THE EFFECTS OF HIGH FAT DIET ON THE STRESS RESPONSE

by

ARIADNE LEGENDRE

(Under the Direction of Ruth Harris)

ABSTRACT

It has been suggested that high-fat diets (HF) exaggerate stress-induced activation of the hypothalamic-pituitary-adrenal axis and glucocorticoid release. Experiments in this thesis investigated whether the exaggerated response in HF-fed Sprague Dawley rats was caused by dietary fat or increased adiposity. We found that exposure to HF-diet and not adiposity exaggerates the glucocorticoid and weight loss response to mild stress but not to a greater stress of repeated restraint. The mechanisms by which HF-diet exaggerates the stress response are unknown, therefore, we measured aspects of the stress pathway. Corticortrophin releasing factor (CRF) mRNA expression in the paraventricular nucleus of the hypothalamus and of urocortin I in the Edinger Westphal nucleus were not increased in HF-fed rats and corticosterone release following third ventricle CRF injection was not changed. This suggests that acute exposure to HF-diet increases stress-responsiveness by modify an aspect of the stress pathway down-stream of the hypothalamic CRF receptors.

INDEX WORDS: High fat diet, HPA axis, CRF, Urocortin, Stress, Weight regulation

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ARIADNE LEGENDRE

B.S., The University of Texas, 2001

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2006

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ARIADNE LEGENDRE

Major Professor:	Ruth Harris
Committee:	Arthur Grider Gaylen Edwards

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2006

ACKNOWLEDGEMENTS

I would like to acknowledge my family and friends who encouraged and supported my commitment to achieving a higher education. Thanks, Mom, for encouraging me when I needed it the most. To my loving husband, Jeff, thank you for always believing in me and making sure I never gave up or lost sight of the big picture. You have made me a better person over the years. I could have never done it without you.

The caring and supportive environment of the Harris lab was the answer for my success. First I would like to thank its inspiring leader, Dr. Ruth Harris. For the last two years she has been a role model for me, always teaching and motivating me to reach my potential and goals, never letting me take the easy way out. She has given me the opportunity of a lifetime. To the rest of the Harris Lab, not only were you an instrumental part of all the work and research but also a great source of mental support. Thank you for all the good laughs, especially during all those long hours on the eighth floor. I would like to give a special thank you to Joyce Power for being by my side all the time. I am so happy to have found someone who shares the same passion for food and flowers. Joyce, you let me know when you want to go into business together.

I would also like to thank Dr. Arthur Grider and Dr. Gaylen Edward for their guidance as members of my thesis committee.

Lastly, thank you to Dr. Mark Wilson, who saw my potential and has opened doors for me. Your generous, caring, and encouraging personality has been very important to me. You have been such an inspiration over the years and I will always be grateful to you.

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CHAPTER 1

INTRODUCTION

In the last 20 years, there has been a dramatic increase in the occurrence of obesity in the United States (www.cdc.gov). Overweight and obesity and their associated health problems have a significant economic impact on the U.S. health care system (1). Overweight and obesity are a result of positive energy balance over a long period of time. The cause of this energy imbalance for each individual may be due to a combination of several factors. Individual behaviors, environmental factors, such as diet, and genetics, all contribute to the complexity of the obesity epidemic. The consumption of dietary fat is one environmental factor that has been linked to the development of obesity (2). Due to its increased palatability and decreased satiety factor, dietary fat is often over-consumed (19, 20). Consequently, the increasing rate of obesity has been correlated to increased dietary fat intake (2, 23). Additionally, in efforts to address both the burden of obesity-associated chronic disease and individual concerns about appearance, interest in weight-loss therapies has been rising. However, weight-loss therapies have not been effective in decreasing and maintaining body weight over time. A review of 6 randomized trials in weight-loss therapies showed an average weight loss of 9.9 kg for a mean of 18 weeks of therapy. Unfortunately, only 66% of the patients had maintained the weight loss at 52 weeks (8). Likewise, Wing et al. (24) showed that only 20% of the people that had lost 10% or more of their initial body weight were successful at maintaining the reduced weight for 1 year. Also associated with weight gain and weight loss, and perhaps the onset and maintenance of obesity in some cases, are certain physiologic states, such as stress (5).

The human body reacts to stress by activating a complex repertoire of physiologic and behavioral adaptive responses, which are regulated by the central nervous system and peripheral adaptive responses. In response to stress, the hypothalamic-pituitary-adrenal (HPA) axis plays a vital role in adaptation of the organism to homeostatic changes by regulating a cascade of hormones. Activation of the HPA axis ultimately results in the release of glucocorticoids, which act at multiple levels to redirect body energy resources (14, 16, 21) including energy mobilization (glycogenolysis) in the liver, suppression of immune systems, inhibition of bone and muscle growth, suppression of reproductive function, and behavioral depression (14, 16). Although these, and other responses, allow an animal or individual to adapt to a change in environment or a socially stressful situation, if inadequate or excessive and/or prolonged, they may have adverse consequences on physiologic functions, such as growth, metabolism, circulation, reproduction, and the inflammatory/immune response (4, 10). Exposure to chronic stress has been acknowledged to be involved in the development of a wide range of physiological and behavioral diseases such as heart disease, depression, anxiety and mood disorders, detrimental weight gain, and anorexia nervosa (25).

Stress acts on the hypothalamus by stimulating the release of corticotropin-releasing factor (CRF). CRF induces the anterior pituitary gland to release adrenocorticotropin hormone (ACTH) into the circulation, which stimulates the adrenal gland to release glucocorticoids, specifically corticosterone in rodents and cortisol in humans. Lastly, free glucocorticoids will bind to glucocorticoid receptors in the pituitary and in the hypothalamus to down-regulate ACTH and CRF, respectively, by negative feedback mechanisms (3). In addition to surges in glucocorticoid concentration, decreases in food intake, decreases in weight gain, and increases in energy expenditure are observed in response to stress (11). These responses are apparent in a

variety of acute stress models in rodents, such as repeated restraint (11) immobilization (15), and social defeat (9).

Previous studies have reported that stress may alter macronutrient selection, and favoring the consumption of high fat and high density foods (5-7). Meanwhile other studies have found that variations in the macronutrient composition of a diet, specifically the fat content, can affect some of the stress responses, including mood and behavior (13), and the neuroendocrine response to stress. Previous studies investigating the effect of high fat diet on the stress response reported that rats fed a HF diet showed an elevated HPA activity in response to restraint stress in comparison to rats fed a LF diet (12, 17, 18, 22). In studies investigating the relationship between stress and dietary fat it is not clear whether increased stress responsiveness is due to diet composition or increased adiposity.

In the following experiments, we hypothesized that a high fat diet acts as a chronic stressor, consequently rats fed a high fat diet will react with an increased response to repeated restraint or mild stress. In order to differentiate between the effects of a high fat diet and the effects of increased adiposity on the endocrine stress response in rats, the present studies fed a high fat diet for only 4 days to Sprague-Dawley rats. Furthermore, we hypothesized that the exaggerated stress response in rats fed a high fat diet is a result of modifications in the activity of the CRF system through either an increased release of CRF or related peptides, or increased responsiveness of CRF receptors or increased receptor number. The hypothesis was tested in two specific aims. The first aim tested whether rats fed a high fat diet increased levels of corticosterone released in response to repeated restraint or mild stress. The second aim tested whether a high fat diet changed the activity of the CRF system or corticosterone levels released in response to intracerebroventricular CRF injections. Messenger RNA expression of CRF and

Ucn I in high fat and low fat fed rats was also measured in the PVN, amygdala, and Edinger Westphal nucleus.

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CHAPTER 2

LITERATURE REVIEW

Stress

Life exists through maintenance of a complex dynamic equilibrium, termed homeostatis, that is constantly challenged by intrinsic or extrinsic adverse forces or stressors (15, 28). Consequently, stress has been defined as a state of threatened homeostasis (80). The human body reacts to stress by activating a complex repertoire of physiologic and behavioral adaptive responses, which are regulated by the central nervous system and peripheral adaptive responses. Remarkably, the responses evoked from different stressors all activate similar pathways but differ in magnitude. These changes are normally adaptive, time limited, and improve the chances of survival for the individual.

Activation of the stress system leads to important endocrine changes including decreases in growth hormone (5, 49), thyroid-stimulating hormone (49), and reproductive hormones (40). The decline in these hormones results in the prioritization of energy needs by the body and the redirection of energy towards maintenance of homeostasis. Although these, and other responses, allow an animal or individual to adapt to a change in environment or a socially stressful situation, they may affect mood and behavior. If the responses are inadequate or are excessive and/or prolonged then they may have adverse consequences on physiologic functions, such as growth, metabolism, circulation, reproduction, and the inflammatory/immune response (15, 28). Exposure to chronic stress has been shown to be involved in the development of a wide range of physiological and behavioral diseases such as heart disease, depression, anxiety, mood disorders, and anorexia nervosa (98).

In most cases acute stressors can be characterized as a one time or short duration event that elicits a strong endocrine and physiological response. For humans, an acute stressor could be defined as major life crisis such as death or illness of a loved one, sudden loss of income, or a serious accident (97). Acute stress is simulated in animal research using techniques such as repeated restraint (32) immobilization (53), and social defeat (25). Contrary to acute stress, chronic stress is not a very well defined concept, partly because the body will habituate and adapt to a stress over time resulting in a decreased endocrine and physiological response. Such chronic stressors are often characterized in everyday human life by problems at work or in relationships and also in patients with chronic disease. Many animal models specifically developed to simulate chronic stress use a series of intermittent daily changing stressors which may include electric foot shocks, restraint, food and water deprivation, and cold exposure (24, 39), rather than the continuous presence of a single stressor.

In experiments described in the thesis, we have used the repeated restraint stress model and the mild stress model to create acute stress in rats. The repeated restraint model consists of placing rats into restraining plastic tubes for 3 hours on 3 consecutive days. The restraint stress model has the benefits of allowing a uniform degree of stress to be applied to a relatively large number of animals simultaneously and allowing blood collection without cannulating or removing the rats from the stressful environment. In addition, the restraining tubes are inexpensive and do not require designated space in the animal facility. The mild stress model consists of a saline intraperitoneal (i.p.) injection and moving the animals to a novel environment for 2 hours. Rats exposed to repeated restraint stress show elevated glucocorticoid levels compared to non-restrained rats (32, 38, 83). Glucocorticoid concentration reaches adequate levels to provide negative feedback, 30 to 60 minutes after the start of the restraint (25), and as

early as the second hour of a 3-hour restraint serum glucocorticoid concentrations have returned to normal (32). The repeated restraint stress model has been shown to reduce food intake, increase energy expenditure, and reduce body weight on the day of the restraint (32). Compared to repeated restraint, the glucocorticoid response to mild stress peaks faster, between 15-30 minutes following the saline injection, and will also return to basal level faster. This mild stress model results in decreased food intake in the subsequent 24 hours (29).

Stress and HPA axis

The hypothalamic-pituitary-adrenal (HPA) axis plays a vital role in adaptation of the organism to homeostatic changes by regulating a cascade of hormones (Figure 1). Activation of the HPA axis ultimately results in the release of glucocorticoids, which act at multiple levels to redirect body energy resources (52, 57, 73) including energy mobilization (glycogenolysis) in the liver, suppression of synthesis of immune proteins such as cytokines and antibodies, inhibition of bone and muscle growth, potentiation of sympathetic nervous system-mediated vasoconstriction, proteolysis and lipolysis, suppression of reproductive function, and behavioral depression (52, 57).

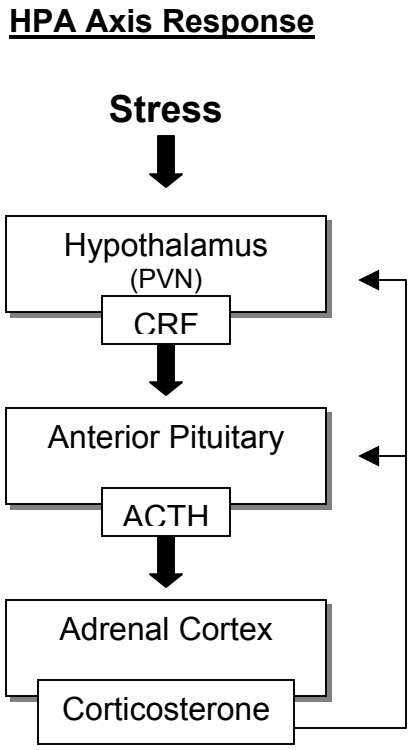

Figure 2.1: Hypothalamic-pituitary-adrenal (HPA) axis response.

The HPA axis acts in a daily rhythm of corticosterone release and is also activated in response to stress. Under relatively unstressed conditions, glucocorticoid secretion undergoes a daily rhythm coordinated by the suprachiasmatic nucleus (20). In nocturnal animals, including the laboratory rat, glucocorticoid levels peak just prior to the onset of the dark period and then decline, reaching a nadir in the morning. In contrast, in diurnal animals such as humans, glucocorticoid levels are highest in the morning and lowest in the evening. Glucocorticoid concentration peaks prior to waking, and has been associated with increased activity and feeding (42) suggesting that increased glucocorticoid secretion may mediate the mobilization of energy required to accommodate the increase in activity associated with waking and foraging (20). Rhythmic glucocorticoid concentrations are important in carbohydrate, protein, and fat

metabolism (84). Glucocorticoids increase the activity of enzymes required to convert amino acids into glucose in the liver cells, resulting in mobilization of amino acids from the extrahepatic tissues, mainly muscle.

The second domain of the HPA axis activity, and our principal interest, is control of corticosteroid secretion in response to stress. Stress acts on the hypothalamus by stimulating the release of corticotropin-releasing factor (CRF). CRF induces the anterior pituitary gland to release adrenocorticotropin hormone (ACTH) into the circulation, which stimulates the adrenal gland to release glucocorticoids, specifically corticosterone in rodents and cortisol in humans. Free corticosterone will bind to glucocorticoid receptors on the pituitary and on the hypothalamus to down-regulate secretions of ACTH and CRF, respectively, representing a negative feedback mechanisms (7).

Since CRF was first characterized in 1981 (89), a growing family of neurotransmitters and receptors has been identified. The CRF system includes the neurotransmitters CRF, urocortin I (Ucn I), Ucn II, and Ucn III, along with two receptor subtypes CRF1 and CRF2 and a CRF binding protein (CRFBP). CRF, a 41-amino acid peptide, is found in many areas of the brain including the paraventricular nucleus (PVN) of the hypothalamus, the central nucleus of the amygdala, and hindbrain regions in the CNS, and in the gut, skin, and adrenal gland in the periphery (7). CRF appears to play a stimulatory role in the stress response through activation of the CRF receptors. CRF binds with equal affinities to both CRF1 and CRF2 receptors (7). Ucn I is predominantly expressed in cell bodies of the Edinger Westphal nucleus (EW) in the brain (92). In the periphery, Ucn I has been found in the gastrointestinal tract (93), testis, cardiac myocytes, thymus, and spleen. Like CRF, Ucn I has been found to have equal affinities for both CRF1 and CRF2 receptors (63). Ucn II is expressed in the hypothalamus, brainstem, and spinal

cord in the CNS, and in the heart, blood cells, and adrenal gland in the periphery (36, 69). Unc III expression has been found in the hypothalamus and amygdala in the CNS, and in the GI and pancreas in the periphery (36, 44). Both Ucn II and Ucn III appear to be more selective for CRF2 receptors than for CRF1 receptors (7).

CRF1 and CRF2 receptors are G protein-coupled receptors and are widely distributed in the brain and peripheral tissues. Central CRF1 receptors are more predominantly expressed throughout the cerebral cortex, cerebellum, olfactory bulb, medial septum, hippocampus, amygdala, and anterior pituitary (91). These areas of the brain are involved in the control of motor and sensory functions (91). Activation of the CRF1 receptors appears to initiate the HPA axis endocrine response. CRF2 receptors are mostly expressed in subcortical hypothalamic structures, but are also widely expressed in peripheral tissues, such as the heart, GI tract, lungs, skeletal muscle, and vasculature (46, 63, 79). Activation of the CRF2 receptors appears to mediate behavioral response to stress, including stress-induced inhibition of food intake (16). It has been suggested that CRF1 receptors may be more involved with the cognitive aspects of behavior, including attention, executive functions, emotions and possibly learning and memory, while CRF2 receptors primarily influence processes necessary for survival including feeding, reproduction, and defense (78). CRF1 and CRF2 receptor mRNA and protein levels are influenced by multiple factors such as CRF, vasopressin, glucocorticoids, and cytokines, which are released during stress to directly or indirectly coordinate the physiological and behavioral response to stress (1, 58). Restraint or other psychological stressors have been shown to increase CRF mRNA in specific areas of the brain (47), which may facilitate an appropriate response to a large or sustained stress.

In addition, CRF and Ucn I, but not Ucn II or Ucn III, are capable of binding with high affinity to the CRFBP (65), which is localized in several distinct brain regions, including the cerebral cortex and subregions of the amygdala (13, 64, 66). As the CRFBP binds to CRF or Ucn I, it prevents them to further bind to the CRF receptors, and may act as a gatekeeper for modulating activation of receptors by CRF. Although the principal function of the CRFBP is still unknown, it has been hypothesized that an increased availability of CRFBP after stress might serve to dampen subsequent responses to stress (75). Consistent with its proposed role to reduce the ligand's availability for CRF receptors mediated actions, it has been shown that 40-60% of CRF located in the human brain is bound by CRFBP (10). Thus, in addition to possibly down regulating HPA axis activity, the CRFBP binding protein may serve as a physiologically relevant reservoir of endogenous CRF or Ucn I if the CRFBP eventually releases its ligands. However, there are also many areas where CRF and Ucn I mRNA do not overlap with CRFBP expression, raising the possibility that CRFBP may have other functions in these areas. Here, it is important to remember that neurotransmitters are not necessarily released at the site of synthesis. Although less is known about CRFBP regulation, restraint and food deprivation have been reported to increase CRFBP mRNA in specific brain areas (45, 85), suggesting that alterations in CRFBP may be part of the stress response. An increase in CRFBP would represent a decrease in CRF and Ucn I available to bind to CRF receptors and may serve as a protective mechanism against hyperactivity of the HPA axis.

In addition to central roles, studies have demonstrated important peripheral functions for CRF-related neuropeptides. The peripheral administration of CRF receptor agonists and antagonists have revealed potent effects on GI motility. Both CRF1 and CRF2 receptors are found in the GI tract and stimulation of either receptor produces significant changes in gastric

emptying. Whereas administration of CRF increases gastric motility, UcnI treatment appears to delay gastric emptying (59, 95). Ucn II also inhibits gastric emptying while not influencing distal colonic transit (55). A second major peripheral action of CRF and CRF-related peptides involves cardiovascular functions. Studies have demonstrated its ability to decrease mean arterial pressure and increase superior mesenteric artery flow when peripherally administered (7).

Although CRF and CRF-related peptides may be considered as some of the most important regulators of the HPA axis, other peptides have also been shown to regulate the HPA axis activity. For example, it has been proposed that vasopressin (VP) may act synergistically with CRF to augment the release of ACTH in rodents and humans, suggesting that VP plays a physiologic role in modulating the ACTH response to stress (2, 4). Produced in the hypothalamic paraventricular and supraoptic nuclei, VP is well recognized for its role on fluid regulation. However, evidence has accumulated indicating that the peptide could contribute to the sensitization of ACTH to a novel stimulus and to maintaining its responsiveness during chronic stress (3, 4, 82).

Stress and Weight Regulation

The long-term weight-related consequences of elevated glucocorticoids in chronically stressed individuals may result in harmful weight gain, abdominal obesity, type II diabetes, increased cardiovascular diseases**,** and finally mortality in humans (18). Although weight gain in humans has been associated with the effects of chronic stress, it is not the case for acute stress. Instead, the effects of acute stress have been demonstrated to down-regulate body weight in humans (30, 32). Similarly numerous acute stress models in rodents, such as repeated restraint stress (32), mild stress (33), immobilization (90), and social defeat (53) have been shown to

decrease body weight. Contrary to the human response to chronic stress, rodents demonstrate a decrease in body weight in response to chronic stress (54, 62, 99).

Changes in body weight represent changes in energy balance, which is determined by the relationship between energy intake and energy output. Therefore, the observed decrease in body weight in response to acute stress can only be a result of a relative decrease in food intake or increase in energy expenditure. Although a large number of studies have focused on the increased appetite and food intake in response to stress some recent data show that stress increases energy expenditure in rats exposed to an acute stress (31). Presently there are no mechanisms suggested to explain the increased energy expenditure during stress. Stress is involved in the regulation of appetite and food intake by influencing appetite and satiety center in the hypothalamus (14). The centers that contribute to hunger and satiety include the hypothalamic arcuate, the paraventricular, the dorsomedial, and the suprachiasmatic nuclei among the most important regions. There has been emerging interest in the role of the neuropeptides present in these areas that inhibit or stimulate feeding behavior (56). CRF, cholecystokinin (CCK), neurotensin, cocaine- and amphetamine-regulated transcript, α-melanocyte-stimulating hormone (α-MSH), and vasopressin are anorexigenic (9, 41, 56, 60), whereas neuropeptide Y (NPY), galanin, Agouti-related protein (AgRP), and melanin-concentrating hormone (MCH) stimulate food intake (17, 72, 77, 87). In addition, these neuropeptides may interact in stimulatory and inhibitory ways with each other and/or with the regulation of the HPA axis. For example, research has shown that NPY and CRF may counter-regulate each other in areas where they overlap (76). Likewise, glucocorticoids enhance the expression of hypothalamic NPY (35, 84), whereas they directly inhibit CRF.

Stress and Obesity

Individuals with eating disorders, whether it is overeating or ingesting most of the daily calories during the night, generally characterize themselves as chronically stressed (81) and are also obese. In contrast, extremely underweight patients, such as those with anorexia nervosa, have elevated basal plasma glucocorticoid levels (26). A high rate of depression is found in both obese and underweight groups, which suggests that disregulation of the stress system may play a role in the energy balance of these patients. Additionally, there appears to be a relationship between elevated levels of glucocorticoids and the presence of abdominal obesity and its co-morbidities in humans (22, 71). Some obese individuals show enhanced responsitivity to stress, although others may actually be hypo-responsive compared with lean individuals (68). The foods that are craved and indulged in response to stress typically have a high fat (HF) and high carbohydrate caloric content. Although the onset of obesity can be attributed to many causes, the consumption of HF diet has been closely correlated with an increase in body fat mass (6, 88). Additionally, recent reports have demonstrated that the consumption of the preferred foods is associated with a decrease in CRF levels in rodents (18).

Because of such conflicting data, the relationship between stress and obesity is unclear and the use of animal models has not clarified this issue appreciably. The obese Zucker rat (*fa/fa*) is a genetic model of obesity with impairment in leptin signaling. The fa/fa rat is characterized by very high circulating levels of corticosterone due to the hyperactivity of the HPA axis (27). High levels of ACTH have been reported in the fa/fa rat, which also exhibit a decrease in the clearance of corticosterone (96). In addition, the fa/fa rat has been reported to over-express CRF when faced with stressful experimental conditions (27, 70, 94). Osborne-Mendel (OM) rats become obese when eating a HF diet. Consequently, they show elevated

levels of corticosterone concentration compared to their low fat (LF) fed counter parts (74). These models suggest that the onset of or maintenance of an obese state may be associated with increased HPA axis activity. Consistently, rodent obesity can be ameliorated by removal of the adrenal gland, and the obesity is reinstated with glucocorticoid replacement (12). All together, this suggests a clear involvement of glucocorticoids in the development of obesity. However, because the rodent stress models described here are genetically obese it is difficult to conclude if obesity is a result of the increased glucocorticoid concentrations or if obesity is causing the increase in HPA axis activity. Furthermore, a variety of rodent models of obesity have shown variable responsiveness to stress (8, 34, 37, 51, 61, 70, 86).

High Fat Diets and Stress

Epel et al. (21, 23) have demonstrated that, under conditions of chronic and acute stress, humans increase their intake of high-density food. Similarly, previous studies in rodents have demonstrated that macronutrient selection may be influenced by stress. Dallman et al. (18) have suggested that chronic stress promotes the consumption of a HF or preferred diet**.** In addition to stress influencing food choice, it has been suggested that variations in the macronutrient composition of a diet can affect mood (48) and neuroendocrine response to stress (38, 61, 67, 83).

Although the human condition suggests that consumption of palatable food is associated with an inhibition of the stress response, Tannenbaum et al. (83) reported that rats fed a HF diet showed an elevated HPA activity in response to restraint stress in comparison to rats fed a LF diet. Tannenbaum et al. (83) reported that rats fed a 40% kcal fat diet for 7 or 21 days had elevated basal and peak levels of corticosterone, increased ACTH release during stress and an

impaired recovery of corticosterone release after 20 minutes of restraint stress. Similarly Kamara at al. (38) reported an exaggerated glucocorticoid response to restraint for 30 minutes in rats fed a HF diet. Also, previous reports in rats fed a HF diet showed increased circulating corticosterone concentrations and hypothalamic catecholamine turnover following a swim stress (61). Additionally, previous studies reported that weight loss and inhibition of food intake in rats exposed to repeated restraint is exaggerated by HF diet (32).

In the experiments just described, the rats were fed the HF diet for different durations and the diets differed in the amount and type of fat. Therefore, it is unclear whether or not some of the rats fed a HF diet were significantly fatter than the rats fed a low fat (LF) diet. As a result, possible explanations for the demonstrated increase in stress response could be attributed to either the effects of obesity or the effects of HF diet. These data do not, however, exclude the possibility that development of obesity can modify stress-responsiveness in addition to an independent effect of dietary fat.

Is High fat diet a Chronic Stressor?

It has been suggested that HF diet acts as a chronic stress on the body and the brain (83). Prolonged elevation of glucocorticoid levels leads to many of the metabolic changes that are induced by a HF diet by altering the balance between insulin and glucocorticoids. Chronically elevated glucocorticoids play an antagonist role against insulin leading to an increase in basal and glucose-stimulated insulin levels and pancreatic β-cell hyperplasia (43, 50). Glucocorticoids antagonize the effects of insulin by decreasing the breakdown of protein, glycogen, and triacylglycerol (11). Therefore, the effects of HF diet are mimicked by those of elevated glucocorticoids, raising the possibility that some of the effects of HF diet might be initiated by

increases in circulating glucocorticoids levels. Furthermore, earlier studies have observed an increase in peak glucocorticoid concentration following restraint stress in rats fed a HF diet (38, 83). Similarly, chronically stressed rats persistently hyper-secrete glucocorticoids during application of a novel stressor (19).

Previous data have shown that chronically stressed rats are more responsive to novel stress (93). Rats exposed to the acute stress of repeated restraint also show an exaggerated HPA response to subsequent mild stressors (30). Corticosterone release during and after mild stress is exaggerated in rats that have previously been stressed compared to rats that have not been previously exposed to repeated restraint (29). Therefore, if rats fed a HF diet are hyper-responsive to repeated restraint, it could insinuate that the HF diet acts as a chronic stressor, and subsequently the second stressor (repeated restraint or mild stress) would increase the HPA response to stress.

Summary

The human body reacts to stress by activating a complex repertoire of physiologic and behavioral adaptive responses, which are regulated by the central nervous system. The HPA axis plays a vital role in adaptation of the organism to homeostatic challenge by regulating a cascade of hormones. One of the physiological responses that is observed in rats in response to stress and activation of the HPA axis is a decrease in appetite and food intake, which results in a decrease in body weight. It was previously demonstrated that stress and activation of the HPA axis not only alter eating behavior but also macronutrient selection. Recently, it has been suggested that HF diet may exaggerate the hormonal and physiologic response to stress (38, 61, 83)**.**

In the following experiments we hypothesized that a HF diet acts as a chronic stressor, consequently rats fed a HF diet would react with an increased response to repeated restraint or mild stress. More specifically, we hypothesized that the exaggerated stress response in rats fed a HF diet results from modifications in the activity of the CRF system through either an increased release of CRF or related peptides, or increased responsiveness of CRF receptors.

The hypothesis was tested in two specific aims. The first aim tested for increased levels of corticosterone release in response to repeated restraint or mild stress in rats fed a HF diet. The second aim tested for the activity of the CRF system in rats fed a HF diet and examined the corticosterone levels released in response to intracerebroventricular CRF injections. Messenger RNA expression of CRF and Ucn I in HF and LF fed rats was also measured in the PVN, amygdala, and Edinger-Westphal nucleus.

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CHAPTER 3

EXAGGERATED RESPONSE TO MILD STRESS IN RATS FED HIGH FAT DIET.[1]

[1] Legendre, A. and R.B. Harris, 2006. Submitted April 4th to the *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology.*

ABSTRACT

It has been suggested that high fat (HF) diet exaggerates the stress-induced release of glucocorticoids due to activation of the hypothalamic-pituitary-adrenal (HPA) axis. In an initial experiment, where rats were fed HF diet for 4 days, we found that HF-fed controls stopped gaining weight indicating that HF-fed rats were hyper-responsive to the mild stress of tail bleeding but responded the same as low fat (LF) fed rats to the more severe stress of restraint. A second experiment confirmed these results when rats fed HF diet for 4 days showed an exaggerated corticosterone release in response to an i.p. injection of saline and movement to a novel cage, compared to LF-fed rats. Experiment 3 tested the same parameters as Experiment 2 but interchanged the diets. This allowed us to differentiate between the effects of the dietary fat and the novelty of the diet. Additionally this experiment determined whether hyper-responsiveness to mild stress in HF-fed rats was sustained during a prolonged exposure to diet. The results confirmed that HF diet, not novelty, exaggerated the endocrine stress response after 9 days on diet but that the effect was no longer present after 23 days on the diet. The hyper-responsiveness of the HPA axis in HF-fed rats is similar to that observed in animals that have been exposed to a significant chronic or acute stress, suggesting that HF diet may initially be perceived as a stressor.

Key words: CRF, energy intake, HPA axis, and weight regulation.

INTRODUCTION

Stress activates different behavioral and physiological systems to allow an animal, or individual, to adapt to, or respond to, a change in environment or a socially stressful situation. The physiological response to stress is, in part, regulated by hormones released by the hypothalamic-pituitary-adrenal (HPA) axis (2, 3, 22). The corticotropin releasing factor (CRF) system is the primary initiator of neurochemical, behavioral, and endocrine response to stress (2). In regard to the HPA axis, stress induced stimulation of the neuropeptide CRF in the hypothalamus, induces the anterior pituitary gland to release adrenocorticotropin hormone (ACTH), which then stimulates the adrenal gland to release glucocorticoids, corticosterone for rodents and cortisol for humans. These responses are apparent in a variety of acute stress models in rodents, such as repeated restraint (11) immobilization (16), and social defeat (8). There is a negative feedback mechanism in place so that free corticosterone acts on the pituitary and hypothalamus to down-regulate expression of ACTH and CRF, respectively. In addition to maintaining homeostasis in response to stress, CRF plays an important regulatory role in energy balance. Rodents exposed to stress have been shown to decrease food intake (24, 25), decrease weight gain (11, 14), and increase energy expenditure (10).

Previous studies have demonstrated that macronutrient selection may be influenced by stress. Dallman et al. (4) have suggested that chronic stress promotes the consumption of a high fat (HF) or preferred diet**.** Similarly, Epel et al. (5, 7) have demonstrated that, under conditions of chronic and acute stress, people increase their intake of high-density food. In addition to stress influencing food choice, it has been suggested that variations in the macronutrient composition of a diet can affect mood (15) and neuroendocrine response to stress (12, 17, 20, 27). Several investigators have shown an exaggerated response to stress in rats fed a HF diet

compared to their low fat (LF) fed counterparts. Tannenbaum et al. (27) reported that rats fed a 40% kcal fat diet for 7 or 21 days had elevated basal levels of corticosterone, increased ACTH release during stress and an impaired recovery of corticosterone release after 20 minutes of restraint stress. Others reported that rats fed a HF diet showed increased circulating corticosterone concentrations and hypothalamic catecholamine turnover following a swim stress (17). We have previously reported that weight loss and inhibition of food intake in rats exposed to repeated restraint is exaggerated by HF diet (11).

In addition to reports of HF diet exaggerating the stress response and to stress influencing macronutrient selection, elevated levels of glucocorticoids are found in obese patients (6, 18, 19, 23). Although the onset of obesity can be attributed to many causes, the consumption of HF diet has been closely correlated with the increase in body fat mass (1, 28). In studies investigating the relationship between stress and dietary fat it is not clear whether increased stress responsiveness is due to diet composition or increased adiposity.

Observations from previous studies suggest that rats fed a HF diet for only 10 days will begin to show a significant weight gain compare to their LF counter parts (11). In the experiments (12, 17, 27) described above it was unclear how long the HF diet was administered before stress and therefore the rats fed a HF diet might have been significantly fatter than the rats fed a LF diet. The present studies investigated the effects of acute exposure to a HF diet on the endocrine stress response in rats. By feeding the rats either HF or LF diet for only 4 days or for several weeks before they were exposed to stress we hoped to be able to separate the changes associated with exposure to HF diet from those caused by the development of obesity.

METHODS

All procedures for care and use of animals were approved by the Institutional Animal Care and Use Committee of the University of Georgia and were in accordance with the APS's Guiding Principles (26).

Experiment 1: Acute Exposure to HF Diet and Repeated Restraint Stress.

Forty two male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing approximately 300g were housed in individual stainless steel cages in a room maintained at 23 ± 1°C on a 12h/12h light/dark cycle with lights on at 7:00 am. Upon arrival the rats were allowed one week to acclimate to the new conditions. All animals had free access to rodent chow (Purina Rodent Chow 5001, Purina Mills, St-Louis, MO) and water.

Following the acclimation period the animals were fed regular chow for one more week, during which daily body weights and food intakes were measured. The animals were divided into two weight-matched group, one group was fed a HF diet (40% kcal fat: Diet D02041901, Research Diets Inc., New Brunswick, NJ) while the other group was fed a LF diet (12% kcal fat: Diet D02041902, Research Diets Inc.). After 4 days each dietary group was sub-divided into two weight-matched groups. One subgroup was exposed to restraint stress for 3 hours on 3 consecutive days (repeated restraint), while the other group was a non-stressed control. The restrained rats were placed in Perpex restraining tubes measuring 6.5 cm in diameter and 21 cm in maximum length (Plas Laboratories, Lansing, MI) for 3 hours, while the control rats were placed in shoebox cages without food or water for the period of the restraint**.** Blood samples from the tail were collected at the end of the first restraint, at 30 minutes intervals (0, 30, 60, 90, 120, 150, 180 min.) during the second restraint and at the end of the third restraint. Additional

blood samples were obtained 2 and 5 days following the restraint stress at a time that would be equivalent to the end of restraint stress. Food and water was removed 3 hours prior to these blood draws. Blood samples were centrifuged and the serum was stored at -80°C until assays could be performed. Corticosterone concentration was measured in all samples (Corticosterone RIA; MP Diagnostics, Costa Mesa, Ca). ACTH concentration (ACTH RIA; Nichols Institute Diagnostics, San Clemente, CA) was measured on the 30 minutes blood sample collected on the second day of restraint. Insulin (Insulin RIA; Linco Research, St. Charles, MO) was measured on the blood samples from the first day of restraint, and those collected 2 and 5 days following the last day of restraint.

Experiment 2: Acute Exposure to HF and Mild Stress.

In Experiment 1, the HF-fed controls stopped gaining weight on the days of restraint when blood samples were collected. This suggested that these rats were more sensitive to handling and tail bleeding than the LF-control group. In contrast, there was no effect of diet on the response to the more severe stress of repeated restraint. Therefore, this experiment tested the effects of HF diet on the endocrine response to a mild stress.

Forty-two male Sprague-Dawley rats, weighing approximately 300g, were housed as described above. Following the acclimation period, all the animals were fed the LF diet for 5 days. After 5 days, the animals were divided into 2 weight-matched groups. One group stayed on the LF diet while the other group was switch to the HF diet for 4 days. On the fifth day, each dietary group was sub-divided into two weight-matched groups. One subgroup from each dietary group was exposed to a mild stress, while the other groups were non-stressed control. The animals exposed

to mild stress received an intra-peritoneal (i.p.) injection of 2 ml saline and were moved to a shoebox in an adjacent testing room for 2 hours. The animals in the control groups were handled and returned to their home cage without access to food or water for 2 hours. Blood was collected from the tail immediately prior to the injection and at +15, +30, +60, +90, +120 minutes following the injection in stressed rats and at equivalent times in controls. Blood samples were centrifuged and the serum was removed and stored at -80°C until it was analyzed for corticosterone concentration.

Experiment 3: Novelty of Diet and Mild Stress.

The previous experiment showed that HF-fed rats gave an exaggerated corticosterone response to mild stress and to tail bleeding. To ensure that this was due to the HF diet and not the novelty of diet, this experiment tested the same parameters as Experiment 2 but interchanged the diets. This allowed us to differentiate between the effects of the dietary fat and those of being offered a new diet. Additionally this experiment determined whether the hyper-responsiveness to mild stress in HF-fed rats in Experiment 1 was sustained during a prolonged exposure to diet.

Thirty-six male Sprague-Dawley rats, weighing approximately 300g, were housed as described above. Following one week of acclimation, all of the animals were fed the HF diet for 5 days. The animals were divided into two weight-matched groups. One group stayed on the HF diet while the other group was switch to the LF diet for 4 days. On the fifth day, each dietary group was sub-divided into two weight-matched groups. One sub-group from each dietary group was exposed to mild stress and the other was left in their home cage without access to water or food for 2 hours. Blood samples were collected as described for Experiment 2. Fourteen days later,

the treatment groups were interchanged, the animals that had been the controls were now exposed to mild stress and the animals previously exposed to mild stress were the controls. Nine days following the second mild stress, the animals in each dietary group were divided into two weight-matched groups. One group from each dietary group was exposed to repeated restraint as described for Experiment 1. Blood from the tail was collected on the first day of restraint at 30 minutes intervals for 3 hours. Animals were decapitated 9 days following the last restraint, trunk blood was collected, fat pads (mesenteric, epididymal, and retroperitoneal) were weighed, and carcass composition was determined as described previously (9). All blood samples were centrifuged and the serum was stored at -80°C until corticosterone concentrations were measured.

Data analysis:

The effect of diet on body weight, energy intake, and repeated measures of corticosterone were determined by repeated measure analysis of variance (ANOVA) using intake or body weight measured immediately before the start of the stress (repeated or mild), or corticosterone measured at time 0 min., as a covariant in the analysis. Statistically significant ($p \leq 0.05$) differences in body weight, food intake and corticosterone levels between groups on specific days or at specific time points were determined by two-way analysis of variance and post-hoc Duncan's Multiple Range test. All statistical procedures were carried out using Statistica software (Statistica Software, Stat Soft, Tulsa, OK). Animals with 2 or more corticosterone values from the timed blood draw that were 2 standard deviations above or below the mean were considered outliers and were not used for any of the data analysis.

RESULTS

Experiment 1:

There were no significant differences between the body weights of the groups of rats at the beginning of the restraint. Rats exposed to repeated restraint lost weight on the days of restraint (Fig. 3.1a) and there was no effect of diet on the amount of weight that was lost (Diet: NS, Stress: $p \leq 0.05$, Interaction: NS). The HF-fed control rats stopped gaining weight on the days of restraint but they returned to their previous rate of gain once the restraint and tail bleeds stopped. In contrast LF-fed controls gained weight steadily throughout the repeated restraint. Animals fed HF diet consumed significantly more calories than rats fed LF diet on the days prior to the MS, most likely related to the increased palatability of the diet (Fig. 3.1b). All groups, except LF controls, consumed less energy on the days of restraint than during the baseline period. The percent change in energy intake from the 3 days during and after restraint compared to the 3 days before (Fig. 3.1c) indicated that the reduced energy intake during the 3 days of restraint did not cause overeating in the 3 days following the restraint (Diet: $p \leq 0.05$, Stress: $p \leq 0.05$, Interaction: NS). In contrast, the HF/Stress group did not return to their baseline intake following the restraint. On day 2 of restraint there were no differences in basal (Time 0) corticosterone between HF and LF-fed groups. Stress caused a significant increase in corticosterone, which peaked between 30 and 60 minutes. There was no effect of diet on the size of this response. At 90 min, HF-fed rats showed a significantly faster recovery of corticosterone concentration (Fig. 3.1d) but by 120 min both HF and LF-fed rats were back to basal concentration. This small difference in corticosterone response did not lead to significant differences in area under the curve between dietary groups. There were no differences in corticosterone concentration measured at the end of day 1 and 3 of restraint or on day 2 and 5 following the restraint. There

were no differences in ACTH levels between the HF-stress and LF-stress at the 30 min. time point on day 2 of restraint (data not shown). There were no differences in insulin levels (data not shown) at the end of the first restraint or on day 2 and day 5 after the last restraint.

Experiment 2:

There were no differences in body weights of the four groups of rats on the day of mild stress. Energy intake was greater in the HF group on the first 2 days of exposure to the diet, but by the fourth day their energy intake was the same as that of the LF group (data not shown). Serum corticosterone concentrations were not different between rats exposed to mild stress and those that were controls, possibly due to the stress caused by tail bleeding. Since there was no statistically significant effect of mild stress, we combined the data from the control and mild stress groups within each dietary treatment (Fig. 3.2a). HF-fed rats showed a significantly greater peak corticosterone release than the LF-fed rats but area under the curve was not significantly changed by diet.

Experiment 3:

There were no differences in body weight of the 4 groups of rats before either of the two exposures to mild stress after the rats were switched from HF to LF diet. There were no significant changes in body weight of any of the rats in response to the first mild stress (Fig. 3.3a). All the groups showed a small weight gain following the manipulations associated with the second mild stress but the HF-Control group showed a significant greater weight gain compared to the other groups (Diet: $p \leq 0.05$, Stress: $p \leq 0.05$, Interaction: NS). Corticosterone concentrations in response to the 1^{st} and 2^{nd} mild stress peaked between 15 and 30 min and were

back to baseline values at 120 min. The HF-Stress group showed an increased corticosterone peak (Fig. 3.3b) in response to the 1st mild stress compare to the LF-Stress group. This difference between diet groups in response to stress was not found in the 2nd mild stress (Fig. 3.3c). After 28 days on the HF-diet there was no effect of diet on the amount of weight lost in response to repeated restraint (Fig. 3.4a). There was no effect of diet or stress on energy intake of any of the groups (data not shown). There were no differences in basal corticosterone, at time 0, between the HF and LF groups and no dietary effect on the response to restraint (Fig. 3.4b). In contrast to the results from Experiment 1, where corticosterone concentration came back down to baseline after 2 hours, corticosterone values peaked at 60 minutes in response to restraint and stayed elevated during the entire restraint. Results from body composition indicated that the HF-fed rats had a significantly increased percent body fat compared to LF-fed rats (HF: 9.19 ± 0.34 % fat; LF: 7.58 ± 0.34 % fat) but that there was no effect of restraint in either group.

DISCUSSION

The combined data from the three experiments described here shows that feeding a HF diet to adult male rats can exaggerate some of the physiological and endocrine responses to stress but that the effect is subtle. Only a mild stress, which does not induce a maximal glucocorticoid response, appears to be affected by a HF diet. Because the rats were fed LF or HF diet for only a short period of time before being stressed we eliminated the possible effects of obesity and clearly demonstrated that HF diet alone was responsible for the observed exaggerated endocrine and body weight response to mild stress.

Previously, others have reported that HF-fed rats exposed to different stressors reacted with an increased endocrine response to stress (12, 13, 27). Tannenbaum et al. (27) used a 20

min one time restraint and both Kamara et al. (12) and la Fleur et al. (13) used a 30 min one time restraint. The duration of the restraint used in the present experiment was significantly longer (3 hours) and was also repeated over 3 days. These experiments all used restraint as a common stressor, however, different durations of restraint and different fat content in the diet were used in each of the experiments. In addition, la Fleur et al. (13) examined the effects of having a choice or no choice between two diets on the stress response, which meant that the amount of energy consumed between each group was significantly different. The different experimental design used by la Fleur et al. (13) from our experiments might explain differences in the results. In our first experiment, rats fed a HF diet for 4 days did not demonstrate an exaggerated response to repeated restraint compare to their LF counterparts. These observations suggest that a HF diet does not affect the endocrine response to repeated restraint. It is likely that the results obtained from previous experiments (12, 13, 27) demonstrating an increase stress response in HF fed animals was caused by side effects of obesity and not diet. We did, however, observe some differences in the energetic response to restraint and tailbleeding in the HF-control group, compared to the LF-control group. These results demonstrate that the manipulations and handling associated with tail bleeding were more stressful to the HF-fed than the LF-fed rats. Because there was no diet effect on response to the greater stress of repeated restraint, it is likely that the increased responsiveness to mild stress was due to a lower threshold for activation than a shift in the response curve. This would make an animal more responsive to the same stress without changing the peak values and/or recovery time. Even though, we did not observe any differences in corticosterone levels during stress, the decrease in energy intake demonstrated by the HF-fed groups along with the reduced weight gain of the HF-control group, suggest that HF diet may act as a stressor and agrees with previous reports from Tannenbaum et al. (27). These

data alone also suggest that the HPA axis response to stress may not play an important role in regulating inhibition of food intake during stress.

In Experiment 2, because of the changes we observed in control HF-fed rats in Experiment 1 we examined the HPA response to mild stress in rats fed HF diet for 4 days. Although, we did not find a specific response to mild stress, after combining the control and mild stress group together we found that the HF-fed rats demonstrated a significantly greater corticosterone release in response to mild stress and/or tail bleeding. This data, combined with the weight differences found in the control HF-fed rats from Experiment 1, suggest that diet may modify the response to small stressors but not to more severe stress. Thus in a high stress situation an appropriate response would be initiated irrespective of diet composition. Here, it is important to point out that in Experiment 1 we observed a decrease in corticosterone in the restrained HF-fed rats at 90 min and decrease in weight gain in the control HF-fed rats. This differed from Experiment 2, where we observed an increase in corticosterone during mild stress in the HF-fed rats compare to the LF-fed rats. Although a diet effect was observed in Experiment 2, a question remained: Is the increase stress response specific to the increase in dietary fat or is it due to the novelty of the diet?

Experiment 3 was designed to address this issue and to determine how long hyper-responsiveness was apparent once the diet had changed. The results from this experiment confirmed that the increased stress response observed in the previous experiment was indeed a diet effect and not caused simply by offering the rats a new diet. It also indicated that the HPA axis hyper-responsiveness elicited by the diet was not sustained. This would contradict the previous hypothesis from Tannenbaum et al. (27) that HF diet may act as a chronic stressor. Our observations would further suggest that the increased glucocorticoid response observed in obese

patients (6, 18, 19, 23) is a consequence of the extra adiposity and not the consumption of a HF diet. If obesity does cause an exaggerated secretion of glucocorticoids then this must be associated with accumulation of significant amounts of fat because we did not find any exaggeration of the HPA response to repeated restraint in HF-fed rats in Experiment 3, which had a modest increase in body fat (20%) compared with their LF-fed counterparts. This suggests that the amount of excess body fat needed to show a difference in glucocorticoid secretion must be considerably larger than 20%.

At the end of Experiment 3, repeated restraint did not produce any differences in the corticosterone response, even though HF-fed rats were significantly fatter then their LF counterparts. In Experiment 1, the corticosterone levels were measured on the second day of restraint and reach a peak at 60 min and were back to baseline levels by the end of the restraint (3 hours). Whereas corticosterone levels in this last experiment were measured on the first day of restraint and peaked at 60 min but failed to return to baseline. The rats in this last experiment were adapted to handling and lost a similar amount of weight following restraint as rats in Experiment 1. Therefore it is unlikely that the stress and tail bleeding procedures were perceived as more extreme than they were by rats in Experiment 1. The difference in pattern of corticosterone release between the two experiments could be due to the different day of restraint that was used to measure corticosterone concentrations. Day 1 of restraint (Experiment 3) may be more stressful than day 2 of restraint (Experiment 3) as the procedure is novel and the rats do not have any expectations of being removed from the restraining tubes.

This series of experiments suggests that HF diet acts as a stressor but contrary to Tannenbaum's (27) hypothesis that HF acts as a chronic stressor, our data show that the stress effects of HF diet only last for 1-2 weeks. In Experiment 3 we did not observed increased stress

responsiveness during the second mild stress or the repeated restraint stress. Additionally, if HF diet acts as a chronic stressor we would expect there to be differences in baseline corticosterone concentration, which was not the case in any of our measurements. It is important to note that in this series of experiments we principally measured HPA activity and that it is possible that other aspects of the stress response may be exaggerated by consumption of a HF diet, as suggested by weight changes caused by mild stress in Experiments 1 and 3.

In the rodent brain there are two major subtypes of CRF receptors: CRFR1 and CRFR2. Previous studies have shown that CRFR1 are more involved in the mediation of the endocrine responses whereas CRFR2 would be responsible for the feeding responses to stress (2). Therefore, if a different ratio of these receptors was created from consuming a HF diet, then we could hypothesize that the endocrine or feeding stress response would also be different. In experiment 1, we showed that rats fed a HF diet did not have an increased corticosterone response to repeated restraint, but we still observed a significant decrease in food intake during the stress. Further studies should examine the effects of HF diets on CRF receptor number and the ratio between CRFR1 and CRFR2. Furthermore, there are at least four neuropeptides with affinity for the CRF receptors: CRF has equal affinity for CRFR1 and CRFR2, whereas urocortin I has a higher affinity for CRFR2 than CRFR1, and urocortin II and III appear to be selective for CRFR2 (21). Therefore, if the amount of CRF or urocortin secreted in response to mild stress was influenced by dietary fat content, then that could have been the cause of the exaggerated response to mild stress in HF-fed rats. CRF and ACTH are down regulated by corticosterone levels feeding back on the hypothalamus and adrenal gland (22). Therefore, it is possible that the HF diet is inhibiting the negative feedback system, resulting in a slightly increased and/or prolonged endocrine response, as suggested from Experiments 2 and 3. Whereas the endocrine

data from Experiment 1 suggests that the HF diet promotes the negative feedback system, decreasing the time needed to reach basal corticosterone values following stress. Even though, ACTH levels did not differ between dietary groups at 30 min following the start of restraint in Experiment 1, one time point is insufficient to conclude that there was no modulation of the negative feedback system that changes ACTH concentrations.

In conclusion, these results suggest that HF diet makes an animal hyper-responsive to other stressors. Whether this is due to HF diet functioning as a stressor and increasing sensitivity to other stressors or whether the HF diet inhibits mechanisms that normally down-regulate different aspects of the stress response needs to be determined. Future studies also should investigate the effects of HF diet on the levels of CRF, CRF related neuropeptides, CRFR1 and CRFR2 receptors in response to stress in areas of the brain specific to eating behavior and energy balance, since we observed an effect of diet on these parameters of the stress response.

Acknowledgement:

The authors wish to thank Joyce Power and Jessica Davenport for their technical assistance.

This work was supported by NIH grant MH068281.

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Figure Legends:

Figure 3.1: Body weights (panel A) and energy intake (panel B) for 3 days prior, during, and after repeated restraint of male Sprague Dawley rats in Experiment 1. Data are means ± sem for groups of 10 rats. The percent change in energy intake from the 3 days during and after restraint compared to the 3 days before (panel C) indicated that the reduced energy intake during the 3 days of restraint did not cause overeating in the 3 days following the restraint. * indicates all groups being different from each other; # indicate HF/Stress and LF/Control to be different from the other groups; % indicate LF/Stress to be statistically significant ($p<0.05$) different from the other groups' body weight. Asterisks indicate statistically significant ($p<0.05$) difference in total energy intake between each group. Blood corticosterone concentrations (panel D) were measured at 30 min. intervals on the second day of restraint. Asterisks indicate statistically significant ($p<0.05$) differences between the restrained groups from each dietary group at specific time points. Insert panel shows the calculated area under the curve.

Figure 3.2: Serum corticosterone concentrations in response to mild stress of Sprague-Dawley male rats in Experiment 2. Since there was no stress effect we combined the mild stress and control groups from the same diet group. Data are means ± sem for groups of 20 rats. Asterisks indicate statistically significant ($p<0.05$) differences between each group at specific time points.

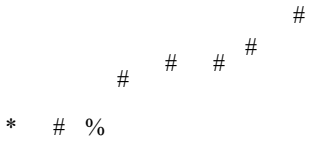
Figure 3.3: Weight change (panel A) after 1st and 2nd mild stress in Experiment 3. Data are means ± sem. Supercripts represent statistically significant ($p<0.05$) difference in weight change in response to the 1st and 2nd mild stress. Blood corticosterone concentrations were measured in response to the 1st (panel B) and the 2nd (panel C) mild stress. Asterisks represent statistically

significant ($p<0.05$) difference between retrained groups from each dietary regimen within a specific time point.

Figure 3.4: Body weights (panel A) in response to repeated restraint of male Sprague Dawley rats in Experiment 3. Rats were fed either HF or LF diet for 29 days and exposed to one mild stress event each before being exposed to repeated restraint. Data are means ± sem for groups of 10 rats. Repeated measures indicated a diet and stress effect, but no interactions. Asterisks represent statistically significant ($p<0.05$) difference between the control and restrained group from each dietary group. Blood corticosterone concentrations (panel B) were measured at 30 min. intervals on the second day of restraint. Asterisks represent statistically significant ($p<0.05$) difference between the HF- and LF-fed rats that were restrained.

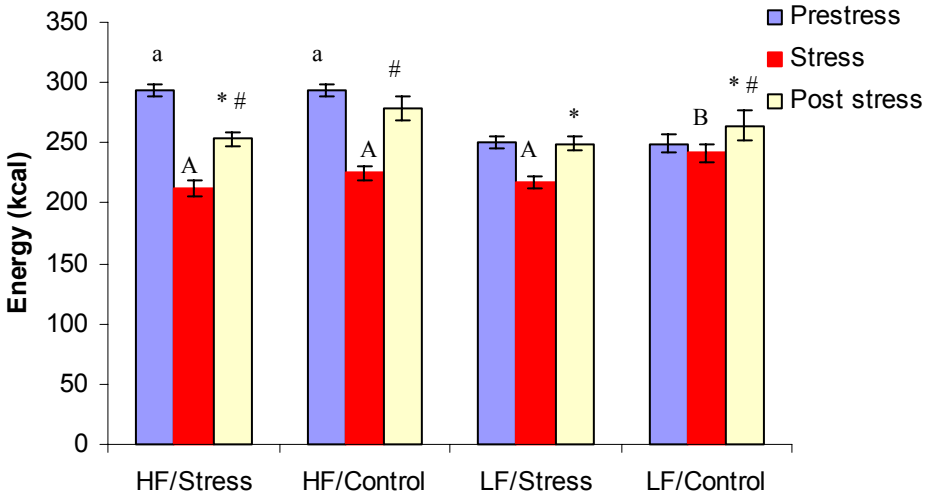
Figure 3.1

A

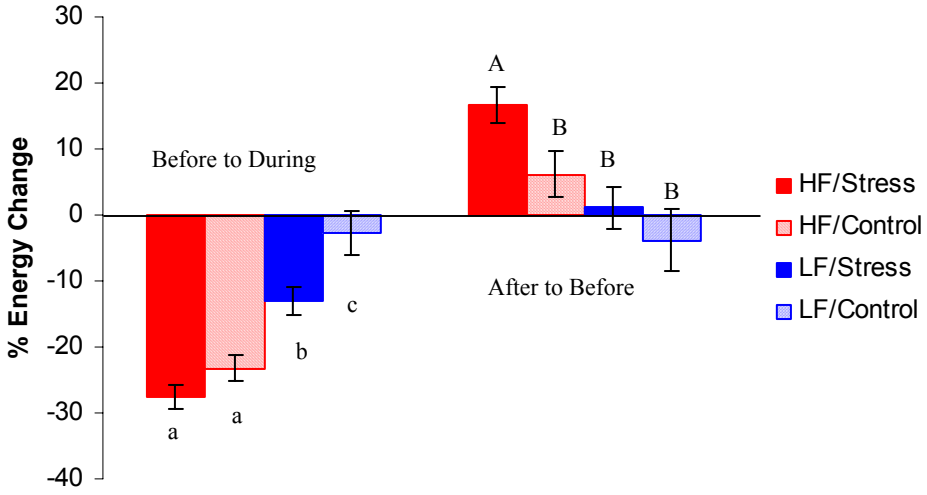


Diet Change Restraint

B



C


30
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0
-10
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-40
% Energy Change
Before to During
After to Before
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B
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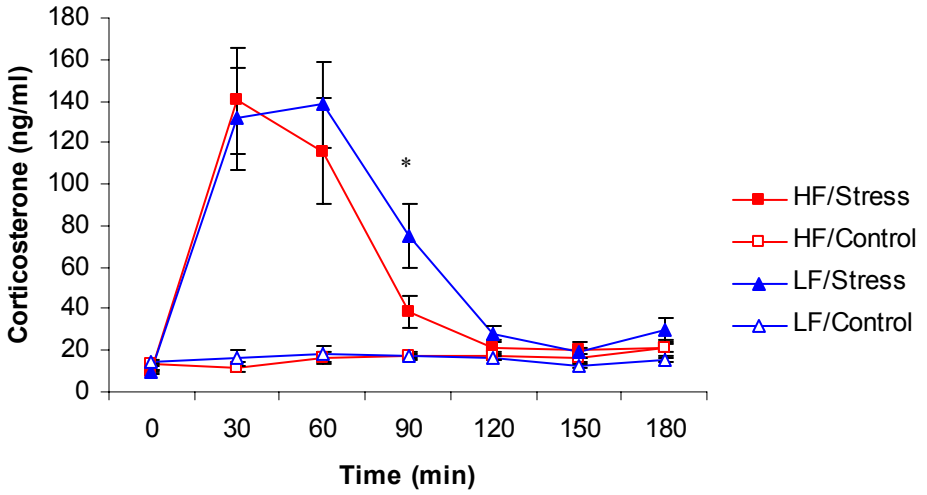
B
a
a
b
c
HF/Stress
HF/Control
LF/Stress
LF/Control

D


180
160
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120
100
80
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40
20
0
Corticosterone (ng/ml)
*
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30
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150
180
Time (min)
HF/Stress
HF/Control
LF/Stress
LF/Control

E

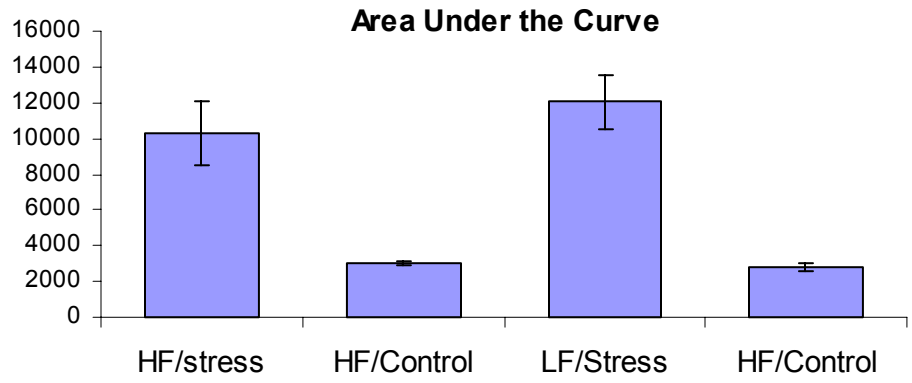

Area Under the Curve
16000
14000
12000
10000
8000
6000
4000
2000
0
HF/stress
HF/Control
LF/Stress
HF/Control

Figure 3.2

A

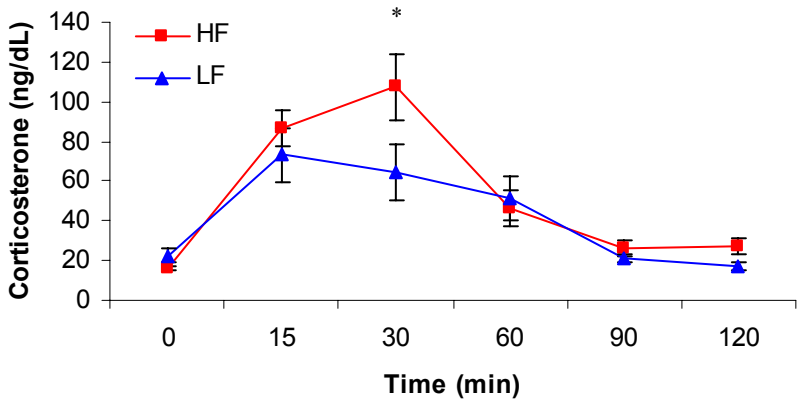
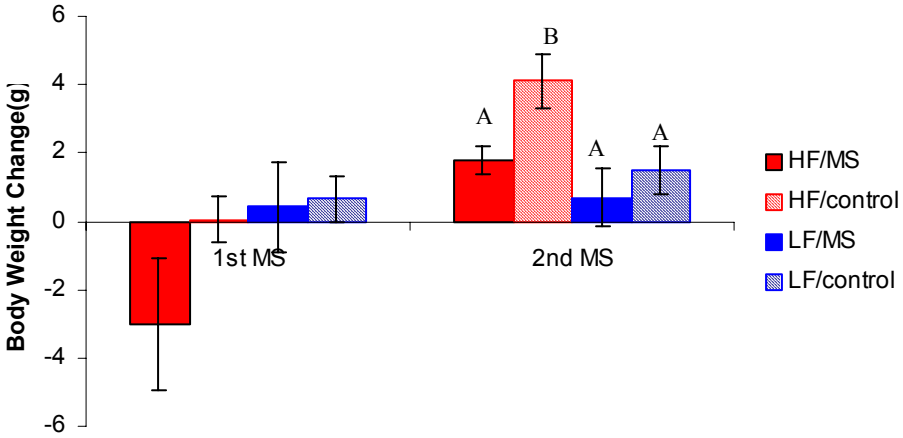
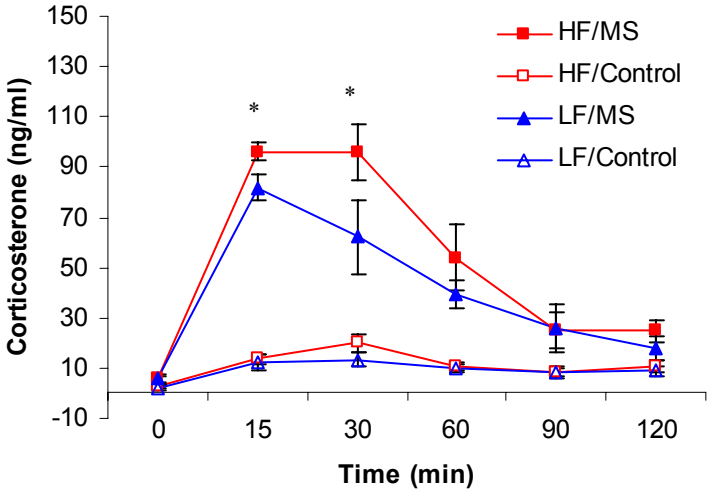

Corticosterone (ng/dL)
140
120
100
80
60
40
20
0
HF
LF
*
0
15
30
60
90
120
Time (min)

Figure 3.3

A


Body Weight Change(g)
6
4
2
0
-2
-4
-6
A
B
A
A
1st MS
2nd MS
HF/MS
HF/control
LF/MS
LF/control

B


Corticosterone (ng/ml)
150
130
110
90
70
50
30
10
-10
*
*
0
15
30
60
90
120
Time (min)
HF/MS
HF/Control
LF/MS
LF/Control

C

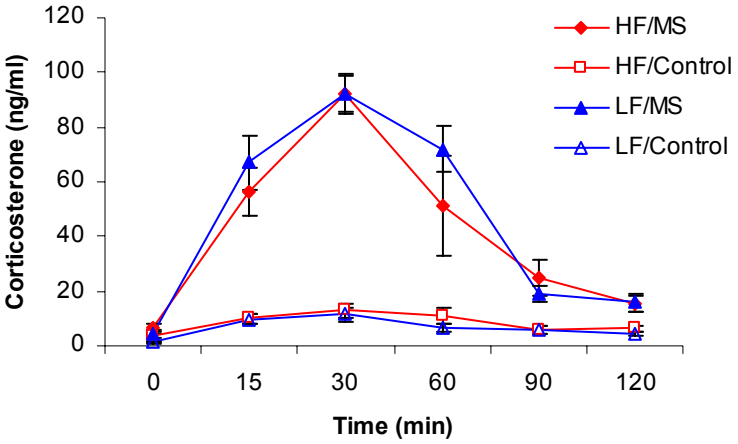
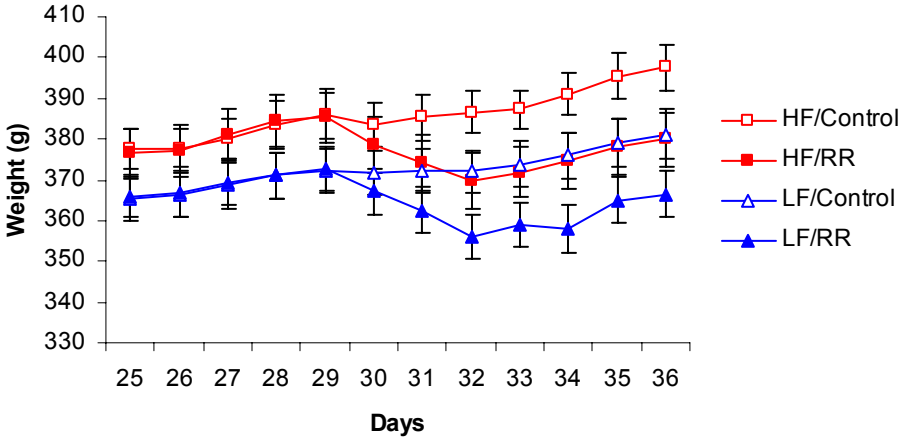
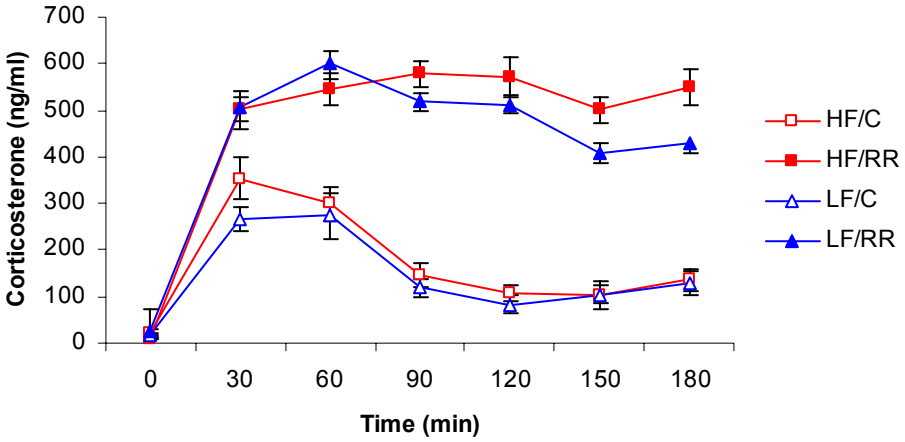

120
100
80
60
40
20
0
Corticosterone (ng/ml)
0
15
30
60
90
120
Time (min)
HF/MS
HF/Control
LF/MS
LF/Control

Figure 3.4

A


410
400
390
380
370
360
350
340
330
Weight (g)
25
26
27
28
29
30
31
32
33
34
35
36
Days
HF/Control
HF/RR
LF/Control
LF/RR

B


700
600
500
400
300
200
100
0
Corticosterone (ng/ml)
0
30
60
90
120
150
180
Time (min)
HF/C
HF/RR
LF/C
LF/RR

CHAPTER 4

HIGH FAT DIET DECREASES UROCORTIN BUT NOT CORTICOTROPIN RELEASING FACTOR mRNA IN THE BRAIN.[2]

[2] Legendre, A., Roy, M.C., Richard, D., Harris, R.B. *To be submitted to the American journal of Physiology: Regulatory, Integrative and Comparative Physiology.*

ABSTRACT

It has been suggested that high fat (HF) diet exaggerates the stress-induced release of glucocorticoids due to hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis. Studies described here demonstrate that a simple increase in release of corticotropin releasing factor (CRF) and the related peptide Urocortin I (Ucn I) are unlikely to be the cause of the exaggerated glucocorticoid response. Rats fed HF diet for 4 days did not demonstrate increased corticosterone levels in response to infusion of 1μg of CRF into the 3rd ventricle. In an additional experiment, in situ hybridization was used to measure mRNA expression of CRF in the paraventricular nucleus (PVN) of the hypothalamus and the central nucleus of the amygdala, and of Ucn I in the Edinger Westphal nucleus (EW) of rats exposed to a mild stress (1 ml i.p. injection of saline and move immediately to new room). HF feeding did not cause any changes in CRF but did decrease Ucn I mRNA expression. These results suggest that the exaggerated glucocorticoid responses to stress in HF-fed rats reported for previous experiments may be caused by a change in ratio of CRF and Ucn I mRNA expression in specific areas of the brain.

Key words: HPA axis, CRF, stress, high fat diet, in situ hybridization

INTRODUCTION

The incidence of obesity has dramatically increased in the last 20 years in the United States and is now perceived as one of the country's leading health problems (**www.cdc.gov**). Overweight and obesity are a result of a positive energy balance over a long period of time. Although such an energy imbalance may be caused by a combination of several factors, common environmental and life-style factors have been shown to play an important role in the development and maintenance of obesity (5). The consumption of dietary fat is one environmental factor that has been linked to the development of obesity (1). Due to its increased palatability and decreased satiety factor, dietary fat is often over-consumed (22, 23). Consequently, the increasing rate of obesity has been correlated to increased dietary fat intake (1, 30).

In response to stress, the hypothalamic-pituitary-adrenal (HPA) axis plays a vital role in adaptation of the organism to homeostatic changes by regulating a cascade of hormones. Activation of the HPA axis ultimately results in the release of glucocorticoids, which act at multiple levels to redirect body energy resources (16, 18, 24) including energy mobilization (glycogenolysis) in the liver, suppression of the immune system, inhibition of bone and muscle growth, potentiation of sympathetic nervous system-mediated vasoconstriction, proteolysis and lipolysis, suppression of reproductive function, and behavioral depression (16, 18). Although these, and other responses, allow an animal or individual to adapt to a change in environment or a socially stressful situation, if inadequate or excessive and/or prolonged they may have adverse consequences on physiologic functions, such as growth, metabolism, circulation, reproduction, and the inflammatory/immune response (3, 7). Exposure to chronic stress has been acknowledged to be involved in the development of a wide range of physiological and behavioral

diseases such as heart disease, depression, anxiety and mood disorders, detrimental weight gain, and anorexia nervosa (33).

Stress acts on the hypothalamus by stimulating the release of corticotropin-releasing factor (CRF). CRF induces the anterior pituitary gland to release adrenocorticotropin hormone (ACTH) into the circulation, which stimulates the adrenal gland to release glucocorticoids, specifically corticosterone in rodents. Free corticosterone will bind to glucocorticoid receptors on the pituitary and on the hypothalamus to down-regulate secretions of ACTH and CRF, respectively, by negative feedback mechanisms (2). In addition to surges in glucocorticoid concentration, decreases in food intake, decreases in weight gain, and increases in energy expenditure are observed in response to stress (8). These responses are apparent in a variety of acute stress models in rodents, such as repeated restraint (8) immobilization (17), and social defeat (6).

Previous studies have found that variations in the macronutrient composition of a diet, specifically the fat content, can affect some of the stress responses, including mood and behavior (15), and the neuroendocrine response to stress (11, 19, 21, 28). Studies investigating the effect of high fat (HF) diet on the stress response show that rats fed a HF diet demonstrated an elevated HPA activity in response to restraint stress in comparison to rats fed a low fat (LF) diet (11, 19, 21, 28). Tannenbaum et al. (28) reported that feeding rats a HF diet resulted in both elevated basal and peak levels of corticosterone, increased ACTH release during stress and an impaired recovery of corticosterone release after 20 minutes of restraint stress. Additionally, rats fed a HF diet responded with an increased weight loss following repeated restraint and failed to gain the weight back (8). All together these observations suggest that HF diet may act as a chronic stressor, and subsequently rats respond in an exaggerated manner to an acute stress. In studies

investigating the relationship between stress and dietary fat it is not clear whether increased stress responsiveness is due to diet composition or increased adiposity. Recent reports (unpublished data) from our lab have shown that rats fed a HF diet for only 4 days demonstrate an exaggerated release of serum corticosterone concentrations when exposed to mild stress but not to a stronger stress such as repeated restraint. Furthermore, we have found that the exaggerated corticosterone in response to mild stress lasted for less than 2 weeks. This suggests that diet alone, not increase in adiposity, is responsible for the exaggerated stress response.

In the following experiments, we hypothesized that HF diet acts as a chronic stressor; consequently rats fed a HF diet will react with an exaggerated response to stress. Furthermore, we hypothesized that the exaggerated stress response in rats fed HF diet results from modifications in the activity of the CRF system through either an increased responsiveness of CRF receptors or an increased expression of CRF and CRF related peptides. In order to differentiate between the effects of a HF diet and the effects of increased adiposity on the endocrine stress response in rats, in the present studies we fed Sprague-Dawley rats a HF diet for only 4 days.

METHOD

All procedures for care and use of animals were approved by the Institutional Animal Care and Use Committee of the University of Georgia and were in accordance with the Guiding Principles of the American Physiological Society (26).

Experiment 1: Acute exposure to HF diet and CRF infusions.

Fifty-six Sprague-Dawley male rats (Harlan Sprague Dawley, Indianapolis, IN), weighing approximately 350g were housed in individual stainless steel cages in a room with controlled temperature (23°C) and a 12h/12h light/dark cycle with lights on at 7:00 am. Upon arrival the

rats were allowed one week to acclimate to the new conditions. All animals had free access to rodent chow (Purina Rodent Chow 5001, Purina Mills, St-Louis, MO) and water.

Following one week of habituation, the animals were anesthetized by intraperitoneal (i.p.) injection of ketamine (90 mg/kg) and of xylazine (10 mg/kg) and implanted with cannulas in the third ventricle using stereotaxic techniques. Guide cannulas (25 gauge, 15 mm long) were placed using the following coordinates applied to a flat skull: anteroposterior –2.8, lateral 0.0, ventral –8.3 from bregma (25). The cannulas were secured in place with machine screws and dental cement. To confirm cannula placement, 5 days following the surgery the animals were infused with 10 ng of angiotensin II and animals that drank less than 3 ml within 5 minutes of infusion were excluded from the study. The day following the angiotensin II infusion, all the animals were offered a LF diet (12% kcal fat: Diet D02041902, Research Diets Inc., New Brunswick, NJ). Body weights and food intake were measured daily throughout the experiment. After 4 days on the LF diet, the animals were divided into 2 weight-matched groups. One group stayed on the LF diet while the other group was switch to the HF diet (40% kcal fat: Diet D02041901, Research Diets Inc., New Brunswick, NJ) for 4 additional days. After 4 days, each dietary group was sub-divided into two weight-matched groups. One group from each dietary group was infused with either 2µl of saline for control (HF/Saline, LF/Saline) or 2µl of saline containing 1µg of CRF (HF/CRF, LF/CRF). Blood samples from the tail were collected immediately prior to the infusion and at 30 minutes intervals following the infusion (time 0, +30, +60, +90, +120, +150, +180 min.). All the animals were decapitated 2 days following the CRF infusions. Blood samples were centrifuged and the serum was stored at -80°C until assays could be performed. Corticosterone concentrations were measured at all time points by RIA

(Corticosterone RIA; MP Diadnostics, Costa Mesa, Ca). ACTH (ACTH RIA; Nichols Institute Diagnostics, San Clemente, CA) was measured on the 30 minutes blood sample. Body weight and food intake were recorded for 2 days after the infusion.

Experiment 2: In situ hybridization

Twenty-four Sprague-Dawley male rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing approximately 300g were housed as described above. Following the acclimation period (1 week) the animals were fed a LF diet (same as Experiment 1) for one week, during which daily body weights and food intakes were measured. The animals were divided into two weight-matched groups, one group was fed a HF diet (same as Experiment 1) while the other group remained on the LF diet. On the fifth day, each dietary group was subdivided into two weight-matched groups. One subgroup from each dietary group was non-stressed for control while the other was subjected to mild stress (HF/MS, HF/Control, LF/MS, LF/Control). The mild stress groups received a 1ml i.p. injection of saline and were moved to individual shoebox cages in a testing room for 1 hour with no access to food or water. The control groups were left in their home cage without access to food or water for 1 hour. Immediately at the end of 1 hour of mild stress the rats were anesthetized with 90 mg/kg ketamine and 10 mg/kg xylazine and then perfused intracardially with 75 ml ice-cold saline followed by 200 ml 4 % paraformaldehyde solution. The brains were collected and stored in 4% paraformaldehyde solution at 4ºC until slicing.

For in situ hybridization the brains were transferred to a 4% paraformaldehyde, 10% sucrose solution for 12 to 16 hours before sectioning. Brain sections were cut using a freezing Vibratome and stored in cold sterile cryoprotectant solution (50 mM phosphate buffer, 30% ethylene glycol, 20% glycerol). For each mRNA that was evaluated one of every 5 sections was mounted on a

poly-L-lysine coated slide, and desiccated overnight under vacuum. The sections were then fixed for 20 minutes in 4% paraformaldehyde, digested for 30 minutes at 37ºC with proteinase K (10 ug/ml in 100 ml Tris HCl containing 50 mM EDTA, pH 8.0) acetylated with acetic anhydride (0.25% in 0.1 M Triethanolamine, pH 8.0) and dehydrated through increasing concentrations of ethanol (50, 70, 95 and 100 %). After vacuum drying for at least 2 hours hybridizing solution containing antisense S^{35}- labelled cRNA probe (10^7 cpm/ml) was spotted onto each slide. The slides were sealed with a coverslip and incubated overnight at 60ºC in a slide warmer. The next day the coverslips were removed and the slides were rinsed four times with 4X saline -sodium citrate (SSC), digested for 30 minutes at 37ºC with RNase A (20 ug/ml in 10 mM Tris, 500 mM NaCl, 1 mM EDTA) and dehydrated through increasing concentrations of ethanol. After drying for 2 hours in a vacuum oven the slides were exposed to an X-ray film for 24 hours. After removal from the autoradiography cassette the slides were defatted in xylene and dipped in NTB-2 nuclear emulsion (Eastman Kodak, Rochester NY). The slides were exposed for 7 minutes before being developed in D19 developer (Eastman Kodak) for 3.5 minutes at 14-15°C and fixed in rapid fixer (Kodak) for 5 minutes. Tissues were rinsed in running distilled water for 1-2 hours, counterstained with 0.25% thionin, dehydrated with increasing concentrations of ethanol, cleared in xylene and coverslipped with DPX (Sigma-Aldridch, St Louis, MO). The hybridization signals revealed on NTB-2-dipped nuclear emulsion slides were analyzed and quantified under a light microscope equipped with a black and white video camera coupled to a computer using image software (AIS 6.0, Imaging Research Inc). The optical density for the hybridization signal was measured under a bright field illumination at a magnification of x25. Brain sections from the different rats were matched for rostrocaudal levels as closely as possible. CRF mRNA expression was quantified in the paraventricular nucleus of the hypothalamus (PVN) and the

central nucleus of the amygdala (CeA), and Ucn I mRNA expression was quantified in the Edinger-Westphal nucleus (EW).

Antisense ^{35}S-labeled cRNA probe.

The CRF cRNA probe was generated from the 1.2 kb *Eco*RI fragment of rat CRF cDNA (Dr. K. Mayo, Northwestern University, IL) subcloned into a pGEM-4 vector and linearized with *Hin*dIII/SP6 and *Eco*RI/T7 for antisense and sense probes, respectively. The UCN cRNA probe was generated from 600 pb *Eco*RI fragment of rat UCN cDNA (Dr. W. Vale, The Salk Institute, La Jolla, CA) subcloned into pBluescript vector and linearized with *Sma* I/T7 and *Hind* III/T3 for antisense and sense respectively. The specificity of each probe was confirmed by the absence of a positive signal in sections hybridized with sense probe. Radioactive riboprobes were synthesized by incubation of 250 ng linearized plasmid in 10 mM NaCl, ATP/GTP/CTP, a-35S-UTP, 40 U Rnasin (Promega, Madison, WI) and 20 U of either T7, SP6 or T3 RNA polymerase for antisense probe, for 60 min at 37°C. The DNA templates were treated with 100 µl of DNAse solution (1 µl DNAse, 5 µl of 5 mg/ml tRNA, 94 µl of 10 8 mM Tris/10 mM $MgCl_2$). The preparation of the riboprobe was completed through a phenol-chloroform extraction and ammonium acetate precipitation.

Data analysis:

The effect of diet on body weight, energy intake, and repeated measures of corticosterone were determined by repeated measure analysis of variance using intake or body weight measured immediately before the start of the stress (CRF infusions), or corticosterone measured at time 0 min., as a covariant in the analysis. Statistically significant ($p \leq 0.05$) differences in body weight,

food intake and corticosterone levels between groups on specific days or at time points were determined by two-way analysis of variance and post-hoc Duncan's Multiple Range test. Single measures were compared by unpaired t-test or by two-way analysis of variance with post-hoc Duncan's Multiple Range test. All statistical procedures were carried out using Statistica software (Statistica Software 99' Edition, Stat Soft, Tulsa, OK). Animals with corticosterone or mRNA density values with 2 standard deviations above or below the mean were considered outliers and were not used for any of the data analysis.

RESULTS

Experiment 1:

There were no significant differences between the body weights of the groups of rats on the day of infusion. There was no effect of diet or CRF on the amount of weight that was lost (Diet: NS, CRF: NS, Interaction: NS) (Fig. 4.1a and 4.1b). There were no differences in basal (0 min.) corticosterone between HF- and LF-fed groups. CRF infusion caused a significant increase in corticosterone, which peaked between 30 and 60 minutes after the infusion (Fig. 4.1c). There was no effect of diet on the size of this response. Both HF- and LF-fed rats infused with CRF showed a decrease in corticosterone concentrations at 90 minutes, and had reached control corticosterone levels by 120 minutes.

Experiment 2:

On the day the brains were collected there were no differences in body weight between each group (data not shown). A positive hybridization signal for CRF mRNA was detected in various regions of the brain including the hypothalamus, the arcuate nucleus, the amygdala, and the

neocortex. Within the hypothalamus, CRF mRNA was abundant in the PVN. PVN CRF mRNA was increased in rats exposed to mild stress (Fig. 4.2a). This increase was significant ($p \leq 0.05$) in the LF-fed rats but it was not significant in the HF-fed rats. PVN CRF mRNA was not different between the HF- and LF-fed rats when compared in non-stressed or in stressed condition. CRF mRNA was also abundant in the CeA. There were no diet or mild stress effects on CRF mRNA expression in the CeA (Diet: NS; Stress: NS; Interaction: NS) (Fig. 4.2b). The HF-fed rat showed a significant increase in Ucn I mRNA expression in the EW when exposed to mild stress (Diet: NS; Stress: NS; Interaction: 0.012). Levels of Ucn I mRNA expression in both groups of LF-fed rats were comparable to the elevated level found in the HF-fed rats exposed to mild stress (Fig. 4.2c).

DISCUSSION

It has been well established that hypothalamic CRF plays an important role in the regulation of basal and stress-induced activation of the HPA axis (2). CRF also affects anxiety-related behaviors, which are mediated through the amygdala and locus coeruleus, and eating behaviors, which are mediated through hypothalamic and brainstem nuclei. Previous research has shown that rats fed HF diet will demonstrate an exaggerated corticosterone concentration in response to restraint stress (11, 28). To date, however, there are no reports investigating the physiological changes causing the exaggerated stress response in HF-fed rats. Therefore, the current experiments were conducted to assess the effects of HF diet on the HPA axis, more specifically the responsiveness to CRF and mRNA expression of CRF and the related peptide Ucn I.

Experiment 1 investigated the effects of 3rd ventricle administration of CRF on the endocrine response in rats fed a HF diet. The dose of CRF (1 μg) used was chosen because in a previous experiment (unpublished) we found that with 1 μg of CRF the animals lost a significant amount of weight compared to the animals infused with saline. In animals exposed to repeated restraint, corticosterone concentrations will peak between 150 and 200 ng/ml. Here, with a CRF dose of 1 μg we obtained a peak corticosterone response between 450 and 500 ng/ml. This represents a much larger response than normally observed physiologically. Dietary fat did not affect corticosterone concentrations in response to administration of CRF into the 3rd ventricle.

An interesting aspect of the current experiments is that 3rd ventricle CRF infusions caused a significant increase in corticosterone concentrations but did not induce weight loss, which is a typical response to stress in rats. The saline-infused rats also showed a significant corticosterone increase compared to basal concentrations, most likely due to the stress involved with the manipulations. These observations reinforced the possibilities that CRF1 and CRF2 receptors are regulating different aspects of the stress response. It has been established that CRF and related peptides, urocortin, will bind to two different types of CRF receptors, CRF1 and CRF2 receptors (2). These receptors are distributed in different areas of the brain and body and are believed to be responsible for regulating different stress response. Central CRF1 receptors are predominantly expressed throughout the cerebral cortex, cerebellum, olfactory bulb, medial septum, hippocampus, amygdala, and anterior pituitary (31). These areas of the brain are involved in the control of motor and sensory functions (31). Activation of the CRF1 receptors appears to initiate the endocrine response (2). CRF2 receptors are mostly expressed in subcortical hypothalamic structures, but are also widely expressed in peripheral tissues, such as the heart, GI tract, lungs, skeletal muscle, and vasculature (13, 20, 27). Activation of the CRF2

receptors appears to mediate behavioral response to stress, including stress-induced inhibition of food intake (4). Here, our results suggest that the mechanisms involved in weight loss in response to stress were activated in both CRF and saline infused groups and were probably responding to the stress of handling but were not responding to CRF specifically. This implies that either the CRF receptors mediating weight loss are not located near the 3rd ventricle or that the half-life of CRF was too short to induce a response. If this second alternative is true than the stress induced by handling must have extended beyond the time taken to infuse the rats.

In Experiment 2, CRF mRNA expression in the PVN was increased in rats exposed to mild stress but this response was not significant in rats fed HF diet. CRF mRNA expression in the central nucleus of the amygdala did not differ across all the groups. Some of the CRF neurons in the PVN project to the brainsteam autonomic systems and are involved in arousal and appetite regulation, whereas the CeA has been shown to play a critical role in fear- and anxiety-related behaviors (12, 32). Taking this into account, the increased CRF mRNA expression in the PVN should decrease appetite and subsequently food intake and weight gain in animals exposed to stress. A decrease in food intake and weight loss has been previously observed in rats exposed to stress (8). The fact that the HF-fed control rats had increased CRF mRNA expression compared with LF-fed controls correlates with our previous observations (unpublished data) from rats fed HF that showed a decrease in weight gain in response to the mild stress of tail bleed compared to LF-fed animals. In contrast to the effect of stress on PVN CRF mRNA, no stress-induced changes in CRF mRNA were observed in the CeA. Data from previous studies demonstrated that restraint or other psychological stressors increase CeA CRF mRNA (10, 14). However, other studies have shown no increases in CeA CRF mRNA following tail shock (9) or have found decreases in CeA CRF mRNA following 24-hour food deprivation (29). Taken

together, these studies suggest that the effects of acute stress on CeA CRF mRNA are variable and are likely to be influenced by numerous factors. Since we did not observe any differences in CeA CRF mRNA between diet regimens, it seems unlikely that dietary fat will have any effect on the regulation of CeA CRF mRNA.

Ucn I mRNA expression in the EW was decreased in HF/Control rats compared to the LF/Control. In addition, whereas levels of Ucn I mRNA did not differ between the LF-fed mild stress or control groups, Ucn I mRNA expression was significantly increased in HF-fed rats exposed to mild stress. This suggests that the exaggerated stress response previously observed in HF-fed rats may be due to a change in the ratio of CRF/Ucn I mRNA expression. CRF binds to both CRF1 and CRF2 receptors, but has high affinity to CRF1 receptors (2). In contrast, Ucn I will bind with equal affinity to both CRF1 and CRF2 receptors (20). If the Ucn I mRNA expression decreases and CRF mRNA expression does not change in rats fed HF, we could expect a greater activation of CRF1 receptors compared to CRF2 receptors in non-stress conditions. If this holds true then we could expect to see a greater endocrine response from activation of CRF1 receptors in rats fed a HF diet because the change in binding required for reaching a threshold for activation is smaller for HF-fed than LF-fed rats.

In conclusion, these experiments provide evidence indicating that dietary fat does not affect CRF mRNA but decreases Ucn I mRNA expression in important brain areas of the brain that are involved in the response to stress. Although, we did not specifically measure CRF receptors in these experiments, the result suggest that CRF receptors are not affected by HF diet. Further studies investigating the mechanisms by which HF diet may exaggerate the stress response should focus on CRF receptors, on mechanisms downstream from the hypothalamus

and CRF, on negative feedback mechanisms, and other possible neuropeptides interacting with CRF receptors and the HPA axis.

Acknowledgements:

The authors wish to thank Hemanth Narr from Emory University for his technical assistance.

This work was supported by NIH grant MH068281.

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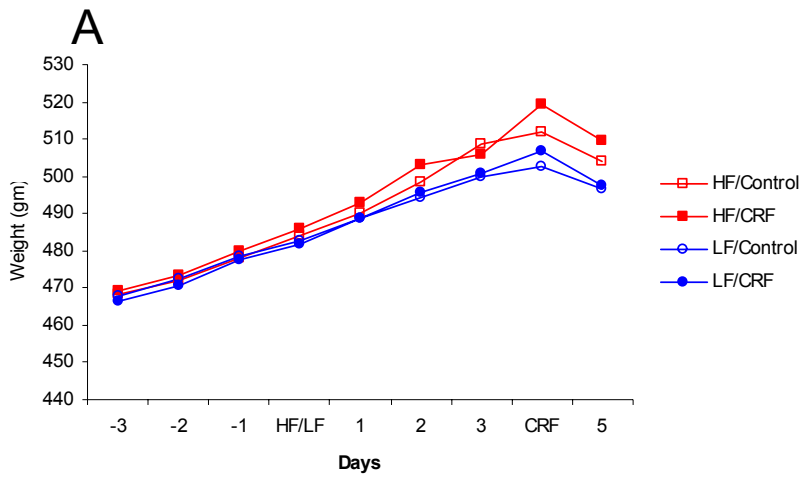
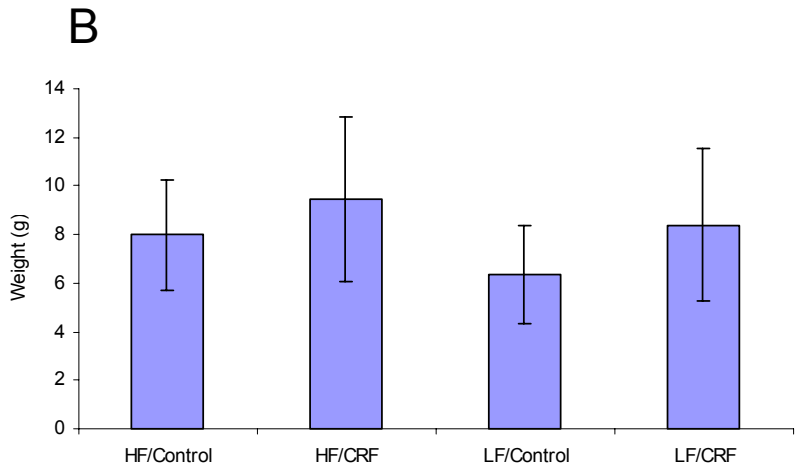
Figure Legend:

Figure 4.1: Body weights (Panel A) and weight loss (Panel B) in response to 3rd ventricle CRF infusion in Sprague-Dawley male rats that were previously fed HF or LF diet for 4 days. Blood corticosterone concentrations (Panel C) were measured at 30 min. intervals following the CRF infusion.

Figure 4.2: CRF mRNA expression was measured in the PVN (Panel A) and CeA (Panel B) of rat fed HF diet for 4 days following 1 hour of mild stress. Similarly, Unc I mRNA expression was measured in the Edinger-Westphal nucleus (Panel C). Superscripts indicate significant differences between groups.

Figure 4.1


A
530
520
510
500
490
480
470
460
450
440
Weight (gm)
-3
-2
-1
HF/LF
1
2
3
CRF
5
Days
HF/Control
HF/CRF
LF/Control
LF/CRF


B
14
12
10
8
6
4
2
0
Weight (g)
HF/Control
HF/CRF
LF/Control
LF/CRF

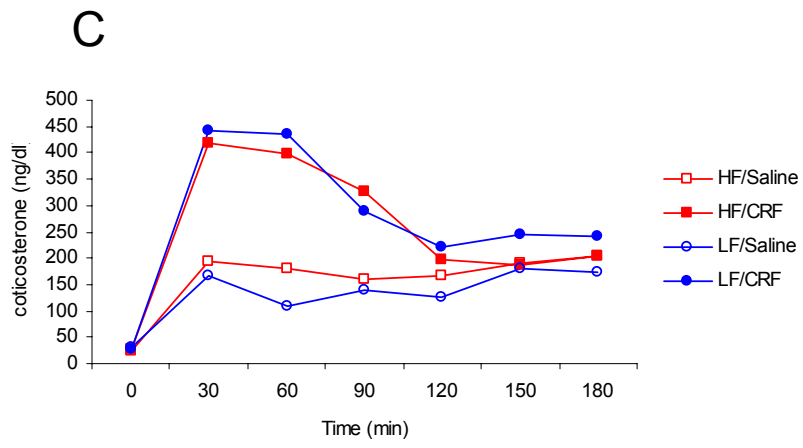
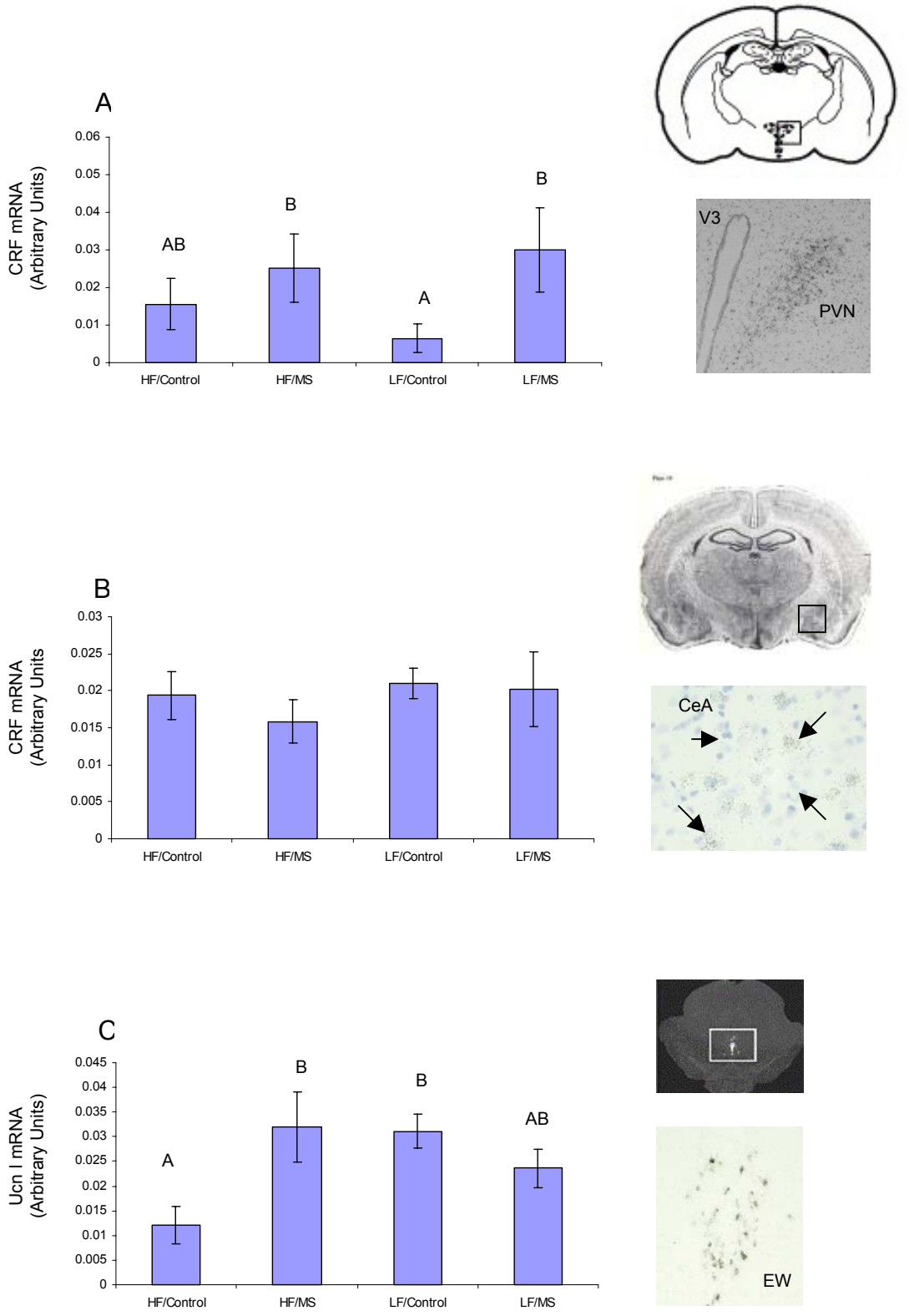

C
500
450
400
350
300
250
200
150
100
50
0
coticosterone (ng/dl
0
30
60
90
120
150
180
Time (min)
HF/Saline
HF/CRF
LF/Saline
LF/CRF

Figure 4.2


A
CRF mRNA (Arbitrary Units)
0.06
0.05
0.04
0.03
0.02
0.01
0
AB
B
A
B
HF/Control
HF/MS
LF/Control
LF/MS
V3
PVN
B
CRF mRNA (Arbitrary Units
0.03
0.025
0.02
0.015
0.01
0.005
0
HF/Control
HF/MS
LF/Control
LF/MS
CeA
C
Ucn I mRNA (Arbitrary Units)
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
A
B
B
AB
HF/Control
HF/MS
LF/Control
LF/MS
EW

CHAPTER 5

SUMMARY AND CONCLUSION

The consumption of dietary fat is one environmental factor that has been linked to the development of obesity (1). Due to its increased palatability and decreased satiety factor, dietary fat is often over-consumed (9, 10). Consequently, the increasing rate of obesity has been correlated to increased dietary fat intake (1, 12). Additionally, in efforts to address both burden of obesity-associated chronic disease and individual concerns about appearance, interest in weight-loss therapies has been rising. However, weight-loss therapies have not been effective in decreasing and maintaining body weight over time. Also associated with weight gain and weight loss, and perhaps the onset and maintenance of obesity in some cases, is certain physiologic states, such as stress (2). The relationship between weight gain and weight loss and stress is still not fully understood. The effects and mechanisms of HF on the HPA axis in response to stress may be helpful in understanding some of the barriers and limitations related to weight loss management.

Previous studies have reported that stress may alter macronutrient selection, and favoring the consumption of high fat (HF) and high density foods (2-4). Meanwhile other studies have found that variations in the macronutrient composition of a diet, specifically the fat content, can affect some of the stress responses, including mood and behavior (6), and the neuroendocrine response to stress. Previous studies investigating the effect of HF diet on the stress response reported that rats fed a HF diet showed an elevated hypothalamic-pituitary-adrenal (HPA) activity in response to restraint stress in comparison to rats fed a low fat (LF) diet (5, 7, 8, 11).

In studies investigating the relationship between stress and dietary fat it is not clear whether increased stress responsiveness is due to diet composition or increased adiposity. In addition, to date, there are no reports investigating the physiological changes causing the exaggerated stress response in HF-fed rats.

The series of experiments in this thesis investigated whether the exaggerated response in HF-fed Sprague-Dawley rats was caused by dietary fat or increased adiposity and investigated aspects of the stress pathway, by measuring CRF mRNA and Ucn I mRNA expression in the brain. The first series of experiments described here demonstrate that HF diet, not obesity, is responsible for the exaggerated corticosterone response in HF-fed rats. This further suggests that HF diet may act as a stressor. The second series of experiments investigated the mechanisms behind the increased stress response in HF-fed rats. Results from these experiments provide evidence indicating that dietary fat does not affect the endocrine response following a CRF infusion into the 3rd ventricle and that CRF mRNA is unchanged, whereas Ucn I mRNA expression is decreased in important brain areas in response to stress in HF-fed rats. Together this data suggest that the exaggerated stress response in HF-fed rats is not caused by an increase in CRF, but rather to a change in the ratio of CRF/Ucn I. Further studies investigating the mechanisms by which HF diet may exaggerate the stress response should focus on CRF receptors, on mechanisms down stream from the hypothalamus and CRF, on negative feedback mechanisms, and other possible neuropeptides interacting with CRF receptors and the HPA axis.

This new information, compiled with previous results, may be helpful in identifying some of the limitations and restrictions involved in weight loss management. Furthermore, this information will be helpful in evaluating the potential effectiveness of certain weight loss therapy.

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