Differential stress responsivity in diet-induced obese and resistant rats

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Levin, Barry E., Denis Richard, Chantal Michel, and Richard Servatius. Differential stress responsivity in diet-induced obese and resistant rats. Am J Physiol Regulatory Integrative Comp Physiol 279: R1357–R1364, 2000.—The relationship between stress and obesity was assessed in male rats selectively bred to develop either diet-induced obesity (DIO) or diet resistance (DR) when fed a high-energy, 31% fat diet for 3 wk followed by 2 wk on a hyperphagic liquid diet (Ensure). One-half of the rats of each phenotype were subjected to moderate daily, unpredictable stress (cage changing, exposure to conspecific, swim, and immobilization stress, intraperitoneal saline injection) during the 5 wk. Both stressed and unstressed DIO rats were 26% heavier and ate 27% more than comparable DR rats at onset and had 48% lower basal morning plasma corticosterone levels. Stressed DR rats gained less weight and had significant elevations of basal morning corticosterone but reduced basal sympathetic activity (24-h urine norepinephrine) over 5 wk compared with their unstressed DR controls. Terminally, there was a 35% increase in the paraventricular nucleus corticotropin-releasing hormone mRNA expression. On the other hand, stressed DIO rats showed only a transient early increase in open-field activity and a terminal increase in basal corticosterone levels as the only effects of stress. Thus DIO rats are hyporesponsive to chronic stress compared with DR rats. This is in keeping with several other known differences in hypothalamicpituitary and autonomic function in this model.

The use of animal models has not clarified this issue appreciably. Rodent obesity can be ameliorated by removal of the adrenal gland, and the obesity is reinstated with glucocorticoid replacement (4). This suggests that glucocorticoids, as part of the overall stress response, have an important role in the development of obesity. However, a variety of rodent models of obesity have shown quite variable responsiveness to stress (2, 9, 10, 23, 25, 28, 35). This suggests that stress itself may not be a primary factor in the development and maintenance of obesity.

Differential stress responsivity in diet-induced obese and resistant rats. Am J Physiol Regulatory Integrative Comp Physiol 279: R1357–R1364, 2000. The relationship between stress and obesity was assessed in male rats selectively bred to develop either diet-induced obesity (DIO) or diet resistance (DR) when fed a high-energy, 31% fat diet for 3 wk followed by 2 wk on a hyperphagic liquid diet (Ensure). One-half of the rats of each phenotype were subjected to moderate daily, unpredictable stress (cage changing, exposure to conspecific, swim, and immobilization stress, intraperitoneal saline injection) during the 5 wk. Both stressed and unstressed DIO rats were 26% heavier and ate 27% more than comparable DR rats at onset and had 48% lower basal morning plasma corticosterone levels. Stressed DR rats gained less weight and had significant elevations of basal morning corticosterone but reduced basal sympathetic activity (24-h urine norepinephrine) over 5 wk compared with their unstressed DR controls. Terminally, there was a 35% increase in the paraventricular nucleus corticotropin-releasing hormone mRNA expression. On the other hand, stressed DIO rats showed only a transient early increase in open-field activity and a terminal increase in basal corticosterone levels as the only effects of stress. Thus DIO rats are hyporesponsive to chronic stress compared with DR rats. This is in keeping with several other known differences in hypothalamic-pituitary and autonomic function in this model.

corticosterone; corticotropin-releasing hormone; norepinephrine; epinephrine; leptin; motor activity; paraventricular nucleus; sympathetic nervous system

There appears to be a relationship between elevated levels of cortisol and the presence of abdominal obesity and its comorbidities in humans (6, 29). Some obese individuals show enhanced responsivity to stress, although others may actually be hyporesponsive compared with lean individuals (26). Because of such conflicting data, the relationship of stress to obesity is less than clear. The use of animal models has not clarified this issue appreciably. Rodent obesity can be ameliorated by removal of the adrenal gland, and the obesity is reinstated with glucocorticoid replacement (4). This suggests that glucocorticoids, as part of the overall stress response, have an important role in the development and maintenance of obesity. However, a variety of rodent models of obesity have shown quite variable responsiveness to stress (2, 9, 10, 23, 25, 28, 35). This suggests that stress itself may not be a primary factor in the development and maintenance of obesity.

Diet-induced obesity (DIO) in the rat has proven useful as a model for human obesity. In this model, about one-half of the rats fed a diet moderately high in fat, sucrose, and energy content [high-energy (HE) diet (20)] become obese. The rest remain lean and are designated as diet resistance (DR). DIO rats share many of the characteristics of human obesity. As in much of human obesity, the DIO model appears to follow a polygenic mode of inheritance (19). DIO rats develop insulin resistance (19) and have abnormalities of centrally mediated sympathetic nervous system function (16). However, their responsiveness to chronic stress has not been evaluated. Here we examined the stress responsivity of DIO and DR rats using substrains, which were selectively bred to reproducibly express their given phenotype (19). A relatively high-density diet that does not cause persistent hyperphagia and a highly palatable one that does induce hyperphagia were used in sequence to evaluate the effect of stress on these two dietary conditions. 

METHODS

Animals and diet. Male rats from the F15 generation of our in-house colony of rats selectively bred to be DIO or DR were used at 2.5 mo of age. These substrains were derived from the outbred Sprague-Dawley strain (Charles River Laboratories) and were bred for high (DIO) and low (DR) weight gain on a HE diet (19). Rats were fed Purina rat chow (No. 5001) and water ad libitum from weaning and were housed on a 12:12-h light-dark schedule with lights out at 1800. At 2.5 mo of age, all rats were switched to a HE diet ad libitum for 1 wk and intake and body weight were measured. The HE diet is composed of 8% corn oil, 44% sweetened condensed milk, and 48% Purina rat chow (Research Diets). It contains 4.47 kcal/g and 21% of the metabolizable energy content as protein, 31% as fat, and 48% as carbohydrate, 50% of which is sucrose (20). Rats were then continued on this diet for an additional 3 wk. At that time, they were given continuous access to HE diet

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plus additional access to a highly palatable liquid diet (Ensure, Ross Products) for an additional 2 wk. Ensure contains 1.06 kcal/ml with 14% of the metabolizable energy content as protein, 22% as fat, and 64% as carbohydrate.

Experimental protocol. After the first baseline week on HE diet, 12 DIO and 12 DR rats were randomized by weight into control (n = 6) and stressed (n = 6) groups, respectively. The stress protocol was designed to present moderate, random, chronic stress daily over a 5-wk period (7). Stressors included
1) restraint for 15 min in a plastic cone open at the end to allow breathing, 2) moving the animal to the cage of another without cleaning the contents, 3) exposure to another conspecific male rat placed in the home cage of the experimental rat for 10 min, 4) 2-min swim stress in a bucket of water at room temperature, which was deep enough that the animals could not touch bottom, and 5) saline injection (1 ml of 0.9% ip). These stresses were presented in a randomized, unpredictable order in which the time of administration during the day was also varied. In addition, several repetitive tests were carried out in both stressed and nonstressed control animals. These included weekly 1) open-field testing for 10 min/session beginning at 0900 in a 40 × 40 × 30.5 cm 16-beam Digiscan activity monitor (Omnitech) (15), 2) weighing, 3) blood sampling by tail nicking for plasma leptin and corticosterone levels (~150 µl), and 4) placement in metabolic cages for collection of 24-h urine for norepinephrine and epinephrine levels at the end of 3 wk of stress on HE diet and the end of 2 wk on HE diet plus Ensure. Terminally, rats were killed by decapitation. Trunk blood was collected, fat pads and livers were weighed, and brains were removed and frozen on dry ice.

Assays. Samples of tail blood were collected into heparinized tubes, and the plasma was removed for radioimmunoassays. Samples for corticosterone were collected at 0800 (2 h after lights on) except for the last week of testing when an additional sample was collected at 1700 (1 h before lights off). Corticosterone was assayed using a double-antibody radioimmunoassay kit (ICN Biomedicals). Leptin was measured by radioimmunoassay (Linco) using authentic rat leptin as a standard. Urine norepinephrine and epinephrine were assayed by HPLC with electrochemical detection (16).

In situ hybridization for corticotropin-releasing hormone. The brains were prepared as previously described (13). Briefly, rats were anesthetized with 1.5 ml of a mixture containing 20 mg/ml of ketamine and 2.5 mg/ml of xylazine. They were perfused intracardially with 30 ml of ice-cold isotonic saline followed by 200 ml of a 4% paraformaldehyde. The brains were removed at the end of perfusion and kept in paraformaldehyde for an additional 7 days. They were then transferred to a solution containing paraformaldehyde and sucrose (10%) before being cut 12 h later using a sliding microtome (Histoslide 2000, Reichert-Jung). Brain sections (30 μm) were collected and stored at ~30°C in a cold sterile cryoprotectant solution containing sodium phosphate buffer (50 mM), ethylene glycol (30%), and glycerol (20%). One of every five brain sections was mounted onto poly-l-lysine-coated slides and allowed to desiccate overnight under vacuum. The sections were then successively fixed for 20 min in paraformaldehyde (4%), digested for 30 min at 37°C with protease K (10 μg/ml in 100 mM Tris-HCl containing 50 mM EDTA, pH 8.0), acetylated with acetic anhydride (0.25% in 0.1 M triethanolamine, pH 8.0), and dehydrated through graded concentrations of alcohol. After vacuum drying for at least 2 h, 90 ml of the hybridization mixture, which contains an antisense 35S-labeled cRNA probe (107 counts/min per ml), were spotted on each slide. The slides were sealed under a coverslip and incubated overnight at 60°C in a slide warmer. The next day, the coverslips were removed and the slides were rinsed four times with 4× saline-sodium citrate (SSC; 20× stock solution: 3 M NaCl, 0.3 M trisodium citrate buffer, pH 7.0), digested for 30 min at 37°C with RNase A (20 µg/ml in 10 mM Tris-500 mM NaCl containing 1 mM EDTA), washed in descending concentrations of SSC (2 × 10 min, 1 × 5 min, 0.5 × 5 min, 0.1 × 30 min at 60°C), and dehydrated through graded concentrations of alcohol. After a 2-h period of vacuum drying, the slides were exposed on an X-ray film (Kodak) for 24 h. Once removed from the autoradiography cassettes, the slides were defatted in xylene and dipped in NTB-2 nuclear emulsion (Eastman Kodak, Rochester, NY). The slides were exposed for 7 min before being developed in D19 developer (Kodak) for 3.5 min at 14–15°C and fixed in rapid fixer (Kodak) for 5 min. Finally, tissues were rinsed in running distilled water for 1–2 h, counterstained with thionin (0.25%), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX (Fluka). The corticotropin-releasing factor (CRF) cRNA probe was generated from the EcoR I fragment of a rat CRF cDNA (Dr. K. Mayo, Northwestern University, Evanston, IL), subcloned into pBluescript SK-1 (Stratagene, La Jolla, CA), and linearized with Hind III (Pharmacia). A radioactive sense cRNA copy also was prepared to verify the specificity of the antisense probe. The hybridization signals revealed on NTB-2-dipped nuclear emulsion slides were analyzed and quantified under a light microscope (Olympus, BX50) equipped with a black and white video camera (Sony, XC-77) coupled to a Macintosh computer (Power PC 7100/66) using Image software (version 1.55 nonFPU, Wayne Rasband, National Institutes of Health, Bethesda, MD). The optical density for the hybridization signal was measured under dark-field illumination at a magnification of ×25. Brain sections from the different groups of rats were matched for rostrocaudal levels as closely as possible. The optical density for each specific region was corrected for the average background signal, which was determined by sampling unlabeled areas outside of the areas of interest. Brain sections were analyzed for semiquantification of optical density.

Statistics. Body weights, food intake, and tail blood corticosterone and leptin levels were measured repeatedly over the entire period. These data were subjected to ANOVA for repeated measures initially to compare intergroup differences. When significant intergroup differences were found (P < 0.05), post hoc Scheffé multiple-comparison tests were carried out at each time point where significant differences across a given phase occurred. Data were analyzed both across the entire 5-wk stress protocol and separately across the 3 wk on HE diet and 2 wk on HE diet plus Ensure. All terminal measures and the two sets of urine catecholamine assays also were prepared to verify the specificity of the antisense probe. The hybridization signals revealed on NTB-2-dipped nuclear emulsion slides were analyzed and quantified under a light microscope (Olympus, BX50) equipped with a black and white video camera (Sony, XC-77) coupled to a Macintosh computer (Power PC 7100/66) using Image software (version 1.55 nonFPU, Wayne Rasband, National Institutes of Health, Bethesda, MD). The optical density for the hybridization signal was measured under dark-field illumination at a magnification of ×25. Brain sections from the different groups of rats were matched for rostrocaudal levels as closely as possible. The optical density for each specific region was corrected for the average background signal, which was determined by sampling unlabeled areas outside of the areas of interest. Brain sections were analyzed for semiquantification of optical density.

RESULTS

Morphometric data. Initially, the DIO rats weighed 26% more and consumed 27% more calories of HE diet than the age-matched DR rats (Table 1). DIO rats also ate more than DR rats over the 3 wk on HE diet, regardless of whether they were stressed [F(1,20) = 11.13; P = 0.003]. This was associated with 28% higher cumulative weight gain [F(1,19) = 41.4; P = 0.001] and 28% higher feed efficiency during this time [F(1,19) = 9.37; P = 0.001]. Over these 3 wk, there was a signif-
significant difference in weight gain between the two phenotypes with regard to their weight gain responses to stress \[F(1,19) = 6.70; P = 0.001\]. Stressed DR rats progressively gained less body weight and weighed 27% less than their unstressed controls by the third week (Fig. 1, Table 1). However, this reduced weight gain was not associated with any decline in intake (Fig. 2). Given their reduced weight gain over this period, stressed DR rats had lower feed efficiency over the entire 3 wk by repeated measures \[F(1,19) = 5.36; P = 0.032\] but cumulatively this did not quite reach statistical significance compared with their unstressed controls. Although there was a tendency for the stressed DIO rats to gain less weight over the 3 wk on HE diet, this did not reach statistical significance compared with their unstressed controls. Nor did their food intake or feed efficiency differ from controls.

Addition of Ensure to the HE diet tended to minimize differences between stressed and unstressed DR rats. Over the 2 wk on this diet combination, all DR and DIO rats increased their food intake. However, this was most marked in the DR rats to the extent that their total intake rose to the same level as DIO rats (Fig. 2, Table 1). Despite this, DR rats still gained 22% less weight than DIO rats, although their feed efficiency was similar (Figs. 1 and 2, Table 1). Addition of Ensure slightly increased the rate of weight gain in stressed DR rats so that their terminal body weights did not differ significantly from

### Table 1. Body weight, food intake, and food efficiency in DR and DIO rats subjected to stress

<table>
<thead>
<tr>
<th></th>
<th>DR-C</th>
<th>DR-S</th>
<th>DIO-C</th>
<th>DIO-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, initial</td>
<td>313 ± 10*</td>
<td>308 ± 12*</td>
<td>395 ± 12†</td>
<td>390 ± 11†</td>
</tr>
<tr>
<td>BW, final</td>
<td>418 ± 12*</td>
<td>395 ± 10*</td>
<td>529 ± 16†</td>
<td>520 ± 10†</td>
</tr>
<tr>
<td>BW gain, HE</td>
<td>41.3 ± 4.2*</td>
<td>34.0 ± 3.3†</td>
<td>74.3 ± 5.4‡</td>
<td>67.5 ± 4.5‡</td>
</tr>
<tr>
<td>BW gain, Ensure</td>
<td>53.2 ± 4.2*</td>
<td>87.3 ± 3.6§</td>
<td>134 ± 4‡</td>
<td>63.0 ± 3.3†</td>
</tr>
<tr>
<td>BW gain, total</td>
<td>105 ± 6*</td>
<td>87.3 ± 3.6§</td>
<td>134 ± 4‡</td>
<td>63.0 ± 3.3†</td>
</tr>
<tr>
<td>FI, initial (on HE) (kcal/d)</td>
<td>65.7 ± 7.3*</td>
<td>63.7 ± 5.4*</td>
<td>85.3 ± 3.5†</td>
<td>79.2 ± 2.5†</td>
</tr>
<tr>
<td>FI, HE</td>
<td>4,772 ± 209*</td>
<td>4,686 ± 168*</td>
<td>6,107 ± 233†</td>
<td>6,103 ± 346†</td>
</tr>
<tr>
<td>FI, Ensure</td>
<td>3,136 ± 208*</td>
<td>3,524 ± 296*</td>
<td>3,550 ± 169†</td>
<td>3,445 ± 149*</td>
</tr>
<tr>
<td>FI, total</td>
<td>7,909 ± 206*</td>
<td>8,210 ± 387†</td>
<td>9,657 ± 293†</td>
<td>9,548 ± 384†</td>
</tr>
<tr>
<td>FE, HE × 10³</td>
<td>10.1 ± 0.7§*</td>
<td>8.13 ± 0.76†</td>
<td>12.1 ± 0.9*</td>
<td>11.2 ± 0.8*</td>
</tr>
<tr>
<td>FE, Ensure × 10³</td>
<td>16.1 ± 1.9*</td>
<td>14.2 ± 0.8*</td>
<td>17.3 ± 1.2†</td>
<td>19.2 ± 1.1*</td>
</tr>
<tr>
<td>FE, total × 10³</td>
<td>8.1 ± 0.3†</td>
<td>7.2 ± 0.2†</td>
<td>10.2 ± 0.1*</td>
<td>9.1 ± 1.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Groups of diet-induced obese (DIO) and diet-resistant (DR) rats were held under control conditions (C, n = 6/phenotype) or stressed repeatedly for 5 wk (S, n = 6/phenotype). They were all kept on high energy (HE) diet for the first 3 wk and then switched to Ensure with or without HE diet for the last 2 wk. BW, body wt (g); FI, food intake (kcal); FE, food efficiency [body wt gained (g)/food intake (kcal)]. Values with different symbols differ by P ≤ 0.05 or less by post hoc test after intergroup differences were found by ANOVA.
unstressed controls. However, their cumulative weight gain over the entire 5-wk period was still 15% lower than DR controls (Fig. 1, Table 1). There were differences in neither energy intake nor feed efficiency over either the 2 wk on Ensure or over the entire 5-wk period between stressed and unstressed DR rats. Ensure intake had no effect on weight gain, intake, or feed efficiency in stressed DIO rats. Nor did these measures differ over the entire 5-wk period from unstressed DIO rats.

Stressed DIO rats had higher morning levels than DIO rats. Although stress had little effect on plasma corticosterone levels in DIO rats, stressed DR rats had significantly higher levels (Fig. 5). Under basal conditions, DR rats had 168% higher morning levels than DIO rats. Stress had no effect on plasma corticosterone levels in DIO rats. Nor did these measures differ over the entire 5-wk period between stressed and unstressed rats. Ensure intake had no effect on weight gain, intake, or feed efficiency in stressed DIO rats. Nor did these measures differ over the entire 5-wk period from unstressed DIO rats.

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Table 2. Final fat pad and liver weights after repeated stress in DR and DIO rats

<table>
<thead>
<tr>
<th></th>
<th>DR-C</th>
<th>DR-S</th>
<th>DIO-C</th>
<th>DIO-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mes, g</td>
<td>6.98 ± 0.92*</td>
<td>8.08 ± 1.92*</td>
<td>13.9 ± 2.4†</td>
<td>15.0 ± 1.8†</td>
</tr>
<tr>
<td>RP, g</td>
<td>7.23 ± 0.83*</td>
<td>8.02 ± 0.17*</td>
<td>13.5 ± 2.2†</td>
<td>15.0 ± 0.9†</td>
</tr>
<tr>
<td>PR, g</td>
<td>1.08 ± 0.11*</td>
<td>0.75 ± 0.17*</td>
<td>1.88 ± 0.48†</td>
<td>1.91 ± 0.32†</td>
</tr>
<tr>
<td>Ing, g</td>
<td>8.53 ± 0.79*</td>
<td>8.10 ± 1.17*</td>
<td>12.9 ± 2.6†</td>
<td>14.3 ± 1.6†</td>
</tr>
<tr>
<td>Total, g</td>
<td>23.8 ± 2.5*</td>
<td>25.0 ± 4.9*</td>
<td>42.3 ± 7.7†</td>
<td>46.1 ± 4.3†</td>
</tr>
<tr>
<td>Total FP/BWf</td>
<td>0.057 ± 0.005*</td>
<td>0.063 ± 0.013*</td>
<td>0.083 ± 0.014†</td>
<td>0.087 ± 0.009†</td>
</tr>
<tr>
<td>Liver, g</td>
<td>18.2 ± 0.78*</td>
<td>18.3 ± 1.5*</td>
<td>21.3 ± 1.8*</td>
<td>17.8 ± 0.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Groups of DIO and DR rats were held under control conditions (n = 6/phenotype) or stressed repeatedly for 5 wk (n = 6/phenotype). They were all kept on HE diet for the first 3 wk and then switched to Ensure with HE diet for the last 2 wk. BWf, final body weight; Mes, mesenteric pad; RP, retroperitoneal pad; PR, perirenal pad; Ing, inguinal pad; Total, total weights of Mes, RP, PR, and Ing pads; Total FP/BWf, ratio of total fat pad weight to final body weight. Values with differing symbols differ by P < 0.05 or less by post hoc test after intergroup differences were found by ANOVA. By 2-way ANOVA, there were significant phenotype effects for BWf (1, 20) = 83.4, P < 0.0001, Mes (1, 20) = 14.1, P = 0.001, RP (1, 20) = 19.1, P = 0.001, PR (1, 20) = 9.92, P = 0.005, Ing (1, 20) = 9.75, P = 0.005, Total (1, 20) = 14.5, P = 0.001, Total FP/BWf (1, 20) = 5.35, P = 0.032. There were no significant treatment (stress) effects for any parameter.

Hormonal changes. There were no differences in urine norepinephrine levels between the DIO and DR phenotypes as a whole during HE diet or HE diet plus Ensure intake when assessed across all 5 wk for both stressed and unstressed animals (Table 3). However, there was a specific stress effect seen in DR rats. Stressed DR rats had 25% lower urine norepinephrine levels during the third week on HE diet and 18% lower levels during the second week on HE diet plus Ensure than their unstressed controls (Table 3). Neither stress nor phenotype had a significant effect on urine epinephrine levels during either of these periods. There were, however, major effects of phenotype and stress on plasma corticosterone levels (Fig. 5). Under basal conditions, DR rats had 168% higher morning levels than DIO rats. Although stress had little effect on plasma corticosterone levels in DIO rats, stressed DR rats had significantly higher levels than their respective unstressed controls over the entire 5-wk period, regardless of diet (F(1,5) = 16.55; P = 0.001). Over this period, corticosterone levels also were higher in stressed than nonstressed animals regardless of diet (F(1,15) = 8.02; P = 0.008).
of phenotype \( F(1,15) = 8.21; P = 0.012 \). This was primarily due to the major effect of stress on DR rats because stress had little effect on plasma corticosterone levels in DIO rats. The only exception to this was seen during the last week of stress when both stressed DR and DIO rats had significantly higher corticosterone levels than their respective controls in both morning and evening samples (Table 4). However, overall DR rats still had higher corticosterone levels than their respective DIO rats during the fifth week.

**Brain CRF mRNA expression.** In keeping with their higher levels of plasma corticosterone, DR rats had higher levels than DIO rats of corticotropin-releasing hormone (CRH) mRNA expression overall in their hypothalamic paraventricular nucleus (PVN) terminally \( F(1,19) = 7.49; P = 0.014 \). Also, stressed DR rats had 35% higher levels than their respective unstressed controls.

**DISCUSSION**

Rats selectively bred to express either the DIO or DR phenotypes reacted very differently to moderate chronic daily, unpredictable stress when fed diets of Table 3. Levels of 24-h urine NE and Epi collected during last week on HE diet (wk 3) and last week on HE plus Ensure (wk 5)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>24-h Urine NE, ( \mu g )</th>
<th>24-h Urine Epi, ( \mu g )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HE Diet</td>
<td>HE + Ensure</td>
</tr>
<tr>
<td>DR control</td>
<td>6</td>
<td>1.47 ± 0.18*</td>
<td>1.40 ± 0.09*</td>
</tr>
<tr>
<td>DR stress</td>
<td>6</td>
<td>1.11 ± 0.12†</td>
<td>1.16 ± 0.08†</td>
</tr>
<tr>
<td>DIO control</td>
<td>6</td>
<td>1.22 ± 0.09**†</td>
<td>1.42 ± 0.07**†</td>
</tr>
<tr>
<td>DIO stress</td>
<td>6</td>
<td>1.39 ± 0.12***†</td>
<td>1.30 ± 0.21***†</td>
</tr>
</tbody>
</table>

Values are means ± SE. NE, norepinephrine; Epi, epinephrine. Means with dissimilar symbols within a given time period differ by \( P < 0.05 \) or less by Scheffé's test.
Fig. 5. Effect of chronic stress on plasma morning corticosterone levels during 3 wk of HE diet followed by 2 wk on HE diet plus Ensure. Groups are as in Fig. 1. Data are means ± SE. Stressed and unstressed DR rats had higher plasma corticosterone levels over the entire 5-wk period than respective DIO rats [*F(1,15) = 16.55; P = 0.001]. *P < 0.05 or less when stressed DR rats were compared with all other groups by t-test after repeated-measures ANOVA showed significant intergroup differences.

Table 4. Plasma corticosterone level under nonstressed (basal) and after 5 wk of chronic stress conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>AM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR control</td>
<td>6</td>
<td>2.51 ± 1.40*</td>
<td>3.05 ± 1.29*</td>
</tr>
<tr>
<td>DR stress</td>
<td>6</td>
<td>3.11 ± 0.82*</td>
<td>7.81 ± 1.96†</td>
</tr>
<tr>
<td>DIO control</td>
<td>6</td>
<td>0.72 ± 0.30*</td>
<td>1.71 ± 0.31†</td>
</tr>
<tr>
<td>DIO stress</td>
<td>6</td>
<td>0.74 ± 0.12*</td>
<td>4.13 ± 1.05*</td>
</tr>
</tbody>
</table>

Values are means ± SE in μg/dl. AM, morning; PM, evening. Means with dissimilar symbols within a given time period differ by *P < 0.05 or less by Bonferroni test.

Fig. 6. Effect of chronic stress on the expression of corticotropin-releasing hormone (CRH) mRNA in the paraventricular nucleus (PVN) after 3 wk of HE diet followed by 2 wk on HE diet plus Ensure. Groups are as in Fig. 1. Data are means ± SE. Bars with differing letters differ from each other at P ≤ 0.05 by post hoc t-test after 2-way ANOVA showed there to be significant intergroup differences.

Relatively high HE density and/or palatability. DIO rats were little affected by the stress, whereas DR rats had the expected response to acute stress but then failed to become tolerant to the stress. Stressed DR rats gained less weight but had no decrease in energy intake and actually decreased their sympathetic nervous system activity. Reduced weight gain in stressed DR rats was associated with elevated basal plasma corticosterone levels during nonstress periods, suggesting a state of chronic, ongoing stress. The origin for this chronic stress state was suggested by the increased expression of CRH mRNA in the PVN. Because elevated plasma corticosterone levels normally provide a negative feedback on PVN CRH expression (37), it is likely that the CRH overexpression per se was the origin of the elevated plasma levels. On the other hand, stressed DR rats showed no indication of adrenomedullary activation (24-h urine epinephrine) and their sympathetic activity (24-h urine norepinephrine levels) was actually decreased during their third and fifth weeks of stress exposure. Finally, the reduced weight gain associated with this chronic stress state in DR rats was not associated with reduced adipose depot weights terminally. Nor were there depot-selective differences in adipose weights as has been reported in stressed outbred rats (27). The lack of effect on terminal body weight and adipose mass was probably due to the fact that stressed DR rats made up much of their lost body weight gain during the final 2 wk on Ensure diet. Despite this relative recovery of body weight, the persistent elevation of plasma corticosterone and PVN CRH mRNA expression suggests that DR rats might be especially stress sensitive.

Whereas stress induces weight loss, this is often (12, 22) but not always (24) associated with reduced food intake. Intraventricular administration of a CRH antagonist blocks stress-induced hypophagia and weight loss, suggesting a role for central CRH pathways (34). Given the elevated PVN CRH expression in our stressed DR rats, it is unclear why they never became hypophagic or exactly what was responsible for their reduced weight gain. Despite the lowered sympathetic activity, it is likely that they had either increased resting and/or activity-derived thermogenesis. The lack of increased activity in an open-field setting does not necessarily rule out such an increase in activity in the home cage. This is particularly true here as open-field activity was assessed during daylight hours and over only a short period of time (10 min). Furthermore, DIO rats have lower activity levels when assessed during the dark phase (15) as was seen for the DIO controls vs. DIO-stressed rats in this study during a short period in the light. Thus an increase in resting and/or diet-induced thermogenesis seems a more likely consequence of their chronic stress state in DR rats. This is merely speculation because no measure of thermogenesis was made here.

Nor is it clear why stressed DR rats failed to habituate to the relatively mild stress paradigm used. The degree to which body weight, sympathoadrenal and hypothalamicpituitary responses habituate to chronic
stress in rats appears to be highly dependent on the severity and timing of the stressors employed (5, 12, 22). However, the response to acute stress often habituates on repeated presentation (5, 7, 12, 33), especially if the stressor is escapable or predictable. However, the stressors used here were neither escapable nor predictable and only ongoing; basal stress responses were examined rather than the direct, proximate response of animals to an acute stressor. Whereas the basal response in DR rats failed to habituate to chronic stress, DIO rats showed virtually no response to the same paradigm. Prior studies have suggested that overfed (2) or high-fat-fed outbred rats (25) show enhanced direct responses to acute stressors. Similarly, genetically obese (fa/la) Zucker rats generally overrespond to acute stressors (2, 28, 35), although obese (cp/cp) JCR:LA corpulent rats show normal responsiveness (23). Finally, the ob/ob mouse has shown both high (10) and low (9) stress responsiveness. Again, the current studies measured only the basal, interstressor responses and not the direct, acute response to the stressor. Thus there are two interesting features of the current studies. First, it is unusual for rats to show an increase in interstressor corticosterone levels, especially when such mild stressors are used (32). Second, there was a pronounced differential response between DIO and DR rats to chronic stress. Although DR rats may be hyperresponsive to chronic stress, DIO rats may actually be hyporesponsive based on a reduced central reactivity.

In fact, DIO rats do show several abnormalities of central nervous system function before they become obese (14, 17, 18, 21), although many of these are normalized after they become fully obese (17, 21, 36). Several of these abnormalities involve systems such as norepinephrine (11), serotonin (3), and neuropeptide Y (8), which play an important role in the regulation of PVN CRH expression. The CRH neurons in the parvocellular PVN are among the most important participants in the stress response. They project to both autonomic (30) and hypothalamopituitary outflow pathways (31). During normal physiological function, adrenal corticoids inhibit PVN CRH production by a negative feedback loop (37). However, stress increases PVN CRH expression (33), leading to increased ACTH and corticosterone release (1). Interestingly, leptin, which is elevated in DIO rats (18), blocks the stress-induced release of ACTH and corticosterone (9). This could be partly responsible for the lack of stress response in DIO rats. Thus the general lack of a stress response in DIO rats might represent another example of the way in which full realization of genetic potential through environmental input produces a new, stable equilibrium for the defense of energy homeostasis at the elevated levels seen in obesity. However, the current results do not support the hypothesis that stress is a primary factor in the development of obesity.

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