Weight loss in rats exposed to repeated acute restraint stress is independent of energy or leptin status

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Harris, Ruth B. S., Tiffany D. Mitchell, Jacob Simpson, Stephen M. Redmann, Jr., Bradley D. Youngblood, and Donna H. Ryan. Weight loss in rats exposed to repeated acute restraint stress is independent of energy or leptin status. Am J Physiol Regulatory Integrative Comp Physiol 282: R77–R88, 2002—Acute release of corticotropin-releasing factor (CRF) during repeated restraint (3-h restraint on each of 3 days) causes temporary hypophagia but chronic suppression of body weight in rats. Here we demonstrated that a second bout of repeated restraint caused additional weight loss, but continuing restraint daily for 10 days did not increase weight loss because the rats adapted to the stress. In these two studies serum leptin, which suppresses the endocrine response to stress, was reduced in restrained rats. Peripheral infusion of leptin before and during restraint did not prevent stress-induced weight loss, although stress-induced corticosterone release was suppressed. Restrained rats were hyperthermic during restraint, but there was no evidence that fever or elevated free interleukin-6 caused the sustained reduction in weight. Restraining food-restricted rats caused a small but significant weight loss. Food-restricted rats fed ad libitum after the end of restraint showed a blunted hyperphagia and slower rate of weight regain than their controls. These results indicate that repeated acute stress induces a chronic change in weight independent of stress-induced hypophagia and may represent a change in homeostasis initiated by repeated acute activation of the central CRF system.

corticotropic-releasing factor; food intake; food restriction; corticosterone

RATS EXPOSED to repeated restraint stress experience a temporary reduction in 24-h food intake but maintain a reduced body weight compared with controls (12). The weight loss is dependent on the acute central release of corticotropin-releasing factor (CRF) (28), but there is no sustained activation of this pathway to account for the maintained suppression of body weight (27). CRF activates the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic nervous system, and serotonergic and catecholamine systems. All of these systems have the potential to inhibit food intake and reduce body weight, but none of them is activated significantly during the hours or days after exposure to repeated restraint (12, 27).

All of the weight loss that occurs on the days of restraint is accounted for by a loss of lean tissue. During the days immediately after the end of repeated restraint, there is a shift in tissue metabolism so that the difference in weight between control and restrained rats is a combination of both lean and fat tissue (30). The reduction in weight is relatively small and represents only 5–10% of body weight (12), but there are aspects of the model that are unique. First, the stressed rats do not make any attempt to overeat once stress is ended, implying that the feedback signals that promote hyperphagia after weight loss due to food restriction (10) are not responding to the loss of weight caused by stress. Second, the maintenance of a reduced body weight for extended periods of time provides a model that can be used to examine mechanisms that normally regulate body weight. Although the stressed rats gain weight once stress has ended, they do not recover the weight that has been lost during the period of restraint and remain significantly lighter than their controls (12). Weight changes induced by environmental conditions such as diet composition (15), food restriction (10), or temperature (22) are usually reversed as soon as the environmental factor is removed. A sustained reduction of body weight in ad libitum-fed animals is found in rats with lesions of the lateral hypothalamus (17) or mice in which gene expression has been modified (16), but this stress model provides an opportunity to examine chronic weight reduction in an intact animal.

The mechanism of the response has not been elucidated, but it appears that the repeated acute stress initiates a cascade of events that resets the homeostatic equilibrium of the animal, with a reduced body weight being a representative end point of the new equilibrium. To have a better understanding of the limits of the response, it was necessary to clarify whether the amount of weight that could be lost during stress was limited or was proportional to the number of exposures to stress. In addition, others have shown that weight loss in rats injected with turpentine, to induce an inflammatory response, correlates with the energy status of the animal (20, 21). Overfed animals have the potential to inhibit food intake and reduce body weight, but none of them is activated significantly during the hours or days after exposure to repeated restraint (12, 27).

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lose more weight and underfed animals lose less weight than ad libitum-fed rats. Therefore, the objective of the first two experiments described here was to test whether weight loss in restrained rats was proportional to the number of episodes of stress they experienced. In the process of conducting these studies, we found that leptin was unexpectedly low in restrained rats. Heiman et al. (14) have reported that leptin, a cytokine released from adipose tissue that may be a negative-feedback signal in the regulation of energy balance (29), suppresses the endocrine response to stress. Additionally, it has been shown that leptin treatment of rats or mice specifically reduces body fat but protects muscle mass (2). All of the initial weight loss in restrained rats is lean body mass (30); therefore, the third experiment tested whether chronic treatment with leptin would prevent stress-induced weight loss or change the composition of the loss. Finally, to determine whether the weight loss of restrained rats was dependent on the energy status of the rats at the time of stress, we tested whether restraint stress inhibited food intake or body weight of rats that were already food restricted and weight reduced.

METHODS

Experiment 1: 10 days of repeated restraint. In previous experiments, rats were exposed to 3 h of restraint stress on each of 3 consecutive days, and this resulted in a chronic downregulation of body weight (12). In this experiment we determined whether additional days of restraint would produce a progressively greater weight loss in the rats.

Twelve Wistar male rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 400 g were housed individually in hanging wire mesh cages with free access to food and water in a room maintained at 23 ± 2°C, with lights on 12 h a day from 6:30 AM. They were adapted for 10 days to a diet containing 40% kcal fat and 16% kcal protein, because a high-fat diet exaggerates the energy balance response to restraint stress (12). Baseline daily food intakes and body weights were recorded for 7 days, and then the rats were divided into two weight-matched groups. One was exposed to 3 h of restraint each morning, from 7:00 to 10:00 AM, on each of 10 consecutive days. The nonstressed controls were moved to the same room as restrained rats and housed without food or water for the period of restraint. On days 1, 3, 5, 7, and 9 of restraint, a small blood sample was collected from the tail of each rat for measurement of corticosterone and leptin. Twelve days after the end of restraint, the rats were decapitated. Trunk blood was collected for measurement of corticosterone and leptin, and the carcass, less gut content, was analyzed for composition (9).

Experiment 2: two bouts of 3 days of repeated restraint. This experiment tested whether weight loss induced during the first bout of repeated restraint represented a maximal response to the stimulus or whether exposure to a second bout of repeated restraint caused additional weight loss in the rats. Serum interleukin (IL)-6 and rectal temperatures were also measured to test whether repeated restraint induced an inflammatory response.

Fourteen male Wistar rats, weighing 400 g, were housed in individual cages with free access to water and the 40%-kcal-fat diet. After 10 days they were divided into two weight-matched groups, and one was exposed to 3 h of restraint on each of 3 days. On the morning before the start of stress, and for the next 5 days, a small tail blood sample was collected at a time that was equivalent to 1 h after the end of restraint. Serum leptin and free IL-6 (Quantikine M IL-6, R&D Systems, Minneapolis, MN) were measured. Rectal temperatures of the rats were measured immediately before and immediately after restraint and at an equivalent time on the 4 days after stress. Seven days after the end of restraint, the rats were exposed to a second bout of repeated restraint. Rectal temperatures were measured and blood samples were collected as before. The experiment ended on day 26, 13 days after the end of the second bout of repeated restraint, and carcass composition was determined (9).

Experiment 3: effect of chronic leptin infusion in rats exposed to repeated restraint. Experiments 1 and 2 indicated that leptin declined during the days after repeated restraint. It is well established that leptin has little effect on lean mass while reducing body fat (2, 7, 8), and it has been reported that leptin inhibits some of the HPA responses to stress (14). Therefore, we tested whether chronic infusion of leptin before and during repeated restraint would prevent weight loss during restraint, which is predominantly lean tissue (30), or influence the body composition of the rats during the post-stress period.

Forty-eight male rats, each weighing 375 g, were housed individually with free access to water and the 40%-kcal-fat diet. Food intakes and body weights were recorded daily for 10 days, after which the rats were divided into three weight-matched groups. Each rat was anesthetized with isoflorurane, and an Alzet miniosmotic pump (model 2 ML4, Durect, Cupertino, CA) delivering 0, 30, or 100 μg/day recombinant rat leptin (R&D Systems) for 28 days was placed in the intra-peritoneal cavity. Two days after pump placement, a small tail blood sample was collected in the morning after a 3-h fast to measure serum leptin and corticosterone.

On day 21 of leptin infusion, the rats within each leptin treatment group were subdivided into two weight-matched groups. One subgroup was exposed to 3 h of restraint on each of 3 days, and the other subgroup was a nonrestrained control. One hour after the start of restraint on the first day of stress, a tail blood sample was collected for measurement of leptin and corticosterone. On the day after the end of stress, the rats were food deprived for 3 h in their home cages, and a blood sample was collected for measurement of leptin and corticosterone. Five days after the end of restraint, the rats were decapitated. Trunk blood was collected for measurement of serum corticosterone, leptin, insulin, glucose, and free fatty acids (NEFA C kit, WAKO Chemicals). Epididymal fat was weighed and snap-frozen, and total RNA was extracted for measurement of leptin mRNA expression by Northern blot test (11). Thymus, adrenal glands, liver, and inguinal, retroperitoneal, perirenal, and mesenteric fat pads were dissected and weighed before being returned to the carcass. The carcass, less gut content, was analyzed for composition (9).

Experiment 4: repeated restraint in weight-reduced, food-restricted rats. In other experimental protocols that induce a prolonged downregulation of body weight, the energy balance status of the animal at the time that weight loss is induced determines the amount of weight that is lost. For example, weight loss caused by an inflammatory response is exagger-
ated if the rats have been overfed before being exposed to turpentine, whereas they gain weight if they are weight reduced at the time of treatment (21). The objective of this experiment was to determine whether weight-reduced rats would lose weight in response to repeated restraint stress.

Sixty male Wistar rats, each weighing ~320 g, were singly housed in suspended stainless steel cages with ad libitum access to water and the 40%-kcal-fat diet. Food intakes and body weights were recorded daily for a week before the rats were divided into three weight-matched groups. One group had ad libitum access to food, and the two remaining groups were restricted to 50% of their voluntary food intake for 10 days, by which time food-restricted rats weighed 75 g less than ad libitum-fed animals. Food-restricted rats were given their food at the onset of the dark cycle.

Each of the three groups was divided into two weight-matched subgroups. One subgroup was exposed to repeated restraint, and the other was a nonstressed control group. A small blood sample was collected from each rat by tail bleeding after the first hour of restraint on the first day of stress for measurement of serum corticosterone. After the third restraint, the ad libitum group continued to eat ad libitum (AL), one group of food-restricted rats remained on 50% voluntary food intake (FR), and the rats in the second food-restricted group were returned to ad libitum feeding (FR-AL). Twelve days after the end of restraint, the rats were killed in the morning, between 9:00 and 11:00 AM. Trunk blood was collected for determination of serum corticosterone, leptin, glucose, insulin, and free fatty acids. The carcass, less gut content, was analyzed for composition.

Statistical analysis. Daily body weight and food intake measures were modeled separately with repeated-measures ANOVA. Initial body weight or food intake at the start of stress was used as a covariate in the analysis. Post hoc comparisons of treatment means at different time points were effected by t-test, and the significance levels reported are unadjusted for multiple comparisons (SAS for Windows, release 6.12, SAS Institute). Differences in single time point measures were determined by t-test, one-way ANOVA, or two-way ANOVA and post hoc Duncan’s multiple range test (Statistica, StatSoft, Tulsa, OK).

RESULTS

Experiment 1: restraint stress on 10 consecutive days. Exposing rats to restraint for 10 days caused a significant loss of body weight (Fig. 1A: restraint, \( P < 0.002; \) day, \( P < 0.0001; \) interaction, \( P < 0.0001 \)), such that restrained rats weighed significantly less than controls from day 3 to the end of the experiment. The extended period of repeated restraint did not increase the amount of weight lost because restrained rats weighed significantly less on day 5 of restraint than day 0, but their body weight was not significantly different on day 10 of restraint compared with day 3. Stress inhibited food intake on the days of restraint, but the intake of restrained rats returned to control levels once stress ended (Fig. 1B: restraint, \( P < 0.02; \) day, \( P < 0.0001; \) interaction, \( P < 0.0001 \)). Restraint stress caused a significant increase in serum corticosterone (Fig. 2A: restraint, \( P < 0.03; \) day, \( P < 0.0001; \) interaction, \( P < 0.0001 \)) and body temperature (Table 1: restraint, \( P < 0.04; \) day, \( P < 0.0009; \) interaction, \( P < 0.004 \)). The rats appeared to be adapting to repeated exposure to stress because corticosterone was significantly higher after restraint on day 1 than on days 7 or 9. Similarly, rectal temperatures were higher on days 1, 4, and 6 than on day 10. Temperatures of the controls were also elevated on day 1, which suggests that being moved to a novel environment was stressful. In contrast, repeated restraint caused a delayed decline in serum leptin (Fig. 2B: restraint, \( P < 0.08; \) day, \( P < 0.005; \) interaction, \( P < 0.0005 \)). Leptin in restrained rats was significantly lower than that in controls from day 7 of restraint to day 17 of the experiment, a week after the stress had ended. The reduced body weight of restrained rats was accounted for by nonsignificant decreases in both fat and lean tissue (control fat, 71 ± 11 g; restrained fat, 52 ± 3 g; control lean mass, 389 ± 7 g; restrained lean mass, 378 ± 7 g).

Experiment 2: two bouts of repeated restraint. Exposing rats to two bouts of repeated restraint had an additive effect on weight loss. Restrained rats weighed significantly less than controls from the last day of the first bout of repeated restraint (day 3) to the end of the experiment (Fig. 3A: restraint, \( P < 0.0001; \) day, \( P < 0.0001; \) interaction, not significant (NS)). The difference in weights between the two groups plateaued...
after the first bout of restraint and was then increased by the second bout of restraint. This response is illustrated in Fig. 3B, which is a plot of the difference between the mean body weight of the two groups of rats on each day of the experiment. Restraint significantly inhibited food intake (Fig. 3C: restraint, P < 0.04; day, P < 0.0001; interaction, NS) but only on the days that stress was applied. Restraint caused a significant elevation of rectal temperature compared with either controls or with prestress temperatures (Fig. 4: restraint, P < 0.0001; day, P < 0.0001; interaction, NS) but only on the days that stress was applied. Restraint caused a significant reduction in body weight that was apparent from day 3 to the end of the experiment. C: *food intake was inhibited only on days rats were restrained.

stress × restraint, P < 0.0001; prestress/poststress × day, P < 0.0001). Hyperthermia was transient and was not present 24 h after the end of stress.

Serum concentrations of free IL-6 were undetectable on day 0, before stress, in all rats, as shown in Fig. 5A. After stress, free IL-6 was significantly increased in both control and restrained animals, with no differences between the two groups (restraint, NS; day, P < 0.0001; interaction, NS), indicating that the daily manipulations involved in this protocol, independent of restraint, were stressful enough to induce release of this cytokine. There were no differences in serum leptin concentrations of control and restrained rats during the first bout of restraint (Fig. 5B). During the second bout of restraint, leptin was lower in restrained than control rats (Fig. 5C: restraint, NS; day, P < 0.0001; interaction, NS).

Table 1. Rectal temperatures of rats exposed to restraint on 10 consecutive days in experiment 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Restraint</th>
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<tbody>
<tr>
<td>Day 1</td>
<td>38.8 ± 0.17A</td>
<td>38.6 ± 0.1a</td>
</tr>
<tr>
<td>Day 4</td>
<td>37.9 ± 0.3R</td>
<td>38.9 ± 0.1ab,a</td>
</tr>
<tr>
<td>Day 6</td>
<td>38.3 ± 0.2AB</td>
<td>38.9 ± 0.1b,a</td>
</tr>
<tr>
<td>Day 10</td>
<td>37.5 ± 0.3R</td>
<td>38.0 ± 0.2a</td>
</tr>
</tbody>
</table>

Data are means ± SE for groups of 6 rats. Values are rectal temperatures (°C) of rats in experiment 1. The rats were restrained for 3 h each day, and temperatures were measured at the end of stress on each of the days indicated. Values within a column that do not share a common superscript are significantly different at P < 0.05. *Significant difference between control and restrained rats.
interaction, \( P < 0.002 \), but post hoc analysis did not show any specific day on which there was a significant difference between the two groups. Carcass analysis did not show any change in the carcass fat content of the rats as all of the weight difference was accounted for as lean mass (water + protein) (control fat, 69 ± 2 g; restrained fat, 69 ± 5 g; control lean mass, 455 ± 11 g; restrained lean mass 430 ± 12 g).

Experiment 3: repeated restraint of leptin-infused rats. There was no effect of peripheral leptin infusion on food intake or body weight during the 21 days before restraint stress in rats consuming a 40%-kcal-fat diet, as shown in Table 2. Serum leptin concentrations, measured 2 days after the start of infusion, were increased ~4-fold in rats receiving 100 \( \mu g \)/day compared with controls (Table 2). There was no effect of leptin on serum corticosterone, glucose, or free fatty acids at this time point. By the end of the study serum leptin was ~7-fold higher in the 100 \( \mu g \)/day group than in controls (see Fig. 7A). All of the rats that were exposed to repeated restraint-stress lost weight, but rats infused with 100 \( \mu g \)/day leptin appeared to regain weight faster than the two other groups (Fig. 6A: leptin, NS; stress, \( P < 0.03 \); day, \( P < 0.0001 \); stress \( \times \) day, \( P < 0.002 \); leptin \( \times \) day, \( P < 0.02 \)). All rats exposed to

![Fig. 4. Repeated measures of rectal temperatures of rats exposed to 2 bouts of repeated restraint on days 1–3 (A) and days 10–12 (B). Temperature was measured before and after a 3-h restraint. Data are means ± SE for groups of 7 rats. *Stress caused a significant elevation of body temperature at the end of 3-h restraint.](http://ajpregu.physiology.org/)

![Fig. 5. Repeated measures of serum free interleukin (IL)-6 (A) during the 1st of 2 bouts of repeated restraint and of leptin (B and C) during both of the bouts of repeated restraint. Data are means ± SE for groups of 7 rats. The manipulations of the study caused significant elevations in IL-6 for all rats, but there was no difference between control and restrained animals. There was a significant interaction between stress and time (\( P < 0.002 \)) for leptin concentrations, determined by repeated-measures analysis, but post hoc tests did not reveal a significant difference on any specific day of the experiment.](http://ajpregu.physiology.org/)
Table 2. Prestress body weights, food intakes, and serum hormone concentrations in rats receiving intraperitoneal infusions of leptin

<table>
<thead>
<tr>
<th>Leptin (µg/day)</th>
<th>0</th>
<th>30</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prestress weight, g</td>
<td>402 ± 4</td>
<td>402 ± 4</td>
<td>402 ± 4</td>
</tr>
<tr>
<td>Prestress food intake, g/21 days</td>
<td>300 ± 5</td>
<td>300 ± 5</td>
<td>297 ± 5</td>
</tr>
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**Leptin concentrations at the end of the experiment (Fig. 8A):**

- Leptin, ng/ml: 4.7 ± 0.6^A^ 6.2 ± 0.5^A^ 16.5 ± 2.6^B^ 16.5 ± 2.6
- Corticosterone, ng/ml: 19 ± 3 15 ± 1 17 ± 2
- Glucose, mmol/l: 5.6 ± 0.3 5.6 ± 0.3 6.0 ± 0.3
- Free fatty acids, µeq/l: 670 ± 33 659 ± 31 651 ± 31

Data are means ± SE for groups of 16 rats. The preinfusion weight is the weight of the rats on the day that leptin infusions started. The prestress weight is the weight of the rats on the morning before the first restraint stress, after 21 days of leptin infusion. Prestress food intake is the total amount of food eaten during the 21 days of leptin infusion before the start of restraint stress. Values for leptin, measured after 2 days of infusion, that do not share a common superscript are significantly different (determined by 1-way ANOVA).

Repeated restraint ate less during stress than their controls. There was no effect of leptin on this response (Fig. 6B: leptin, NS; stress, P < 0.002; stress x leptin, NS; day, P < 0.0001; stress x day, P < 0.009), and intakes returned to control levels once restraint ended. Serum corticosterone measured during the first restraint was lower in both control and restrained rats receiving 100 µg/day leptin than in comparable treatment groups receiving 0 or 30 µg/day leptin (Fig. 7B: leptin, P < 0.005; stress, P < 0.06; interaction, NS). There were no differences in corticosterone measured the day after the end of repeated restraint (Fig. 7B), but at the end of the experiment, 5 days after the end of restraint, corticosterone was lower in all rats that had been restrained than in controls (Fig. 8A: leptin, NS; stress, P < 0.01; interaction, NS). There were no differences in serum glucose or insulin at this time point (data not shown). Stress had no effect on serum leptin concentrations at the end of the experiment (Fig. 8B), but there was a significant interaction between leptin and stress (P < 0.05) on leptin mRNA expression in epididymal fat (Fig. 8C). Expression was inhibited in restrained 100 µg/day rats, compared with their controls, even though there were no differences in serum leptin concentrations of the two groups.

Carass weights of restrained rats were lower than those of controls (leptin, NS; stress, P < 0.009; interaction, NS), and this difference was a combination of lean and fat tissue for all treatment groups (data not shown). The 100 µg leptin/day control group of rats was nonsignificantly fatter than the other animals, and there were significant increases in epididymal and retroperitoneal fat pad weights (epididymal: 100 µg/day controls 9.2 ± 0.5 g, all other groups 7.2–7.8 ± 0.5 g, P < 0.05; retroperitoneal: 100 µg/day controls 4.8 ± 0.5 g, all other groups 3.4–3.8 ± 0.3 g, P < 0.05). There were no differences in adrenal gland weight, but stress significantly reduced thymus weight (leptin, NS; stress, P < 0.01; interaction, NS), especially in the 100 µg/day leptin group (control 376 ± 35 mg; restrained 285 ± 21 mg, P < 0.02).

**Experiment 4: restraint of food-restricted rats.** Restraint stress caused a significant reduction in body weight, independent of the rat's feeding status (Fig. 9A: feeding status, P < 0.0001; restraint, P < 0.0001; day, P < 0.0001; feeding status x restraint, P < 0.0008; feeding status x restraint x day, P < 0.01). Restrained AL and FR-AL rats weighed significantly less than their respective nonstressed controls on all poststress days. Restrained FR rats weighed significantly less than nonstressed FR rats for the last 5 days.

Fig. 6. Change in body weight (A) and daily food intake (B) of rats infused with PBS (0 µg leptin/day groups) or leptin for 21 days before being exposed to repeated restraint. Data are means ± SE for groups of 8 rats. • stress caused a significant weight loss in all groups of rats; # on the last 2 days of the experiment, the difference in weight was significant only for the 0 and 30 µg leptin/day groups. B: food intakes of the rats were inhibited by restraint. This difference was significant for all 3 groups on days 22 and 24 (#) but only for the 0 and 100 µg leptin/day groups on day 23 (Ψ).
of the recovery period. Restrained rats gained less weight than their nonstressed controls during the 12-day poststress period (days 3–14), independent of feeding status (Fig. 10A: feeding status, \( P < 0.0001 \); restraint, \( P < 0.0001 \); interaction, NS).

Both groups of restrained rats that had free access to food during the poststress period ate significantly less than their control group (Fig. 9B: restraint, \( P < 0.001 \); feeding status, \( P < 0.0001 \); day, \( P < 0.003 \); feeding status \( \times \) restraint, \( P < 0.0001 \); feeding status \( \times \) restraint \( \times \) day, \( P < 0.003 \)). Restrained and control FR-AL rats were initially hyperphagic when they returned to ad libitum feeding, but this was reversed earlier in the restrained than the control animals. The effect of restraint on food intake was also apparent when cumulative food intake between days 3 and 14 of the experiment was compared for all groups of animals (Fig. 10B: feeding status, \( P < 0.0001 \); restraint, \( P < 0.001 \); feeding status, NS; interaction, NS), but the response was greater in FR than AL rats. Twelve days after the end of repeated restraint, serum corticosterone was higher in all groups of rats that had been restrained than in their respective control group (Table 3: restraint, \( P < 0.05 \); feeding status, NS; interaction, NS). The effect was only significant in the FR animals. Free fatty acids were lower in FR and FR-AL rats than
AL rats, but there was no effect of restraint (restraint, NS; feeding status, \( P < 0.0001 \); interaction, NS). Similarly, serum insulin concentrations were lower in FR rats than the two other groups of animals (restraint, NS; feeding status, \( P < 0.0002 \); interaction, NS). Serum glucose and leptin levels measured at the end of the experiment were significantly reduced by both restraint and food restriction (restraint, \( P < 0.01 \); feeding status, \( P < 0.0001 \); interaction, NS). Glucose was lower in FR rats than the two other groups and tended to be lower in restrained than control rats in all three treatment groups, but the difference was only significant for AL rats. Leptin was substantially reduced in both control and restrained FR rats compared with the other animals, and in both AL and AL-FR rats, serum leptin concentrations were lower in restrained than control rats.

Carcass weight, carcass fat, and lean mass (protein + water) were all significantly reduced by restraint stress (Table 3: restraint, \( P < 0.0001 \); feeding status, \( P < 0.04 \); interaction, NS), but post hoc analysis did not detect any significant differences between restrained and control rats within any of the feeding treatments. The effect of feeding status was significant for all aspects of body composition except for carcass ash.

**DISCUSSION**

Exposing rats to restraint stress causes weight loss, and the stressed rats subsequently maintain a body

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**Fig. 9.** Daily body weight (A) and food intakes (B) of rats that were fed ad libitum (AL) or were food restricted (FR) throughout the experiment or were food restricted to 50% voluntary intake for 10 days before the experiment and returned to ad libitum feeding at the end of restraint (FR-AL). Data are means ± SE for groups of 10 rats. *Significant difference between restrained rats and their controls, determined by repeated-measures ANOVA and post hoc t-test.

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**Fig. 10.** Weight change (A) and food intake (B) of AL, FR-AL, and FR rats during 12 days that followed the end of repeated restraint. Data are means ± SE for groups of 10 rats. C: serum corticosterone concentrations measured after 1 h of restraint on the 1st day of repeated restraint in AL (n = 10) and FR rats (n = 20). Values for a specific parameter that do not share a common superscript (A-F) are significantly different at \( P < 0.05 \).
weight that is 5–10% less than that of nonrestrained controls for periods as long as 40 days after stress has ended (12, 27). This response is blocked by infusion of a CRF receptor antagonist, αhCRF, into the third ventricle immediately before restraint (28), suggesting that the chronic downregulation of body weight is initiated by acute release of CRF in an area of the brain close to the hypothalamus. Chronic weight loss is not limited to restraint stress as similar changes have been found in rats exposed to a single social defeat (25) or limited to restraint stress as similar changes have been found in rats exposed to a single social defeat (25) or chronic mild stress (13). The restraint protocol, however, allows us to expose a relatively large number of animals to a standardized stressor. The experiments described here were carried out to provide further clarification of physiological conditions that influence the chronic response to repeated acute stress. Previous experiments have shown that exposure to 3 h of restraint on each of 3 days (12) causes a greater weight loss than that produced by one 3-h period of restraint (27). The results of experiments 1 and 2 demonstrate that exposing rats to a second bout of repeated restraint increased the amount of weight lost by stressed rats, but increasing the number of consecutive days that the rats were restrained did not exaggerate the weight loss caused by 3 days of restraint. This may be explained by the rats adapting to the stress in experiment 1, such that stress-induced activation of central mechanisms that initiate the chronic response was ameliorated on successive days of restraint, and the subsequent effect on body weight was limited. This concept is supported by the gradual decline in stress-induced corticosterone release during the 10 days of exposure to restraint stress. In contrast, when the rats were exposed to two bouts of restraint, with an interval of 1 wk between bouts, the hyperthermic response to stress was as great during the second bout of restraint as the first, implying that the central response was also intact and induced further loss of body weight in the rats.

In both experiments 1 and 2 we found a delayed decline in serum leptin concentrations during the poststress period. When rats were restrained daily for 10 days, the reduction in leptin was significant after 5 days of restraint and was maintained for 1 wk after the stress had ended. The time delay between onset of stress and the drop in leptin implies that it did not result from direct inhibition of expression by activation of either the HPA axis or stress-induced release of catecholamines. In addition, these results do not support the concept of physiological concentrations of leptin having an inhibitory effect on corticosterone release in stressed animals (14), because both leptin and corticosterone were significantly reduced on the last 5 days of restraint. If leptin provided a tonic inhibition of the HPA axis, corticosterone release in response to stress would be expected to increase, or at least be maintained, once leptin concentrations declined. It is interesting to note that although leptin concentrations were reduced at the end of restraint and after stress had ended, there was no evidence of poststress hyperphagia to compensate for the period of hypophagia during restraint. The failure to show compensatory overeating in restrained rats may contribute to their maintenance of a reduced body weight compared with controls. Although leptin is hypothesized to be a circulating satiety signal (29), and, on the basis of this hypothesis, food intake would be expected to increase when leptin levels decline, the results from these experiments clearly demonstrate that leptin is not responsible for either the inhibition of intake during stress or for the failure to overeat once stress has ended.

We have previously reported that weight loss during restraint is exclusive to lean tissue, but during the days after the end of stress, the weight difference between control and restrained rats shifts to a combination of both lean and fat tissue (12, 30). In experiments 1 and 2, body composition of the rats was measured almost 2 wk after the end of stress; therefore it was not possible to determine whether the decline in leptin coincided with changes in body fat content of the rats. Unlike previous experiments (12, 30) or experiments

Table 3. Serum hormones and body composition of food-restricted rats exposed to repeated restraint

<table>
<thead>
<tr>
<th></th>
<th>AL</th>
<th></th>
<th>FR</th>
<th></th>
<th>FR-AL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Restrainted</td>
<td>Control</td>
<td>Restrainted</td>
<td>Control</td>
<td>Restrainted</td>
</tr>
<tr>
<td>Day 12 recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Corticosterone, ng/ml</td>
<td>35 ± 10A</td>
<td>57 ± 16AB</td>
<td>70 ± 16B</td>
<td>153 ± 70B</td>
<td>55 ± 12AB</td>
<td>117 ± 35B</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.6 ± 0.2A</td>
<td>2.5 ± 0.3A</td>
<td>1.3 ± 0.1C</td>
<td>1.5 ± 0.2BC</td>
<td>2.4 ± 0.2A</td>
<td>1.8 ± 0.3B</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>26 ± 2A</td>
<td>19 ± 3A</td>
<td>4 ± 0.5C</td>
<td>4 ± 0.4C</td>
<td>20 ± 2B</td>
<td>14 ± 2D</td>
</tr>
<tr>
<td>FFA, meq/l</td>
<td>0.60 ± 0.05A</td>
<td>0.51 ± 0.05A</td>
<td>0.45 ± 0.02B</td>
<td>0.44 ± 0.04B</td>
<td>0.39 ± 0.04B</td>
<td>0.48 ± 0.03AB</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>289 ± 15A</td>
<td>231 ± 9B</td>
<td>216 ± 10C</td>
<td>200 ± 13A</td>
<td>260 ± 12A</td>
<td>241 ± 15AB</td>
</tr>
<tr>
<td>Carcass composition, g/rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Weight</td>
<td>466 ± 16A</td>
<td>434 ± 17A</td>
<td>323 ± 7B</td>
<td>314 ± 7B</td>
<td>416 ± 13AC</td>
<td>391 ± 12C</td>
</tr>
<tr>
<td>Protein</td>
<td>146 ± 5A</td>
<td>140 ± 6A</td>
<td>98 ± 3BC</td>
<td>95 ± 9C</td>
<td>135 ± 6AB</td>
<td>112 ± 9B</td>
</tr>
<tr>
<td>Water</td>
<td>260 ± 7A</td>
<td>249 ± 7A</td>
<td>201 ± 4B</td>
<td>194 ± 7B</td>
<td>240 ± 8AC</td>
<td>233 ± 9C</td>
</tr>
<tr>
<td>Fat</td>
<td>47 ± 6A</td>
<td>32 ± 6BC</td>
<td>10 ± 5B</td>
<td>6 ± 1B</td>
<td>29 ± 4C</td>
<td>26 ± 2C</td>
</tr>
<tr>
<td>Ash</td>
<td>14 ± 0.9</td>
<td>13 ± 0.4</td>
<td>13 ± 0.7</td>
<td>12 ± 0.6</td>
<td>13 ± 0.7</td>
<td>13 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± SE for groups of 10 rats killed 12 days after the end of repeated restraint. Rats were fed ad libitum (AL) or were food restricted (FR) throughout the experiment or were food restricted to 50% voluntary intake for 10 days before the experiment and returned to ad libitum feeding at the end of restraint (FR-AL). FFA, free fatty acids. Values for a specific parameter that do not share a common superscript are significantly different at P < 0.05 (determined by 2-way ANOVA and post hoc Duncan’s multiple range test).
ment 1, all of the weight loss in animals that had been exposed to two bouts of repeated restraint was accounted for by lean body mass. This suggests that a second exposure to stress does not promote the same cascade of metabolic events initiated by the first exposure to stress.

It has been established in normal mice that leptin protects lean mass while selectively reducing body fat content (2, 6), and in vitro studies have shown a direct inhibition of cortisol secretion from adrenocortical cells (19). Therefore, in experiment 3, we determined whether treating the rats with leptin before and during exposure to repeated restraint would moderate, or prevent, stress-induced weight loss. The rats in all of the experiments described here were fed a high-fat diet, and peripheral leptin had no effect on food intake or body weight during the prestress infusion. Thus the response to stress was not confounded by rats treated with different doses of leptin having different starting body weights or body compositions. The results of the experiment demonstrate that, although the highest dose of leptin did inhibit stress-induced release of corticosterone during restraint, it did not influence the amount or composition of weight that was lost. Thus a fivefold elevation of circulating concentrations of leptin was able to suppress glucocorticoid release from the adrenal gland, whereas endogenous leptin in experiment 1 did not appear to exert any limiting effect on corticosterone release. Although the high dose of leptin inhibited stress-induced corticosterone release, it did not prevent stress-induced hypophagia or weight loss, confirming previous observations that the chronic response to stress is independent of glucocorticoid release (28).

Five days after the end of restraint, corticosterone, measured in blood collected between 9:00 AM and 12:00 PM, was lower in all of the rats exposed to restraint than in their controls. These results are contrary to those from other investigators who have reported a stress-induced chronic elevation of basal corticosterone during the trough of the diurnal cycle (1, 4). Because only a single measure was made in our experiment, it is not possible to determine whether there was a sustained inhibition of activity of the adrenal cortex or whether stress had modified the circadian rhythm of corticosterone release. Dallman et al. (3) have proposed that a small elevation of basal corticosterone may promote abdominal obesity due to the metabolic effects of glucocorticoids. On the basis of this hypothesis, a reduction in the lowest diurnal level of corticosterone release would not be expected to have any substantial effect on metabolism or body composition. If, however, the pattern of release of corticosterone had shifted, this would disrupt the dynamic equilibrium between catabolic and anabolic hormones and could account for the change in body weight of the rats. Leptin expression was increased in the epididymal fat of control 100 μg leptin/day rats compared with all other groups, although there were no differences in serum leptin concentrations between this group and the restrained rats that received the same dose of leptin. The increase in leptin expression reflected the significant enlargement of epididymal depots in these animals. It is possible that stress had reversed a similar degree of adiposity in the restrained 100 μg leptin/day rats and had also changed the rate of leptin clearance, as there were no differences in circulating concentrations of leptin in control and restrained rats infused with 100 μg leptin/day.

In the final experiment described here, we determined whether rats that were already weight reduced would show the same chronic response to repeated restraint as ad libitum-fed rats. Restrained rats that remained food restricted after the end of stress lost only a small amount of weight, suggesting that there is an innate protective mechanism that prevents an additive response to two simultaneous stressors. These results are similar to those reported by Gursoy et al. (5), who found that daily immobilization stress did not reduce the body weight of food-restricted rats. In experiment 4, the restrained FR-AL rats were initially hyperphagic and gained weight once stress had ended. The rate at which they gained weight was not as rapid as that in the control FR-AL rats, and food intake of restrained FR-AL rats returned to control levels before that of the FR-AL control rats. These results imply that, although the weight-reduced rats did not lose weight in response to restraint, the mechanisms responsible for initiating a chronic downregulation of body weight was intact and caused restrained FR-AL rats to gain less weight than their nonrestrained counterparts during refeeding. Similar results have been reported for weight loss after lesions of the lateral hypothalamus. Rats that have been weight reduced have a lower energy expenditure (18) and lose less weight than ad libitum-fed animals so that both groups of lesioned animals reach the same postlesion weight (17). Other investigators (20, 21) have shown that the metabolic response to acute inflammation caused by turpentine injection is also related to the energy status of the rats at the time of injection. Overfed rats become hypermetabolic and anorexic and lose weight, whereas weight-reduced rats given ad libitum access to food after the injection are hyperphagic and gain weight so that the postinflammation weight is the same for all of the rats, independent of their energy status at the onset of inflammation. Thus it appears that both restraint stress and inflammation initiate a series of events that lead to the rats defending a reduced body weight. Because we did not find any evidence that restraint stress caused an independent stimulation of IL-6 or development of fever, it is not clear whether the two different types of stress cause weight loss through the same mechanisms or whether different mechanisms result in similar changes in the two models.

In summary, exposing rats to repeated restraint stress causes a chronic downregulation of body weight initiated by acute activation of central CRF receptors (28). Exposing the rats to a second bout of restraint caused further weight loss, whereas extending the
number of days of restraint within one bout did not, possibly because the rats adapted to the stressor. Although there was a delayed decline in serum leptin concentrations of restrained rats, infusing the rats with leptin before and during exposure to restraint did not protect against weight loss even though stress-induced corticosterone release was inhibited by high concentrations of leptin. Stress-induced weight loss was inhibited in weight-reduced rats, but animals that were allowed to refeed after the stress has ended gained less weight than their nonrestrained controls. These results indicate that the reduced body weight is not dependent on stress-induced hypophagia.

Perspectives

The effects of stress on body weight are determined both by the severity of the stress and by an individual’s perception of the stress. In animals, mild stressors, such as tail pinch, increase food intake (23), whereas more severe stressors, such as restraint or immobilization, inhibit food intake (24) and have long-term effects on body weight and behavior (12, 25). In humans, extreme stress, such as combat, inhibits food intake (26), but the chronic effects on food intake and body composition have not been determined. Experiments described here demonstrate that the sustained reduction in body weight caused by acute stress is not a direct result of hypophagia, but on the basis of the response of food-restricted rats, it appears that repeated restraint changes some aspect of the metabolic equilibrium. This change is induced in weight-reduced animals but is only expressed as a difference in body weight in ad libitum-fed animals. Further studies are needed to identify other chronic behavioral and biochemical changes present in these animals. Elucidation of the mechanisms that cause this disruption of homeostasis would provide new information on the regulation of body composition and body weight and may also be valuable in developing new treatments for trauma patients.

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REFERENCES


