Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets

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Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1928–R1938, 1998.—Exposure to the moderate stressor of 3-h restraint for 3 consecutive days causes a temporary drop in food intake but a permanent reduction in body weight in adult rats. Young rats did not show the same response. Food intake of adult rats exposed to repeated restraint was significantly lower than that of controls for 4 days after the end of stress, and there was no rebound hyperphagia. Body weight remained significantly lower for at least 40 days after stress. When the rats were fed a high-fat diet of 80% chow and 20% vegetable shortening (48% kcal fat, 16% protein), lean body mass accounted for all of the weight loss in stressed rats. When the experiment was repeated with a purified high-fat diet containing corn oil and coconut oil as the source of fat (41% kcal fat, 16% protein), weight loss consisted of both lean and fat tissue. There were no sustained changes in single time point measures of corticosterone, insulin, or leptin that could account for the reduced body weight in these rats.

Dallman et al. (8) suggest that the balance between corticosterone and insulin is a primary determinant of food intake and nutrient utilization because the two hormones have opposing effects on feeding, when administered centrally, and on nutrient partitioning in the periphery. Diurnal rhythms in activity of the hypothalamic-pituitary-adrenal (HPA) axis, and resulting changes in corticosterone feedback regulation of insulin secretion, may normally regulate energy balance and be disrupted by stress-related activation of the HPA axis (8). However, in addition to promoting release of corticosterone, stress, and its activation of the HPA axis, promotes release of inflammatory cytokines (33) and prolactin (2) and suppresses release of growth hormone (19), all of which have also been shown to influence feeding. Therefore, the effect of stress on energy balance probably involves interactions between multiple systems.

Changes in food intake and body weight of rats exposed to acute, repeated, or chronic stress are well documented (20, 26, 27); there is less information available concerning the recovery of rats during the period following stress, although it has been noted that rats exposed to a single extreme stress failed to return to control weight 3 wk after the end of stress (21). Accidental or surgical trauma, which may be considered a severe stress, results in a prolonged anorexia and weight loss that is proportional to the severity of the injury (23). In experiments described here we have found prolonged effects on food intake and body weight in rats exposed to the moderate stress of repeated restraint. In addition, it appears that diet composition has the potential to influence the degree of body weight response to this stress paradigm.

METHODS

Experiment 1. Twelve male Sprague Dawley rats, ~11 wk old and weighing 350 g, were purchased from Harlan Sprague Dawley (Houston, TX) and housed in individual wire-mesh cages with free access to water and rodent chow (Purina rodent chow 5001; Purina Mills, St. Louis, MO). Daily body weights and food intakes were recorded for 10 days, and then the rats were divided into two weight-matched groups. One group was exposed to repeated restraint and the other group was nonrestrained controls. Restrained rats were moved to an experimental room and placed in plastic restraining tubes (Plas Laboratories, Lansing, MI) for 3 h, and controls were moved to the same room and placed in shoe box cages without food or water. At the end of restraint all animals were returned to their home cages. Rats were restrained for 3 h on three consecutive days, from 0700 to 1000, and daily body weights and food intakes were recorded for 40 days after the end of restraint. To determine whether stress had any effect

STRESS HAS MULTIPLE EFFECTS on the physiology, neurochemistry, and behavior of animals and humans. Inhibition of food intake and weight loss in rats exposed to stress is well established, although the specific feeding effect may be modulated by the severity of stress used, the duration and frequency of exposure, and the time of day that the stress is applied (15, 20, 26). The mechanisms responsible for this suppression of food intake have not been fully elucidated and appear to be complex. Stress is characterized by release of hypothalamic corticotropin-releasing factor (CRF), which in turn triggers release of pituitary and adrenal hormones (13), central catecholamines (28), and serotonin (25) and activation of the peripheral sympathetic nervous system (34). Central administration of CRF inhibits food intake, probably by reducing expression of the orexigenic protein neuropeptide Y (NPY) in the hypothalamus (12). Serotonergic mechanisms have also been implicated (6), and it appears that these three neurotransmitters interact in positive and negative feedback loops.

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on responses to pleasurable stimuli, preference for 5 mM saccharin solution over water was determined in 24-h, two-bottle preference tests for 7 days, starting from the end of the last restraint. Preference was expressed as a ratio of saccharin intake to total fluid intake. Therefore, values greater than 0.5 indicate a preference for saccharin over water. Statistically significant differences in daily body weights, food intakes, and saccharin preference were determined by repeated-measures analysis of variance using treatment (control vs. restrained) as the independent variable. Differences between treatment groups on specific days of the experiment were determined by post hoc calculation of least significant difference (P < 0.05) (Statistica; StatSoft, Tulsa, OK). All animal procedures were approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

Experiment 2. The results from the first experiment indicated that repeated restraint caused a prolonged suppression of food intake and body weight in mature rats. In this experiment we determined whether repeated restraint could initiate a similar response in young animals.

Sixteen young male Sprague-Dawley rats, age ~ 3 wk, were obtained from Harlan Sprague Dawley and housed as described above. Body weights and food intakes were recorded for 8 days, by which time the rats were 30 days old and weighed an average of 106 g. They were divided into two weight-matched groups, and one group was exposed to repeated restraint and the other group was controls. Eight days after the end of stress, body composition was determined as described previously (9), and serum corticosterone concentration was measured (corticosterone RIA; ICN Pharmaceuticals, Costa Mesa, CA). Statistically significant differences in food intake and body weight were determined as described above. Other parameters were compared by two-tailed unpaired t-test, assuming equal variances.

Experiment 3. Because exp 1 demonstrated a prolonged suppression of food intake and body weight in mature rats exposed to repeated restraint, we determined whether feeding adult rats a high-fat diet, which has the potential to induce weight gain, would prevent the stress-induced weight loss.

Thirty-two male Sprague-Dawley rats (~350 g, 11 wk of age) were housed individually with free access to powder chow. They were adapted to handling, and body weights were recorded daily for 1 wk before they were divided into two weight-matched groups. One group continued to eat chow (12.4% kcal fat, 28.7% kcal protein, 3.3 kcal/g, based on manufacturer’s formulation), and the other was offered a high-fat diet of 80% powdered chow mixed with 20% vegetable shortening (48.1% kcal fat, 16.9% kcal protein, 4.4 kcal/g). Daily food intakes and body weights were recorded for a further 9 days, and then the animals on each diet were subdivided into two weight-matched groups of eight rats. One group in each dietary treatment was exposed to repeated restraint and the other group acted as controls. On the first day that rats were stressed, food intakes were recorded at regular intervals to determine when intake was suppressed in relation to the light-dark cycle. Daily food intakes and body weights were measured until 5 days after the end of stress, when the rats were killed and trunk blood was collected for measurement of serum corticosterone, insulin (rat insulin RIA; Linco, St. Louis, MO), and glucose (Sigma kit 510; Sigma Chemical, St. Louis, MO). Adrenal glands, thymus, and epididymal fat pads were dissected and weighed. The fat was snap frozen for measurement of leptin mRNA expression by Northern blot analysis, as described previously (11). The other organs were returned to the exsanguinated carcass for determination of carcass composition. Day 5 was chosen on the basis of results from exp 1, which indicated that by this time the food intake of rats exposed to repeated restraint had returned to control levels but body weight remained significantly lower than that of control animals.

Experiment 4. The objectives of this experiment were to determine whether the difference in body composition of restrained rats fed low- or high-fat diets was due to a difference in the composition of tissue lost in response to stress or a difference in recovery of tissue following stress. Thirty-eight male Sprague-Dawley rats (~350 g, 11 wk old) were housed as described above with free access to chow. They were adapted to handling, and body weights were recorded for 5 days before they were divided into two weight-matched groups, one of 14 rats and one of 24 rats. The 14 rats were adapted to the high-fat diet, described in exp 3, for 9 days and were subdivided into two groups of 7, and one group was subjected to repeated restraint for 3 days. A small blood sample (150 µl) was collected by tail bleeding from restrained and control rats at the end of restraint on each of the 3 days for measurement of serum corticosterone. The day after the end of restraint, the rats were killed for determination of body composition. Adrenal glands and thymus glands were weighed, and serum corticosterone was measured.

The 24 rats were divided into four weight-matched subgroups, and two of these groups were given the high-fat diet described in exp 3; the remainder of the animals continued to eat powdered chow. After 9 days, one high-fat-fed subgroup and one chow-fed subgroup were exposed to repeated restraint; the others were controls. Five days after the end of stress the rats were killed for determination of body composition, and spleen, thymus, and adrenal gland weights were recorded. Day 5 was chosen on the basis of the results of exps 1 and 3, which indicated that this was the time at which restrained rats had a similar food intake but reduced body weight compared with controls.

Experiment 5. In the two previous experiments, the high-fat diet was made by combining vegetable shortening with chow, which, in addition to raising the fat content and caloric density, diluted the micronutrient and protein content of the diet. This experiment was carried out to determine whether the failure to gain protein in stressed rats fed high-fat diet was due to a low protein-to-calorie ratio in the diet. The experimental design was the same as that in exp 3 except that the rats were fed purified low- or high-fat diets that both delivered 16% kcal protein, but the low-fat diet contained 10% kcal fat whereas the high-fat diet contained 41% kcal fat (see Table 1). In addition to the change in ratio of protein to fat calories from that of diets in expts 3 and 4, which were based on chow, diets in this experiment also contained different sources of fat, protein, and carbohydrate.

Thirty-six male Sprague-Dawley rats (325–350 g, 11 wk of age) were adapted to the low-fat diet. They were then divided into two weight-matched groups, and one group continued to receive the low-fat diet while the other group was fed the high-fat diet described in Table 1. After 7 days on these diets, the rats were further subdivided into two weight-matched groups within each dietary treatment and one group was exposed to repeated restraint and the other was control. Corticosterone was measured in small blood samples collected from each rat by tail bleeding at 30-min intervals during the 3-h stress on the last day of restraint. Five days after the end of restraint the rats were killed for determination of body composition, serum insulin, and glucose.
RESULTS

Experiment 1. Daily body weights of chow-fed adult rats exposed to repeated restraint are shown in Fig. 1A. Restrained rats experienced significant weight loss during stress and continued to weigh significantly less than controls throughout the recovery period, 40 days after the end of restraint. Food intakes of the rats are shown in Fig. 1B. Stress caused a transient inhibition of food intake that was significant up to day 7, 4 days after the end of stress. Restrained rats did not show any compensatory hyperphagia after stress, so that cumulative intake during the entire experimental period was significantly different (P, 0.01) between the two groups (control: 5931 + 612 g/43 days, restrained: 5872 + 615 g/43 days), and 46% of the difference in total intake was accounted for by the hypophagia on days 1–7. Preference for 5 mM saccharin solution is shown in Fig. 1C. Both control and stressed rats greatly preferred saccharin over water, as indicated by a preference ratio greater than 0.5, and there was no effect of stress on preference, indicating that suppression of food intake was not associated with a loss of sensitivity to pleasurable stimuli.

Experiment 2. The daily body weights and food intakes of young rats exposed to repeated restraint are shown in Fig. 2. Repeated restraint caused a small, but significant, inhibition of weight gain that was compensated for by the end of the experiment. Food intake was also transiently inhibited but returned to control levels 2 days after the end of stress, and there was no significant difference in cumulative intake of control and stressed rats during the poststress period (control: 169 ± 3 g·rat⁻¹·8 days⁻¹, restrained: 166 ± 3 g·rat⁻¹·8 days⁻¹). As shown in Table 2, there was no effect of stress on body composition of young rats, measured 8 days after the end of the restraint, and there were no significant differences in thymus or adrenal weights or in serum corticosterone.

Experiment 3. The body weights and cumulative energy intakes of adult rats fed low- or high-fat chow-based diets are shown in Fig. 3. Energy content of the diet was calculated from diet composition, and energy

Table 1. Diet composition experiment 5

<table>
<thead>
<tr>
<th></th>
<th>High-Fat Diet</th>
<th>Low-Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>146.4</td>
</tr>
<tr>
<td>Vitamin mix AIN 76</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mix AIN 76</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Corn oil</td>
<td>125</td>
<td>26.3</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>100</td>
<td>18.7</td>
</tr>
<tr>
<td>Starch</td>
<td>243.5</td>
<td>360.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>243.5</td>
<td>360.3</td>
</tr>
<tr>
<td>Alphacel</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>dL-Methionine</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Dietary energy, kcal/g</td>
<td>3.66</td>
<td>5.0</td>
</tr>
<tr>
<td>%kcal Fat</td>
<td>41</td>
<td>10</td>
</tr>
<tr>
<td>%kcal Protein</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Composition of high-fat diet used in expt 5 in g/kg unless otherwise indicated. All dietary components were obtained from ICN Pharmaceuticals, Costa Mesa, CA. Dietary energy content was calculated from diet composition.
intake was determined from daily food intakes. Repeated restraint caused a significant reduction in energy intake and weight loss in rats from both dietary treatments. Measurements of energy intake during the 19 h following the first restraint (Fig. 4) showed an effect of diet on the pattern of response. Repeated-measures analysis of variance indicated a significant effect of diet \( (P, 0.007) \), treatment \( (P, 0.0001) \), and time \( (P, 0.0001) \) but no significant interactions \( (diet \times time: P, 0.07) \). Restraint caused a reduction in energy intake of high-fat-fed rats immediately after stress and again at the start of the dark cycle. In rats fed the low-fat diet, there was no significant difference in intake during any specific time period, although total intake over the 19-h period was significantly different \( (P, 0.003) \) for the two groups. This implied a consistent reduction in energy intake throughout the 19 h for rats fed low-fat diet, whereas reduced intake of high-fat-fed animals was predominantly accounted for by reductions in intake at specific time periods during the diurnal cycle. The only time periods in which energy intake of rats fed low-fat diet were different from those of high-fat-fed controls were immediately after the end of stress and at the start of the dark period, the same intervals at which restraint had a significant effect on

### Table 2. Body composition of young rats exposed to repeated restraint in experiment 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Restrained</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Weight, g</td>
<td>168 ± 3</td>
<td>169 ± 2</td>
</tr>
<tr>
<td>Protein, g</td>
<td>31.9 ± 0.6</td>
<td>33.0 ± 0.3</td>
</tr>
<tr>
<td>Water, g</td>
<td>119.7 ± 2.4</td>
<td>119.8 ± 1.6</td>
</tr>
<tr>
<td>Fat, g</td>
<td>11.1 ± 0.4</td>
<td>10.8 ± 0.4</td>
</tr>
<tr>
<td>Ash, g</td>
<td>5.1 ± 0.2</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Thymus, mg</td>
<td>719 ± 32</td>
<td>655 ± 20</td>
</tr>
<tr>
<td>Adrenals, mg</td>
<td>32 ± 1</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>Corticosterone, ng/ml</td>
<td>21 ± 7</td>
<td>19 ± 7</td>
</tr>
</tbody>
</table>

Data are means ± SE for groups of 8 young male Sprague-Dawley rats that were 30 days old when they were exposed to repeated restraint. Body composition was determined 8 days after the end of restraint, when rats were 41 days old. There were no significant effects of repeated restraint on any of the parameters measured at the end of the experiment.
food intake in high-fat-fed rats. There was no significant difference in total 19-h energy intake of high-fat- and low-fat-fed restrained rats.

Cumulative 24-h intake of the rats during the 3 days of restraint and the 5 days after the end of stress remained significantly lower in restrained than control rats, and control rats fed the high-fat diet consumed more energy than those fed chow (see Fig. 3B). Analysis of serum collected 5 days after the end of stress revealed no significant effect of restraint on corticosterone or insulin, although serum glucose was lower in restrained than control rats, possibly due to their reduced energy intake (see Table 3). There was no effect of diet or restraint on epididymal fat leptin mRNA expression (Table 3). Body composition of the rats is shown in Table 3. In rats fed chow there were no statistically significant differences between control and restrained rats, although carcass fat, protein, and water all tended to be lower in restrained than control animals. In rats fed the high-fat diet there was no significant difference in carcass fat content, but lean body mass (protein + water) was reduced in rats exposed to repeated restraint.

**Experiment 4.** For adult rats fed high-fat, chow-based diet and killed 1 day after the end of restraint, pre-stress and final body weights, recorded on the morning before the first restraint and the morning of death, respectively, and cumulative energy intake during the prestress or the stress plus poststress days are shown in Table 4. Rats fed the high-fat diet gained more weight than those on chow but repeated restraint caused significant weight loss in both dietary groups. Statistical analysis of daily body weights of the rats killed 1 day after the end of stress indicated a significant effect of day (P < 0.0001) but no effect of stress, and the weights of the two groups of animals were not different on any day of the experiment. For rats killed 5 days after the end of stress, there was no significant effect of diet or stress on body weight but a significant effect of day (P < 0.0001) and significant interactions

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**Table 3.** Serum hormones and carcass composition of restrained rats fed low- and high-fat diets in experiment 3

<table>
<thead>
<tr>
<th>Carcass Composition</th>
<th>LF Control</th>
<th>LF Rest</th>
<th>HF Control</th>
<th>HF Rest</th>
<th>Two-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g/rat</td>
<td>384 ± 5a</td>
<td>370 ± 4a</td>
<td>403 ± 7b</td>
<td>383 ± 5b</td>
<td>D: NS; St: P &lt; 0.003; Int: NS</td>
</tr>
<tr>
<td>Protein, %</td>
<td>23.0 ± 0.3</td>
<td>23.4 ± 0.4</td>
<td>23.8 ± 0.4</td>
<td>23.9 ± 0.4</td>
<td>D: P &lt; 0.006; St: P &lt; 0.005; Int: NS</td>
</tr>
<tr>
<td>Water, g/rat</td>
<td>65.9 ± 0.5a</td>
<td>66.4 ± 0.6a</td>
<td>66.5 ± 0.5b</td>
<td>64.1 ± 0.5a</td>
<td>D: P &lt; 0.003; St: NS; Int: NS</td>
</tr>
<tr>
<td>Lean tissue (water + protein), g/rat</td>
<td>341 ± 5b</td>
<td>332 ± 4a</td>
<td>356 ± 6b</td>
<td>337 ± 4a</td>
<td>D: P &lt; 0.06; St: NS; Int: NS</td>
</tr>
<tr>
<td>Fat, %</td>
<td>7.5 ± 0.4a,b</td>
<td>6.4 ± 0.4b</td>
<td>8.4 ± 0.7a,b</td>
<td>8.4 ± 0.6a</td>
<td>D: P &lt; 0.01; St: NS; Int: NS</td>
</tr>
<tr>
<td>Ash, %</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>D: P &lt; 0.05; St: NS; Int: NS</td>
</tr>
<tr>
<td>Adrenals, mg</td>
<td>50 ± 4</td>
<td>52 ± 6</td>
<td>54 ± 4</td>
<td>51 ± 6</td>
<td>D: P &lt; 0.005; St: NS; Int: NS</td>
</tr>
<tr>
<td>Thymus, mg</td>
<td>264 ± 59</td>
<td>266 ± 52</td>
<td>335 ± 60</td>
<td>270 ± 56</td>
<td>D: P &lt; 0.01; St: NS; Int: NS</td>
</tr>
</tbody>
</table>

Data are means ± SE for groups of 8 rats killed 5 days after the end of restraint stress. Statistical significance was determined by 2-way analysis of variance and post-hoc calculation of least significant difference (P < 0.05). LF, low fat; HF, high fat; Rest, restrained; D, diet; St, stress; Int, interaction. Values for a given parameter that do not share a common superscript are significantly different.
between diet and day (P < 0.001) and between stress and day (P < 0.008). Post hoc analysis revealed a difference in body weights of restrained and control low-fat-fed rats from the second day of restraint to the end of the experiment (P < 0.03). On the high-fat diet, restrained rats weighed significantly less than their controls from the last day of restraint to the end of the experiment (P < 0.02). There was a significant effect of stress (P < 0.01) but not of day on energy intakes of rats fed high-fat diet and killed the first day after the end of stress. Energy intake was significantly reduced in restrained compared with control rats on all 3 days after stress, and cumulative intake over the 3 days was also significantly reduced (see Table 4). In rats killed 5 days after the end of stress there were significant effects of stress (P < 0.02) and of day (P < 0.001) and interactions between diet and day and between stress and diet (P < 0.003). Rats fed high-fat diet had significantly higher energy intakes than those fed low-fat diet before stress but not after stress. Cumulative energy intake during stress and the 5 days after stress showed a significant effect of restraint but no effect of diet composition (see Table 4).

Body composition is shown in Table 5. There were nonsignificant reductions in carcass weight, fat, and water of rats fed high-fat diet and killed 1 day after the end of restraint. By day 5 after the end of stress, restrained rats had significantly less carcass protein than their controls. In rats fed chow, there was a nonsignificant reduction in carcass fat and a significant reduction in carcass water of restrained rats, compared with their controls. Restrained rats fed high-fat diet and killed 5 days after the end of stress had significantly more fat (expressed either as grams or percent) than those killed on day 1 (P < 0.05), whereas control rats killed on day 5 had the same amount of carcass fat but more protein than those killed on day 1. As shown in Fig. 5A, serum corticosterone measured at the end of 3 h of restraint on each of 3 days was higher in restrained rats than controls, and there was no interaction between day and stress. Serum corticosterone measured 1 day after stress was significantly higher in restrained than control rats, but there were no differences between groups of rats killed 5 days after the end of stress (see Table 4). Serum insulin was not different between groups on day 1 after stress, but there was a significant effect of diet on insulin in rats killed 5 days after the end of stress (see Table 4).

Experiment 5. As in previous experiments, repeated restraint of rats fed low- or high-fat purified diet caused reduced food intake and body weight, as shown in Fig. 6A. There was a significant effect of day (P < 0.001) and a significant interaction between diet and day (P < 0.0001) and between treatment and day (P < 0.0001) for body weight. Restrained rats in both dietary groups weighed less than their respective controls from the second day of restraint to the end of the experiment. There was a significant effect of diet on energy intake (diet: P < 0.001, treatment: NS, day: P < 0.0001, diet × day: P < 0.005), with rats fed the 40% kcal fat diet consuming more energy than those fed the 10% kcal fat diet on all days except the last 3 days of the experimental period. When considering only the days that included or followed restraint stress, there were significant (P < 0.0001) effects of diet, treatment, and day and significant interactions between diet and day (P < 0.04) and treatment and day (P < 0.0001). For rats on both diets, stress caused a significant reduction in energy intake that was reversed by the end of the experiment (Fig. 6B). The only statistically significant differences in body composition of the two groups of rats were in carcass weight (diet: P < 0.08, stress: P < 0.02, interaction: NS) and fat content (see Table 6). The rats fed high-fat diet were significantly fatter than those on low-fat diet, and restraint caused a significant reduction in fat content of restrained rats fed high-fat diet (diet: P < 0.0001, stress: P < 0.01, interaction: NS). Corticosterone measured during the last period of

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### Table 4. Body weights, food intakes, and serum insulin and corticosterone of rats fed low- and high-fat diets in experiment 4

<table>
<thead>
<tr>
<th></th>
<th>LF Control</th>
<th>LF Rest</th>
<th>HF Control</th>
<th>HF Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prestress weight, g</td>
<td>387±5</td>
<td>387±5</td>
<td>389±5</td>
<td>394±5</td>
</tr>
<tr>
<td>Poststress weight, g</td>
<td>244±8</td>
<td>239±5</td>
<td>383±4</td>
<td>388±5</td>
</tr>
<tr>
<td>Prestress intake, kcal/3 days</td>
<td>213±1</td>
<td>192±7</td>
<td>239±5</td>
<td>680±11b</td>
</tr>
<tr>
<td>Poststress intake, kcal/3 days</td>
<td>579±8b</td>
<td>538±16b</td>
<td>658±19b</td>
<td>538±16b</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.4±0.2</td>
<td>1.9±0.2</td>
<td>2.2±0.2</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>12±1</td>
<td>26±4</td>
<td>12±1</td>
<td>26±4</td>
</tr>
</tbody>
</table>

Values for rats killed on day 1 that do not share a common superscript are significantly different.
The mechanisms responsible for restraint-induced changes in food intake and body composition were not explored in detail in these experiments. The model can be considered to consist of three distinct components: the period of weight loss during stress, the period of normalized food intake but reduced body weight continuing for extended periods after the end of stress, and the period of normal food intake following restraint and reduced body weight continuing for extended periods after the end of stress. Changes in neurotransmitters during stress have been extensively investigated. Both CRF and a related neuropeptide, urocortin (UCN), inhibit food intake. The failure to induce a sustained suppression of food intake, and these may be the mechanisms responsible for the sustained, poststress inhibition of food intake.

The results from these experiments demonstrate that exposing adult rats to repeated restraint causes a temporary suppression of food intake and sustained reduction in body weight, compared with nonrestrained rats. The reduction in food intake could not be attributed to anhedonia because there was no effect of repeated restraint on preference for saccharin solution during the 7 days immediately following stress. The body-weight response is similar to that observed in rats that have received lesions of the lateral hypothalamus or in which inflammation has been induced by turpentine injection (16). The similarities between these models suggest that repeated restraint may provide a new model for investigating physiological and metabolic responses to trauma. The failure to induce a sustained reduction in body weight of young rats in exp 2 suggests either that the mechanisms mediating stress-associated weight loss in adult rats are immature in fast-growing animals, or that factors promoting rapid growth in young rats override any inhibitory effects induced by stress. This aspect of the model will only be elucidated once the mechanisms activated by stress in older rats have been identified.

Restraint caused a significant elevation in serum corticosterone concentration, which was back to control levels 120 min after the initiation of restraint, 60 min before the end of stress. There was a significant effect of diet on corticosterone concentration, which was back to control levels 120 min after the end of stress (diet: P < 0.009, interaction: NS) measured 5 days after the end of repeated restraint.

The mechanisms responsible for restraint-induced changes in food intake and body composition were not explored in detail in these experiments. The model can be considered to consist of three distinct components: the period of weight loss during stress, the period of reduced food intake following the end of stress, and the period of normalized food intake but reduced body weight continuing for extended periods after the end of stress. Changes in neurotransmitters during stress have been extensively investigated. Both CRF and a related neuropeptide, urocortin (UCN), inhibit food intake when infused centrally (30), and it has been demonstrated that the reduction in food intake that immediately follows a period of restraint can be partially prevented by the CRF receptor antagonist α-helical CRF (14). Activation of the CRF system by stress also results in increased peripheral corticosterone and sympathetic activity (13), release of cytokines (32), and activation of central catecholamine pathways (28); increased hypothalamic release of leutinizing hormone (3) and prolactin; and decreased release of growth hormone (18). Within the brain, stress increases serotonin turnover (25) and this may be responsible for some of the changes in hypothalamic hormone release (3). Serotonin, catecholamines, and cytokines have all been shown to suppress food intake, and these may be the mediators of the initial hypophagia of restrained rats. However, because we were unable to find elevations of serum corticosterone during the days following restraint, it is unlikely that CRF or UCN are directly responsible for the sustained, poststress inhibition of food intake.

In a previous study, we found that a large portion of the drop in 24-h food intake following exposure to a...
A single bout of restraint stress occurred at the start of the dark period, irrespective of the time of day that the rats were restrained (26). However, we were unable to correlate this change in food intake with central concentrations of either serotonin or catecholamines. In experiments described here, the stress-induced suppression of food intake was reversed within a week of the end of repeated restraint, but there was no indication of rebound hyperphagia. In contrast, animals that have lost weight due to food restriction overeat to compensate for the period of negative energy balance once food becomes available (10). This difference between restrained and food-restricted rats implies that stress downregulates feedback systems that normally defend body weight. The initial hypophagia of restrained rats accounted for only 46% of the difference in cumulative intake over the 40-day recovery period in exp 1; therefore, there must have been a sustained reduction in daily intake that was too small to be detected on a 24-h basis. This small, but maintained, reduction in intake may have been secondary to the smaller body size of restrained rats, rather than a specific suppression of food intake.

Carcass analysis indicated that the body composition of repeatedly restrained rats was dependent on the protein concentration of the diet. When dietary protein was diluted with fat in exp 4, the restrained rats gained fat but not lean tissue during the 5 days following restraint. Because there were only 4 days between the two time periods compared, the changes in body composition were small and need to be confirmed by measurements of protein turnover during the period following repeated restraint. When a purified diet was used in exp 5, the rats gained more fat during the baseline period before stress, and body fat accounted for 44% of the difference in carcass weight of control and restrained rats at the end of the experiment.

Fig. 5. A: serum corticosterone of control and restrained rats fed high-fat diet measured at the end of 3 h of restraint on each day of restraint in exp 4. Corticosterone was significantly higher in stressed rats compared with controls as indicated by different letters. Data are means ± SE for groups of 7 rats, and blood samples were collected by tail bleeding. B: serum corticosterone measured in blood samples collected by tail bleeding at intervals during 3 h of restraint in control and stressed rats of exp 5 on 3rd day of restraint. Values are means ± SE for groups of 5 rats fed high-protein, high-fat diet. *Corticosterone levels were significantly higher (P < 0.05) than at time 0, within either restrained or control treatment groups. Corticosterone concentrations in stressed rats returned to control levels by 120 min, 1 h before end of restraint.

Fig. 6. A: daily body weight of rats in exp 5. B: daily energy intake of control and stressed rats in exp 5. Food intake of all rats declined during period of stress. Arrows indicate days on which rats were restrained. *Significant difference (P < 0.05) between control and restrained rats.
Because of the differences in protein, carbohydrate, and fat sources in chow-based and purified high-fat diets, it is possible that all of these factors, rather than protein-to-fat ratio alone, contributed to the increased adiposity of rats fed high-fat purified diet compared with those fed the chow-based high-fat diet. However, because the amount of available energy was not restricted in any experiment but the restrained rats maintained a reduced body weight, these results suggest that repeated restraint results in a metabolic state that favors energy storage as fat, rather than protein accretion, and inhibits feedback signals that are normally active during, or following, periods of negative energy balance. These results are similar to those from other studies in which stress resulted in accretion of fat, and specifically abdominal fat (8, 17). It is possible that the relative enlargement of body fat stores was also responsible for the maintenance of a reduced body weight following the end of repeated restraint. Because body fat produces circulating feedback signals that regulate energy balance and control body weight (31), adipose tissue in restrained rats may provide erroneous signals of appropriate body composition, preventing both overeating and weight gain.

It is established that glucocorticoids inhibit protein synthesis (29), making corticosterone an obvious candidate for the mediator of change in body composition of restrained rats. Measurements of serum corticosterone during or after the repeated restraint in expts 4 and 5 support a role for this hormone in reducing lean tissue in stressed rats. In expt 4, when all of the weight loss was lean tissue, serum corticosterone remained elevated in restrained rats compared with controls at the end of each of the three periods of restraint and on the day after the end of restraint. In contrast, in expt 5, serum corticosterone of restrained rats fed the high-protein, high-fat diet was back to control levels before the end of the period of restraint, although all rats on the high-fat diet had higher corticosterone levels than those on the low-fat diet, consistent with previous reports that corticosterone is essential for selection of dietary fat (4, 5). In these rats, weight loss was distributed between both lean and fat tissue. Other circumstantial evidence that implicates involvement of corticosterone comes from a previous study in which we found that restraining rats in the morning had a greater effect on body weight than exposing them to the same stress at the end of the afternoon (26). Although we did not find differences in a single time-point measure of serum corticosterone 5 days after the end of stress, when body weight was still below that of controls, it is possible that exposure to repeated restraint disrupts the circadian pattern of glucocorticoid release and that this results in a resetting of the equilibrium that is maintained by the rats. This possibility is supported by observations that repeated exposure to restraint plus intermittent tail shock resulted in elevations of morning, but not evening, corticosterone in rats for 2 days after the end of stress (24). Alternatively, because stress also inhibits growth hormone (1) and promotes prolactin (2), it is possible that a change in the relative pattern of release of these hormones is responsible for the change in body weight and body composition. Others have demonstrated significant changes in body weight and lipid deposition in hamsters, mice, rats (7), and humans (22) in which the relationship between circulating concentrations of corticosterone and prolactin are modulated. Dallman et al. (8) have proposed that the promotion of fat storage by chronic stress results from a change in the relative ratios of corticosterone and prolactin. Further studies of the circadian pattern of release of insulin, corticosterone, growth hormone, and prolactin are needed to determine their importance in mediating the change in body weight of repeatedly restrained rats.

In conclusion, the results from these experiments show that repeated exposure of adult rats to a relatively mild stress results in a sustained reduction in body weight that may not be corrected for extended periods after the end of the stress. This change in level at which body weight is maintained is similar to that seen in rats that have experienced acute inflammation and may provide a new model for investigating the metabolic response to trauma. Further studies are needed to determine the importance of different aspects of the HPA axis in mediating the response and the importance of stress-induced changes in circadian release of hypothalamic and adrenal hormones.

### Perspectives

Repeated restraint is a moderate, mixed physical and psychological stressor that may provide a new model for investigating the mechanisms responsible for meta-
bolic aspects of accidental or surgical trauma. In hu-
mans it has been shown that weight loss and inhibition
of protein synthesis is proportional to the degree of
trauma (23). It is well established that stress or trauma
causes weight loss, but little attention has been paid to
changes in body weight during the recovery period.
In these studies we were able to manipulate the amount
of lean body mass lost, and recovered, by restrained rats
by changing the protein-to-calorie ratio of the diet.
Mechanisms responsible for weight loss were not elu-
dated, but, because repeated restraint did not cause
the same chronic reduction in body weight in young rats as
in older animals, it is possible that the mechanisms
inducing weight loss were either overridden by factors
that promote rapid growth or that the system that was
modulated by stress had not matured in the young
animals. Further studies are needed to determine
whether temporal changes in the release of adrenal and
hypothalamic hormones are responsible for the sus-
tained reduction in body weight or whether it results
from erroneous feedback signals originating in the
periphery due to the retention of body fat by stressed
rats.

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