Exaggerated response to mild stress in rats fed high-fat diet

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Exaggerated response to mild stress in rats fed high-fat diet. Am J Physiol Regul Integr Comp Physiol 291: R1288–R1294, 2006. First published June 22, 2006; doi:10.1152/ajpregu.00234.2006.—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, in which rats were fed HF diet for 4 days, we found that HF-fed controls stopped gaining weight, indicating that they were hyperresponsive 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 a HF diet for 4 days showed an exaggerated corticosterone release in response to an intraperitoneal injection of saline and movement to a novel cage, compared with 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 hyperresponsiveness to mild stress in HF-fed rats was sustained during a prolonged exposure to diet. The results confirmed that a HF diet, not novelty, exaggerated the endocrine stress response after 9 days on the diet but that the effect was no longer present after 23 days on the diet. The hyperresponsiveness 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 the HF diet may initially be perceived as a stressor.

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 of the American Physiology Society (1).

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Experiment 1: acute exposure to HF diet and repeated restraint stress. Forty-two male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing ~300 g were housed in individual stainless steel cages in a room maintained at 23 ± 1°C on a 12:12 h light-dark cycle with lights on at 7:00 AM. Upon arrival, the rats were allowed 1 wk 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.

After the acclimation period, daily body weights, and food intakes, corrected for spillage, were measured for a week, during which all of the animals continued to have free access to chow. The animals were divided into two weight-matched groups, one group was fed a HF diet (40% kcal fat: Diet D02041901, Research Diets, New Brunswick, NJ), while the other group was fed a LF diet (12% kcal fat: Diet D02041902, Research Diets). The experimental diets were pelleted; dietary fat was a mixture of corn oil and coconut oil that remained in the same ratio in the two diets but was partially replaced with equal amounts of starch and sucrose in the LF diet (12). After 4 days, each dietary group was subdivided into two weight-matched groups. One subgroup was exposed to restraint stress for 3 h on 3 consecutive days (repeated restraint), while the other group was a nonstressed control. Starting at ~8:30 AM each day, 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 h, 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 min 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 after the restraint stress at a time that would be equivalent to the end of restraint stress. Food and water were removed 3 h before these blood collections. Blood samples were centrifuged, and the serum was stored at −80°C until assays could be performed. Corticosterone concentration was measured in all samples collected on the days of restraint (corticosterone RIA; MP Diagnostics, Costa Mesa, CA). ACTH concentration (ACTH RIA; Nichols Institute Diagnostics, San Clemente, CA) was measured on the 30-min blood sample collected on the second day of restraint. Insulin (rat 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 after the last day of restraint as an indirect index of the development of obesity and insulin resistance and because it has been reported that the combination of elevated corticosterone and adequate insulin will promote consumption of preferred foods (6).

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 they were simply placed in new cages in the experimental room, and 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 a HF diet on the endocrine response to a mild stress. Forty-two male Sprague-Dawley rats, weighing ~300 g, were housed as described above. After the acclimation period, all of the animals were fed the LF diet for 5 days. After 5 days, the animals were divided into two weight-matched groups. One group stayed on the LF diet, while the other group was switched to the LF diet for 4 days. On the fifth day, each dietary group was subdivided into two weight-matched groups. One subgroup from each dietary group was exposed to the same MS as described for experiment 1 and the other was left in their home cage without access to water or food for 2 h. Stress was initiated at 8:00 AM, and blood samples were collected as described for experiment 1. Fourteen days later, the treatment groups were interchanged, the animals that had been the controls were now exposed to MS, and the animals previously exposed to MS were the controls. Nine days after the second MS, 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-min intervals for 3 h. Animals were decapitated 9 days after the last restraint, trunk blood was collected, fat pads (mesenteric, epididymal, and retroperitoneal) were weighed, and carcass composition was determined as described previously (10). 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-measures 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 < 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 ANOVA and post hoc Duncan’s multiple range test. All statistical procedures were carried out using Statistica software (Statistica Software, StatSoft, Tulsa, OK). Animals with two or more corticosterone values from the timed blood withdrawal 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. Rats switched from chow to a LF diet had a reduced energy intake and lost some weight on the first day that they were offered the diet, possibly because it was found to be less palatable than chow. The rats regained their normal energy intake by the second day on the diet but did not compensate for the initial weight loss; however, there were no statistically significant differences between the body weights of the groups of LF- and HF-fed rats at the beginning of the restraint. Rats exposed to repeated restraint lost weight on the days of restraint (Fig. 1A), and there was no effect of diet on the amount of weight that was lost [Diet: not significant (NS), Stress: P ≤ 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 a HF diet consumed significantly more calories than rats fed a LF diet on the days before restraint stress, most likely related to the increase in palatability of the diet (Fig. 1B). Fig. 1C shows energy intake of the rats during and after stress expressed as a percent change from baseline (3 days before stress). All groups, except LF controls, consumed less energy on the days of restraint than during the baseline period. None of the rats overate after stress ended, and the HF/stress group had the lowest intake on the 3 days after the restraint (Diet: *P < 0.05, stress: *P < 0.05, interaction: NS).

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 min. 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. 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. There were no differences in ACTH levels between the restrained groups at specific time points. Inset: calculated area under the curve.

Experiment 2. 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 (Table 2). There were no differences in body weights of the four groups of rats on the day of MS (Table 2). Serum corticosterone concentra-
tions were not different between rats exposed to MS and those that were controls, possibly due to the stress caused by tail bleeding. Because there was no statistically significant effect of MS, we combined the data from the control and MS groups within each dietary treatment (Fig. 2). 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. There was no significant effect of MS or of diet on the weight change of the rats during the 24 h after MS (Table 2).

Experiment 3. There were no differences in body weights of the four groups of rats before either of the two exposures to MS 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 MS (Fig. 3A). All of the groups showed a small weight gain after the manipulations associated with the second MS, but the HF-control group showed a significant greater weight gain compared with the other groups (Diet: P ≤ 0.05, Stress: P ≤ 0.05, Interaction: NS). Corticosterone concentrations in response to the 1st and 2nd 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. 3B) in response to the 1st MS compared with the LF-stress group. This difference between diet groups in response to stress was not found in the 2nd MS (Fig. 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. 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. 4B). In contrast to the results from experiment 1, in which corticosterone concentration came back down to baseline after 2 h, corticosterone values peaked at 60 min 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 with 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 show 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 release, 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 diet alone was responsible for the observed exaggerated endocrine and body weight response to MS.

Previously, others have reported that HF-fed rats exposed to different stressors react with an increased endocrine response to stress (13, 14, 28). Tannenbaum et al. (28) used a 20-min one-time restraint and both Kamara et al. (13) and la Fleur et al. (14) used a 30-min one-time restraint. The duration of the restraint used in the experiments described here is significantly longer (3 h) and was also repeated over 3 days. In addition, la Fleur et al. (14) examined the effects of having a choice or no choice between two diets on the stress response and found that rats fed a mixed HF diet with 50% of energy from lard demonstrated a corticosterone response to restraint similar to that of HF-fed rats exposed to mild stress in the experiments described here, in that corticosterone was elevated in the HF-fed rats at the end of a 30-min restraint compared with restrained rats fed chow (14). In contrast, rats that had a choice of eating chow or lard for 7 days and consumed 64% of their calories from lard had a blunted corticosterone response to stress. These results suggest that the matrix in which fat is consumed influences the endocrine response to stress; however, more information is needed to clarify the relative importance of percent calories as fat, food matrix, energy intake, and body fat mass, all of which were different between the two dietary groups.
groups (14). In our first experiment, rats fed a LF or HF diet for 4 days showed the same pattern of corticosterone release during repeated restraint. The difference between these results and those from others who found a greater corticosterone release during 20 or 30 min of restraint (13, 14, 28) may be due to the duration of the stress or the side effects of obesity and not diet. In a study in which rats were offered only chow or a choice of chow, lard, and sucrose and were exposed to 30 min of restraint stress on each of 5 days, Pecoraro et al. (20) found no difference in the corticosterone response to restraint on the first 3 days of stress, although it was decreased on day 5. In contrast, area under the curve for ACTH was lower on days 1 and 3, but not day 5, of restraint for the rats given a choice of foods. We measured ACTH only at one time point on the second day of restraint and cannot exclude the possibility of a difference between the LF- and HF-fed rats; however, because the rats were fed a high-fat diet and not a choice of foods, it is unlikely that we would have observed the same changes as those reported by Pecoraro et al. (20). Even though Pecararo et al. (20) found a reduced HPA response to restraint when rats had a choice of foods to consume, restraint stress inhibited energy intake and caused weight loss, indicating selectivity in the dietary modulation of stress-induced endocrine and energetic responses.

Although we did not find any substantial effect of acute exposure to HF diet on corticosterone response to repeated restraint stress in experiment 1, we did observe some differences in the energetic response to restraint and tail-bleeding in the HF-control group, compared with the LF-control group.

Fig. 3. Weight change (A) after 1st and 2nd MS in experiment 3. Data are expressed as means ± SE. Superscripts represent statistically significant (P < 0.05) difference in weight change in response to the 1st or 2nd MS. Serum corticosterone concentrations were measured in response to the 1st (B) and the 2nd (C) mild stress. Asterisks represent statistically significant (P < 0.05) difference between retrained groups from each dietary regimen within a specific time point.

Fig. 4. Body weights (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 MS event each before being exposed to repeated restraint. Data are means ± SE for groups of 10 rats. Repeated measures indicated a diet and stress effect, but no interactions. #HF/stress and LF/control are different from the other groups. Blood corticosterone concentrations (B) were measured at 30-min intervals on the first day of restraint. *Statistically significant (P < 0.05) difference between the HF- and LF-fed rats that were restrained.
These results demonstrate that the manipulations and handling associated with tail bleeding and being placed in a new cage were more stressful to the HF-fed than to the LF-fed rats. Because there was no diet effect or 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. 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. (28). These data also suggest that the HPA axis response to stress may not play an important role in regulating inhibition of food intake during stress and are consistent with those of Pecararo et al. (20) who found an attenuation of HPA activation but a reduction in energy intake and weight gain of rats offered a choice of chow, lard, and sucrose on the days that they were exposed to restraint stress. We did not measure mRNA expression of any of the stress-related neuropeptides in this study, but it would be of interest to know whether the separation of endocrine and body weight responses in the stressed animals was due to differences in level or pattern of expression of CRF or of one of the urocortins or whether it was due to subtype-specific changes in receptor and/or postreceptor activation.

In experiment 2, because of the changes we observed in control HF-fed rats in experiment 1, we examined the HPA response to MS in rats fed HF diet for 4 days. Although we did not find a specific response to MS, after combining the control and MS 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, which differed from experiment 2, where we observed an increase in corticosterone during mild stress in the HF-fed rats compared to the LF-fed rats. There are a number of factors that may have contributed to these different results, including the severity of the stressor and the acute nature of MS compared with 3 h of restraint. Also, we measured corticosterone on day 2 of restraint in experiment 1, whereas the MS was entirely novel. Although a diet effect was observed in experiment 2, a question remained: Is the increased 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 the exaggerated response 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 increase in HPA axis activity elicited by the diet was not sustained and confirmed results from a study by Kamara et al. (13), who found higher levels of serum corticosterone at the end of a 30-min restraint stress in rats fed diets containing 54% kcal fat for 2 wk than in rats fed chow but no effect of diet composition on stress-induced corticosterone after 10 wk on the HF diet. This would contradict the previous hypothesis from Tannenbaum et al. (28) that a HF diet may act as a chronic stressor. Our observations would further suggest that the increased glucocorticoid response observed in obese individuals (8, 19, 21, 25) 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%. In contrast to the exaggerated glucocorticoid response to restraint stress in obese HF-fed rats, Bulwvalda et al. (4) reported that social defeat produced smaller changes in body temperature and locomotion in rats made obese on a 61% kcal fat diet compared with their Chow-fed controls. These observations suggest that HF diet-induced obesity suppresses stress responsiveness, but the nature of the stress and the end points being measured may function through different pathways than those associated with activation of the HPA axis.

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 than their LF-fed counterparts. In experiment 1, the corticosterone levels measured on the second day of restraint reached a peak at 60 min and were back to baseline levels by the end of the restraint (3 h). In contrast, 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 after restraint as rats in experiment 1. Therefore, it is unlikely that the stress and tail-bleeding procedures were perceived by the rats to be more extreme in experiment 3 than 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. It is possible that day 1 of restraint (experiment 3) is more stressful than day 2 of restraint (experiment 1) as the procedure is novel and the rats do not have any expectations of being removed from the restraining tubes. In experiment 1, we found that corticosterone was same in restrained and control rats at the end of the 3 h of restraint stress; therefore, it is possible that the combination of novel exposure to restraint combined with tail-bleeding (experiment 3) was much more stressful than tail-bleeding during a second exposure to restraint (experiment 1). Although rats in experiment 3 had been fed the experimental diets for longer than the rats in experiment 1, this would not explain the failure of corticosterone to return to baseline levels in LF-fed rats.

This series of experiments suggests that a HF diet acts as a stressor, but contrary to Tannenbaum’s (28) hypothesis that HF acts as a chronic stressor, our data show that the stress effects of HF diet last for less than 3 wk. In experiment 3, we did not observe 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 MS 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 (3), whereas CRFR2 would be responsible for the feeding responses to stress (3). In experiment 1, we showed that rats fed a HF diet had differential effects on corticosterone and food intake during the stress. Further studies should examine the effects of HF diets on CRF receptor number and function. There are at least four neuropeptides with affinity for the CRF receptors: CRF, urocortin I and urocortin II and III, each of which have different affinities for the two receptor subtypes (23). If a HF diet changes the relative amounts of CRF or urocortin secreted in response to mild stress this could change the corticosterone and food intake response to mild stress. In addition, CRF and ACTH are downregulated by corticosterone levels feeding back on the hypothalamus and adrenal gland (24). Thus it is possible that the HF diet is modifying the negative feedback system; however, we found a slightly increased/prolonged endocrine response in HF-fed rats in experiments 2 and 3, whereas the HF diet in experiment 1 decreased the time for corticosterone to return toward baseline following stress.

In conclusion, these results suggest that a HF diet makes an animal hyperresponsive to mild 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 downregulate 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, as we observed an effect of diet on these parameters of the stress response.

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