Differences in response to corticotropin-releasing factor after short- and long-term consumption of a high-fat diet

Ariadne Legendre, Emilia Papakonstantinou, Marie-Claude Roy, Denis Richard, and Ruth B. S. Harris

1Department of Foods and Nutrition, University of Georgia, Athens, Georgia; and 2Department of Anatomy and Physiology, Université Laval, Quebec City, Quebec, Canada

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Legendre A, Papakonstantinou E, Roy M-C, Richard D, Harris RB. Differences in response to corticotropin-releasing factor after short- and long-term consumption of a high-fat diet. Am J Physiol Regul Integr Comp Physiol 293: R1076–R1085, 2007. First published June 20, 2007; doi:10.1152/ajpregu.00592.2006.—We previously reported an exaggerated endocrine and weight loss response to stress in rats fed a high-fat (HF) diet for 5 days. Others report blunted stress-induced anxiety in rats made obese on a HF diet. Experiments described here tested whether sensitivity to stress-related peptides was changed in obese and nonobese HF-fed rats. Third ventricle infusion of corticotropin-releasing factor (CRF) in rats made obese on HF diet (40% kcal fat) produced an exaggerated hypophagia, which is thought to be mediated by CRF2 receptors. Obese rats responded to a lower dose of CRF for a longer time than rats fed a low-fat (LF) diet (12% kcal fat). CRF-induced release of corticosterone, which is thought to be mediated by CRF1 receptors, was not exaggerated in obese HF-fed rats. In contrast, rats fed HF diet for 5 days showed the same food intake and corticosterone response to CRF as LF-fed rats. CRF mRNA expression in the paraventricular nucleus of the hypothalamus was stimulated by mild stress (ip saline injection and placement in a novel cage) in LF-fed rats but not in rats fed HF diet for 5 days because of a nonsignificant increase in expression in nonstressed HF-fed rats. In addition, nonsellected levels of urocortin (UCN) I mRNA expression in the Edinger-Westphal nucleus were significantly inhibited in HF-fed rats. These data suggest that rats that have become obese on a HF diet show a change in responsiveness to stress peptides, whereas the increased stress response in nonobese HF-fed rats may be associated with changes in basal CRF and UCN I mRNA expression.

SEVERAL INVESTIGATORS have reported that animals fed a high-fat (HF) diet show an exaggerated response to stress compared with their low-fat (LF)-fed counterparts. Tannenbaum et al. (35) found that rats fed a 40% kcal fat diet for 7 or 21 days had elevated basal levels of corticosterone compared with rats fed a 20% kcal fat diet, with a greater effect of diet at 7 days than at 21 days. There also was an increased adrenocorticotropic hormone (ACTH) release during 20 min of restraint stress and an impaired recovery of corticosterone release after stress for up to 12 wk of HF feeding. Moreover, the delayed recovery was not associated with insensitivity to the negative feedback of glucocorticoids, although a reduction in hypothalamic glucocorticoid receptor number was found after 1 wk, but not after 9 wk, of HF feeding (35). Similar exaggerated glucocorticoid responses were found in rats fed HF diets for 2 wk and restrained for 30 min (16). After 10 wk on the diet there was no longer an exaggeration of corticosterone release during restraint, but recovery of corticosterone to baseline levels was slower in HF-fed rats than LF-fed rats (16). We previously reported (12) that rats fed a HF (40% kcal fat) diet and exposed to repeated restraint stress lose more weight than those fed a LF (11% kcal fat) diet. These studies all suggest that rats adapted to a HF diet are more responsive to stress than those adapted to a LF diet, but more recently Dallman’s group (17, 27) reported that if fat is presented as a dietary choice, rather than as part of a composite diet, stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis is muted. These data imply that the matrix in which dietary fat is presented, or the ability of the animal to select how nutrients are consumed, also may influence the stress responsiveness of the HPA axis.

In contrast to these experiments that have largely focused on the endocrine response to stress, studies that tested the behavioral responses to stress have demonstrated that rats fed extremely HF diet (90% kcal fat) for 5 days show less anxiety-type behaviors in an elevated plus maze than LF-fed rats (28) and that rats fed a 61% kcal fat diet for 4 mo show less anxiety-type behavior, a faster recovery of body temperature, and less hypersensitization of serotonin receptors following either psychosocial or physiological stress (5). Similarly, diet-induced obese rats showed less anxiety and less disruption of the diurnal rhythm of corticosterone release than obesity-resistant rats exposed to 5 wk of unpredictable stress (21). It is not clear whether the conflicting reports for the effects of HF diets on stress responsiveness are due to the end point measures that were made, differences in the composition of the HF diets, differences in the duration of HF feeding, or differences in body composition of HF-fed and LF-fed rats.

In the studies described above HF-fed rats were significantly fatter than those fed a LF diet (5, 12, 17, 28, 35), and the effects of diet composition were not separated from those of obesity. We recently reported (19) that rats fed a HF diet for only 5 days demonstrate an exaggerated release of serum corticosterone concentrations and weight loss when exposed to mild stress but not to a more extreme stress such as 3 h of restraint on each of 3 days. Furthermore, we found (19) that the exaggerated response to mild stress lasted for <3 wk. This suggests that diet alone, not an increase in adiposity, is responsible for the increased sensitivity toward mild stressors in HF-fed rats, but that it is a transitory effect. Thus the increased stress responsiveness of rats that have been fed a HF diet for longer periods

Address for reprint requests and other correspondence: R. B. S. Harris, Dept. of Foods and Nutrition, Univ. of Georgia, Athens, GA 30602 (e-mail: harrisrb@uga.edu).

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of time may be associated with obesity rather than consumption of increased amounts of fat.

The exaggeration of the stress response may result from modifications in the activity of the corticotropin-releasing factor (CRF) system through either an increased responsiveness to stress peptides at a receptor or postreceptor level or an increased expression of CRF and CRF-related peptides, such as urocortin (UCN) (34). The experiments reported here tested whether feeding rats a HF diet associated with, or independent of, the development of obesity increased their responsiveness to central injections of CRF, a neuropeptide that is thought to initiate many of the endocrine, behavioral, and physiological responses to stress (8, 20). The first experiment tested the effect of central administration of increasing doses of CRF on food intake in rats that had been fed a HF diet for 60 days and were significantly fatter than their LF-fed controls. To differentiate between the effects of a HF diet and the effects of increased adiposity on the response to CRF, we subsequently tested rats that had been fed a HF diet for 5 days and measured food intake or corticosterone release during the hours immediately following the CRF injection. In addition we determined whether 5 days of HF feeding influenced stress-induced CRF mRNA or UCN I mRNA expression in specific brain areas and also confirmed that there were no differences in body composition of rats fed LF or HF diets for 5 days.

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 “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society (1).

Experiment 1: Effect of CRF on energy intake of rats made obese on HF diet. The objective of this study was to determine whether rats that had become obese on a HF diet were more or less responsive to central infusions of CRF than rats fed a LF diet. Twenty male rats, weighing ~350 g (Harlan Sprague Dawley, Indianapolis, IN) were housed individually in hanging wire mesh cages in a temperature-controlled room at 23°C with lights on for 12 h/day from 7:00 AM. All animals had free access to rodent chow (Purina Rodent Chow 5001, Purina Mills, St. Louis, MO) and water. After 1 wk of adaptation the rats were divided into two groups, and 10 rats were offered a HF diet (40% kcal fat, 4.58 kcal/g; Diet D02041901, Research Diets, New Brunswick, NJ). The remaining animals were fed a LF diet (12% kcal fat, 3.04 kcal/g; Diet D02041902, Research Diets). After 44 days, when the weight of the HF-fed rats was ~75 g greater than that of the LF-fed animals, the rats were anesthetized with intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) and implanted with cannulas by stereotaxic techniques. Guide cannulas aimed at the third ventricle (25 gauge, 11 mm long) were placed with the following coordinates applied to a flat skull: anteroposterior −2.8, lateral 0.0, ventral −8.3 from bregma (26). The cannulas were secured in place with machine screws and dental cement. Cannula placement was tested 5 days later by infusing 5 ng of angiotensin II and monitoring drinking behavior. Two LF-fed rats that drank <3 ml within 5 min of injection were excluded from the study.

Five days after cannula placement was tested the rats were fasted overnight before receiving a 2-μl third ventricle infusion of 0, 0.25, 0.5, 1, or 3 μg of CRF. Energy intake was recorded 1, 2, and 4 h after injection. Over a period of 17 days, with 3 days between each overnight fast and infusion, each rat was treated with each of the doses of CRF in random order. We confirmed that the body weights of the rats had returned to pretest levels between consecutive tests. Four days after the last CRF injection the rats were killed and fat pads were weighed.

Experiment 2: Effect of CRF on energy intake of rats fed HF diet for 5 days. The previous study indicated that CRF caused a greater suppression of food intake in rats made obese on the HF diet than rats fed the LF diet; therefore, the objective of this study was to determine whether rats exposed to the HF diet for only a short period of time were more or less responsive to central CRF than rats fed a LF diet. Thirty-two male rats (Harlan Sprague Dawley) were housed as described for experiment 1 and adapted to the environment for 1 wk before being fitted with third ventricle cannulas as described above. One week after the angiotensin test the rats were divided into two weight-matched groups, and one group was fed the HF diet and the other was fed the LF diet for 4 days. The rats were fasted overnight, and on the morning of the fifth day the rats within each dietary group were divided into two weight-matched groups and received a third ventricle infusion of either 2 μl of saline (LF-Saline, HF-Saline) or 2 μl of saline containing 1 μg of CRF (LF-CRF, HF-CRF). Energy intake was recorded 1, 2, 4, and 6 h after the infusion.

Experiment 3: Effect of different doses of CRF on energy intake of rats fed HF diet for 5 days. Experiment 1 indicated that 1 μg CRF was a threshold dose for inhibiting food intake of rats made obese on a HF diet, but the results from experiment 2 showed that an intracerebroventricular infusion of 1 μg of CRF inhibited food intake equally in rats fed LF or HF diet for only 5 days. Therefore, this study tested whether rats fed the HF diet for only 5 days were more sensitive to low doses of CRF than rats fed the LF diet. Forty-two male Sprague-Dawley rats were fitted with third ventricle cannulas, and cannula placement was tested as described above. One week after testing with angiotensin the rats were placed on the LF diet for 5 days; on the fifth day the rats were divided into two weight-matched groups, and one group was offered ad libitum access to the HF diet and the other half remained on the LF diet. Body weights were recorded daily. Twenty-five hours after the fourth-hour food intake was measured on day 3 of HF feeding. Food was removed from the cages at 10:00 PM on day 4 of HF feeding. Starting at 9:00 AM on the fifth day, each rat was infused with 0, 0.25, or 0.5 μg of CRF in a total volume of 2 μl delivered over 2 min. Energy intake was measured 1, 2, 4, 6, and 24 h after infusion.

Experiment 4: Effect of CRF on corticosterone release of rats fed HF diet for 5 days. This study tested whether there was a difference in corticosterone release after central administration of CRF. Fifty-six Sprague-Dawley male rats, weighing ~350 g, were housed as described above and were fitted with third ventricle cannulas. One day after cannula placement was verified, all animals were offered the LF diet. Body weights and food intakes were measured daily throughout the experiment. After 4 days on the LF diet, the animals were divided into two weight-matched groups. One group stayed on the LF diet, while the other group was switched to the HF diet. After 5 days, each dietary group was subdivided into two weight-matched groups and food was removed from the cages at 7:00 AM. Starting at 9:00 AM, one group from each dietary group was infused with 2 μl of saline (HF-Saline, LF-Saline) and the other group was infused with 2 μl of saline containing 1 μg of CRF (HF-CRF, LF-CRF). Blood samples (~50 μl) from the tail were collected immediately before the start of the infusion and at 30-min intervals after the infusion (0, 30, 60, 90, 120, 150, 180 min). 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. ACTH was measured (ACTH RIA; Nichols Institute Diagnostics, San Clemente, CA) on the 30-min blood sample. Body weights were recorded for 2 days after the infusion.
Experiment 5: Effect of different doses of CRF on corticosterone release of rats fed HF diet for 5 days. Experiment 4 showed that 1 μg of CRF induced a high level of serum corticosterone in both LF- and HF-fed rats. Therefore, this study tested whether there was any difference in HPA response of rats fed LF or HF diet infused with lower doses of CRF. Forty-eight rats were fitted with third ventricle cannulas as described above. The rats were adapted to the LF diet for 5 days, and then half of the rats were switched to the HF diet. At 8:00 AM on day 5 of HF feeding rats were moved from their home cages to individual shoe box cages in a testing room. Starting at 11:00 AM rats were infused with 0, 0.25, or 0.5 μg of CRF in a volume of 2 μl infused over 2 min. Tail blood samples were collected immediately before infusion (0 min) and at 15, 30, 60, 90, 120, and 180 min after infusion for subsequent measurement of serum corticosterone concentration.

Experiment 6: Measurement of CRF and UCN I mRNA expression in rats fed HF diet for 5 days. Experiment 5 did not show any differences in response to infusion of CRF in rats exposed to HF or LF diet for 5 days, although we previously found (19) that rats fed the HF diet for 5 days show an exaggerated release of corticosterone and weight loss in response to mild stress. Therefore, this experiment measured mRNA expression of CRF and of UCN I in rats fed HF or LF diet for 5 days before being subjected to mild stress. Twenty-four Sprague-Dawley male rats, weighing ~300 g, were housed as described above. After the acclimation period (1 wk) the animals were fed LF diet for 1 wk, during which daily body weights and food intakes were measured. The animals were divided into two weight-matched groups: one group was fed the HF diet, 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 nonstressed as control (LF-Control, HF-Control), while the other was subjected to mild stress (LF-MS, HF-MS). The mild stress rats received a 1-ml intraperitoneal injection of saline and were moved to individual shoe box cages in a testing room for 1 h with no access to food or water. The control rats were left in their home cage without access to food or water for 1 h. Immediately at the end of 1 h of mild stress the rats were anesthetized with 90 mg/kg ketamine and 10 mg/kg xylazine and then perfused intracardially with 75 ml of ice-cold saline followed by 200 ml of 4% paraformaldehyde solution. The brains were collected and stored in 4% paraformaldehyde solution at 4°C until being sliced.

On the basis of reports of distribution of the mRNA for stress-related peptides (31, 39) together with information on the involvement of specific areas of the brain in the control of food intake or the HPA axis, in situ hybridization was used to quantify CRF mRNA expression in the paraventricular nucleus of the hypothalamus (PVN) and the central nucleus of the amygdala (CeA) and UCN I mRNA expression in the Edinger-Westphal nucleus (EW), as described in detail previously (11). 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). The optical density for the hybridization signal was measured under bright-field illumination at a magnification of ×25. Brain sections from the different rats were matched for rostrocaudal levels as closely as possible.

The CRF cRNA probe was generated from the 1.2-kb EcoRI fragment of rat CRF cDNA (Dr. K. Mayo, Northwestern University, Evanston, IL) subcloned into a pGEM-4 vector (Promega) and linearized with HinI/SP6 and EcoRI/T7 for antisense and sense probes, respectively. The UCN cRNA probe was generated from a 600-bp EcoRI fragment of rat UCN cDNA (Dr. W. Vale, The Salk

Fig. 1. The effect of corticotropin-releasing factor (CRF) on energy intake of rats made obese on a high-fat (HF) diet. A and B: cumulative intake of HF-fed (A) and low-fat (LF)-fed (B) rats. C and D: food intake during each of the 3 time intervals after CRF infusion in experiment 1. Data are means ± SE for 8 or 10 rats. A superscript letter indicates a significant effect of CRF dose on intake during a specific time interval.
Institute, San Diego CA) subcloned into pBluescript vector and linearized with Smal/I7 and HindIII/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 of linearized plasmid in 10 mM NaCl, ATP/GTP/CTP, α35-S-UTP, 40 U of RNAsin (Promega), 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 of DNase, 5 μl of 5 mg/ml tRNA, 94 μl of 10 mM Tris-10 mM MgCl2). The preparation of the riboprobe was completed through a phenol-chloroform extraction and ammonium acetate precipitation.

Experiment 7: Body composition of rats fed HF diet for 5 days. This experiment tested whether rats fed a HF diet for 5 days had a different body composition from those fed a LF diet. Sixteen male rats, weighing ~360 g, were housed as described above. After 1 wk of adaptation to the environment they were divided into two weight-matched groups. One group was fed the LF diet and the other group the HF diet for 4 days. On the fifth day, the rats were killed and carcass composition was determined as described previously (10).

Statistical analysis. The effect of diet on body weight, intevaled energy intake, and repeated measures of corticosterone were determined by repeated-measures analysis of variance using intake or body weight immediately before the start of the stress (CRF infusions), or corticosterone measured at time 0, as a covariant in the analysis. Statistically significant (P ≤ 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. 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 with Statistica software (Stat Soft, Tulsa, OK).

RESULTS

Experiment 1. In this dose-response study 3 μg of CRF reliably inhibited cumulative energy intake of the rats compared with saline [Fig. 1, A and B; diet: P < 0.001, CRF dose: P < 0.0001, interaction: nonsignificant (NS) for each time interval]. This inhibition was significant for both obese HF- and LF-fed rats 1 h after injection. The effect of 3 μg of CRF on cumulative energy intake was significant for the obese HF rats (P < 0.007) but not the LF rats (P < 0.08) at 2 h. The 1-μg CRF dose also inhibited cumulative energy intake of the obese HF rats at both the 1 (P < 0.003) and 2 (P < 0.03)-h time points. CRF injection had no significant effect on 4-h cumulative energy intake of LF rats. Four-hour cumulative energy intake of obese HF rats infused with 3 μg of CRF was not different from that of saline-infused rats but was lower than that of rats that had been infused with 0.25 or 0.5 μg of CRF. When food intake was analyzed by time interval the 1- and 3-μg doses of CRF inhibited food intake for a longer period of time in obese HF rats than in LF-fed rats (Fig. 1, C and D; diet: P < 0.01, CRF dose: NS, time: P < 0.0001, diet × time: P < 0.0001, dose × diet: P < 0.0001). The highest dose of CRF inhibited food intake of LF rats during the first hour after infusion, and these animals were compensating by overeating between 2 and 4 h after infusion. In obese HF rats both 1 and 3 μg of CRF inhibited food intake at 1 h, and food intake was still inhibited by 3 μg of CRF between 1 and 2 h after infusion. There was no evidence of compensatory hyperphagia in obese HF rats 4 h after infusion.

In contrast to the effect of CRF on food intake, corticosterone release following injection of 1 μg of CRF was blunted in obese HF rats compared with LF-fed animals, but the difference did not reach statistical significance either when considered as corticosterone concentration over time (Fig. 2A; diet: NS, time: P < 0.0001, interaction: NS) or when the area under the curve was compared (LF fed = 2,175 ± 690 units, HF fed = 4,514 ± 786 units; P < 0.09). Because no vehicle-infused controls were included in this study the results do not differentiate between diet-related differences in response to the stress of handling and differences in response to CRF infusion. At the end of the experiment the obese HF rats were significantly heavier than LF-fed rats (LF: 447 ± 14 g, obese HF: 499 ± 14 g; P < 0.001), and the fat depots of obese HF fed rats were significantly larger than those of the LF rats (Fig. 2B; P < 0.04). The combined weight of the five fat depots that were dissected was 42% greater in obese HF rats than in LF rats (LF: 43 ± 3 g, obese HF: 60 ± 6 g; P < 0.001).

Experiment 2. Rats offered the HF diet for 4 days ate significantly (P < 0.01) more than those offered the LF diet during the days before CRF injection (HF rats: 151 ± 2 kcal/day, LF rats: 115 ± 5 kcal/day). A third ventricle injection of 1 μg of CRF inhibited energy intake of both LF- and HF-fed rats compared with their saline-injected controls (Fig. 3A). This effect was significant for cumulative energy intake at both 1 h (diet: P < 0.007, CRF: P < 0.0009, interaction: NS) and 2 h (diet: NS, CRF: P < 0.01, interaction: NS) after injection, but there was no difference in cumulative energy intake of the four
groups of rats 4 or 6 h after injection (Fig. 3A). When the energy intakes of the rats during each interval were compared, CRF initially inhibited energy intake (Fig. 3B; diet: NS, CRF: NS, time: P < 0.0001, diet × CRF: P < 0.02, CRF × time: P < 0.0001). HF-Saline rats ate more than any other group during the first hour after injection, but the CRF infusion reduced the intake of the HF-fed rats to the same level as that of CRF-treated LF-fed rats (diet: P < 0.001, CRF: P < 0.0001, interaction: NS). There were no differences in energy intake of the four groups during the second hour after injection. Both of the CRF-treated groups ate more than their controls between 2 and 4 h after injection, but the effect was significant only for the LF-fed rats (diet: P < 0.07, CRF: P < 0.007, interaction: NS). During the time interval between 4 and 6 h after infusion there were no differences in energy intake of the groups, although both CRF-infused groups tended to eat more than the saline-injected rats (diet: NS, CRF: P < 0.06, interaction: NS).

**Experiment 3.** Similar to the results from experiment 2, rats fed the HF diet had a higher daily energy intake than LF-fed rats, both on day 3 of exposure to HF diet and during the 24 h following CRF infusion (Fig. 4, A and B; P < 0.001). There was no effect of 0.25 or 0.5 μg of CRF on energy intake of LF-fed rats at any time interval measured or on cumulative energy intake during the 24 h after CRF infusion (Fig. 4A). Cumulative energy intake of HF-fed rats infused with 0.5 μg of CRF was significantly less (P < 0.04) than that of rats infused with saline at 4 and 24 h after infusion. There was a trend for inhibition of intake at 6 h after infusion, but the difference did not reach significance (P < 0.09). There was no significant difference in intake between groups when individual time

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**Fig. 3.** Effect of 1 μg of CRF on energy intake of rats fed HF diet for 5 days. Cumulative energy intake (A) and intake during each time interval (B) after a 3rd ventricle infusion of 1 μg of CRF in experiment 2 are shown. Data are means ± SE for groups of 16 rats. Superscript letters indicate significant (P < 0.05) differences between groups at a specific time point.

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**Fig. 4.** Effect of different doses of CRF on energy intake of rats fed HF diet for 5 days. Cumulative energy intake of rats fed LF diet (A) or HF diet (B) after infusion of 0, 0.25, or 0.5 μg of CRF in experiment 3. Data are means ± SE for groups of 7 rats. *Significant difference in energy intake of 0.5 μg-infused HF-fed rats compared with their saline-infused controls (P < 0.05). C. change in body weight during the 24 h following infusion. Superscripts indicate significant differences between treatment groups (P < 0.05).
intervals were compared (data not shown), and there was no significant difference in 24-h intake after infusion compared with that measured on day 3 for any LF-fed or HF-fed treatment group. During the 24 h after infusions HF-fed rats treated with saline gained the most weight and HF-fed rats infused with 0.5 μg of CRF lost weight. There was no effect of CRF on weight change of the LF-fed rats (Fig. 4C; diet: NS, CRF: P < 0.03, interaction: P < 0.03).

Experiment 4. 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 min after the infusion, but there was no effect of diet on the size of this response (Fig. 5; diet: NS, CRF: P < 0.0001, interaction: NS). Corticosterone concentrations had decreased by 90 min in both HF-CRF and LF-CRF rats and had returned to control levels by 120 min after CRF infusion. CRF infusion caused a significant increase in serum ACTH concentration measured 30 min after infusion, but there was no effect of diet on ACTH concentration (Fig. 5; diet: NS, CRF: P < 0.0001, interaction: NS). There was no effect of diet or CRF on the amount of weight that was lost during the 24 h following the CRF infusion, and the average weight loss for all groups was between 6 and 7 g per rat.

Experiment 5. There was no effect of diet on basal serum corticosterone measured at time 0 min (11:00 AM), but infusion of 0.25 or 0.5 μg of CRF caused a significant increase in serum corticosterone concentrations of both LF-fed and HF-fed rats (Fig. 6, A and B), as illustrated by calculation of area under the curve for corticosterone measured up to 90 min after infusion (Fig. 6C; diet: NS, infusion: P < 0.03, interaction: NS). Corticosterone concentrations did not return to baseline for any group of rats, but the final blood samples were collected at about 3:00 PM so it is possible that we were sampling during the start of the circadian elevation of corticosterone.

Experiment 6. On the day the brains were collected there were no differences in body weights of the rats on LF and HF diets (LF = 341 ± 4 g, HF = 342 ± 3 g). PVN CRF mRNA was increased in rats exposed to mild stress (Fig. 7A). This increase was significant (P < 0.05) between saline- and CRF-infused rats at a specific time point. Bottom: adrenocorticotropic hormone (ACTH) measured 30 min after CRF infusion. Superscripts indicate significant differences between control and CRF-infused rats.

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**Fig. 5.** Effect of 1 μg of CRF on corticosterone release of rats fed HF diet for 5 days. Top: serum corticosterone after a 3rd ventricle infusion of 1 μg of CRF in experiment 3. Data are means ± SE for groups of 6 rats. *Significant difference (P < 0.05) between saline- and CRF-infused rats at a specific time point. Bottom: adrenocorticotropic hormone (ACTH) measured 30 min after CRF infusion. Superscripts indicate significant differences between control and CRF-infused rats.

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**Fig. 6.** Effect of different doses of CRF on corticosterone release of rats fed HF diet for 5 days. A and B: serum corticosterone concentrations of LF-fed or HF-fed rats in experiment 5 after a 3rd ventricle infusion of 0, 0.25, or 0.5 μg of CRF. C: area under the curve for the different treatment groups, calculated up to 90 min after the infusion. Superscripts indicate significant differences between groups (P < 0.05).
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.

Experiment 7. There were no differences in carcass composition or in the weights of different fat depots of rats fed LF or HF diet for only 5 days (Table 1).

DISCUSSION

Previous studies have shown that rats fed a HF diet demonstrate an exaggerated corticosterone release during 20 or 30 min of restraint stress (16, 35) or the mild stress of an intraperitoneal injection of saline and placement in a novel cage (19). Little is known, however, of the mechanistic basis of the exaggerated stress response in HF-fed rats or whether some of the differences in response between HF-fed and LF-fed rats are due to the development of obesity rather than the increase in fat consumption. The present experiments were conducted to test the effects of chronic and acute exposure to a HF diet on the response to third ventricle administration of CRF, to determine whether the exaggerated HPA activation could potentially be explained by an increased sensitivity to stress-related neuropeptides. In addition, we measured mRNA expression of CRF and the related peptide UCN I in rats that had been fed the HF diet and were exposed to mild stress to determine whether the increased corticosterone release and exaggerated weight loss following mild stress in HF-fed rats were associated
Table 1. Body composition of rats fed either LF or HF diet for 5 days in experiment 7

|                      | LF Fed   | HF Fed
|----------------------|----------|--------
| Weight before diet   | 397±4    | 393±5  |
| Weight after 5 days  | 421±8    | 421±7  |
| Carcass weight       | 382±7    | 382±6  |
| Carcass fat           | 34±4     | 37±4   |
| Carcass water         | 248±5    | 243±4  |
| Carcass protein       | 91±3     | 94±3   |
| Carcass ash           | 8.9±1.1  | 8.8±0.8|

Data (in g) are means ± SE for groups of 8 rats. LF, low fat; HF, high fat.

There were no significant differences in body weight or body composition of rats in the 2 dietary groups, as determined by unpaired Student’s t-test.

with increased central release of stress-related neuropeptides.

In experiment 1, rats that had become obese on the HF diet were more sensitive to the hypophagic effects of CRF than rats fed the LF diet. Food intake was inhibited by a lower dose of CRF in obese HF-fed than in LF-fed rats, and the duration of inhibition of food intake was extended in obese HF-fed rats. These results suggest that rats made obese on the HF diet have an increased responsiveness to stress-related neuropeptides.

Other investigators, however, have shown that diet-induced obese rats are less responsive to stress than their controls. Levin et al. (21) found reduced anxiety-type behavior and less change in basal corticosterone in diet-induced obese rats subjected to unpredictable stress for 5 wk compared with diet-resistant rats subjected to the same stressors. Similarly, Waldla et al. (5) found that rats fed a 61% kcal fat diet for 4 mo showed less anxiety-type behavior, a faster recovery of body temperature, and less hypersensitization of serotonin receptors following either psychosocial or physiological stress. One obvious difference between these studies and experiment 1 described here was that we infused fixed amounts of CRF into the third ventricle whereas others exposed rats to stress (5, 21, 37). Stress induces the release of multiple endogenous peptides in a large number of brain nuclei that would not be influenced by CRF infused into the third ventricle but would influence behavior and endocrinology in stressed rats.

Another difference between the two studies is the end point that was measured. There are two major subtypes of the CRF receptor expressed in the rodent brain, CRF₁ receptors and CRF₂ receptors (7). CRF binds to both, with a higher affinity for CRF₁ than CRF₂ receptors (4). CRF₁ receptor mRNA is widely distributed in the rat brain, whereas CRF₂ receptor mRNA is primarily expressed in subcortical hypothalamic structures, the brain stem, and the posterior pituitary gland (38). Advances in the development of CRF receptor-specific antagonists (42) and of transgenic mice (3, 6) have made it possible to differentiate between stress-related events that are mediated by CRF₁ and CRF₂ receptors. It appears that the CRF₂ receptor is the primary mediator of stress-induced anorexia (3, 41), whereas CRF₁ receptors predominantly mediate the activation of the HPA axis and anxiety-type behaviors (32). Tannenbaum et al. (35) suggested that a HF diet acts as a chronic stressor, and it has been clearly demonstrated that stress increases expression of both CRF₁ receptors and CRF in the PVN (22, 29, 36, 38) and that CRF₁ receptor mRNA expression is upregulated by both CRF (24) and UCN 1 (25).

In contrast, stress has been shown to downregulate CRF₂ receptor mRNA in the ventromedial nucleus of the hypothalamus (11) and the pituitary gland (15), and glucocorticoids inhibit expression in the pituitary gland (15) and in cardiac tissue (2). If the food intake response to stress is primarily mediated by CRF₂ receptors but the HPA response is mediated by CRF₁ receptors, then the results of experiment 1 would suggest that obesity increases the CRF₂ receptor and/or postreceptor response to stress peptides.

The extended effect of CRF in rats fed the HF diet may be explained by a failure to downregulate activity of the receptor following ligand binding. Tannenbaum et al. (35) did not find any change in the negative feedback regulation of the HPA axis by glucocorticoids in HF-fed rats, but the duration of activation of G protein-coupled receptors also is limited by regulators of G protein signaling (RGS) proteins that accelerate signal termination (40). In vitro studies have shown that expression of RGS proteins is increased by cellular stress and by activation of specific signaling pathways (33), either of which could potentially be modified by chronic exposure to a HF diet. The results of increased responsiveness to CRF in experiment 1 are in contrast to observations by others that rats made obese on high-calorie diets show less anxiety-type behavior than LF-fed controls when they are stressed (5) and that rats that have become obese on a palatable diet have reduced anxiety-type behavior and less change in basal corticosterone compared with nonobese rats (21). This may imply that the increased sensitivity is specific to the CRF₂ receptor subtype because, as noted above, both anxiety-type behaviors and activation of the HPA axis are thought to be mediated by CRF₁ receptors. The nonsignificant reduction in CRF-induced corticosterone release of obese HF-fed rats in experiment 1 described here was also consistent with reduced activation of CRF₁ receptors, but a full-dose response study and inclusion of rats infused with vehicle are needed to confirm a difference in CRF responsiveness of the HPA axis. Because we did not find an exaggeration of the feeding response to 1 μg of CRF in rats fed the HF diet for only 5 days it is possible that the change in CRF sensitivity in obese HF-fed rats was secondary to the development of obesity, and, because all rats in our study received the same doses of CRF, it is likely that the change in responsiveness was at the receptor or postreceptor level.

Rats that had been exposed to the HF diet for only 5 days did not show any increase in body fat content and showed the same degree of HPA activation as LF-fed rats in response to even low-dose infusions of CRF in the third ventricle. When food intake was used as the end point measure, there was no effect of feeding HF diet for 5 days on the initial hypophagia caused by CRF, but the HF diet may have extended the time for which food intake was inhibited. The rebound feeding that compensated for an initial inhibition by the highest dose of CRF may have been delayed in HF-fed rats compared with their LF-fed counterparts, and at lower doses of CRF there was an inhibition of 24-h food intake and weight gain of HF-fed but not LF-fed rats. These observations suggest that our previous findings (19) of increased corticosterone release during mild stress in rats fed a HF diet for a short period of time were not due to an increased responsiveness to stress-related peptides at the receptor or postreceptor level. It should be noted, however, that the concentrations of corticosterone measured in CRF-infused rats in experiments 4 and 5 were higher than those we previ-
ously found (19) in rats subjected to mild stress but similar to levels found in rats exposed to a more severe stress, such as restraint stress. Therefore, without completing a more comprehensive dose-response study we cannot entirely exclude a difference in sensitivity to CRF as the cause of increased stress responsiveness in nonobese HF-fed rats. If there is a change in sensitivity then it would be different from that found in the obese HF-fed rats, which have a blunted corticosterone response to stress, because rats fed HF diet for only 5 days show an exaggerated corticosterone response to mild stress (19). In contrast, both obese HF-fed and 5-day HF-fed rats showed an extended inhibition of food intake in response to CRF compared with their LF-fed counterparts, although the response was much more dramatic in obese HF-fed rats. In addition, rats exposed to HF diet for only 5 days lost more weight after mild stress (19) and central infusion of CRF than rats fed LF diet. Therefore, it is possible that the HF diet modifies the pattern of release of CRF receptor ligands to extend the effects of stress on energy balance.

In experiment 6, CRF mRNA expression in the PVN was increased in rats exposed to mild stress, but this was significant only for LF-fed rats because CRF mRNA was already nonsignificantly elevated in the HF-Control rats, either because of a direct effect of diet or because these animals were hyperresponsive to stress and they found the simple procedures of being handled and returned to their cage stressful. Some of the CRF neurons in the PVN project to the brain stem autonomic systems and are involved in arousal and appetite regulation. Thus a hyperresponsiveness would be consistent with our previous observation (19) that rats fed HF diet for 5 days stopped gaining weight after the stress of tail bleeding, whereas LF-fed rats did not. In contrast to the effect of stress on PVN CRF mRNA, no stress- or diet-induced changes in CRF mRNA were observed in the CeA, which plays a critical role in fear- and anxiety-related behaviors (18, 30). Other investigators have reported a variety of stress-induced changes in CeA CRF mRNA expression. Restraint or other psychological stressors have been shown to increase expression (14, 23), whereas tail shock had no effect (13) and food deprivation decreased expression (37). Thus there appear to be many factors that modify CeA CRF mRNA expression, but the results from this study suggest either that a short exposure to HF diet and mild stress do not have any effect or that changes occurred at a time after stress other than at which we made the measures. The 5 days of HF feeding caused a significant inhibition of EW UCN I mRNA expression in HF-Control rats compared with LF-Control rats, and mild stress did not effect UCN I expression in LF-MS rats but stimulated expression in HF-MS rats to the same levels as those found in both groups of LF-fed rats. One could hypothesize that the exaggerated stress response previously observed in HF-fed rats is due to changes in CRF and UCN I mRNA expression. CRF has a higher affinity for CRF1 than CRF2 receptors (4), whereas UCN I will bind with equal affinity to both receptor subtypes (9). If it is assumed that mRNA expression for UCN I and CRF is representative of the respective protein concentrations at their respective sites of release and UCN I mRNA expression decreases in rats fed HF diet but CRF mRNA does not, then this would imply that, in nonstressed conditions, there is a greater activation of CRF1 receptors compared with CRF2 receptors in HF-fed than LF-fed rats. In addition, in stressful conditions the change in peptide production required to reach a threshold for activation of the HPA axis and anxiety-type behaviors would be less for HF-fed than for LF-fed rats. Further studies of endogenous levels of stress peptides and of receptor activation are needed to determine whether this potential difference in basal levels of receptor activation could account for differences in response to stress between rats fed LF and HF diets.

In summary, the experiments described here and those published by others (5, 21, 37) provide evidence that long-term exposure to a HF diet and/or development of obesity selectively changes the responsiveness to stress. The results from experiment 1 show that CRF produces an extended hypophagia in obese rats, which is a behavior that is thought to be mediated by activation of CRF2 receptors. In contrast, others have shown that stress responses that are primarily mediated by CRF1 receptors, such as activation of the HPA axis and anxiety behavior, are blunted. These divergent observations suggest that rats made obese on HF diets show selective changes in sensitivity to, or release of, stress peptides that may be receptor specific. In contrast, a relatively short-term exposure to HF diet for 5 days changes the levels of expression of CRF mRNA and UCN I mRNA in nonstressed conditions. A suppression of UCN I mRNA expression may be indicative of relatively low activation of CRF2 receptors, assuming that mRNA is proportional to peptide production. Similarly, an elevation of CRF mRNA expression, and presumably CRF protein, may be indicative of a relative increase in CRF1 receptor activation in nonstressed conditions. An increase in basal levels of receptor activation would minimize the change that is needed to reach the threshold level of peptide release required for initiation of a stress response. Our previous observations (19) that rats fed HF diet for only 5 days show an exaggerated HPA response to mild stress would be consistent with this hypothesis.


