Effect of leptin on energy balance does not require the presence of intact adrenals

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Arvaniti, Konstantinia, Yves Deshaies, and Denis Richard. Effect of leptin on energy balance does not require the presence of intact adrenals. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R105–R111, 1998.—The present study was conducted to assess the effects of leptin on food intake and energy balance in the presence or absence of corticosterone. Three cohorts of C57BL/6 mice differing in their corticosterone status [nonadrenalectomized (intact), adrenalectomized (ADX), and ADX with corticosterone replacement] were infused with either saline or leptin at a dose of 150 μg·kg⁻¹·day⁻¹. Throughout the study, mice had free access to both a high-starch and a high-fat diet. At the end of the experimental period, mice were decapitated and their carcasses were processed for the determination of energy, protein, and lipid contents. Leptin significantly reduced body gains in weight, fat, and energy, whereas corticosterone therapy significantly promoted all of these gains. Leptin and ADX significantly reduced food intake and gross energetic efficiency, whereas corticosterone therapy significantly increased these variables. The effects of leptin, ADX, and corticosterone on food intake were accounted for by changes in the intake of the high-fat diet. Leptin also attenuated the preference for fat that developed quickly in mice simultaneously exposed to the high-starch and high-fat regimen. Altogether, the results of this study 1) emphasize the abilities of leptin and corticosterone to, respectively, decrease and increase energy deposition and ingestion of fat, 2) do not substantiate any leptin-corticosterone interaction in the regulation of energy balance, and 3) demonstrate that leptin can produce its effect on energy and fat gains in the absence of an intact hypothalamic-pituitary-adrenal axis.

adrenalectomy; body composition; body weight; dietary starch; dietary fat; food intake; obesity; energy expenditure

THE MOST IMPORTANT peripheral hormones in the regulation of energy balance are probably leptin (7, 40) and corticosteroids (6, 44), together with insulin (6, 44). Leptin, which is secreted by the adipocytes in proportion to the size of the fat mass, has been reported to reduce food intake and stimulate thermogenesis (7, 40). These anorectic and thermogenic attributes appear particularly strong in the genetically obese (ob/ob) mouse, in which a nonsense mutation in the coding region of the leptin gene prevents the production of a functional protein (48). Corticosteroids, whose circulating levels could to some extent be controlled by the size of the fat mass (42), are known, in contrast to leptin, to increase food intake (37) and decrease energy expenditure (14, 36). The influence of corticosterone in the regulation of energy balance has been principally emphasized in experiments showing the ability of corticosterone to reverse the effects of adrenalectomy (ADX), a procedure that blocks the development of most forms of experimental obesity in laboratory animals (10, 28, 29, 34, 38, 39, 45). Both leptin and corticosterone are currently regarded as peripheral signals capable of informing the brain about the state of the fat reserves, so that appropriate adaptations in energy intake and in energy expenditure can occur to maintain the energy reserves stable (7, 40).

The fact that ADX can prevent the development of obesity in the ob/ob mouse despite a deficiency in leptin raises the possibility that leptin could exert its effect on energy balance through interacting with the secretion or the action of corticosterone and suggests that the effect of leptin in the regulation of energy balance requires the presence of intact adrenals. In this respect, we designed the present study to investigate the effects of a chronic infusion of leptin on energy balance in the presence and absence of corticosterone. In vivo studies of the relationship between leptin and glucocorticoids in the regulation of energy balance are further justified by results of recent reports (1, 4, 17, 41) emphasizing the complex role of leptin in the control of the hypothalamic-pituitary-adrenal (HPA) axis.

MATERIALS AND METHODS

Animals and treatments. Lean male C57BL/6 mice, weighing 22–24 g and aged 6–8 wk, were individually housed in plastic cages. The cages were placed in an isolated, temperature-controlled room (25 ± 1°C) under a 12:12-h light-dark cycle (0700–1900). The animals were allowed unrestricted access to food and water. Throughout the study mice were concomitantly offered a high-starch and a high-fat diet. The macronutrient composition of the two diets is shown in Table 1. The choice between the two diets was offered because of the possible involvement of the diet composition in the effects of leptin and corticosterone on energy balance. The mice were accustomed to the diets during a pretreatment period of 6 days, after which they were subjected to the experimental treatments, which lasted 7 days.

Mice were assigned to a 2 × 3 factorial design. Three cohorts of mice differing in their corticosterone status [non-adrenalectomized (intact), adrenalectomized (ADX), and ADX with corticosterone replacement] were infused with either saline or leptin. The six experimental groups formed were labeled as follows: 1) intact-saline, 2) intact-leptin, 3) ADX-saline, 4) ADX-leptin, 5) ADX-corticosterone-saline, and 6) ADX-corticosterone-leptin. The bilateral removal of adrenals was achieved through two small lateral skin incisions made under isoflurane anesthesia. The total procedure was completed within 15 min. Each adrenal was removed, and the incisions were thereafter appropriately sutured. Sham-operated animals were handled in the same way as ADX animals except that adrenals were not excised. Sterile saline and recombinant mouse leptin (r-MuLeptin) at a dose of 150 μg·kg⁻¹·day⁻¹ were infused using Alzet osmotic minipumps (model 2001, ALZA, Palo Alto, CA). Leptin was kindly provided by Dr. Frank Collins (Amgen, Thousand Oaks, CA). Corticosterone was administered using 100-mg cholesterol-based pellets, which were prepared in our laboratory and contained either no corticosterone or 30% corticosterone (70
nd) Maple Leaf (Canada Packers, Toronto, Canada), casein purified (Best Foods; Canada Starch, Montreal, Canada), Tenderflake (pure ICN Nutritional Biochemicals, Cleveland, OH), dextrose monohydrate, and cellulose, alphacel nonnutritive bulk (ICN Nutritional Biochemicals, Montreal, Canada).

Test Diets, Madison, WI, AIN 76 mineral mixture (ICN Nutritional Biochemicals, Montreal, Canada), cellulose, alphacel nonnutritive bulk (ICN Nutritional Biochemicals, Cleveland, OH).

mg cholesterol plus 30 mg corticosterone). The pumps and pellets were subcutaneously implanted during the same anesthesia that allowed the removal (or the sham removal) of adrenals. After surgery all groups were provided with drinking water supplemented with NaCl (0.9%).

Body weight, food intake, and body gains in energy, fat, and protein. Throughout the whole study body weight and the amount of food ingested from each of the two diets offered to the mice were monitored every day. Food spilled on the absorbent paper was carefully collected, allowed to dry, and accounted for in the food intake calculations. Feces were also collected on a daily basis. At the end of the experimental treatment, digestible energy (DE) intake was determined by subtracting energy content of the feces from gross energy intake. Gross energy intake was calculated by multiplying cumulative intakes of each of the two diets by the respective energy density of each of the diets. The gross energy density of the diets, as well as that of the feces, was determined by adiabatic bomb calorimetry (Parr Instruments, Moline, IL).

Energy, protein, and fat gains were determined as previously described (27). At the end of the experimental treatments, mice were exsanguinated by decapitation between 1030 and 1200. On the day of decapitation, food was removed from the cages 4 h before the mice were killed. Carcasses were autoclaved at 125 kPa for 15 min. This procedure, which had been reported not to affect energy yield (22), was used to soften hard tissues. Once autoclaved, carcasses were homogenized in a volume of water corresponding to two times their weight. The homogenized carcasses were then freeze-dried, pending the determination of their energy and nitrogen contents. Carcass energy content was determined by adiabatic bomb calorimetry, whereas carcass nitrogen was determined in 250- to 300-mg samples of dehydrated carcasses using the Kjeldahl procedure. Carcass protein content was computed by multiplying the carcass nitrogen content by 6.25. The energy as protein was subtracted from total carcass energy to determine energy as nonprotein matter. Because carbohydrate represents a negligible part of carcass total energy, energy from nonprotein matter was assumed to be essentially that of fat. Such an assumption tends to be confirmed by studies in which energy, fat, and protein were directly determined (2). Values of 23.5 and 39.3 kJ/g were used for the calculation of the energy content of protein and fat, respectively (43). Initial energy, fat, and protein contents of carcasses were estimated from the live body weight of the mice with reference to the baseline group of mice killed at the beginning of the experimental period. Such estimates allow gains in energy, fat, and protein to be determined for the treatment period. The five mice in the baseline group were killed at the beginning of the energy balance trial, and the carcass of each animal was analyzed for energy, protein, and fat. The body weight densities in energy (kJ energy/g body wt), protein (g protein/g body wt), and fat (g fat/g body wt) were then computed and averaged. The average densities were then multiplied by the initial body weight of each mouse ascribed to the experimental groups. Mice in the initial group were identical in every respect (strain, age, and gender) to those of the six experimental groups. Apparent energy expenditure was calculated by subtracting the energy gain from DE intake. Gross energetic efficiency was expressed as the ratio of energy gain to DE intake multiplied by 100.

Statistical analysis. A 2 × 3 factorial ANOVA was used to determine the main and interaction effects of infusion (saline or leptin) and corticosterone status (intact, ADX, or ADX with corticosterone). Regression analyses were also performed to assess the relationship existing between energy gain and fat or protein gain.

RESULTS

Body weight and food intake. Figure 1 illustrates the effects of leptin on body weight in mice with intact adrenals, in ADX mice, and in ADX mice treated with corticosterone. Before surgery (pretreatment period), all groups of mice exhibited similar growth curves (Fig. 1A). From the start to the end of the treatment period, leptin induced a reduction in body weight gain regardless of the corticosterone status of the mice (main effect of infusion, P < 0.05; no infusion times corticosterone status interaction; Fig. 1B). ADX reduced body weight gain, whereas the replacement therapy with corticosterone elevated the value of this variable at the levels of the mice with intact adrenals.

At the end of the pretreatment period mice had already developed a preference for the high-fat diet. This is emphasized in Table 2. Throughout the treatment period leptin-infused mice ate less of the high-fat diet than mice treated with saline. In addition, leptin attenuated the preference for the fat regimen as the proportion of the total kilojoules ingested as the high-fat diet was less in leptin-infused mice than in saline-infused animals. Intact and ADX mice consumed less of the high-fat diet than mice treated with corticosterone.

Energy balance. The effects of leptin on energy gain and DE intake in mice with intact adrenals, in ADX mice, and in ADX mice treated with corticosterone are
illustrated in Fig. 2. Body energy gain (Fig. 2A) and DE intake (Fig. 2B) were lower in leptin-treated mice compared with saline-infused animals. On the other hand, ADX animals treated with corticosterone exhibited higher energy gain and DE intake than mice with intact adrenals and ADX mice. There was no interaction effect of leptin and corticosterone status on these variables. Figure 2 also demonstrates the effects of leptin on apparent energy expenditure (Fig. 2C) and gross energetic efficiency (Fig. 2D) in function of the corticosterone status. Leptin did not affect apparent energy expenditure but significantly reduced gross energetic efficiency. ADX animals treated with corticosterone had a larger gross energetic efficiency compared with mice with intact adrenals and with ADX mice.

Fat and protein gain. The various treatments of the present study did not affect protein gain (Fig. 3A). However, ANOVA revealed strong main effects of leptin and corticosterone status on fat gain (Fig. 3B). Fat gain in mice treated with leptin was lower than that in mice infused with saline. ADX mice supplemented with corticosterone gained significantly more fat than mice with intact adrenals and ADX mice. There was no significant correlation between protein and energy gains (Fig. 3C), whereas a very strong correlation was observed between energy and fat gains (Fig. 3D).

Glucose and corticosterone. Serum levels of glucose and corticosterone are shown in Table 3. Leptin had no significant effect on circulating corticosterone. The corticosterone replacement therapy brought the levels of circulating corticosterone significantly above the levels measured in mice with intact adrenals. Neither leptin nor corticosterone status affected fasting glucose levels. The levels of leptin were not measured in this study. Recent experiments carried out by Harris et al. (16) have shown that constant infusions of leptin at rates similar or slightly above the one used in this study bring leptin levels slightly above those of untreated mice.

**DISCUSSION**

The present results confirmed the opposite effects of leptin (7, 40) and corticosterone (6, 44) on energy intake and energy balance. Whereas leptin and ADX retarded energy deposition, corticosterone promoted energy gain.

**Table 2.** Effects of leptin on mean daily intake of high-fat and high-starch diets in mice with intact adrenals, in ADX mice, and in ADX mice treated with corticosterone

<table>
<thead>
<tr>
<th></th>
<th>High-Fat Diet</th>
<th></th>
<th>High-Starch Diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kJ</td>
<td>% of total kJ ingested</td>
<td>kJ</td>
<td>% of total kJ ingested</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Before</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All mice</td>
<td>39</td>
<td>51.43 ± 2.73</td>
<td>73.93 ± 3.61</td>
<td>17.78 ± 2.44</td>
</tr>
<tr>
<td>Intact-saline</td>
<td>6</td>
<td>65.69 ± 5.27</td>
<td>89.28 ± 4.37</td>
<td>7.39 ± 2.89</td>
</tr>
<tr>
<td>Intact-leptin</td>
<td>8</td>
<td>50.53 ± 4.24</td>
<td>75.91 ± 6.81</td>
<td>16.58 ± 4.80</td>
</tr>
<tr>
<td>ADX-saline</td>
<td>5</td>
<td>53.37 ± 2.38</td>
<td>83.82 ± 4.24</td>
<td>10.70 ± 2.99</td>
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<tr>
<td>ADX-leptin</td>
<td>4</td>
<td>41.74 ± 6.17</td>
<td>69.87 ± 8.85</td>
<td>17.43 ± 4.58</td>
</tr>
<tr>
<td>ADX-cort-saline</td>
<td>8</td>
<td>75.02 ± 5.11</td>
<td>84.88 ± 5.53</td>
<td>13.74 ± 5.23</td>
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<tr>
<td>ADX-cort-leptin</td>
<td>8</td>
<td>68.09 ± 5.64</td>
<td>79.45 ± 4.89</td>
<td>17.30 ± 4.30</td>
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<td><strong>ANOVA</strong></td>
<td></td>
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</tr>
<tr>
<td>Infusion</td>
<td>0.0143</td>
<td>0.0366</td>
<td>0.1049</td>
<td>0.0366</td>
</tr>
<tr>
<td>Corticosterone status</td>
<td>0.0003</td>
<td>0.6353</td>
<td>0.7157</td>
<td>0.6353</td>
</tr>
<tr>
<td>Interaction (I × C)</td>
<td>0.6935</td>
<td>0.7116</td>
<td>0.8078</td>
<td>0.7116</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of observations. ADX, adrenalectomized; cort, corticosterone; I, infusion; C, corticosterone status.
The effects of corticosterone and leptin on energy gain reflect those on fat gain. Neither leptin nor corticosterone affected protein gain. The effects of leptin and corticosterone on fat gain were consistent with the effects that these hormones exert on the insulin secretion and lipoprotein lipase activity (13, 26). The effects of leptin and corticosterone on food intake were largely accounted for by the effects these hormones exert on fat intake. The inclusion in this protocol of a high-fat regimen given concomitantly with a high-starch diet may have accentuated the effects of leptin on energy balance, which are generally less striking in lean than in obese animals (8, 15, 25). Interestingly, leptin-treated mice demonstrated less preference for fat than saline-injected animals. The mechanisms whereby leptin can affect the preference for fat are not known.

The effects of corticosterone and leptin on energy gain also reflect those on energetic efficiency. Indeed, the variations in body energy gain induced by the various treatments used were closely related to the changes in energetic efficiency ($r^2 = 0.975, P = 0.0001$), suggesting that the opposite actions of leptin and corticosterone on energy balance were due to effects simultaneously exerted on energy intake and thermogenesis. Although leptin did not increase apparent energy expenditure, it was nonetheless shown to maintain energy expenditure at the level of saline-infused animals in the presence of a reduced energy intake, indicating that it could have stimulated thermogenesis. Previous results have indicated the stimulating effects of leptin on thermogenesis and suggested brown adipose tissue as the thermogenic effector for this action (21). In contrast to leptin, corticosterone status affected energy expenditure; ADX mice exhibited a low apparent energy expenditure compared with animals with intact adrenals and to ADX mice treated with corticosterone. The reduction in apparent energy expenditure in ADX mice was not, however, sufficient to prevent the fall in energy gain seen in these animals. Corticosterone has been reported to reduce and ADX to enhance thermogenic activity in brown adipose tissue of obese animals (14, 18, 35, 46).

The results of this study indicate that the ability of leptin to inhibit body gains in energy and fat is not dependent on the presence of corticosterone. The effects of leptin were observed in ADX mice and in mice with various circulating levels of corticosterone; no significant infusion (leptin) times corticosterone status interaction was observed on any of the variables assessed in the study. The observation that leptin may affect energy balance in the absence of an intact HPA axis should, however, not preclude the possibility that leptin could contribute to curtail fat deposition by reducing HPA axis activity in some circumstances. In fasted lean

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**Fig. 2.** Effects of leptin on body energy gain (A), digestible energy (DE) intake (B), apparent energy expenditure (C), and gross energetic efficiency (energy gain/DE intake; D) in mice with intact adrenals, in ADX mice, and in ADX mice treated with corticosterone. Number of observations per group and ANOVA determinations are the same as in legend for Fig. 1. Energy gain: I, $P = 0.0011$; C, $P = 0.0003$; and I × C, $P = 0.0709$. DE intake: I, $P = 0.0416$; C, $P = 0.0001$; and I × C, $P = 0.8707$. Apparent energy expenditure: I, $P = 0.8289$; C, $P = 0.0509$; and I × C, $P = 0.9812$. Gross energetic efficiency: I, $P = 0.0001$; C, $P = 0.0001$; and I × C, $P = 0.3678$. Please refer to the original source for detailed data and statistical analyses.
and in ob/ob mice with intact adrenals, leptin has been reported to attenuate the response of the HPA axis to various challenges (1, 17, 19). In addition, leptin has also been shown in in vitro experiments to decrease the release of cortisol from adrenocortical cells (5). Thus, given the ability of corticosterone to promote accretion of the fat mass, any reduction in the levels of corticosterone is obviously susceptible to reduce energy deposition. Besides, the administration of leptin did not prevent the effects of corticosterone in accentuating energy deposition in the present study. Regardless of whether they were treated with leptin or not, ADX mice treated with corticosterone were much fatter than intact or ADX mice.

Recent results have provided evidence that the removal of the adrenals could accentuate the anorectic effect of an acute brain injection of leptin (47). Although these results are consonant with ours by demonstrating that the presence of intact adrenals is not required for the effects of leptin to occur, they are nonetheless not in total agreement with the present results by suggesting that the lack of corticosterone enhances the effects of leptin. Whether this discrepancy can be explained by differences in diet, the chronic versus acute duration of leptin treatment, or its peripheral versus central mode of administration remains to be investigated. In the present study, energy balance measurements were performed over a relatively long period of time and no significant leptin-corticosterone interaction was observed on key energy balance-related variables such as DE intake (I, P = 0.9791; C, P = 0.3109; and I × C, P = 0.8545), body fat gain (I, P = 0.0010; C, P = 0.6785), and in ADX mice treated with corticosterone (energy gain, −35 kJ; fat gain, −33 kJ) and in ADX mice treated with corticosterone (energy gain, −35 kJ; fat gain, −33 kJ).

Table 3. Effects of leptin on serum glucose and corticosterone in mice with intact adrenals, in ADX mice, and in ADX mice treated with corticosterone

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Corticosterone, µmol/l</th>
<th>Glucose, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact-saline</td>
<td>6</td>
<td>0.11 ± 0.03</td>
<td>13.02 ± 0.50</td>
</tr>
<tr>
<td>Intact-leptin</td>
<td>8</td>
<td>0.07 ± 0.04</td>
<td>12.43 ± 0.27</td>
</tr>
<tr>
<td>ADX-saline</td>
<td>5</td>
<td>0.03 ± 0.01</td>
<td>12.81 ± 0.68</td>
</tr>
<tr>
<td>ADX-leptin</td>
<td>4</td>
<td>0.02 ± 0.01</td>
<td>13.25 ± 0.27</td>
</tr>
<tr>
<td>ADX-cort-saline</td>
<td>8</td>
<td>0.48 ± 0.04</td>
<td>13.36 ± 0.16</td>
</tr>
<tr>
<td>ADX-cort-leptin</td>
<td>8</td>
<td>0.48 ± 0.07</td>
<td>13.18 ± 0.73</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Infusion (I)</td>
<td></td>
<td>0.7092</td>
<td>0.6878</td>
</tr>
<tr>
<td>Corticosterone (C)</td>
<td></td>
<td>0.0001</td>
<td>0.8219</td>
</tr>
<tr>
<td>Interaction (I × C)</td>
<td></td>
<td>0.8921</td>
<td>0.3277</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of observations.

The lack of interaction of leptin and corticosterone on energy balance-related variables in this study suggests that each of these hormones acts in parallel in the control of food intake and energy expenditure. Although the lack of interaction of leptin and corticosterone status on energy balance disproves the hypothesis that one of these hormones can affect energy balance regulation through effects exerted on the receptor-mediated action of the other, it does not rule out the possibility that the actions of both of these hormones converge on the same mechanism to control food intake and thermogenesis. Given the importance of insulin...
sensitivity in the regulation of energy balance (32) and that leptin (33) and corticosterone (11) can, respectively, enhance and deteriorate insulin sensitivity, it can be argued that the latter hormones affect energy balance through influencing insulin sensitivity. In addition, leptin and corticosterone, respectively, have been reported to reduce (23, 31) and to increase (20) the synthesis of hypothalamic neuropeptide Y, whose action in the control of food intake and thermogenesis favors energy deposition (3). The effects of leptin and corticosterone on neuropeptide Y could represent one, although not the only, central mechanism through which leptin and corticosterone could cancel out each other’s actions on energy balance. There is indirect evidence that corticotropin-releasing hormone (30) and proopiomelanocortin (12) could also be involved.

In conclusion, the results of the present study demonstrate that leptin and corticosterone have opposite effects on major energy balance-related variables. Leptin reduces body gains in weight, fat, and energy, whereas corticosterone therapy significantly promoted all of these gains. The effects of leptin, ADX, and corticosterone on food intake were accounted for by a reduction in the intake of dietary fat. Leptin attenuated the preference for fat, which seems to develop quickly in mice simultaneously offered high-fat and regular regimens. The present results also provide evidence that peripheral administration of leptin does not require the presence of intact adrenal to reduce energy and fat gains. In fact, leptin reduced the fat and energy gain as efficiently in ADX animals as in animals with intact adrenals or in mice treated with corticosterone. Finally, the study did not reveal any leptin-corticosterone interaction in the regulation of energy balance. The effects of leptin on energy and fat gain were instead of the same magnitude regardless of the presence or absence of corticosterone.

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REFERENCES


