Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA

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Solano, Joel M., and Lauren Jacobson. Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E708–E716, 1999.—Glucocorticoid stimulation of appetite and leptin expression conflicts with leptin inhibition of food intake and suggests that glucocorticoids reduce sensitivity to leptin. To determine if glucocorticoids impair feeding and metabolic responses to leptin, we measured leptin-induced changes in food intake, body weight, hormones, carcass fat, and hypothalamic neuropeptide Y (NPY) mRNA in adrenalectomized mice with and without corticosterone replacement. Leptin infusion (0.5 µg/h) significantly decreased food intake and body weight in adrenalectomized mice. Corticosterone replacement approximating normal 24-h mean levels restored food intake but did not permit weight gain equivalent to PBS-infused controls. Corticosterone levels comparable to stress-induced production completely reversed leptin-induced reductions in weight gain and body fat, despite significant attenuation by leptin of corticosterone-induced increases in plasma insulin levels. Glucocorticoid replacement increased food intake without reversing leptin inhibition of hypothalamic NPY mRNA levels. We conclude that glucocorticoid levels within the physiological range can interfere with leptin action and that glucocorticoid effects are at least partly independent of NPY.

adrenal cortex; feeding behavior; obesity; body composition; metabolism

There is considerable evidence that glucocorticoids stimulate both food intake and leptin production. Glucocorticoid secretion is linked with meal times in normal humans and rodents (8, 38), and increased appetite is associated with glucocorticoid excess induced by Cushing’s disease or glucocorticoid administration to normal volunteers (30, 37). Glucocorticoids stimulate leptin gene expression and secretion independently of effects on food intake, although increases in insulin or lipogenesis associated with food intake may contribute to leptin production (6, 13, 27, 36).

The dual stimulation of appetite and leptin by glucocorticoids seems paradoxical in light of the ability of leptin to inhibit food intake (11). We (19) and others (31) have not observed appetite inhibition by sustained, glucocorticoid-induced elevations in plasma leptin in normal animals or humans. Moreover, in Cushing’s syndrome, a primary disorder of glucocorticoid production, increased appetite is frequently reported despite increased leptin levels (24, 30).

These observations suggest that glucocorticoids might contribute to obesity by reducing the sensitivity of appetite to leptin regulation. Exaggerated adrenocortical axis activity has been reported in several (22, 33, 40), although not all (18, 23, 26), populations of obese rodents and humans. In rodents with hypothalamic lesions or mutations in either the leptin or leptin receptor genes, hyperphagia and weight gain have been shown to be largely glucocorticoid dependent (40).

In light of the relative scarcity of leptin or leptin receptor mutations in overweight humans, leptin resistance has been assumed to be a major cause of clinical obesity (11). Although elevated glucocorticoids in obesity could result from resistance to the proposed inhibitory effects of leptin on glucocorticoid secretion and glucocorticoid-induced food intake (6, 15), it is also possible that glucocorticoids are a cause as well as an effect of leptin resistance. We have focused on the apparent interference of glucocorticoids with leptin action as a model to understand mechanisms possibly contributing to leptin resistance. To test the hypothesis that glucocorticoids impair leptin action, we have measured leptin-induced changes in food intake, body weight, and body fat content in adrenalectomized (ADX) C57BL/6 mice with and without corticosterone replacement. Because glucocorticoids and leptin affect insulin sensitivity (4, 30, 34), which, in turn, may influence appetite and metabolic regulation (8, 39), we have also examined the effects of combined hormone treatment on plasma insulin levels. We have previously shown that the negative relationship between food intake and endogenous leptin depends more on glucocorticoids than on the putative anorexigen, corticotropin-releasing hormone (CRH; 19). To investigate other potential mediators of glucocorticoid effects, we have further measured hypothalamic expression of the potent appetite stimulant neuropeptide Y (NPY), because NPY has been shown to be a part of the central leptin-sensing pathway and to exhibit positive regulation by glucocorticoids (10, 38, 39).

METHODS

Animals. All experimental procedures were approved by the Children’s Hospital Institutional Animal Care and Use Committee. Male C57BL/6 mice were 2-4 mo old (25-30 g) at the time of use. Mice were individually housed in standard Plexiglas shoebox cages with bedding and given conventional pelleted rodent chow to eat (RMH 3000, Agway, Syracuse, NY). Food intake was measured daily as the change in weight, to the nearest 0.01 g, of a premeasured amount of chow pellets placed in the food holder. In preliminary experiments, we determined that mouse food consumption was sufficiently low relative to the size of the chow pellets, that spillage was minimal, and that accurate and consistent food

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intake measurement could be made with this approach. Food intake and body weight were measured in the morning within 2 h of the same time each day. Daily food intake measurements were normalized to body weight for that day; normalization to preadrenalectomy body weight gave comparable results.

All mice were adrenalectomized via a dorsal approach under 2.5% tribromoethanol anesthesia (19) and allowed to recover for at least 3 days before leptin treatment. To minimize compensatory endocrine and metabolic changes associated with surgical removal of glucocorticoids (20), mice were given 10 µg/ml corticosterone in 0.2% ethanol and 0.9% saline to drink after adrenal surgery. At the time of corticosterone pellet implantation surgery in each experiment, all mice were switched to drinking steroid-free saline. Recombinant murine leptin (generous gift of Dr. Frank Collins, Amgen, Thousand Oaks, CA) or sterile phosphate-buffered saline (PBS) vehicle (GIBCO, Gaithersburg, MD) was delivered by osmotic minipumps (Alza, Palo Alto, CA) implanted subcutaneously under ether anesthesia.

Experiments. In experiment 1, ADX mice were simultaneously implanted subcutaneously with 1) a minipump delivering leptin (0.5 µg/h) or PBS and 2) either no corticosterone (ADX) or subcutaneous 40-µg pellets containing either 10% (ADX + 10% corticosterone) or 50% (ADX + 50% corticosterone) corticosterone-cholesterol by weight (19). At the time of minipump and corticosterone pellet implantation, all mice were switched to drinking steroid-free saline. Food intake and body weight were monitored daily. Mice were killed in the morning after 4 days of combined hormone treatment for determination of plasma hormones and carcass composition.

In experiment 2, ADX mice were first infused with 0.5 µg/h leptin or PBS for 4 days while being maintained on 10 µg/ml corticosterone in the drinking fluid. Mice were then given either sham surgery or a 50% corticosterone subcutaneous pellet under ether anesthesia and were all switched to drinking steroid-free saline. Food intake and body weight were monitored while infusions continued for an additional 2 days after pellet implantation; plasma and hypothalami were collected when mice were killed on the morning of day 2.

Northern analysis. Hypothalami were dissected from coronal brain slices extending from the optic chiasm to the mammillary bodies. Hypothalamic blocks bounded laterally by the optic tract and extending dorsally to the depth of the anterior commissure were removed from this slice, snap-frozen in liquid nitrogen, and stored at −80°C until RNA isolation. Total RNA was prepared, blotted, and hybridized with cRNA probes (60°C) as previously described (21). NPY mRNA was detected by hybridization with a cRNA probe to the 486-bp Fsp I-Eco R I fragment of plasmid pBLNPY-1 (16), kindly provided by Dr. Steven Sabol (National Heart, Lung, and Blood Institute, Bethesda, MD). The RNA detected by this probe exhibits the size by Northern blot and the brain distribution by preliminary in situ hybridization studies expected for NPY (J acobson, unpublished observations). The β-actin plasmid has been previously described (21). Band intensities were quantitated on a Phosphorimager (Molecular Dynamics, Sunnyvale, CA) after sequential hybridization and exposure for NPY and β-actin; the NPY signal was normalized to that of β-actin to control for variations in loading.

Assays. Plasma corticosterone, leptin, and insulin were measured with commercial kits as previously described (19). Plasma glucose was assayed with Trinder reagent according to the instructions of the manufacturer (Sigma, St. Louis, MO). Body composition analysis (14) was performed on decapitated carcasses after the removal of minipumps and corticosterone pellets. Carcasses were first dried to constant weight at 65°C. Body water was calculated as the percent difference between wet and dry carcass weight. After drying, carcasses were subjected to hydrolysis and saponification at 60°C in 40 ml of 2:1 ethanol-30% potassium hydroxide and then diluted to 100 ml with 50% ethanol. Protein content was assayed with biuret reagent with BSA as a standard. Triglyceride content was assayed by enzymatic conversion to glycerol with a commercial kit (GPO-Trinder, Sigma) according to the instructions of the manufacturer.

Statistics. Data were analyzed by two-way ANOVA (Super-ANOVA, Abacus Concepts, Berkeley, CA) for leptin and corticosterone treatment effects, with post hoc testing by t-test with Bonferroni correction. Significance was defined in experiment 1 at P < 0.0167 to account for three planned comparisons between groups: 1) leptin vs. PBS at the same corticosterone replacement level, 2) ADX vs. ADX + 10% corticosterone, and 3) ADX vs. ADX + 50% corticosterone at the same leptin level. In experiment 2, significance was set at P < 0.0167 for three comparisons in the analysis of food intake: 1) leptin vs. PBS at the same corticosterone replacement level, 2) 50% vs. no corticosterone pellet replacement at the same leptin level, and 3) intake vs. 0 time levels in the same mice. For all other endpoints measured in experiment 2, significance was defined at P < 0.025 to account for comparisons between 1) leptin and PBS at the same corticosterone replacement level and 2) ADX mice with or without a 50% corticosterone pellet at the same leptin level. The relationships between food intake or body fat content and plasma leptin were evaluated by linear regression of the raw or log-transformed data, respectively (Statview, SAS Institute, Cary, NC). Data are reported throughout as means ± SE. Where no error bars are visible in graphs, the scale of the error was less than that of the symbol.

RESULTS

Experiment 1: effects of simultaneous manipulation of leptin and glucocorticoids. To test the hypothesis that glucocorticoids impair responses to leptin, we treated ADX mice with one of three levels of corticosterone while simultaneously infusing either leptin (0.5 µg/h) or PBS vehicle. Sham-ADX animals were not examined because our hypothesis specifically addressed the effects of glucocorticoids independent of all other adrenal factors. Leptin significantly decreased food intake in ADX mice not given corticosterone replacement (ADX) over the 4-day course of the infusion. Subcutaneous implantation of either a 10% (ADX + 10% corticosterone) or a 50% corticosterone pellet (ADX + 50% corticosterone) implant did not significantly change food consumption in ADX mice infused with PBS. However, both replacement doses abolished leptin-induced decreases in feeding (Fig. 1A). Cumulative food intake was similar during leptin infusion between ADX + 10% corticosterone and ADX + 50% corticosterone mice and indistinguishable from that in their PBS-infused counterparts (Fig. 1A), as was daily and peak food intake (data not shown).

ADX mice infused with PBS maintained body weight. PBS-infused ADX mice given corticosterone pellet replacement tended to gain weight, although this was not significant relative to ADX mice in the PBS group (Fig. 1B). Infusion of 0.5 µg/h leptin in ADX mice without corticosterone replacement significantly decreased body
weight more than 15% in 4 days. Providing ADX mice with a 10% corticosterone pellet at the time of leptin treatment significantly attenuated weight loss but did not permit weight gain to the level of PBS-treated, ADX mice. Replacement with a 50% corticosterone pellet completely prevented leptin effects on body weight, permitting weight gain that was significantly greater than that in either of the other leptin-treated groups and indistinguishable from that in PBS-infused, ADX + 50% corticosterone mice (Fig. 1B).

Corticosterone and leptin had overall stimulatory and inhibitory effects on body fat content, respectively (Fig. 2). In PBS-infused mice, both corticosterone replacement doses significantly increased carcass triglyceride levels relative to those in ADX mice without glucocorticoid treatment. Concurrent leptin infusion significantly reduced carcass fat content associated with the lower (ADX + 10% corticosterone) but not the higher (ADX + 50% corticosterone) corticosterone replacement, even though food intake was similar between these two groups (compare Fig. 1A). As with body weight, ADX + 50% corticosterone mice had similar carcass triglyceride levels, regardless of leptin treatment (Fig. 2). Consistent with their greater fat content relative to ADX or ADX + 10% corticosterone mice, ADX + 50% corticosterone mice exhibited significantly reduced carcass water content (Table 1). Body water and fat content accounted for differences in carcass weight between all groups; there were no significant between-group differences in carcass protein content (data not shown).

**Table 1.** Body water content and plasma corticosterone and leptin values in mice from experiment 1.

<table>
<thead>
<tr>
<th></th>
<th>Body Water, %</th>
<th>Plasma Corticosterone, µg/dl</th>
<th>Plasma Leptin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADX</td>
<td>67.5 ± 0.4</td>
<td>1.1 ± 0.08</td>
<td>2.8 ± 0.4</td>
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<tr>
<td>ADX + 10% Cort</td>
<td>66.2 ± 1.1</td>
<td>5.2 ± 0.5†</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>ADX + 50% Cort</td>
<td>62.8 ± 1.0†‡</td>
<td>27.5 ± 3.4†</td>
<td>10.6 ± 1.0†‡</td>
</tr>
<tr>
<td>Leptin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADX</td>
<td>69.3 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>35.0 ± 3.5*</td>
</tr>
<tr>
<td>ADX + 10% Cort</td>
<td>68.6 ± 1.1</td>
<td>6.4 ± 1.0†</td>
<td>10.5 ± 2.1†</td>
</tr>
<tr>
<td>ADX + 50% Cort</td>
<td>63.0 ± 0.6‡</td>
<td>30.5 ± 2.5†‡</td>
<td>21.6 ± 0.9‡†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4–7/group. Male C57BL/6 mice were adrenalectomized (ADX) and implanted 3 days later with 1) an osmotic minipump delivering PBS or 0.5 µg/h murine leptin and 2) either no or a 10% or 50% corticosterone (Cort) pellet, as described in METHODS. Plasma hormones and carcass composition were determined after 4 days of combined leptin and glucocorticoid treatment. *P < 0.05 vs. PBS in same corticosterone replacement group; †P < 0.05 vs. ADX in same leptin group; ‡P < 0.05 vs. ADX + 10% Cort in same leptin group.
tein levels over the timeframe of the experiment (Table 1 and data not shown).

To determine if the effects of corticosterone replacement on carcass fat content could be attributed to changes in insulin or insulin sensitivity, we measured plasma insulin and glucose levels in mice from experiment 1. Corticosterone replacement had a significant overall effect to increase plasma insulin that was most evident in mice replaced with the 50% pellet. Plasma insulin levels in ADX + 50% corticosterone mice were disproportionately elevated relative to the similar food intake between the ADX + 10% corticosterone and ADX + 50% corticosterone groups (Figs. 1A and 3A). Leptin significantly decreased plasma insulin levels overall. In contrast to the maintenance of food intake, body weight, and body fat by the 50% corticosterone pellet, plasma insulin was significantly inhibited by leptin even at the high replacement dose (Fig. 3A). To test if high values in ADX + 50% corticosterone mice may have obscured differences between other groups, we performed two-way ANOVA on data only from ADX and ADX + 10% corticosterone mice. This analysis revealed a significant increase in plasma insulin between ADX and ADX + 10% corticosterone mice in the PBS group, as well as a significant decrease in insulin associated with leptin treatment in ADX + 10% corticosterone mice (bracketed symbols, Fig. 3A).

Plasma glucose was unchanged despite elevated insulin levels in ADX + 50% corticosterone mice, but it averaged <50 mg/dl in ADX mice treated only with leptin (Fig. 3B). After exclusion of ADX + 50% corticosterone mice from analysis as described previously, these trends became significant. Plasma glucose values were significantly lower in leptin-treated, ADX mice compared with either PBS-treated, ADX mice or with leptin-treated, ADX + 10% corticosterone mice (bracketed symbols, Fig. 3B). Statistical analysis of other experimental endpoints was not altered by omitting the ADX + 50% corticosterone groups.

Plasma corticosterone levels in a given replacement group were equivalent, regardless of leptin treatment. Replacement of ADX mice with a 10% corticosterone pellet produced plasma concentrations of ~5–6 µg/dl at 4 days after implantation. Replacement with a 50% corticosterone pellet proportionately increased plasma corticosterone to 25–30 µg/dl (Table 1).

Plasma leptin levels varied not only as a result of leptin infusion but also in association with corticosterone replacement (Table 1). Replacement with a 50% corticosterone pellet significantly increased plasma leptin levels in PBS-treated mice. Leptin concentrations in this group were comparable both to those in ADX + 10% corticosterone mice infused with exogenous leptin and to the difference in leptin between ADX + 50% and ADX + 10% corticosterone mice in the leptin-treated group. Plasma leptin was further elevated in ADX mice infused with leptin (Table 1).

To confirm that apparent greater sensitivity of ADX mice to leptin was not due solely to higher leptin levels, we plotted food intake and carcass triglyceride content against plasma leptin for each mouse in a given glucocorticoid replacement group. The initial results of this approach revealed a significant negative correlation between food intake and plasma leptin in ADX, but not in ADX + 10% or ADX + 50% corticosterone mice, consistent with the preservation of food intake in all leptin-treated mice given corticosterone (Fig. 1A). Because the 10% corticosterone pellet produces constant plasma concentrations in mice corresponding to the 24-h mean level in normal rodents, which represents an important physiological setpoint for normalizing many glucocorticoid-sensitive parameters (7, 8, 19, 28), we sought to determine if glucocorticoid levels at or above this level were associated with less effective inhibition of food intake by leptin. For simplicity, we refer to this plasma corticosterone level in Fig. 4 as the 24-h mean. We defined the range of the 24-h mean operationally as the 95% confidence limits of corticosterone levels in the ADX + 10% corticosterone group. Mice with plasma corticosterone below the 24-h mean, or below the 95% confidence limit of levels in the ADX + 10% corticosterone group, exhibited a significant negative relationship between food intake and plasma leptin (corticosterone < 24-h mean; Fig. 4A). However, mice with plasma

![Fig. 3. Plasma insulin (A) and glucose (B) in mice from experiment 1. Samples were collected from nonfasted mice in the morning after 4 days of combined corticosterone and/or leptin treatment. Main effects of leptin, corticosterone, and the interaction between leptin and corticosterone treatment were all significant for plasma insulin by 2-way ANOVA (P < 0.05); main effects of leptin and corticosterone treatment were significant for plasma glucose; n = 4–7/group. Symbols are as in Fig. 1, with significant differences after analyses omitting ADX + 50% corticosterone mice indicated in brackets (see RESULTS). [*]P < 0.05 vs. PBS in same corticosterone replacement group. [†]P < 0.05 vs. ADX in same corticosterone replacement group. [‡]P < 0.05 vs. PBS in same corticosterone replacement group. [§]P < 0.05 vs. ADX in same corticosterone replacement group.]
corticosterone at or above this concentration did not (corticosterone ≥ 24-h mean; Fig. 4A).

In contrast to food intake, there was a negative exponential relationship between body fat content and plasma leptin in ADX + 10% corticosterone as well as ADX mice. Because the relationships of body fat to plasma leptin could not be distinguished for ADX and ADX + 10% corticosterone mice, we pooled mice with plasma corticosterone levels at or below the 24-h mean corticosterone levels at or below those of ADX + 10% corticosterone mice, equivalent to levels at or below the 24-h corticosterone mean, as defined above. Regression analysis revealed a significant negative correlation between carcass triglyceride levels and plasma leptin for mice with plasma corticosterone levels at or below the 24-h mean (corticosterone ≤ 24-h mean; Fig. 4B) but no significant relationship for mice with plasma corticosterone above this concentration (corticosterone > 24-h mean; Fig. 4B). Thus these analyses indicated that significant negative relationships between food intake or body fat and leptin depended on plasma glucocorticoids and not just on plasma leptin levels. Similar results were obtained by analysis of covariance and stepwise multiple regression analysis (not shown).

Experiment 2: effects of sequential manipulation of leptin and glucocorticoids. To determine if elevated glucocorticoids could reverse preexisting inhibition of food intake by leptin, ADX mice were infused with PBS or 0.5 µg/h leptin for 4 days before subcutaneous implantation of either no or a 50% corticosterone pellet. All mice were maintained on a minimal level of corticosterone in the drinking fluid until pellet implantation surgery, at which time they were switched to corticosterone-free drinking fluid (see METHODS). Food intake was significantly lower in mice infused with leptin before 50% corticosterone pellet implantation (Fig. 5B; day 0). Provision of a 50% corticosterone pellet significantly increased food intake in all mice relative to their sham-operated counterparts, regardless of prior leptin treatment. This increase in feeding was significant compared with day 0 intake in leptin-treated mice (Fig. 5).

NPY is a potent stimulator of food intake that has been reported to be reciprocally regulated by leptin and glucocorticoids and to mediate some of the hyperphagia and obesity due to leptin deficiency (10, 38). To determine if in increasing food intake, corticosterone treatment might also reverse leptin inhibition of hypothalamic NPY expression (1), we performed Northern analysis of total hypothalamic RNA. Leptin treatment significantly reduced hypothalamic NPY mRNA levels relative to those in PBS-infused controls (Fig. 6). This
Insulin and hypothalamic NPY gene expression. These results corroborate the lack of appetite inhibition by glucocorticoid-induced increases in leptin in clinical studies (31) and extend our previous findings that glucocorticoids attenuate the negative relationship between endogenous leptin levels and food intake in ADX mice independently of CRH (19). Our current evidence is consistent with the hypothesis that glucocorticoids reduce sensitivity to leptin and indicates that this impairment of leptin action, depending on the level of glucocorticoids, can affect both appetite and fat mobilization.

We have found that glucocorticoids can both prevent and reverse decreases in food intake induced by systemic leptin infusion. These results agree with the appetite-preserving effects of dexamethasone treatment in mice injected intracerebroventricularly with leptin (41). Despite variations in circulating leptin between groups in experiment 1, regression of matched data for individual mice demonstrated that significant inhibition of food intake by leptin only occurred in ADX mice with plasma corticosterone below 24-h mean levels in normal rodents (7, 19, 28). Notably, suppression of food intake by leptin in ADX mice without corticosterone replacement was sufficiently profound to produce hypoglycemia, even though comparable decreases in plasma glucose typically elicit compensatory feeding in normal rodents (12). Although ADX, leptin-infused mice also had some of the highest leptin levels, we attribute these high levels to decreases in distribution volume or clearance associated with the greater weight loss in this group (21). In support of this interpretation, leptin infusion produced similar plasma levels when body weight did not diverge significantly in experiment 2 (Table 2).

Even moderate glucocorticoid concentrations of 5–6 µg/dl prevented inhibition of food intake by leptin levels that were more than three times the circadian maximum (1). Although the relatively high leptin dose used did inhibit food intake when mice were initially maintained on 10 µg/ml corticosterone in the drinking fluid (experiment 2), this form of replacement was ineffective (Table 2). Body weight increased over the 2 days after subcutaneous pellet surgery in ADX + 50% corticosterone vs. ADX mice infused with PBS; however, relative body weight changes were not significantly influenced by corticosterone treatment in leptin-infused mice (Table 2). As in experiment 1, plasma corticosterone levels in a given replacement group were unaffected by leptin treatment; plasma insulin and glucose levels also exhibited similar leptin- and glucocorticoid-associated changes (data not shown).

**DISCUSSION**

We have shown that glucocorticoids can overcome the inhibitory effects of exogenously administered leptin on food intake, body weight, and fat stores. This reversal occurs despite leptin-induced reductions in plasma suppression was not relieved by corticosterone treatment, in contrast to the marked increase in food intake observed in leptin-infused, ADX + 50% corticosterone mice (Fig. 6). Similar inhibition of NPY expression by leptin and lack of reversal by corticosterone were observed in mice treated simultaneously with leptin and corticosterone for 4 days in experiment 1 (data not shown). In all cases, β-actin expression was not changed by any of the hormonal treatments (data not shown).

Plasma leptin 2 days after corticosterone pellet surgery and after 6 days of leptin infusion was equivalent in all ADX mice infused with leptin, regardless of corticosterone treatment. In PBS-infused mice, the 50% corticosterone pellet also increased plasma leptin levels comparable to those in the leptin-treated groups (Table 2). Body weight increased over the 2 days after subcutaneous pellet surgery in ADX + 50% corticosterone vs. ADX mice infused with PBS; however, relative body weight changes were not significantly influenced by corticosterone treatment in leptin-infused mice (Table 2). As in experiment 1, plasma corticosterone levels in a given replacement group were unaffected by leptin treatment; plasma insulin and glucose levels also exhibited similar leptin- and glucocorticoid-associated changes (data not shown).

**Table 2. Plasma leptin and body weight data for mice from experiment 2**

<table>
<thead>
<tr>
<th></th>
<th>Plasma Leptin, ng/ml</th>
<th>Change in Body Weight, %</th>
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<tbody>
<tr>
<td>PBS</td>
<td></td>
<td></td>
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<tr>
<td>ADX</td>
<td>3.2 ± 0.2</td>
<td>-4.85 ± 1.85</td>
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<tr>
<td>ADX + 50% Cort</td>
<td>16.3 ± 2.0†</td>
<td>3.35 ± 1.29†</td>
</tr>
<tr>
<td>Leptin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADX</td>
<td>13.3 ± 4.1</td>
<td>-2.93 ± 1.80</td>
</tr>
<tr>
<td>ADX + 50% Cort</td>
<td>17.0 ± 0.6</td>
<td>0.41 ± 0.86</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3–4/group. Adrenalectomized mice were infused with either PBS or 0.5 µg/h leptin for 4 days while being maintained on 10 µg/ml corticosterone in the drinking fluid, and were then implanted with either no (ADX) or a 50% corticosterone pellet (ADX + 50% Cort) and switched to steroid-free saline. Plasma leptin was assayed from samples that were collected at time of death, 2 days after corticosterone pellet implantation surgery. Change in weight is expressed as % change in weight after pellet implantation surgery. *P < 0.05 vs. ADX in same leptin group.
below that estimated to provide 24-h mean levels of replacement (20) and may have been even less effective if fluid consumption was reduced in parallel with food intake (8). Our results suggest that at the levels normally observed across the 24-h cycle, glucocorticoids may be a more important regulator than leptin of food intake.

Plasma glucocorticoid levels required to prevent leptin-induced loss of body weight and fat, on the other hand, were well above 24-h mean levels and corresponded to stress-induced production. Sustained exposure to glucocorticoid levels above the 24-h mean results in symptoms of glucocorticoid excess, including insulin resistance and glucose intolerance (8, 30), which were reflected by the marked increases in plasma insulin and lack of corresponding decreases in plasma glucose in ADX + 50% corticosterone mice (Fig. 3). Even though leptin treatment significantly decreased plasma insulin across all corticosterone doses, insulin in ADX + 50% corticosterone mice was still twice the level observed in mice with lower plasma corticosterone or in normal, non-ADX mice in our colony (19). The preservation of body fat in the ADX + 50% corticosterone group, despite leptin-induced reductions in insulin, is consistent with the high sensitivity of lipolysis to inhibition by insulin (9). Although antagonism of the diabetogenic effects of glucocorticoids is a novel aspect of leptin-induced improvements in insulin sensitivity, our data indicate that glucocorticoids can increase insulin levels sufficiently to prevent some effects, such as fat depletion, that have been proposed to explain leptin effects on insulin action (4, 34).

During preparation of this manuscript, two recent publications by Arvaniti et al. (2, 3) came to our attention. These studies concluded from effects of a lower leptin dose in both C57BL/6 and ob/ob male mice that glucocorticoids and leptin do not influence the effects of each other on food intake, body weight, or body fat content (2, 3). In contrast, we have found that there is a significant interaction between glucocorticoids and leptin on all of these endpoints and on plasma insulin, with food intake and body composition exhibiting differential sensitivity to glucocorticoids. The lack of interaction reported by Arvaniti et al. in ob/ob mice could be due, as we have previously suggested (19), to the likelihood that glucocorticoids act independently of leptin-signaling pathways, because glucocorticoids restore hyperphagia and obesity in rodents lacking leptin or its receptor (40). We further suspect that beginning leptin infusion at the time of adrenal surgery was a stress that, when combined with the more limited range of corticosterone replacement in the studies by Arvaniti and colleagues (2, 3), obscured the full spectrum of glucocorticoid effects.

We have further shown that glucocorticoid-stimulated feeding in leptin-treated mice could not be attributed to increased hypothalamic NPY gene expression, even though NPY has been proposed to mediate leptin and glucocorticoid effects on appetite and has been shown to contribute to the obesity and hyperphagia of leptin deficiency (10, 38, 39). Although we did not observe increased NPY mRNA with corticosterone treatment alone, the literature is not consistent on this effect in freely feeding animals (32), and glucocorticoid induction of NPY in PBS-treated mice might have been limited by their large increase in endogenous leptin or insulin (1, 39). As with any use of mRNA levels to deduce neurotransmitter release, we cannot exclude the possibility that glucocorticoids induced feeding via increased NPY secretion (38). However, differential regulation of NPY peptide and mRNA levels seems less likely over the 6-day period in which leptin was infused. Our data complement recent findings that factors besides NPY must be invoked to account for the full expression of hyperphagia in ob/ob, AY (agouti), and gold thioglucose-treated mice (10, 17).

Central and peripheral mechanisms for glucocorticoid-induced leptin resistance remain to be defined. Leptin-resistant obesity and hyperphagia are associated with genetic defects in melanocortin pathways (5, 18) and brown fat (26). Although these genetic forms of leptin resistance may not require elevated glucocorticoids (5, 18, 23, 26, 35), glucocorticoids could regulate components of the melanocortin or uncoupling protein systems, as suggested by corticosterone-induced reductions in uncoupling protein 1 (3), to produce similar results in normal animals. Despite evidence that hypothalamic CRH is responsive to leptin, is a putative inhibitor of food intake, and is clearly regulated by glucocorticoids (11, 39), we feel that CRH is unlikely to mediate the present effects because glucocorticoids increase food intake relative to leptin independently of CRH (19).

In summary, our data indicate that the effects of leptin levels approximating those in obesity (25) can be counteracted by plasma glucocorticoid levels within the physiological range. Although leptin could be the primary regulator of glucocorticoid effects through its reported ability to inhibit adrenocortical axis activity (15), the evidence for this role of leptin rests on the use of bolus leptin doses more than four times the daily infusion dose used here. Our current findings that low levels of glucocorticoids preserve food intake and higher glucocorticoid levels defend fat stores indicate that appropriate regulation of glucocorticoids will be critical to leptin effects. Our data also suggest that if glucocorticoids preserve fat deposits at relatively high leptin levels, then increased glucocorticoid production, as may be observed in chronic catabolic states (8), could contribute to the preferential regain of fat when nutritional repletion is used to treat cachexia (29). It will be of further interest to determine why, despite elevated glucocorticoid levels, food intake is often inadequate for metabolic needs in such wasting syndromes. Elucidation of mechanisms of glucocorticoid effects on appetite should provide important insights into the physiology and pathophysiology of appetite and metabolic regulation.

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