phase 1), a standard laboratory chow diet was provided (no choice). The second dietary phase (Phase 2) permitted a dietary choice between a more palatable diet and the standard chow (choice). The final dietary phase (Phase 3) eliminated the dietary choice and a chow-only environment was reestablished (no choice).

The two diets utilized in the study were selected to resemble the Prudent and Western profiles used in human epidemiological studies (168). The chow diet contains 3.60 kcal/gram (12% fat, 72% carbohydrate, and 16% protein; Of the total 2.59 kcal/gram of carbohydrates, 2.44 are derived from fiber and 0.15 from sugar; Purina #5038, re-pelleted by Research Diets). The palatable diet contains 4.74 kcal/gram (Research Diets, D07091204; 40% fat, 44% carbohydrate, and 16% protein; Of the total 2.08 kcal/gram of carbohydrates, 0.6 are derived from fiber and 2.02 from sugar). Both diets contain similar and appropriate vitamin and mineral fortification.

Food intake at the individual level was recorded continuously using previously validated automated feeders (119). Prior to the study onset, unique radio frequency identification (RFID) microchips were implanted subcutaneously in the wrists of each animal. When an

animal placed her hand in a feeder, a Datamars reader surrounding the food dispenser detected the microchip and sent a signal to a remote computer that identified the monkey and triggered the delivery of a single food pellet. Two feeders were attached to each housing unit. During the first and final dietary phases, both feeders dispensed pelleted chow (no-choice conditions). During the second dietary phase, when animals were given a dietary choice, one feeder contained pelleted chow while the other dispensed a pelleted high-fat, high sugar diet. This system allowed for continual quantification of caloric intake of individual monkeys embedded in social groups. Total caloric intake was measured during the 24 hours following exposure to the acute stressor and during two comparable 24-hour control periods in the absence of experimental manipulation. Acute stressor exposures took place during weeks 3 through 5 of each dietary phase with the timing randomized across ranks. The no acute stressor, control conditions occurred during weeks 2 and 6 of each dietary phase. An average of the caloric intake during the two control conditions was used in the statistical analyses.

Social rank of subjects in the present study was determined by the outcome of unequivocal dyadic agonistic interactions (84). Formalized, half-hour observations of each social group were conducted once per week throughout the 24-week study period, and all occurrences of agonistic (i.e., aggressive and submissive) behavior were recorded during each sampling interval in the format of actor–behavior–recipient. All observations were conducted by two previously trained observers who maintained an inter-observer reliability of >92%. Each animal within each social group assumed a rank from 1 through 6. Females were further grouped into high (ranks 1 and 2), mid (ranks 3 and 4), and low (ranks 5 and 6) rank categories to improve statistical power of the planned statistical

analyses. At specified intervals during each of the three dietary phases, subjects were exposed to an acute stressor paradigm, described as follows, and cortisol responsivity was assessed in response to each exposure.

Acute Stressor and Behavioral Response

Once during each of the three dietary phases, animals were subjected to an acute, psychosocial stressor, which involved temporary removal of each animal from her social group followed by exposure to the Human Intruder (HI) task. The HI task is a standardized 30-minute behavioral paradigm used to assess the emotional response of a monkey to threatening stimuli that consists of three challenging conditions (169). Initially, the animal is alone for ten minutes. At this point, an unfamiliar human then enters the room and presents his/her facial profile to the monkey for a ten-minute interval. During a final ten-minute period, the intruder makes continuous eye contact with the animal. During the alone condition, the subject typically responds with cage exploration, vocalizations, and pacing. During the profile condition monkeys typically become behaviorally inhibited (freeze) while scanning the environment and intruder. The stare condition typically elicits anxiety-like and agonistic behaviors, including both threats and submissive gestures towards the intruder. Evidence indicates that animals do not habituate to the HI task (189), which facilitated its utility across the three repeated trials. Each test was video recorded and behavior was summarized using a data acquisition program on a netbook computer following an established ethogram (190). Total frequencies of anxiety behaviors and durations of behavioral inhibition were summed across all three conditions of the HI task (i.e. alone, profile, stare) for each dietary phase to assess behavioral responsivity to repeated trials of an acute, psychogenic

stressor. Repeated exposures to the HI task were separated by 8 weeks, and the time of testing was held constant for each animal across repeated trials. Physiological stress reactivity was assessed via a series of repeated blood sampling prior to and following exposure to the acute stressor, and food intake was quantified during the 24-hours immediately following exposure to the acute stressor.

Cortisol Responsivity

Between 0900 and 1030 hour on the day of the Human Intruder tests, animals were accessed for blood collection (T0, 3 mL) from the saphenous vein. All animals used in this study were trained for conscious venipuncture following procedures previously described (170). Animals were then transferred to an adjacent behavioral testing room and exposed to the Human Intruder (HI) acute stressor task. A second blood sample was collected (3 mL) immediately following completion of the task (T30), and animals were promptly returned to their social groups. Animals were accessed for additional blood samples (3 mL) at 1 hour and 4 hour post-stressor intervals to assess recovery of serum cortisol, an indicator of the sensitivity of the negative feedback regulatory mechanism of the LHPA axis. The sampling protocol for blood collection was a modification of previously reported sampling intervals (14). Blood samples were centrifuged at 4°C for 15 minutes and the serum layer was pipetted into Cryovials (Fisher Scientific, Atlanta, GA) and stored at -40°C until assay. Serum cortisol concentrations were determined by liquid chromatography-mass spectrometry as previously described (171).

Statistical Methods

The effects of stressor exposure and availability of a palatable diet on the total 24-hour caloric intake were determined by repeated measures ANOVA in SPSS using rank

category as a between-subjects variable. The effects of sampling time and diet availability on cortisol response and the effects of diet availability on behavioral response to the HI task (i.e. summed anxiety behaviors and total duration of freezing) were assessed in a similar manner. Post-hoc pairwise comparisons were generated to assess all main effects. Results are presented as the mean ± standard error, and p-values < 0.05 were considered significant.

Regression analyses were performed to assess the predictive value of cortisol with regard to cumulative caloric intake during each 24-hour post-stressor period in the three dietary phases. Analyses included baseline, immediate post-stressor, 1-hour post-stressor, and 4-hours post-stressor cortisol values as well as change in cortisol from baseline to immediate post-stressor. The predictive value of cortisol on caloric intake from both of the palatable diet and the chow diet were also assessed separately during the second dietary phase, when subjects had a dietary choice.

Because exposure to the acute stressor took place during weeks three through five of each dietary phase, additional regression analyses were performed to assess whether the duration of availability of the palatable diet prior to stressor exposure predicted cortisol values at baseline, immediate post-stressor, 1-hour post-stressor, and 4-hours post-stressor cortisol values as well as change from baseline to immediate post-stressor, change from baseline to 1-hour post-stressor, and change from baseline to 4-hours post-stressor. The predictive value of the duration of availability of the palatable diet prior to stressor exposure was also assessed with regard to total caloric intake and caloric intake from each available diet at each hourly interval following stressor exposure. P-values < 0.05 were considered significant.

Results

Food Intake

Exposure to the acute stressor significantly altered food intake relative to the control condition ($F_{1,36} = 47.341$, $p < 0.001$). A significant rank by experimental condition interaction ($F_{2,36} = 9.123$, $p = 0.001$, Figure 4.1) revealed that high ranking and mid ranking animals consumed significantly more calories ($p=0.044$ and $p=0.016$, respectively) than low ranking animals during the control condition. However, following exposure to the stressor, caloric intake did not differ as a function of social status ($p \geq 0.243$) due to significant reductions in caloric intake among high ranking ($p<0.001$) and mid ranking ($p<0.001$) animals compared to the control condition. Low ranking animals did not alter caloric intake in response to the stressor ($p=0.514$). A significant main effect of dietary phase ($F_{2,72} = 62.617$, $p<0.001$) also emerged. Animals consumed significantly more calories in Phase 2 relative to Phase 1 ($p=0.028$) and Phase 3 ($p<0.001$) and significantly fewer calories in Phase 3 relative to Phase 1 ($p<0.001$). However, there was no two-way interaction between experimental condition and dietary phase ($p=0.575$) nor was there three-way interaction between experimental condition, dietary phase, and social status ($p=0.799$).

During the dietary choice phase, Phase 2, a significant main effect of diet emerged ($F_{1,39} = 24.300$, $p < 0.001$, Figure 4.2) in that all animals preferred the palatable diet and consumed significantly more kilocalories from the palatable diet relative to chow. Consumption of either diet did not differ as a function of rank category ($p=0.842$) and there was no significant interaction between diet and experimental condition ($p=0.562$), suggesting that both dominant and subordinate subjects preferred the palatable diet and

did not alter dietary preferences following the acute stressor relative to control conditions.

Behavioral Outcomes

Analyses of behavior during the HI task revealed significant effects of diet phase on anxiety behavior ($F_{2,74} = 8.762$, $p<0.001$) and behavioral inhibition ($F_{2,74} = 12.608$, $p<0.001$) (Table 4.1). Rates of anxiety behavior were highest during Phase 1 (6.452 ± 1.031) compared to Phase 2 (3.156 ± 0.807, p=0.001) and Phase 3 (3.234 ± 0.911, p=0.005). In each case, there was no main effect of rank category or rank by diet phase interaction (all $p > 0.29$). Durations of behavioral inhibition were lower during Phase 1 (12.586 ± 0.751) relative to Phase 2 (15.701 ± 1.026, p=0.001) and Phase 3 (16.319 ± 0.775, $p<0.001$). A significant main effect of rank category emerged ($F_{2,37} = 4.587$, p=0.017, Table 4.1) in that mid ranking animals demonstrated greater durations of behavioral inhibition (17.828 ± 1.273) than high ranking (12.570 ± 1.227, p=0.005) but not low ranking (14.207 ± 1.273, p=0.052) animals independent of dietary phase.

Cortisol Responsivity

Exposure to the acute stressor significantly elevated serum cortisol in each dietary phase (Fig 1, $F_{3,111} = 368.320$, $p < 0.001$, Figure 4.3). Post-stressor cortisol was significantly elevated relative to baseline levels ($p<0.001$). One hour following exposure to the acute stressor, cortisol levels significantly decreased ($p<0.001$) from levels measured immediately post-stressor but remained significantly elevated relative to baseline. At 4 hours post-stressor, cortisol levels were significantly lower ($p<0.001$) than levels measured at all previous time points. A significant main effect of dietary phase emerged in that cortisol levels, collapsed across time, were significantly lower in the final chow-

only dietary phase relative to the first two dietary phases ($F_{2,74}$ = 32.827, $p < 0.001$). Furthermore, a significant diet phase by time interaction emerged ($F_{6,222}$ = 4.605, $p < 0.001$). Post-hoc pairwise comparisons revealed that cortisol levels were significantly lower at all four time points in Phase 3 relative to the first two phases ($p<0.001$). Additionally, cortisol levels at four-hours post-stressor exposure were significantly lower in Phase 2 relative to Phase 1 ($p=0.047$). Serum cortisol did not differ by rank category ($p=0.337$) nor was there significant interaction between rank category and sampling interval ($p=0.919$) or diet phase ($p=0.861$).

An additional analysis assessing change in cortisol, rather than absolute concentrations, from baseline to measures at the three subsequent sampling time points revealed a significant effect of dietary phase on cortisol reactivity ($F_{2,76}$=4.116, $p=0.020$, Figure 4.4) in that animals had a greater elevation in cortisol relative to baseline levels during phase 3, when a chow-only environment was reestablished, relative to phase 1 ($p=0.015$) but not relative to phase 2 ($p=0.060$). Additionally, animals demonstrated more rapid return to baseline at 4 hours post stressor during the dietary choice phase ($F_{2,74}$ =8.554, $p<0.001$) relative to both phase 1 ($p=0.016$), the initial chow-only phase, and phase 3 ($p<0.001$), the final chow-only phase (Fig 4.4).

Linear regression revealed that baseline cortisol, but not cortisol measures at any subsequent post-stressor time point, significantly predicted cumulative caloric intake from the palatable diet during the 24 hours following exposure to the stressor ($r=0.39$, $p=0.010$, Figure 4.5). Cortisol measures were not predictive of chow intake during the 24-hour post-stressor interval during any dietary phase ($p>0.056$) nor were cortisol measures predictive of total caloric intake during the dietary choice condition ($p>0.084$).

Duration of exposure to the palatable diet prior to exposure to the HI acute stressor ranged from 14 to 30 days (22.25 ± 0.71 days) and was not predictive of cortisol values at any time point assessed during the choice dietary phase nor was it predictive of change in cortisol from baseline at any time point assessed ($p>0.185$). Additionally, weeks palatable diet availability prior to exposure to the acute stressor was not predictive of cumulative caloric intake from the palatable diet ($p>0.053$) or the chow diet ($p>0.156$) at any time interval assessed in the 24-hour period following stressor exposure.

Discussion

The present study examined the hypothesis diet availability would determine caloric consumption following an acute stressor and that this would vary by social status. The results provided only partial support for this hypothesis. During control conditions the lowest ranking animals consumed significantly fewer calories than the high and mid ranking animals regardless of dietary phase. However, following all three exposures to the acute stressor, high and mid ranking animals significantly reduced caloric intake while the lowest ranking animals did not alter their dietary patterns, eliminating detectable differences in caloric intake as a function of social status during the 24-hours post-stressor. Although exposure to the acute stressor did not increase total caloric intake during the dietary choice condition, higher baseline cortisol, a marker of basal stress axis activity, significantly predicted greater consumption of the palatable diet in the 24 hours following exposure to the acute stressor. Additionally, animals with access to the palatable diet, presented as a choice, demonstrated a more rapid return to baseline cortisol concentrations following exposure to the acute stressor compared to dietary phases 1 and 3, when only a chow diet was available. Behavioral responses to the HI task differed

across dietary phases in that females exhibited significantly fewer anxiety behaviors and significantly greater behavioral inhibition during the second and third exposures to the stressor relative to the first. However, repeated exposure to the HI task consistently evoked robust activation of the LHPA axis, suggesting that animals did not habituate to the stressor.

The findings that low ranking animals did not alter their food intake in response to the stressor and that stressor exposure reduced food intake among high ranking and mid ranking animals, regardless of the availability of palatable food, did not support our hypothesis that stressor exposure would result in increased caloric intake when animals had access to the preferred, more calorically dense food. Additionally, the observation that post-stressor caloric intake did not differ from control conditions among the lowest ranking animals also contradicted our hypothesis that lower ranking animals would be most affected by the acute stressor. Expectations were based on previous observations that prior stressor exposure or adverse circumstances increase the behavioral and neurochemical impact of subsequent stressors (191, 192). However, there are circumstances in which prior stress experience blunts the impact of subsequent stressors (193).

The present results shed light on the potential importance of the intensity and duration of stressors in shaping effects on food intake. Indeed, severe, acute stressors have induced hypophagia even when a palatable diet is available among humans and animals (68, 185, 194). Further, while the primary objective of this investigation focused on the effects of acute stressor exposure on short-term caloric intake, the observation that the lowest ranking animals were significantly hypophagic relative to high and mid ranking animals

during the control condition warrants discussion. Although, investigations into the effects of chronic social subordination stress on food intake within a rich dietary environment are few, investigations evaluating these effects on other appetitive behaviors, including self administration of drugs of abuse and ethanol, have highlighted the importance of the frequency or intensity of the stressor on neural adaptation to the stressor and the subsequent behavioral response (181). For example, studies of male rats show brief, yet recurring episodes of social defeat stress escalate and prolong self-administration of addictive substances whereas continuous subordination stress leads to anhedonia-like effects (182). Thus, given that the animals used in the present study were members of newly formed groups, the lowest ranking animals in this investigation may have been demonstrating classical anhedonia as a consequence of repetitive, unrelenting reinforcement of their subordinate status and were thus unaffected by the additional exposure to the acute stressor. This response contrasts the subordinate phenotype of excess caloric intake in a choice dietary environment shown by subordinates in long-term, stable groups (120).

Despite an overall decrease in food intake among high ranking and mid ranking animals in response to the stressor during the dietary choice phase, baseline cortisol levels, but not cortisol reactivity, positively predicted consumption of the palatable diet in the hours following exposure to the acute stressor regardless of social status. These findings suggest that elevated basal glucocorticoids play a significant role in acute stress-related preference for palatable foods (195). Thus, it is possible that chronic stressor exposure may interact with acute stressor exposure to influence short-term eating behaviors. Indeed, neuroimaging among humans has demonstrated that amygdala and orbitotfrontal

cortex responses to palatable foods following acute stressor exposure are dependent upon baseline cortisol levels, an indicator of chronic stressor exposure (196). Assessment of the relationship between baseline cortisol and 24-hour food intake in the absence of acute stressor exposure would have permitted the determination of whether such interaction exists. However, the present sampling protocol did not facilitate determination of this effect.

The mechanisms through which stress affects ingestive behaviors are not fully understood. Nonetheless, an additional mechanistic hypothesis linking consumption of highly palatable foods to stressor exposure suggests that this coping mechanism directly attenuates LHPA activation (67). The observation that animals demonstrated a more rapid decrease in cortisol levels in the presence of a palatable diet supports this hypothesis. However, general findings regarding the effects of diet composition on stress-responsivity have been inconsistent. Data from some rodent studies indicate that exposure to a palatable diet directly reduces acute LHPA axis activation and adverse behavioral effects following acute stress exposure (173-175, 188). Conversely, other rodent data demonstrate ingestion of a high-fat, high-sugar diet increases both basal and stress-induced LHPA activity (11, 72). Conflicting results of these studies may be a result of variations in diet composition. Additionally, the effects of diet composition on stress responsivity may be contingent upon the duration of exposure to the experimental diet. For example, among male rats, one week of exposure to a high-fat diet resulted in elevated corticosterone secretion in response to restraint stress relative to chow-fed controls. However, this effect disappeared when animals were exposed to a second stressor after nine weeks of exposure to the diet (71). Likewise rats fed a high-fat diet for

four days showed an exaggerated corticosterone response to an intraperiotoneal injection of saline and relocation to a novel cage relative to rats fed a low-fat diet. However, this effect also appeared to be transient as no elevations were noted after 23 days of exposure to the high-fat diet (72). Animals in this study were exposed to the acute stressor following, on average 22.25 ± 0.71 days (range 14 to 30 days access to the palatable diet. However, regression analyses showed that duration of exposure to the palatable diet prior to stressor exposure was not a significant predictor of cortisol values or change in cortisol from baseline levels at any time point assessed.

Of final note, although animals demonstrated significantly greater anxiety behaviors during the first exposure to the HI task relative to the second and third diet phases, the LHPA axis was nonetheless robustly activated following each acute stressor exposure. Previous work with rats has produced similar results in that progressive familiarization of the animals with the novel environment reduced stress-related behaviors across repeated trials. However, repeated trials did not appear to reduce the stressful properties of the situation as evaluated by ACTH release (197). Thus, these results and the present findings highlight the importance of incorporating objective measures of the physiological stress response into all investigations attempting to delineate the neurobiological mechanisms underlying stressor-induced alterations in dietary patterns.

Conclusion

Findings from the present study suggest that stressor intensity, normally gauged by the level of LHPA activation (6), and duration may be critical factors in the bidirectional relationship between stressor exposure and food intake given that the lowest ranking animals were hypophagic relative to more dominant animals during control conditions

and exposure to the acute psychosocial stressor induced hypophagia among high and mid ranking animals regardless of the dietary condition. Additionally, investigations into the short-term effects of acute stressors on behavioral outcomes must consider the subjects' concurrent chronic stressors and history of stressor exposure. Animals in this experiment were undergoing the process of new group formation, which is a significant chronic stressor for all animals in the group (91). Results may have been quite different if subjects were members of stable social groups in which more dominant animals experience greater control and predictability (48) and prolonged social subordination can result in LHPA dysregulation, evidenced by reduced GC negative feedback and hypercortisolemia (83, 89-93).

The finding that basal cortisol levels were predictive of caloric intake from the palatable diet following exposure to the acute stressor supports a role of glucocorticoids in the stressor-induced consumption of comfort foods. Further, enhanced recovery following exposure to the acute stressor during the dietary choice condition suggests that occasional indulgence in highly palatable foods in response to a stressful event may be an advantageous coping strategy. Perhaps acutely, consumption of palatable foods attenuates the LHPA axis and may be beneficial in response to isolated, intermittent stressors while habitual consumption of highly palatable, energy-dense foods in response to chronic low-grade stressors may promote excess energy consumption and the accumulation of body fat. Longitudinal studies that incorporate assessment of transitional effects of acquired social subordination stress combined with repeated measures of caloric intake, weight gain, and stress responsivity among animals with access to a palatable diet can help address the remaining questions related to stress-induced emotional eating and may

ultimately inform strategies to circumvent undesirable behavioral and physiological consequences of stressor exposure.

Table 4.1
Behavioral inhibition (duration, minutes) and anxiety (frequency) behavior in response to the HI task as a function of dietary phase and rank category. Bold values are statistically significantly different from values in the same category at the p<0.01 (**) or p<0.05 (*) level. Results are presented as mean (±SEM).

Rank Category	Phase 1 (no choice)		Phase 2 (choice)		Phase 3 (no-choice)		All Phases	
	Anxiety (count)	**Freezing (duration)**	**Anxiety (count)**	**Freezing (duration)**	**Anxiety (count)**	**Freezing (duration)**	**Anxiety (count)**	**Freezing (duration)**
High Rank	7.357 (1.742)	11.359 (1.269)	3.929 (1.364)	13.071 (1.734)	3.857 (1.540)	13.281 (1.309)	5.048 (1.287)	12.570** (1.227)
Mid Rank	7.692 (1.808)	14.416 (1.317)	3.692 (1.415)	19.292 (1.799)	4.308 (1.598)	19.776 (1.358)	5.231 (1.335)	**17.828** (1.273)
Low Rank	4.308 (1.808)	11.982 (1.317)	1.846 (1.415)	14.739 (1.799)	1.538 (1.598)	15.901 (1.358)	2.564 (1.335)	14.207 (1.273)
Total	**6.452** (1.031)	**12.586** (0.751)	3.156** (0.807)	15.701* (1.026)	3.234** (0.911)	16.391** (0.775)		

Figure 4.1
Caloric intake in the 24-hours post-stressor exposure compared to the average of two comparable 24-hour control periods stratified by social status and dietary phase. An asterisks (*) indicates a statistically significant difference ($p<0.05$) between rank categories for the given experimental condition.

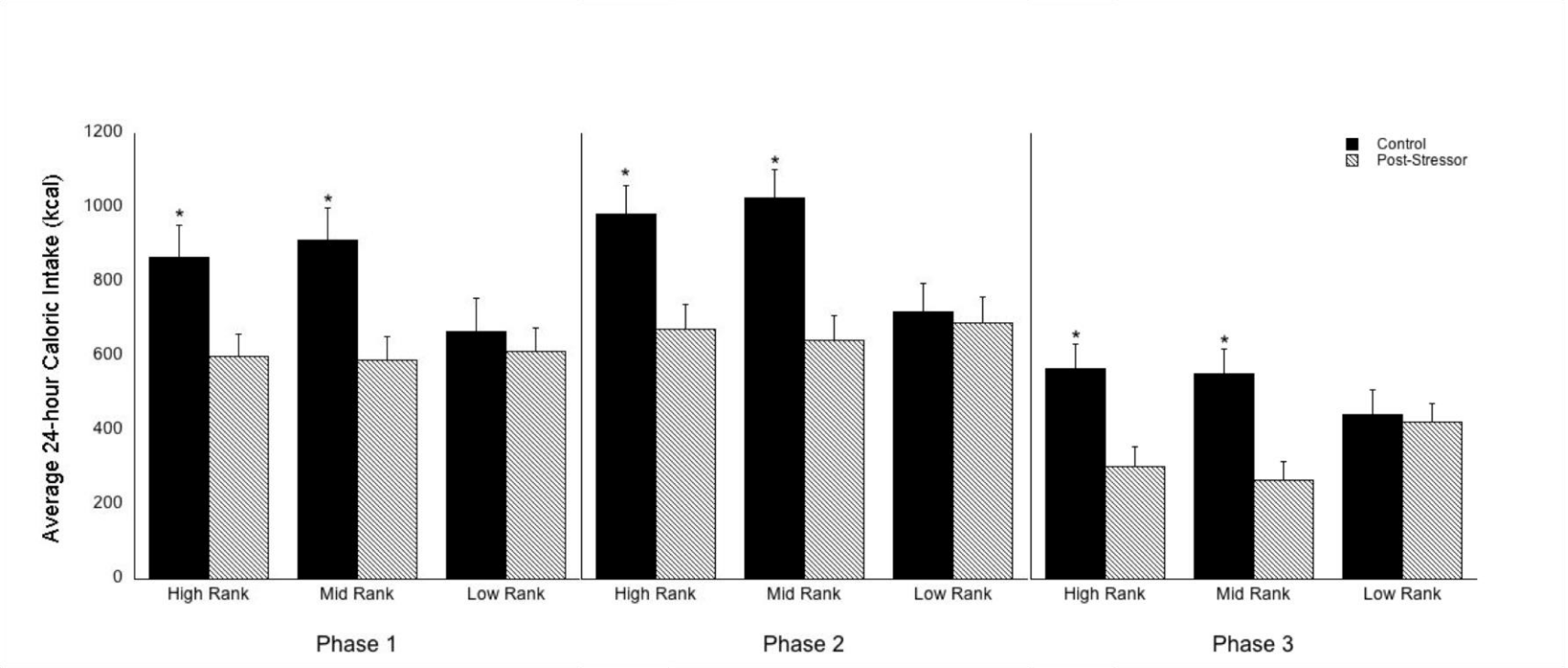


Figure 4.2
Caloric intake during the dietary choice phase (Phase 2) in the 24-hours post-stressor exposure compared to the average of two comparable 24-hour control periods stratified by diet and social status. An asterisks (*) indicates a statistically significant difference ($p<0.05$) in calories consumed from each diet. Different alphanumeric characters indicate statistically significant differences between experimental condition.

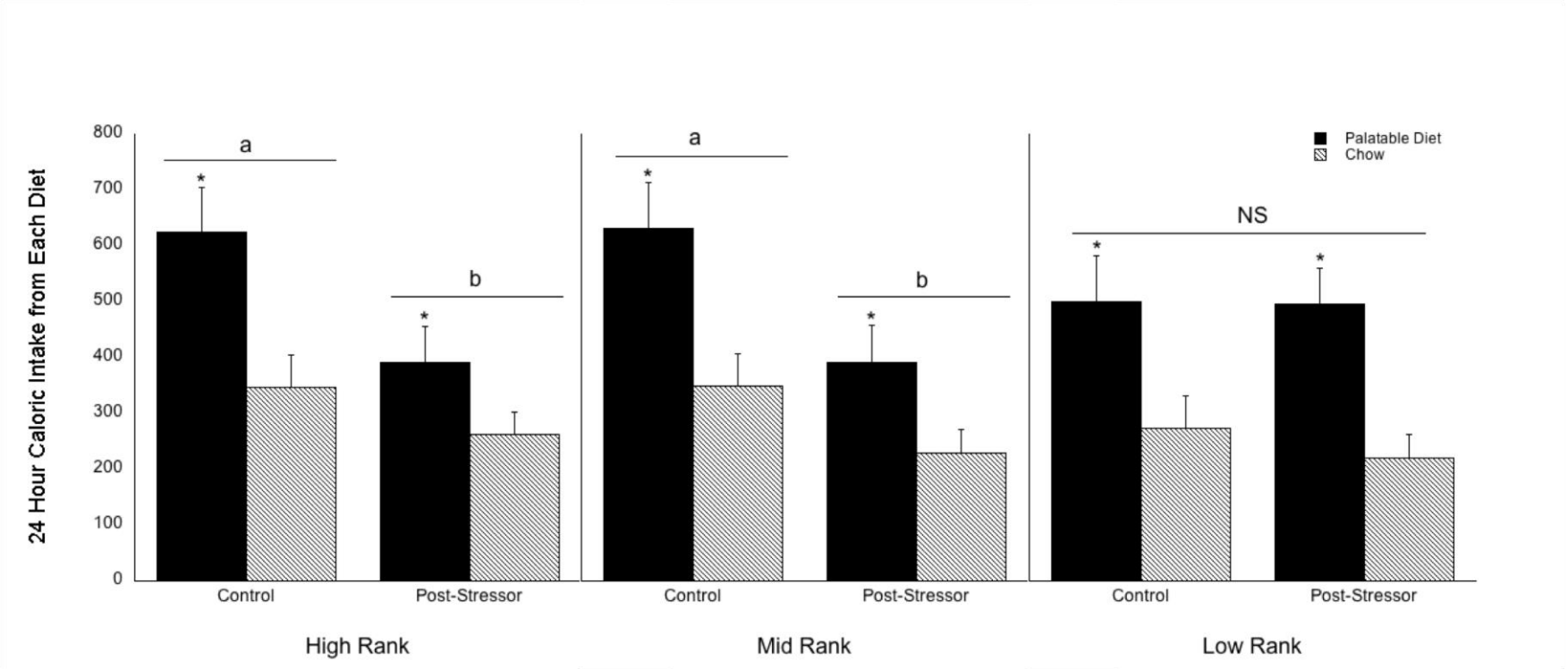


Figure 4.3
Serum cortisol levels (μg/dL) pre-stressor (baseline), immediate post-stressor (30 min), and 1-hour and 4-hours post-stressor stratified by dietary phase and collapsed for rank category. Results are presented as the mean ± SEM. Different alphanumeric characters indicate statistically significant differences ($p<0.05$) between dietary phases. Different typographical symbols indicate statistically significant differences ($p<0.05$) between sampling intervals.

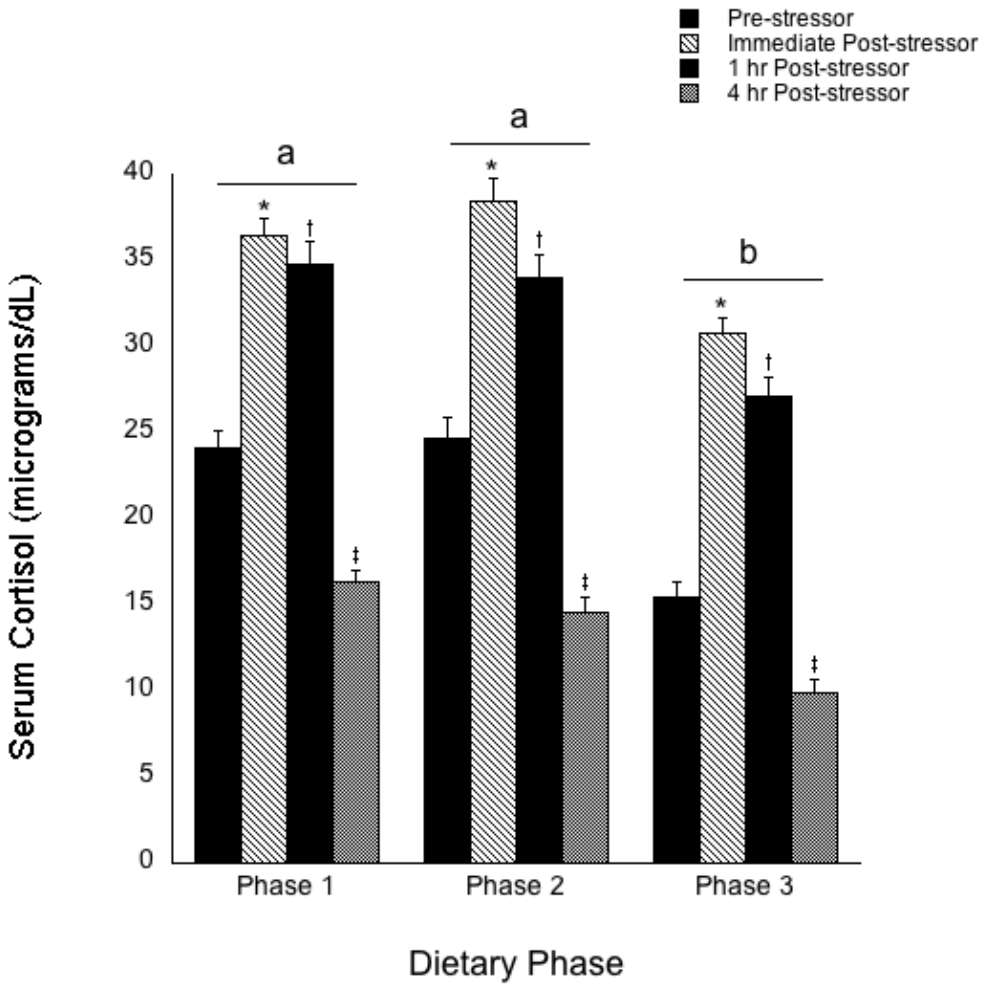


Figure 4.4
Change in serum cortisol levels (μg/dL) immediately following exposure to the acute stressor (30 min) and at 1-hour and 4-hours post-stressor exposure relative to baseline levels (pre-stressor exposure), stratified by dietary phase and collapsed for rank category. Results are presented as the mean ± SEM. Different alphanumeric characters indicate statistically significant differences ($p<0.05$) between dietary phases.

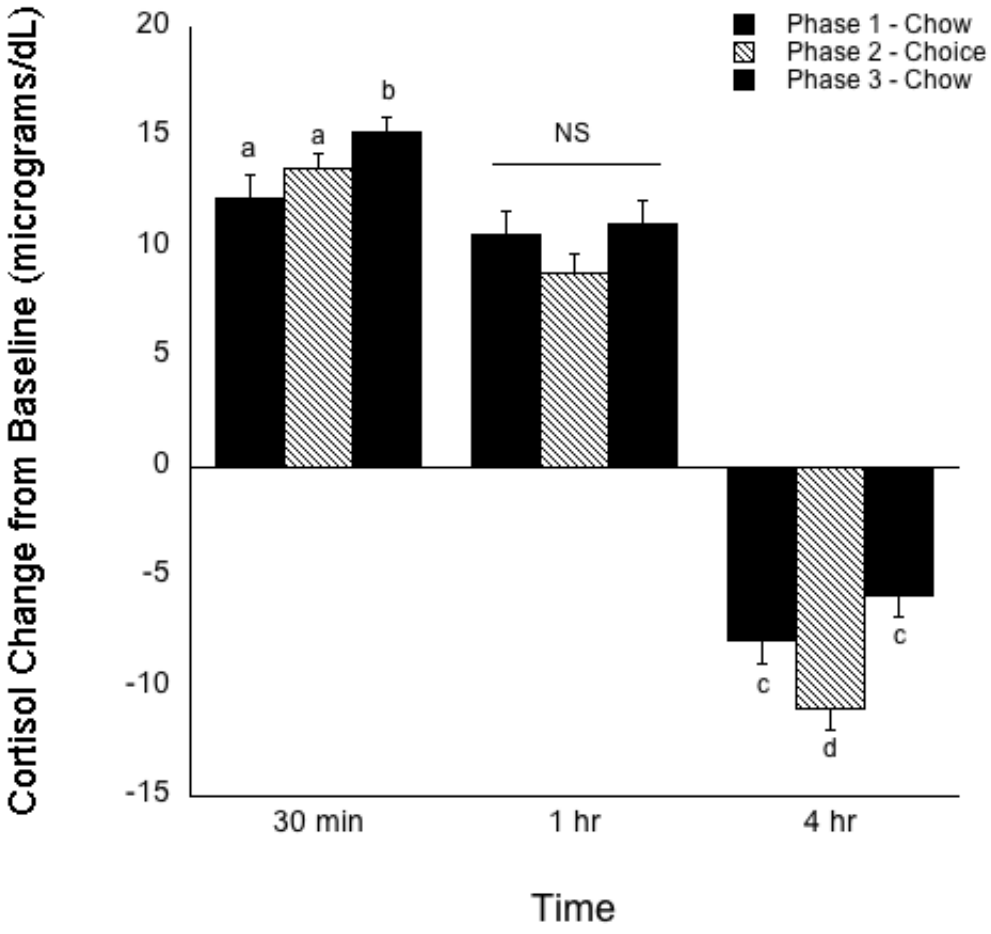
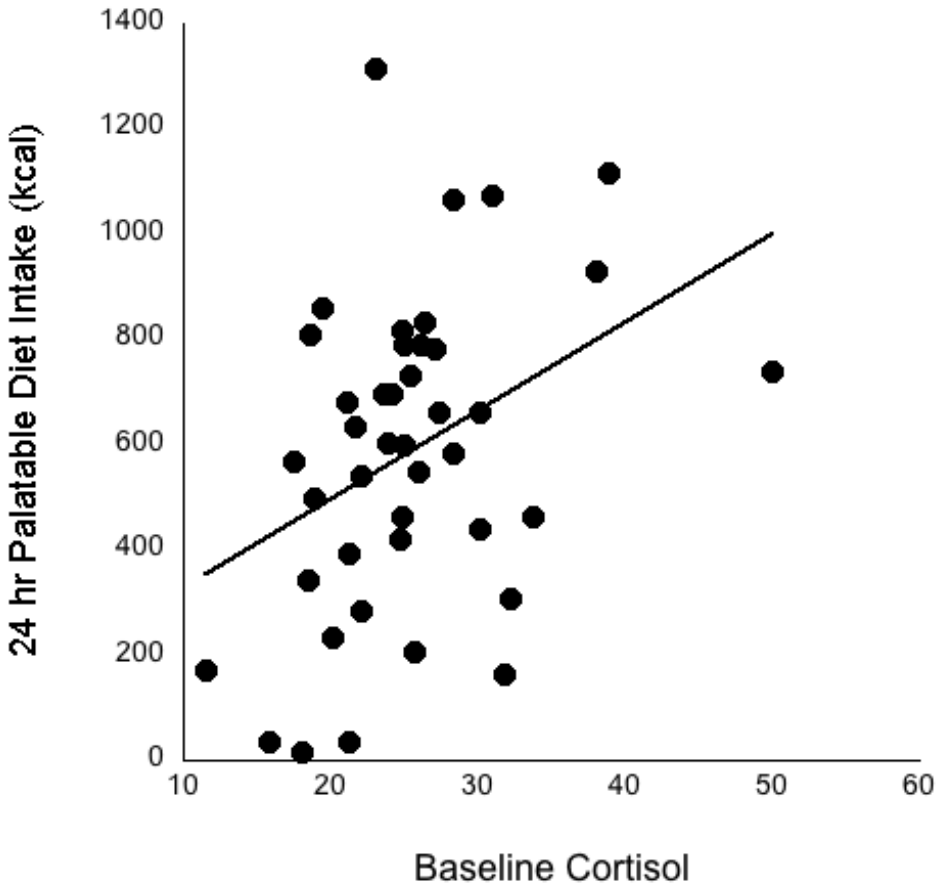


Figure 4.5
Correlation of baseline cortisol (μg/dL) with caloric intake from the palatable diet in the 24 hours following exposure to the acute stressor during the choice dietary condition.



CHAPTER 5

Summary of Findings

The factors that account for the emergence of an obese phenotype are vast and complex. Importantly, factors that promote eating in the absence of hunger have garnered substantial attention in both the scientific community as well as the mainstream media, and the phenomenon colloquially termed "stress-eating" has been investigated as a possible contributor to the current obesity epidemic (4). Findings presented in this collection of work using adult female rhesus monkeys as a model for women were designed to fill existing gaps in our understanding of how stressor exposure initiates and sustains emotional eating in females.

Several of the findings reported herein support a number of the prevailing hypotheses regarding the relation of stress and appetite and its impact on body composition. With respect to the data presented in Chapter 2, the observation that caloric intake was attenuated following administration of a CRF_1 antagonist among long-term, subordinate females living in stable social groups in a rich dietary environment supports the involvement of activation of central CRF_1 receptors for sustaining this stress-induced phenotype. These findings substantiate numerous epidemiologic studies that have linked chronic social stress with excess consumption of high caloric diets, obesity, and metabolic disorders (157-161). In addition, data from Chapter 4 showing that basal cortisol levels were predictive of caloric intake from the palatable diet in the 24-hour interval following exposure to an acute stressor support the well-established role of glucocorticoids in stressor-induced consumption of comfort foods (58, 59). Further, enhanced recovery following exposure to the acute stressor during the dietary choice

condition supports the mechanistic hypothesis that ingestion of sweet, high-fat food directly attenuates LHPA activation (67, 173-175, 188). Notably, however, findings with regard to this particular hypothesis have been inconsistent and some data actually suggest that consuming energy dense, palatable diets may increase basal and stress-induced LHPA activity (11, 14, 72).

Conversely, some investigational outcomes of the present series of studies were unexpected, particularly with regard to food intake and changes in body weight among animals in newly formed social groups, as reported in Chapter 3. Although the observed weight loss among all animals following group formation is consistent with previous data indicating that new social organization is initially a stressor for all animals involved (65, 91), we anticipated that clear effects of social subordination would emerge with regard to caloric intake and weight gain in the presence of a highly palatable diet once social hierarchies were clearly established. However, when the palatable diet was introduced, all animals consumed significantly more calories and increased body weight regardless of social status. This finding contradicted established work (14, 15) and findings from our pharmacological intervention in Chapter 2 that utilized long-standing stable social groups. In these long-established groups, subordinate animals became significantly hyperphagic in the presence of a palatable diet, high in fat and sugar, while dominant animals regulated caloric intake regardless of the dietary environment (14, 15). Further, the status differences that did emerge within newly formed social groups were opposite our hypothesis, in that the most dominant animals gained significantly more weight during the choice dietary condition relative to the lowest ranking animals, and the lowest ranking animals weighed significantly less than high and mid ranking animals at the end

of each dietary phase. These findings suggest that the lowest ranking animals may have been demonstrating classical anhedonia as a consequence of repetitive, unrelenting reinforcement of their subordinate status, similar to the response demonstrated among male rodents exposed daily to a new more dominant animal (182). These divergent results, coupled with behavioral observations of elevated anxiety-like behaviors among dominant and subordinate animals relative to subjects in stable groups, suggest that the recency of social disruption and reorganization may be a stressor for all involved. The lack of status-related differences in cortisol responsivity or recovery in response to an acute stressor at any point throughout the course of the study further supports this explanation. Thus, for these rhesus monkey groups, reorganization of social groups can function as a significant stressor for even the most dominant subjects within a social hierarchy until a greater sense of control and predictability is established (48).

Although these findings were unexpected, they are certainly not without significant value. A recent systematic review that assessed the strength of evidence linking social subordination within nonhuman primate societies to greater risk of cardiovascular disease concluded that the extent to which social dominance, and thus relatively less social stress, was protective against heart disease is limited at best (198). However, the majority of reviewed studies that contradicted the hypothesized protective effect of social dominance on cardiovascular disease were conducted in social groups that were undergoing or had recently undergone social reorganization (198), a situation directly comparable to the model used in Chapters 3 and 4. While there is substantial evidence that prolonged social subordination within stable social hierarchies results in LHPA axis dysregulation, evidenced by reduced GC negative feedback and hypercortisolemia (83, 89-93), the point

at which status-related differences emerge with regard to stress-dependent phenotypes, including emotional feeding, has yet to be determined. Additionally, it is unknown whether identifiable individual differences, such as a history of adverse social experience or a particular polymorphism in genes related to stress physiology, predispose some individuals to becoming anhedonic while others become hyperphagic in response to social stress. Clearly, the broader implications of these findings illustrate that prospective studies must ensure that interventions that are designed to impose different stress phenotypes are accurately defined and measured in order to draw meaningful conclusions.

Finally, the finding in Chapter 4 that high and mid ranking animals significantly reduced caloric intake in response to the acute stressor while the lowest ranking animals did not are at odds with our hypothesis that animals would be hyperphagic in a rich dietary environment following exposure to the stressor as well as human laboratory studies showing that high cortisol reactivity to an acute stressor promotes increases in post-stressor caloric intake – particularly from high-fat, sweet foods – relative to control conditions (44, 195). However, collective evidence from animal and human studies indicates that stressor exposure may induce decreases as well as increases in food intake (6), and stressor intensity, normally gauged by the level of LHPA activation, may be a critical factor in the bidirectional relationship between stressor exposure and food intake. Nonetheless, although animals did not increase caloric intake following exposure to the acute stressor, higher baseline cortisol, a marker of basal stress axis activity, significantly predicted greater consumption of the palatable diet in the 24 hours following exposure to the acute stressor. Taken together, these results support the notion that people may shift

preferences to sweet, high-fat snack foods and potentially forgo traditional meals in response to stressors (157-161), which may or may not lead to significant alterations in total caloric intake.

Strengths of Study Design

The present work is greatly strengthened by the use of an ethologically relevant model of chronic, psychosocial stress. Indeed, any investigation of the biobehavioral effects of stress as it relates to the development of human disease should ultimately focus on stressors that are likely to be shared by humans. Notably, the psychosocial stress imposed by social subordination in macaques provides a translational model for understanding the health burden imposed by stress in humans (118, 199), and the critical feature of this model is the psychogenic nature of the stressor (85-87). Additionally, the use of female subjects makes a significant contribution to the existing literature since animal modeling of stress-induced alterations in food intake generally use male subjects (78) despite the growing evidence highlighting significant gender differences in the stress-eating-obesity relationship (9-12). Further, the effects of acute and chronic stressor exposure on subsequent caloric intake were explored in the context of both a standard laboratory chow environment and a dietary environment that provided additional access to highly palatable foods. This is critical given that humans are embedded within a rich dietary environment, and rodent studies often ignore potential interaction between stressor exposure and the availability of palatable food.

The present study design also circumvented many of the limitations of the human literature since longitudinal studies of this magnitude cannot be conducted in human populations due to practical and ethical issues related to stressor exposure (7) and

limitations of current dietary assessment methods (43). The continuous quantification of caloric intake in the present investigation permitted assessment of causal relationships between stressor exposure, food intake, and changes in body weight. Many epidemiological studies exploring the association between perceived stress and dietary patterns and/or obesity among humans are cross-sectional. Because obesity results from a prolonged period of positive energy imbalance, assessment of dietary behaviors at a single point in time does not permit inferences related to diet and obesity (166). Additionally, even longitudinal investigations among people are limited by the failure of current dietary assessment methods to accurately quantify long-term dietary intake (43). Given these limitations of dietary assessment methods, some human investigations have simply assessed indicators of diet quality (e.g., low fruit and vegetable consumption, high fat intake) and dietary behaviors (e.g., skipping breakfast) as potential correlates of stressor exposure. While it appears that stress may lead to consumption of less nutritionally balanced diets, we cannot assess whether diet mediates a relationship between stress and obesity because it is possible to consume energy-dense foods without exceeding daily caloric intake recommendations (166).

An unexpected strength of the present studies was the realization that consequences of social subordination in this nonhuman primate species are complex. The present results suggest that the use of this model provides a unique opportunity to systematically investigate the neurobiological mechanisms of the emergence of stress-induced phenotypes from anhedonia through addiction, including emotional feeding. Although the long-term effects of social stressors on food intake have not been well explored, particularly within the context of a rich dietary environment, a series of well-designed

rodent studies has demonstrated the significance of the intermittency and duration of social stressors in shaping other appetitive behaviors including self-administration of cocaine and consumption of ethanol (180-182). Specifically, continuous, unrelenting social subordination leads to anhedonia-like behavioral profiles while brief, intermittent episodes of defeat stress are associated with increased appetite for and consumption of pleasurable substances (180-182). It is plausible that social conditions within newly formed social groups resemble the chronic, unrelenting social subordination stress demonstrated in these rodent studies while conditions within stable groups that produced excess consumption of a palatable diet among socially subordinate animals may be more analogous to episodic social defeat stressors. However, future studies are warranted to assess neurobiological and behavioral measures to support or disprove this theory.

Study Limitations

Despite the strengths of these investigations, no model is without limitations (142). In the pharmacological intervention discussed in Chapter 2, peripheral effects of Antalarmin on serum cortisol were not measured. Thus, we cannot determine whether the attenuation of caloric intake among subordinate animals treated with Antalarmin resulted from antagonism of central CRF_1 receptors alone or changes in peripheral cortisol release. Additionally, animals used in this study were ovariectomized and were untreated during the feeding assessments. Both estradiol and progesterone are known to affect appetite and meal size (152-154). Thus, the generalizability is somewhat limited.

New group formation, detailed in Chapter 3, appeared to be a stressor for all animals involved regardless of social status. Thus, the lack of a no stress or minimally stressed group prevented the determination of independent and/or synergistic effects of stressor

exposure and the dietary environment on food intake among these subjects. Specifically, the increase in caloric intake among all animals during the dietary choice condition when the palatable diet was available may have been a result of stress-induced over indulgence in "comfort foods." Indeed, we hypothesized that this interaction would emerge among socially subordinate females in this environment, and we speculate that with continued time, dietary patterns of dominant and subordinate animals would diverge and stress-induced emotional feeding would be pronounced in subordinate females. Conversely, however, the greater energy density of the preferred diet may have simply compensated for the reduction in appetite characteristic of stress-induced anhedonia. Lack of neuroimaging and repeated measures of cerebrospinal fluid levels of CRF and plasma concentrations of ACTH, and cortisol limit our ability to draw conclusions regarding the mechanistic underpinnings of these results, and future studies must employ these methods to clearly delineate when status-related differences in LHPA axis function and neural reward circuitry emerge among socially housed rhesus monkeys.

In response to the acute stressor in Chapter 4, high ranking and mid ranking animals decreased food intake and low ranking animals did not alter caloric intake regardless of diet availability. Importantly, caloric intake for the subordinate females following the acute stressor was not different compared with the control period. However, during the dietary choice phase, baseline cortisol levels, but not cortisol reactivity, positively predicted consumption of the palatable diet in the hours following exposure to the acute stressor. These findings suggest that elevated basal glucocorticoids play a significant role in acute stress-related preference for palatable foods, indicating that chronic stressor exposure may interact with acute stressor exposure to influence short-term eating

behaviors. Assessment of the relationship between baseline cortisol and 24-hour food intake in the absence of acute stressor exposure would have permitted the determination of whether such interaction exists. However, the present sampling protocol did not facilitate determination of this effect.

Finally, there are cognitive and behavioral factors that are relevant to human dietary patterns that cannot be modeled in animals. The social desirability of a shapely physique drives many individuals to consciously restrict dietary intake through various means in an effort to achieve or maintain a desired body weight, a practice known as dietary restraint or "dieting" (32). Despite a lack of consistent general effects regarding the influence of stress on food intake, some intriguing individual differences have emerged, particularly among women, regarding interaction between stress and dietary restraint. Specifically, within non-clinical populations, cognitive dietary restraint is a consistent predictor of overeating in response to laboratory stressor exposure (61). Additionally, emotional eating may be a conscious coping strategy that is driven by cultural norms, and learned associations may actually be reinforced through synaptic plasticity to become unconscious habits (200). While these psychological factors are acknowledged as a limitation, they can also be viewed as strengths in that animal modeling permits the evaluation of the neurobiology underlying stress-induced alterations in food intake without these potential confounding factors.

Broader Implications of Study Findings

Findings from the present investigations highlight the bidirectional relationship between stressor exposure and food intake, which has been well established but remains poorly understood (6). Thus, given that stressor exposure can induce increases or decreases in

food intake, the direct implications of this work with regard to policy and interventions targeting obesity treatment and prevention strategies are not fully evident. Given the complexity of systems and contexts that drive eating behavior, delineating the influence of any single factor on emergent dietary patterns is difficult, if not impossible. Thus, investigations that attempt to investigate food intake as a function of a manipulated exposure must interpret results within the context of all synergistic and competing factors that influence appetite.

The superficial conclusion from investigation demonstrating that caloric intake was attenuated among subordinate animals in a rich dietary environment following administration of a CRF_1 antagonist (Chapter 2), assuming results could be replicated with a longer duration of treatment, is that pharmacological alleviation of stress may attenuate appetite and hold promise as a potential treatment for obesity among individuals reporting high levels of stress exposure. Indeed, pharmacological treatment with agents such as sleep aids, anxiolytics, and antidepressants holds value for addressing the negative consequences of chronic stressor exposure. However, these therapies come with significant side effects. Any drug treatment may either inhibit the beneficial effects of certain pathways or perturb other systems that interact with modified systems in a manner that promotes unwanted side effects (42). Thus, the more holistic or practical approach to incorporating findings from this investigation is to evaluate the efficacy of alternative stress management techniques in reducing emotional eating. For example, cognitive behavioral therapy, physical activity, and the provision and/or receipt of social support, have been shown to attenuate the stress response in laboratory settings (201).

Findings from investigations among newly formed social groups (Chapters 3 and 4), clearly demonstrated the bidirectional nature of the stress-appetite association. Thus, severe, acute stressors or unrelenting stressors may in fact reduce appetite and, in a sense, be protective against obesity. However, we must not get so focused on a single outcome that we ignore the damaging effects of stressor exposure on other systems and conditions, including mental illness, stroke and cardiovascular disease, reproductive dysfunction, and impaired immune function (55). Additionally, stress-induced anhedonia can be detrimental to health as it may lead to malnutrition and increased risk of illness, complications, and mortality among high-risk populations including elderly adults and individuals with certain chronic diseases (183).

In formulating strategies to reduce the damaging health effects of social stress, it is important to recognize that stressor vulnerability is as important as stressor exposure in shaping outcomes. Stress-related vulnerability is determined by genetic, biobehavioral, and environmental factors that interact over time to influence individual risk trajectories (202). Further, stressors can be perceived as "stimulating," "tolerable," or "toxic" depending upon the degree to which an individual has control over a given stressor and has support systems and resources in place for handling a given stressor (202). Thus, improving access to health care and supportive resources and promoting a greater sense of control and participation within society are potential, albeit abstract, strategies for reducing vulnerability to unavoidable stressors, which could potentially transform overwhelming, destructive stressors into tolerable inconveniences or, at best, stimulating challenges (7). In closing, while interventions that target dietary practices and physical activity levels have been the obvious targets for treatment and prevention of obesity,

findings in humans and animals suggest that other potential risk factors may work through diet and physical activity or through other means and should be incorporated into public health research (203). The collective work outlined in this composition highlights the complexity of the relationship between the physiological stress response and subsequent food intake, indicating that stressor type and duration are extremely important in shaping ingestive behaviors.

References

1. World Health Organization Obesity and Overweight Fact Sheet. 2011.
2. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA : the journal of the American Medical Association*. 2012; **307**(5): 491-7.
3. Adamczak M, Wiecek A. The adipose tissue as an endocrine organ. *Semin Nephrol*. 2013; **33**(1): 2-13.
4. Adam TC, Epel ES. Stress, eating and the reward system. *Physiol Behav*. 2007; **91**(4): 449-58.
5. Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, Bell ME, Bhatnagar S, Laugero KD, Manalo S. Chronic stress and obesity: a new view of "comfort food". *Proc Natl Acad Sci U S A*. 2003; **100**(20): 11696-701.
6. Maniam J, Morris MJ. The link between stress and feeding behaviour. *Neuropharmacology*. 2012; **63**(1): 97-110.
7. Scott KA, Melhorn SJ, Sakai RR. Effects of Chronic Social Stress on Obesity. *Current obesity reports*. 2012; **1**(1): 16-25.
8. Tamashiro KL, Nguyen MM, Sakai RR. Social stress: from rodents to primates. *Front Neuroendocrinol*. 2005; **26**(1): 27-40.
9. Kitraki E, Soulis G, Gerozissis K. Impaired neuroendocrine response to stress following a short-term fat-enriched diet. *Neuroendocrinology*. 2004; **79**(6): 338-45.
10. Soulis G, Kitraki E, Gerozissis K. Early neuroendocrine alterations in female rats following a diet moderately enriched in fat. *Cell Mol Neurobiol*. 2005; **25**(5): 869-80.
11. Soulis G, Papalexi E, Kittas C, Kitraki E. Early impact of a fat-enriched diet on behavioral responses of male and female rats. *Behav Neurosci*. 2007; **121**(3): 483-90.
12. Grunberg NE, Straub RO. The role of gender and taste class in the effects of stress on eating. *Health Psychol*. 1992; **11**(2): 97-100.
13. Cohen S, Janicki-Deverts D. Who's Stressed? Distributions of Psychological Stress in the United States in Probability Samples from 1983, 2006, and 2009. *J Appl Soc Psychol*. 2012; **42**(6): 1320-34.
14. Arce M, Michopoulos V, Shepard KN, Ha QC, Wilson ME. Diet choice, cortisol reactivity, and emotional feeding in socially housed rhesus monkeys. *Physiol Behav*. 2010; **101**(4): 446-55.
15. Michopoulos V, Toufexis D, Wilson ME. Social stress interacts with diet history to promote emotional feeding in females. *Psychoneuroendocrinology*. 2012.
16. Woods SC, Seeley RJ. Adiposity signals and the control of energy homeostasis. *Nutrition*. 2000; **16**(10): 894-902.
17. Lowe MR, Butryn ML. Hedonic hunger: a new dimension of appetite? *Physiol Behav*. 2007; **91**(4): 432-9.
18. Berthoud HR. Vagal and hormonal gut-brain communication: from satiation to satisfaction. *Neurogastroenterol Motil*. 2008; **20 Suppl 1**64-72.
19. de Graaf C, Blom WA, Smeets PA, Stafleu A, Hendriks HF. Biomarkers of satiation and satiety. *Am J Clin Nutr*. 2004; **79**(6): 946-61.

20. Harrold JA, Dovey TM, Blundell JE, Halford JC. CNS regulation of appetite. *Neuropharmacology*. 2012.
21. Inui A, Asakawa A, Bowers CY, Mantovani G, Laviano A, Meguid MM, Fujimiya M. Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ. *FASEB J*. 2004; **18**(3): 439-56.
22. Jackson HC, Needham AM, Hutchins LJ, Mazurkiewicz SE, Heal DJ. Comparison of the effects of sibutramine and other monoamine reuptake inhibitors on food intake in the rat. *Br J Pharmacol*. 1997; **121**(8): 1758-62.
23. Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. *Neuron*. 2002; **36**(2): 199-211.
24. Hebebrand J, Hinney A, Knoll N, Volckmar AL, Scherag A. Molecular genetic aspects of weight regulation. *Deutsches Arzteblatt international*. 2013; **110**(19): 338-44.
25. Cutler DM, Glaeser EL, Shapiro JM. Why have Americans become more obese? *J Econ Perspect*. 2003; **17**(3): 93-118.
26. Perello M, Zigman JM. The role of ghrelin in reward-based eating. *Biol Psychiatry*. 2012; **72**(5): 347-53.
27. Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin modulates brain activity in areas that control appetitive behavior. *Cell metabolism*. 2008; **7**(5): 400-9.
28. Neary MT, Batterham RL. Gaining new insights into food reward with functional neuroimaging. *Forum Nutr*. 2010; **63**152-63.
29. Figlewicz DP, Benoit SC. Insulin, leptin, and food reward: update 2008. *Am J Physiol Regul Integr Comp Physiol*. 2009; **296**(1): R9-R19.
30. Wansink B. Environmental factors that increase the food intake and consumption volume of unknowing consumers. *Annu Rev Nutr*. 2004; **24**455-79.
31. Vartanian LR, Herman CP, Polivy J. Consumption stereotypes and impression management: how you are what you eat. *Appetite*. 2007; **48**(3): 265-77.
32. Johnson F, Pratt M, Wardle J. Dietary restraint and self-regulation in eating behavior. *Int J Obes (Lond)*. 2012; **36**(5): 665-74.
33. Oliver G, Wardle J. Perceived effects of stress on food choice. *Physiol Behav*. 1999; **66**(3): 511-5.
34. Gibson EL. Emotional influences on food choice: sensory, physiological and psychological pathways. *Physiol Behav*. 2006; **89**(1): 53-61.
35. Torres SJ, Nowson CA. Relationship between stress, eating behavior, and obesity. *Nutrition*. 2007; **23**(11-12): 887-94.
36. Bast ES, Berry EM. Laugh Away the Fat? Therapeutic Humor in the Control of Stress-induced Emotional Eating. *Rambam Maimonides medical journal*. 2014; **5**(1): e0007.
37. McCann BS, Warnick GR, Knopp RH. Changes in plasma lipids and dietary intake accompanying shifts in perceived workload and stress. *Psychosom Med*. 1990; **52**(1): 97-108.
38. Wardle J, Steptoe A, Oliver G, Lipsey Z. Stress, dietary restraint and food intake. *J Psychosom Res*. 2000; **48**(2): 195-202.
39. Bellisle F, Louissylvestre J, Linet N, Rocaboy B, Dalle B, Cheneau F, Lhinoret D, Guyot L. Anxiety and Food-Intake in Men. *Psychosom Med*. 1990; **52**(4): 452-7.

40. Pollard TM, Steptoe A, Canaan L, Davies GJ, Wardle J. Effects of academic examination stress on eating behavior and blood lipid levels. *Int J Behav Med*. 1995; **2**(4): 299-320.
41. O'Connor DB, Jones F, Conner M, McMillan B, Ferguson E. Effects of daily hassles and eating style on eating behavior. *Health Psychol*. 2008; **27**(1 Suppl): S20-31.
42. McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol*. 2008; **583**(2-3): 174-85.
43. Johnson RK. Dietary intake--how do we measure what people are really eating? *Obes Res*. 2002; **10 Suppl 1**63S-8S.
44. Epel E, Lapidus R, McEwen B, Brownell K. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. *Psychoneuroendocrinology*. 2001; **26**(1): 37-49.
45. Appelhans BM, Pagoto SL, Peters EN, Spring BJ. HPA axis response to stress predicts short-term snack intake in obese women. *Appetite*. 2010; **54**(1): 217-20.
46. Oliver G, Wardle J, Gibson EL. Stress and food choice: a laboratory study. *Psychosom Med*. 2000; **62**(6): 853-65.
47. Linden W, Rutledge T, Con A. A case for the usefulness of laboratory social stressors. *nn Behav Med*. 1998; **20**(4): 310-6.
48. Sapolsky RM. Social Status and Health in Humans and Other Animals. *Annual Review of Anthropology*. 2004; **33**393-418.
49. Bohnen N, Nicolson N, Sulon J, Jolles J. Coping style, trait anxiety and cortisol reactivity during mental stress. *J Psychosom Res*. 1991; **35**(2-3): 141-7.
50. Torres SJ, Turner AI, Nowson CA. Does stress induce salt intake? *Br J Nutr*. 2010; **103**(11): 1562-8.
51. Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA*. 1992; **267**(9): 1244-52.
52. Marmot MG, Wilkinson RG. *Social determinants of health* Oxford ; New York: Oxford University Press, 2006.
53. Habib KE, Gold PW, Chrousos GP. Neuroendocrinology of Stress. *Neuroendocrinology*. 2001; **30**(3): 695-727.
54. Patterson ZR, Abizaid A. Stress induced obesity: lessons from rodent models of stress. *Frontiers in neuroscience*. 2013; **7**130.
55. Beckie TM. A systematic review of allostatic load, health, and health disparities. *Biological research for nursing*. 2012; **14**(4): 311-46.
56. George SA, Khan S, Briggs H, Abelson JL. CRH-stimulated cortisol release and food intake in healthy, non-obese adults. *Psychoneuroendocrinology*. 2010; **35**(4): 607-12.
57. Wung PK, Anderson T, Fontaine KR, Hoffman GS, Specks U, Merkel PA, Spiera R, Davis JC, St Clair EW, McCune WJ, Stone JH, Wegener's Granulomatosis Etanercept Trial Research G. Effects of glucocorticoids on weight change during the treatment of Wegener's granulomatosis. *Arthritis Rheum*. 2008; **59**(5): 746-53.
58. Zakrzewska KE, Cusin I, Stricker-Krongrad A, Boss O, Ricquier D, Jeanrenaud B, Rohner-Jeanrenaud F. Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat. *Diabetes*. 1999; **48**(2): 365-70.

59. Warne JP. Shaping the stress response: interplay of palatable food choices, glucocorticoids, insulin and abdominal obesity. *Mol Cell Endocrinol*. 2009; **300**(1-2): 137-46.
60. Greeno CG, Wing RR. Stress-induced eating. *Psychol Bull*. 1994; **115**(3): 444-64.
61. Hagan MM, Wauford PK, Chandler PC, Jarrett LA, Rybak RJ, Blackburn K. A new animal model of binge eating: key synergistic role of past caloric restriction and stress. *Physiol Behav*. 2002; **77**(1): 45-54.
62. Foster MT, Solomon MB, Huhman KL, Bartness TJ. Social defeat increases food intake, body mass, and adiposity in Syrian hamsters. *Am J Physiol Regul Integr Comp Physiol*. 2006; **290**(5): R1284-93.
63. Solomon MB, Foster MT, Bartness TJ, Huhman KL. Social defeat and footshock increase body mass and adiposity in male Syrian hamsters. *Am J Physiol Regul Integr Comp Physiol*. 2007; **292**(1): R283-90.
64. Tamashiro KL, Hegeman MA, Sakai RR. Chronic social stress in a changing dietary environment. *Physiol Behav*. 2006; **89**(4): 536-42.
65. Tamashiro KL, Nguyen MM, Ostrander MM, Gardner SR, Ma LY, Woods SC, Sakai RR. Social stress and recovery: implications for body weight and body composition. *Am J Physiol Regul Integr Comp Physiol*. 2007; **293**(5): R1864-74.
66. Dalla C, Antoniou K, Drossopoulou G, Xagoraris M, Kokras N, Sfikakis A, Papadopoulou-Daifoti Z. Chronic mild stress impact: are females more vulnerable? *Neuroscience*. 2005; **135**(3): 703-14.
67. Dallman MF, Pecoraro NC, la Fleur SE. Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav Immun*. 2005; **19**(4): 275-80.
68. Pecoraro N, Reyes F, Gomez F, Bhargava A, Dallman MF. Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology*. 2004; **145**(8): 3754-62.
69. Legendre A, Papakonstantinou E, Roy MC, Richard D, Harris RB. Differences in response to corticotropin-releasing factor after short- and long-term consumption of a high-fat diet. *Am J Physiol Regul Integr Comp Physiol*. 2007; **293**(3): R1076-85.
70. Tannenbaum BM, Brindley DN, Tannenbaum GS, Dallman MF, McArthur MD, Meaney MJ. High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. *Am J Physiol*. 1997; **273**(6 Pt 1): E1168-77.
71. Kamara K, Eskay R, Castonguay T. High-fat diets and stress responsivity. *Physiol Behav*. 1998; **64**(1): 1-6.
72. Legendre A, Harris RB. Exaggerated response to mild stress in rats fed high-fat diet. *Am J Physiol Regul Integr Comp Physiol*. 2006; **291**(5): R1288-94.
73. Harfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikstrom AC, Okret S, Yu ZY, Goldstein M, Steinbusch H, et al. Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. *Proc Natl Acad Sci U S A*. 1986; **83**(24): 9779-83.
74. Sauvage M, Steckler T. Detection of corticotropin-releasing hormone receptor 1 immunoreactivity in cholinergic, dopaminergic and noradrenergic neurons of the murine basal forebrain and brainstem nuclei--potential implication for arousal and attention. *Neuroscience*. 2001; **104**(3): 643-52.

75. Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology*. 1983; **36**(3): 165-86.
76. Izzo E, Sanna PP, Koob GF. Impairment of dopaminergic system function after chronic treatment with corticotropin-releasing factor. *Pharmacol Biochem Behav*. 2005; **81**(4): 701-8.
77. Blum K, Sheridan PJ, Wood RC, Braverman ER, Chen TJ, Cull JG, Comings DE. The D2 dopamine receptor gene as a determinant of reward deficiency syndrome. *J R Soc Med*. 1996; **89**(7): 396-400.
78. Shively CA, Clarkson TB. The unique value of primate models in translational research. Nonhuman primate models of women's health: introduction and overview. *Am J Primatol*. 2009; **71**(9): 715-21.
79. Kaplan JR, Adams MR, Clarkson TB, Manuck SB, Shively CA, Williams JK. Psychosocial factors, sex differences, and atherosclerosis: lessons from animal models. *Psychosomatic Medicine*. 1996; **58**(6): 598-611.
80. Morgan D, Grant KA, Gage HD, Mach RH, Kaplan JR, Prioleau O, Nader SH, Buchheimer N, Ehrenkaufer RL, Nader MA. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nat Neurosci*. 2002; **5**(2): 169-74.
81. Kaplan JR, Manuck SB. Ovarian dysfunction, stress, and disease: a primate continuum. *ILAR*. 2004; **45**89 - 115.
82. Gust DA, Gordon TP, Wilson ME, Ahmed-Ansari A, Brodie AR, McClure HM. Formation of a new social group of unfamiliar female rhesus monkeys affects the immune and pituitary adrenocortical systems. *Brain Behav Immun*. 1991; **5**(3): 296-307.
83. Paiardini M, Hoffman J, Cervasi B, Ortiz AM, Stroud F, Silvestri G, Wilson ME. T-cell phenotypic and functional changes associated with social subordination and gene polymorphisms in the serotonin reuptake transporter in female rhesus monkeys. *Brain Behav Immun*. 2009; **23**(2): 286-93.
84. Bernstein IS. Dominance, aggression and reproduction in primate societies. *J Theor Biol*. 1976; **60**(2): 459-72.
85. Bernstein IS, Gordon TP. The function of aggression in primate societies. *Am Sci*. 1974; **62**(3): 304-11.
86. Bernstein IS, Gordon TP, Rose RM. Aggression and social controls in rhesus monkey (Macaca mulatta) groups revealed in group formation studies. *Folia Primatol (Basel)*. 1974; **21**(2): 81-107.
87. Shively C, Kaplan J. Effects of social factors on adrenal weight and related physiology of Macaca fascicularis. *Physiol Behav*. 1984; **33**(5): 777-82.
88. Abbott DH, Keverne EB, Bercovitch FB, Shively CA, Mendoza SP, Saltzman W, Snowdon CT, Ziegler TE, Banjevic M, Garland T, Sapolsky RM. Are subordinates always stressed? a comparative analysis of rank differences in cortisol levels among primates. *Horm Behav*. 2003; **43**(1): 67-82.
89. Wilson ME, Fisher J, Fischer A, Lee V, Harris RB, Bartness TJ. Quantifying food intake in socially housed monkeys: social status effects on caloric consumption. *Physiology & Behavior*. 2008; **94**586-94.

90. Wilson ME, Pazol K, Legendre A, Fisher J, Chikazawa K. Gonadal steroid modulation of the limbic - hypothalamic - pituitary - adrenal (LHPA) axis is influenced by social status in female rhesus monkeys. *Endocrine*. 2005; **26**(2).
91. Jarrell H, Hoffman JB, Kaplan JR, Berga S, Kinkead B, Wilson ME. Polymorphisms in the serotonin reuptake transporter gene modify the consequences of social status on metabolic health in female rhesus monkeys. *Physiol Behav*. 2008; **93**(4-5): 807-19.
92. Shively CA, Laber-Laird K, Anton RF. Behavior and physiology of social stress and depression in female cynomolgus monkeys. *Biological Psychiatry*. 1997; **41**(8): 871-82.
93. Shively CA. Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. *Biological Psychiatry*. 1998; **44**(9): 882-91.
94. Troisi A. Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. *Stress*. 2002; **5**(1): 47-54.
95. Schino G, Troisi A, Perretta G, Monaco V. Measuring anxiety in nonhuman primates: effect of lorazepam on macaque scratching. *Pharmacol Biochem Behav*. 1991; **38**(4): 889-91.
96. Troisi A, Schino G, D'Antoni M, Pandolfi N, Aureli F, D'Amato FR. Scratching as a behavioral index of anxiety in macaque mothers. *Behav Neural Biol*. 1991; **56**(3): 307-13.
97. Troisi A. Gender differences in vulnerability to social stress: a Darwinian perspective. *Physiol Behav*. 2001; **73**(3): 443-9.
98. Kalin NH, Shelton SE. Nonhuman primate models to study anxiety, emotion regulation, and psychopathology. *Ann N Y Acad Sci*. 2003; **1008**189-200.
99. Shively CA, Register TC, Friedman DP, Morgan TM, Thompson J, Lanier T. Social stress-associated depression in adult female cynomolgus monkeys (Macaca fascicularis). *Biol Psychol*. 2005; **69**(1): 67-84.
100. Willard SL, Shively CA. Modeling depression in adult female cynomolgus monkeys (Macaca fascicularis). *Am J Primatol*. 2012; **74**(6): 528-42.
101. Zahn LM, Jasny BR, Culotta E, Pennisi E. A barrel of monkey genes. *Science*. 2007; **316**(5822): 215-.
102. Bauer SA, Arndt TP, Leslie KE, Pearl DL, Turner PV. Obesity in rhesus and cynomolgus macaques: a comparative review of the condition and its implications for research. *Comparative medicine*. 2011; **61**(6): 514-26.
103. Kemnitz JW, Francken GA. Characteristics of spontaneous obesity in male rhesus monkeys. *Physiol Behav*. 1986; **38**(4): 477-83.
104. Harwood HJ, Jr., Listrani P, Wagner JD. Nonhuman primates and other animal models in diabetes research. *Journal of diabetes science and technology*. 2012; **6**(3): 503-14.
105. Warne JP. Shaping the stress response: Interplay of palatable food choices, glucocorticoids, insulin and abdominal obesity. *Mol Cell Endocrinol*. 2009; **300**(1-2): 137-46.
106. Marti O, Marti J, Armario A. Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. *Physiol Behav*. 1994; **55**(4): 747-53.

107. Tomiyama AJ, Dallman MF, Epel ES. Comfort food is comforting to those most stressed: Evidence of the chronic stress response network in high stress women. *Psychoneuroendocrinology*. 2011.
108. Scott KA, Moran TH. The GLP-1 agonist exendin-4 reduces food intake in nonhuman primates through changes in meal size. *American journal of physiology Regulatory, integrative and comparative physiology*. 2007; **293**(3): R983-7.
109. Bonaz B, Rivest S. Effect of a chronic stress on CRF neuronal activity and expression of its type 1 receptor in the rat brain. *Am J Physiol*. 1998; **275**(5 Pt 2): R1438-49.
110. Schulkin J, Gold PW, McEwen BS. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology*. 1998; **23**(3): 219-43.
111. Bale TL, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol*. 2004; **44**525-57.
112. Gillespie CF, Nemeroff CB. Corticotropin-releasing factor and the psychobiology of early-life stress. *Curr Dir Psychol Sci*. 2007; **16**(2): 85-9.
113. Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. Corticotrophin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci*. 1996; **17**(4): 166-72.
114. McEwen BS, Wingfield JC. What is in a name? Integrating homeostasis, allostasis and stress. *Horm Behav*. 2010; **57**(2): 105-11.
115. Schulkin J, McEwen BS, Gold PW. Allostasis, amygdala, and anticipatory angst. *Neurosci Biobehav Rev*. 1994; **18**(3): 385-96.
116. Makino S, Smith MA, Gold PW. Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. *Endocrinology*. 1995; **136**(8): 3299-309.
117. Bhatnagar S, Dallman M. Neuroanatomical basis for facilitation of hypothalamic-pituitary-adrenal responses to a novel stressor after chronic stress. *Neuroscience*. 1998; **84**(4): 1025-39.
118. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. *Ann N Y Acad Sci*. 1998; **840**33-44.
119. Wilson ME, Fisher J, Fischer A, Lee V, Harris RB, Bartness TJ. Quantifying food intake in socially housed monkeys: social status effects on caloric consumption. *Physiol Behav*. 2008; **94**(4): 586-94.
120. Michopoulos V, Higgins M, Toufexis D, Wilson ME. Social subordination produces distinct stress-related phenotypes in female rhesus monkeys. *Psychoneuroendocrinology*. 2012; **37**(7): 1071-85.
121. Michopoulos V, Shepard KN, Arce M, Whitley J, Wilson ME. Food history and diet choice affect food intake in monkeys. *Appetite*. 2009; **52**(3): 848.
122. Michopoulos V, Toufexis D, Wilson ME. Social stress interacts with diet history to promote emotional feeding in females. *Psychoneuroendocrinology*. 2012; **37**(9): 1479-90.

123. Michopoulos V, Wilson ME. Body weight decreases induced by estradiol in female rhesus monkeys are dependent upon social status. *Physiology & Behavior*. 2011; **102**(3-4): 382-8.
124. Michopoulos V, Reding KM, Wilson ME, Toufexis D. Social subordination impairs hypothalamic-pituitary-adrenal function in female rhesus monkeys. *Horm Behav*. 2012; **62**(4): 389-99.
125. Michopoulos V, Berga SL, Kaplan JR, Wilson ME. Social subordination and polymorphisms in the gene encoding the serotonin transporter enhance estradiol inhibition of luteinizing hormone secretion in female rhesus monkeys. *Biol Reprod*. 2009; **81**(6): 1154-63.
126. Collura LA, Hoffman JB, Wilson ME. Administration of human leptin differentially affects parameters of cortisol secretion in socially housed female rhesus monkeys. *Endocrine*. 2009.
127. Herod SM, Pohl CR, Cameron JL. Treatment with a CRH-R1 antagonist prevents stress-induced suppression of the central neural drive to the reproductive axis in female macaques. *American journal of physiology Endocrinology and metabolism*. 2011; **300**(1): E19-27.
128. Herod SM, Dettmer AM, Novak MA, Meyer JS, Cameron JL. Sensitivity to stress-induced reproductive dysfunction is associated with a selective but not a generalized increase in activity of the adrenal axis. *American journal of physiology Endocrinology and metabolism*. 2011; **300**(1): E28-36.
129. Seymour PA, Schmidt AW, Schulz DW. The pharmacology of CP-154,526, a non-peptide antagonist of the CRH1 receptor: a review. *CNS Drug Rev*. 2003; **9**(1): 57-96.
130. Berridge KC, Ho CY, Richard JM, DiFeliceantonio AG. The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. *Brain Res*. 2010; **1350**43-64.
131. Iemolo A, Blasio A, St Cyr SA, Jiang F, Rice KC, Sabino V, Cottone P. CRF-CRF Receptor System in the Central And Basolateral Nuclei Of The Amygdala Differentially Mediates Excessive Eating Of Palatable Food. *Neuropsychopharmacology*. 2013.
132. Cottone P, Sabino V, Roberto M, Bajo M, Pockros L, Frihauf JB, Fekete EM, Steardo L, Rice KC, Grigoriadis DE, Conti B, Koob GF, Zorrilla EP. CRF system recruitment mediates dark side of compulsive eating. *Proc Natl Acad Sci U S A*. 2009; **106**(47): 20016-20.
133. Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am J Psychiatry*. 2007; **164**(8): 1149-59.
134. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology*. 2001; **24**(2): 97-129.
135. Parylak SL, Cottone P, Sabino V, Rice KC, Zorrilla EP. Effects of CB1 and CRF1 receptor antagonists on binge-like eating in rats with limited access to a sweet fat diet: lack of withdrawal-like responses. *Physiol Behav*. 2012; **107**(2): 231-42.
136. Ghitza UE, Gray SM, Epstein DH, Rice KC, Shaham Y. The anxiogenic drug yohimbine reinstates palatable food seeking in a rat relapse model: a role of CRF1 receptors. *Neuropsychopharmacology*. 2006; **31**(10): 2188-96.

137. Smagin GN, Howell LA, Redmann S, Jr., Ryan DH, Harris RB. Prevention of stress-induced weight loss by third ventricle CRF receptor antagonist. *Am J Physiol*. 1999; **276**(5 Pt 2): R1461-8.
138. Mastorakos G, Zapanti E. The hypothalamic-pituitary-adrenal axis in the neuroendocrine regulation of food intake and obesity: the role of corticotropin releasing hormone. *Nutritional neuroscience*. 2004; **7**(5-6): 271-80.
139. Pecina S, Schulkin J, Berridge KC. Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: paradoxical positive incentive effects in stress? *BMC Biol*. 2006; **4**8.
140. Parylak SL, Koob GF, Zorrilla EP. The dark side of food addiction. *Physiol Behav*. 2011; **104**(1): 149-56.
141. Sanchez MM, Young LJ, Plotsky PM, Insel TR. Autoradiographic and in situ hybridization localization of corticotropin-releasing factor 1 and 2 receptors in nonhuman primate brain. *J Comp Neurol*. 1999; **408**(3): 365-77.
142. Anisman H, Matheson K. Stress, depression, and anhedonia: caveats concerning animal models. *Neurosci Biobehav Rev*. 2005; **29**(4-5): 525-46.
143. Volkow ND, Fowler JS, Wang GJ. The addicted human brain: insights from imaging studies. *J Clin Invest*. 2003; **111**(10): 1444-51.
144. Volkow ND, Wise RA. How can drug addiction help us understand obesity? *Nat Neurosci*. 2005; **8**(5): 555-60.
145. Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, Fowler JS. Brain dopamine and obesity. *Lancet*. 2001; **357**(9253): 354-7.
146. Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci*. 2010; **13**(5): 635-41.
147. Moore CJ, Lowe J, Michopoulos V, Ulam P, Toufexis D, Wilson ME, Johnson Z. Small changes in meal patterns lead to significant changes in total caloric intake. Effects of diet and social status on food intake in female rhesus monkeys. *Appetite*. 2013; **62**60-9.
148. Shively C, Kaplan J, Adams M. Effects of ovariectomy, social instability and socia status on female *Macaca fascicularis* social behavior. *Physiology & Behavior*. 1986; **36**1147 - 53.
149. Brent LJ, Semple S, Dubuc C, Heistermann M, Maclarnon A. Social capital and physiological stress levels in free-ranging adult female rhesus macaques. *Physiol Behav*. 2011; **102**(1): 76-83.
150. Herod SM, Pohl CR, Cameron JL. Treatment with a CRH-R1 antagonist prevents stress-induced suppression of the central neural drive to the reproductive axis in female macaques. *American journal of physiology Endocrinology and metabolism*. 2011; **300**(1): E19-27.
151. Herod SM, Dettmer AM, Novak MA, Meyer JS, Cameron JL. Sensitivity to stress-induced reproductive dysfunction is associated with a selective but not a generalized increase in activity of the adrenal axis. *American journal of physiology Endocrinology and metabolism*. 2011; **300**(1): E28-36.
152. Gray JM, Greenwood MR. Time course of effects of ovarian hormones on food intake and metabolism. *The American journal of physiology*. 1982; **243**(5): E407-12.

153. Asarian L, Geary N. Modulation of appetite by gonadal steroid hormones. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2006; **361**(1471): 1251-63.
154. Buffenstein R, Poppitt SD, McDevitt RM, Prentice AM. Food intake and the menstrual cycle: a retrospective analysis, with implications for appetite research. *Physiol Behav*. 1995; **58**(6): 1067-77.
155. Johnson ZP, Lowe J, Michopoulos V, Moore CJ, Wilson ME, Toufexis D. Oestradiol differentially influences feeding behaviour depending on diet composition in female rhesus monkeys. *J Neuroendocrinol*. 2013; **25**(8): 729-41.
156. Asarian L, Geary N. Modulation of appetite by gonadal steroid hormones. *Philos Trans R Soc Lond B Biol Sci*. 2006; **361**(1471): 1251-63.
157. Burdette AM, Hill TD. An examination of processes linking perceived neighborhood disorder and obesity. *Soc Sci Med*. 2008; **67**(1): 38-46.
158. Cartwright M, Wardle J, Steggles N, Simon AE, Croker H, Jarvis MJ. Stress and dietary practices in adolescents. *Health Psychol*. 2003; **22**(4): 362-9.
159. George GC, Milani TJ, Hanss-Nuss H, Freeland-Graves JH. Compliance with dietary guidelines and relationship to psychosocial factors in low-income women in late postpartum. *J Am Diet Assoc*. 2005; **105**(6): 916-26.
160. Hellerstedt WL, Jeffery RW. The association of job strain and health behaviours in men and women. *Int J Epidemiol*. 1997; **26**(3): 575-83.
161. Lallukka T, Lahelma E, Rahkonen O, Roos E, Laaksonen E, Martikainen P, Head J, Brunner E, Mosdol A, Marmot M, Sekine M, Nasermoaddeli A, Kagamimori S. Associations of job strain and working overtime with adverse health behaviors and obesity: evidence from the Whitehall II Study, Helsinki Health Study, and the Japanese Civil Servants Study. *Soc Sci Med*. 2008; **66**(8): 1681-98.
162. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. *JAMA*. 2010; **303**(3): 235-41.
163. Haslam DW, James WP. Obesity. *Lancet*. 2005; **366**(9492): 1197-209.
164. Hall KD. Modeling metabolic adaptations and energy regulation in humans. *Annu Rev Nutr*. 2012; **32**35-54.
165. Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. The epidemiology of obesity. *Gastroenterology*. 2007; **132**(6): 2087-102.
166. Moore CJ, Cunningham SA. Social position, psychological stress, and obesity: a systematic review. *Journal of the Academy of Nutrition and Dietetics*. 2012; **112**(4): 518-26.
167. Arce M, Michopoulos V, Shepard KN, Ha QC, Wilson ME. Diet choice, cortisol reactivity, and emotional feeding in socially housed rhesus monkeys. *Physiol Behav*. 2010; **101**446 - 55.
168. Fung TT, Rimm EB, Spiegelman D, Rifai N, Tofler GH, Willett WC, Hu FB. Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. *Am J Clin Nutr*. 2001; **73**(1): 61-7.
169. Kalin NH. Nonhuman primate studies of fear, anxiety, and temperament and the role of benzodiazepine receptors and GABA systems. *J Clin Psychiatry*. 2003; **64 Suppl 3**41-4.

170. Walker ML, Gordon TP, Wilson ME. Reproductive performance in capture-acclimated female rhesus monkeys (Macaca mulatta). *J Med Primatol*. 1982; **11**(5): 291-302.
171. Franke AA, Custer LJ, Morimoto Y, Nordt FJ, Maskarinec G. Analysis of urinary estrogens, their oxidized metabolites, and other endogenous steroids by benchtop orbitrap LCMS versus traditional quadrupole GCMS. *Anal Bioanal Chem*. 2011; **401**(4): 1319-30.
172. Sullivan EL, Koegler FH, Cameron JL. Individual differences in physical activity are closely associated with changes in body weight in adult female rhesus monkeys (Macaca mulatta). *Am J Physiol Regul Integr Comp Physiol*. 2006; **291**(3): R633-42.
173. Bell ME, Bhatnagar S, Liang J, Soriano L, Nagy TR, Dallman MF. Voluntary sucrose ingestion, like corticosterone replacement, prevents the metabolic deficits of adrenalectomy. *J Neuroendocrinol*. 2000; **12**(5): 461-70.
174. Prasad A, Prasad C. Short-term consumption of a diet rich in fat decreases anxiety response in adult male rats. *Physiol Behav*. 1996; **60**(3): 1039-42.
175. Teegarden SL, Bale TL. Effects of stress on dietary preference and intake are dependent on access and stress sensitivity. *Physiol Behav*. 2008; **93**(4-5): 713-23.
176. Shively CA, Grant KA, Ehrenkaufer RL, Mach RH, Nader MA. Social stress, depression, and brain dopamine in female cynomolgus monkeys. *Ann N Y Acad Sci*. 1997; **807**574-7.
177. Grant KA, Shively CA, Nader MA, Ehrenkaufer RL, Line SW, Morton TE, Gage HD, Mach RH. Effect of social status on striatal dopamine D2 receptor binding characteristics in cynomolgus monkeys assessed with positron emission tomography. *Synapse*. 1998; **29**(1): 80-3.
178. Morgan D, Grant KA, Gage HD, Mach RH, Kaplan JR, Prioleau O, Nader SH, Buchheimer N, Ehrenkaufer RL, Nader MA. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nature Neuroscience*. 2002; **5**(2): 169-74.
179. Kaplan JR, Manuck SB, Fontenot MB, Mann JJ. Central nervous system monoamine correlates of social dominance in cynomolgus monkeys (Macaca fascicularis). *Neuropsychopharmacology*. 2002; **26**(4): 431-43.
180. Miczek KA, Covington HE, 3rd, Nikulina EM, Jr., Hammer RP. Aggression and defeat: persistent effects on cocaine self-administration and gene expression in peptidergic and aminergic mesocorticolimbic circuits. *Neurosci Biobehav Rev*. 2004; **27**(8): 787-802.
181. Miczek KA, Nikulina EM, Shimamoto A, Covington HE, 3rd. Escalated or suppressed cocaine reward, tegmental BDNF, and accumbal dopamine caused by episodic versus continuous social stress in rats. *J Neurosci*. 2011; **31**(27): 9848-57.
182. Miczek KA, Yap JJ, Covington HE, 3rd. Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. *Pharmacol Ther*. 2008; **120**(2): 102-28.
183. Nieuwenhuizen WF, Weenen H, Rigby P, Hetherington MM. Older adults and patients in need of nutritional support: review of current treatment options and factors influencing nutritional intake. *Clin Nutr*. 2010; **29**(2): 160-9.

184. Cottone P, Sabino V, Steardo L, Zorrilla EP. Intermittent access to preferred food reduces the reinforcing efficacy of chow in rats. *Am J Physiol Regul Integr Comp Physiol*. 2008; **295**(4): R1066-76.
185. Heatherton TF, Herman CP, Polivy J. Effects of physical threat and ego threat on eating behavior. *J Pers Soc Psychol*. 1991; **60**(1): 138-43.
186. Morley JE, Levine AS, Rowland NE. Minireview. Stress induced eating. *Life Sci*. 1983; **32**(19): 2169-82.
187. Laitinen J, Ek E, Sovio U. Stress-related eating and drinking behavior and body mass index and predictors of this behavior. *Prev Med*. 2002; **34**(1): 29-39.
188. Buwalda B, Blom WA, Koolhaas JM, van Dijk G. Behavioral and physiological responses to stress are affected by high-fat feeding in male rats. *Physiol Behav*. 2001; **73**(3): 371-7.
189. Kalin NH, Shelton SE. Ontogeny and stability of separation and threat-induced defensive behaviors in rhesus monkeys during the first year of life. *Am J Primatol*. 1998; **44**(2): 125-35.
190. Wilson ME, Bounar S, Godfrey J, Michopoulos V, Higgins M, Sanchez M. Social and emotional predictors of the tempo of puberty in female rhesus monkeys. *Psychoneuroendocrinology*. 2013; **38**(1): 67-83.
191. Johnson JD, O'Connor KA, Deak T, Spencer RL, Watkins LR, Maier SF. Prior stressor exposure primes the HPA axis. *Psychoneuroendocrinology*. 2002; **27**(3): 353-65.
192. Pelton GH, Lee Y, Davis M. Repeated stress, like vasopressin, sensitizes the excitatory effects of corticotropin releasing factor on the acoustic startle reflex. *Brain Res*. 1997; **778**(2): 381-7.
193. Christianson JP, Drugan RC, Flyer JG, Watkins LR, Maier SF. Anxiogenic effects of brief swim stress are sensitive to stress history. *Prog Neuropsychopharmacol Bol Psychiatry*. 2013; **44**17-22.
194. Popper R, Smits G, Meiselman HL, Hirsch E. Eating in combat: a survey of U.S. Marines. *Mil Med*. 1989; **154**(12): 619-23.
195. Tomiyama AJ, Dallman MF, Epel ES. Comfort food is comforting to those most stressed: evidence of the chronic stress response network in high stress women. *Psychoneuroendocrinology*. 2011; **36**(10): 1513-9.
196. Rudenga KJ, Sinha R, Small DM. Acute stress potentiates brain response to milkshake as a function of body weight and chronic stress. *Int J Obes (Lond)*. 2013; **37**(2): 309-16.
197. Gagliano H, Fuentes S, Nadal R, Armario A. Previous exposure to immobilisation and repeated exposure to a novel environment demonstrate a marked dissociation between behavioral and pituitary-adrenal responses. *Behav Brain Res*. 2008; **187**(2): 239-45.
198. Petticrew M, Davey Smith G. The monkey puzzle: a systematic review of studies of stress, social hierarchies, and heart disease in monkeys. *PloS one*. 2012; **7**(3): e27939.
199. Sapolsky RM. The influence of social hierarchy on primate health. *Science*. 2005; **308**(5722): 648-52.
200. Dallman MF. Stress-induced obesity and the emotional nervous system. *Trends in endocrinology and metabolism: TEM*. 2010; **21**(3): 159-65.

201. Foley P, Kirschbaum C. Human hypothalamus-pituitary-adrenal axis responses to acute psychosocial stress in laboratory settings. *Neurosci Biobehav Rev*. 2010; **35**(1): 91-6.
202. McEwen BS, Gianaros PJ. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Ann N Y Acad Sci*. 2010; **1186**190-222.
203. Klimentidis YC, Beasley TM, Lin HY, Murati G, Glass GE, Guyton M, Newton W, Jorgensen M, Heymsfield SB, Kemnitz J, Fairbanks L, Allison DB. Canaries in the coal mine: a cross-species analysis of the plurality of obesity epidemics. *Proceedings Biological sciences / The Royal Society*. 2011; **278**(1712): 1626-32.