Reduced central leptin sensitivity in rats with diet-induced obesity

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Levin, Barry E., and Ambrose A. Dunn-Meynell. Reduced central leptin sensitivity in rats with diet-induced obesity, Am J Physiol Regul Integr Comp Physiol 283: R941–R948, 2002; 10.1152/ajpregu.00245.2002.—On low-fat chow diet, rats prone to diet-induced obesity (DIO) have increased arcuate nucleus neuropeptide Y (NPY) expression but similar leptin levels compared with diet-resistant (DR) rats (19). Here, body weight and leptin levels rose in DIO rats, and they defended their higher body weight after only 1 wk on a 31% fat high-energy (HE) diet. However, DIO NPY expression did not fall to DR levels until 4 wk when plasma leptin was 168% of DR levels. When switched to chow, DIO rats lost carcass fat (18). By 10 wk, leptin levels fell to 148% and NPY expression again rose to 150% of DR levels. During 4 wk of food restriction, DIO leptin fell by ~50% while NPY increased by 30%. While both returned to control levels by 8 wk, DIO rats still regained all lost weight when fed ad libitum. Finally, the anorectic effect of intracerebroventricular leptin (10μg) was inversely correlated with subsequent 3-wk weight gain on HE diet. Thus NPY expression and food intake are less sensitive to the leptin’s suppressive effects in DIO rats. While this may predispose them to develop DIO, it does not fully explain their defense of a higher body weight on HE diet.

NEUROPEPTIDE Y (NPY) neurons in the hypothalamic arcuate nucleus (ARC) play a critical role in regulating energy homeostasis in the body (3, 11, 30). NPY released from these neurons has a potent anabolic effect. It increases intake (3), reduces thermogenic capacity (3, 32), and reduces the oxidation of dietary fat (25). These neurons contain leptin receptors (2), and their expression of NPY mRNA is inversely related to plasma leptin levels and/or leptin signaling under a variety of conditions. Short-term fasting or food restriction for up to 2 wk reduces plasma leptin levels and increases ARC NPY expression (18, 19). This increase can be reversed by central leptin injections (28).

In genetically obese ob/ob mice with no leptin production (26) or in rodents with defects in leptin signaling (13, 14, 26), ARC NPY mRNA expression is increased. Rats predisposed to develop diet-induced obesity (DIO prone), a polygenic disorder (21), also exhibit raised ARC NPY expression, despite the fact that their leptin levels are comparable to diet-resistant (DR) rats when both are kept on low-fat chow from weaning (18). When fed a 31% fat, high-energy (HE) diet, DIO-prone rats gain more weight and carcass adiposity and become hyperleptinemic and hyperinsulinemic compared with DR rats (20, 24). Despite raised leptin levels, DIO-prone rats continue to overexpress ARC NPY mRNA for up to 2 wk on HE diet (19) and fail to increase their ARC NPY expression normally with fasting or food restriction (19). However, after 12 wk on HE diet, DIO rats become quite obese with high leptin levels. At that point, their ARC NPY expression is actually lower than both comparably obese and non-obese DR rats, and expression increases normally during food restriction (18). Once obese, DIO rats avidly defend their elevated body weight against chronic food restriction and/or lowering the fat content of the diet and rapidly regain lost weight when allowed ad libitum intake (20, 24). These data suggest an important interaction between ARC NPY expression and plasma leptin in the control of body weight in DIO rats.

Based on the available data from prior studies (18–20, 24), we postulated that the elevated ARC NPY expression in DIO-prone rats predisposes them to become obese when sufficient dietary fat and calories are provided. Furthermore, ARC NPY might play a role in the defense of a higher body weight once they become obese. However, there is no information as to when DIO rats begin to defend their higher body weight on HE diet. The first set of current studies was designed to examine this issue and to determine the parallel changes in plasma leptin and ARC NPY expression associated with early weight gain. The next set of studies utilized brains from rats subjected to long-term food restriction [morphometric and metabolic data previously published (20)] to determine how this affected the relationship between plasma leptin levels and ARC NPY expression with respect to their regaining of lost body weight when subsequently fed ad libitum. Finally, when it became apparent that ARC NPY expression was elevated in DIO rats as long as their leptin levels were >50% of DR levels, a histological analysis of DIO rats was undertaken to determine the parallel distribution of ARC NPY neurons in DIO rats with respect to their regaining of lost body weight. Based on the above-mentioned studies, a hypothesis was then generated to determine how the relationship between plasma leptin levels and ARC NPY expression changes with respect to the regaining of lost body weight in DIO rats.
levels were less than ∼150% of comparable DR rats, the final set of studies was carried out to determine if this apparently raised threshold for leptin detection was associated with a reduced sensitivity to the anorectic effects of leptin.

METHODS

Animals and diet. Male Sprague-Dawley rats (Charles River Labs, Kingston, NY), 300–425 g, were used for all experiments. Animal usage was in compliance with the animal care committee of the East Orange Veterans Affairs Medical Center (East Orange, NJ). Animals were fed Purina rat chow (no. 5001) ad libitum from weaning and were housed on a 12:12-h light-dark schedule with lights out at 1800. Depending on the experiment, rats were fed either chow or an HE diet. Purina rat chow contains 3.30 kcal/g with 23.4% as protein, 4.5% as fat, and 72.1% as carbohydrate, which is primarily in the form of complex polysaccharide (23). HE diet is composed of 8% corn oil, 44% sweetened condensed milk, and 48% Purina rat chow (Research Diets no. C11024F, New Brunswick, NJ). It contains 4.47 kcal/g with 21% of the metabolizable energy content as protein, 31% as fat, and 48% as carbohydrate, 50% of which is sucrose (23). For the majority of experiments, rats were weighed weekly and had food intake measured biweekly. At the end of each study, rats were decapitated and trunk blood was taken for plasma glucose, insulin, and leptin levels. Epididymal, retroperitoneal, perirenal, and mesenteric fat pads were removed and weighed.

Experiment 1: establishment of the defended body weight. One group of 18 rats was placed on HE diet for 1 wk (Fig. 1). At that time, the six highest weight gainers were designated as DIO and the six lowest gainers as DR. These rats were then switched to chow for an additional 3 wk and killed. A second group of 24 rats was placed on HE diet for 2 wk. At that time, the 12 highest weight gainers were designated as DIO and the 6 lowest gainers as DR (Fig. 2). All rats were switched to chow. Half the DIO rats were then restricted to 50% of their caloric intake during the second week on HE diet (Restrict), and their body weight fell to that of DR rats within 3 days. The restricted rats were then fed chow ad libitum (Ad Lib), and all rats were continued on chow for a total of 3 wk on chow before death.

Experiment 2: relationship between ARC NPY mRNA and plasma leptin. A total of 84 rats was used for this study. Three groups of 18 rats were fed HE diet for 1, 2, or 4 wk, and the highest and lowest tertiles of weight gainers were identified as DIO and DR, respectively. They were decapitated at each of these times, and their brains were quickly removed and frozen on dry ice for in situ hybridization. Trunk blood was collected for leptin assay, and fat pads were removed and weighed. In addition to these rats studied here at 1, 2, and 4 wk on HE diet, we also analyzed ARC NPY expression in the brains of rats for which morphometric and metabolic data have already been reported (20). In that study, 30 rats were placed on HE diet for 4 wk, and the 12 highest weight gainers were designated as DIO rats and the 6 lowest as DR rats. At that time, all rats were switched to chow, and half the DIO rats were restricted to 50% of their caloric intake during the fourth week on HE diet. The body weight of these rats fell to that of the DR rats within 1 wk. They were then allowed ad libitum access to chow, and all rats were continued on chow for a total of 3 wk before death.

Fig. 1. A group of 18 rats was fed high-energy (HE) diet for 1 wk; the 6 highest weight gainers were designated as diet-induced obesity (DIO) rats and the 6 lowest gainers as diet-resistant (DR) rats. These rats were then switched to chow for an additional 3 wk.

Fig. 2. A group of 24 rats was fed HE diet for 2 wk; the 12 highest weight gainers were designated as DIO rats and the 6 lowest gainers as DR rats. All rats were switched to chow. Half the DIO rats were then restricted to 50% of their caloric intake during the 2nd week on HE diet (Restrict), and their body weight fell to that of DR rats within 3 days. The restricted rats were then fed chow ad libitum (Ad Lib), and all rats were continued on chow for a total of 3 wk on chow before death.

Fig. 3. A group of 24 rats was fed HE diet for 4 wk, and the 12 highest weight gainers were designated as DIO rats and the 6 lowest as DR rats. At that time, all rats were switched to chow, and half the DIO rats were restricted to 50% of their caloric intake during the 4th week on HE diet. The body weight of the restricted rats fell to DR levels within 1 wk. They were then fed chow ad libitum, and all rats were continued on chow for a total of 3 wk before death.
placed on HE diet. After 12 wk, the 12 highest weight gainers were designated as DIO and the 6 lowest weight gainers as DR, and all rats were switched to chow. After 2 wk on chow, half the DIO rats had their intake restricted to 50% of their previous week's caloric intake. Their body weights fell to those of chow-fed DR rats in 2 wk, and then their weights were matched to those of DR rats for eight additional weeks. At this time, all groups (DR, DIO ad libitum, DIO restricted) had been on chow for a total of 10 wk. They were then all decapitated, and their brains were removed and frozen on dry ice for in situ hybridization for use in this study. Plasma leptin and insulin levels, body weights, and fat pad weights of these rats at various time intervals were previously reported (20).

Experiment 3: relationship between leptin-induced anorexia and the development of DIO. Three-month-old, chow-fed rats had their food intake and body weights measured daily for 3 days. They were then anesthetized with chloropent, and unilateral 21-gauge guide cannulas were implanted in the lateral ventricle (AP −1.2 mm bregma; lateral 1.4 mm, depth 3.3 mm from brain surface with jaw bar at −3.3 mm) and secured to the skull surface. An inner trocar was left within the guide cannula. Within 2–3 days of surgery, rats had the inner trocar removed, and an inner injection cannula attached to polyethylene tubing was lowered to 1 mm below the bottom of the guide cannula. Rats had sham infusions with 10 μl saline on 5 of 7 days for a minimum of 1 wk until their body weights regained preoperative levels. They were then injected intracerebroventriculatly with 10 ng of human angiotensin II (Calbiochem, San Diego, CA) in 3 μl of sterile saline. All rats that drank a minimum of 5 ml within 60 min were considered to have proper cannula placement. Three days later, the animals were fasted for 24 h, and intake and body weight were measured 4 and 24 h after the return of their food at 1100 as a baseline control. After a 3-day rest, they were fasted again for 24 h and injected intracerebroventricularly with 10 μl of artificial cerebrospinal fluid (aCSF, pH 7.2 (in mM): 2.5 KCl, 147 NaCl, 1.3 CaCl2, 0.9 MgCl2, 7.0 Na2HPO4, and 0.9 KH2PO4) with measurement of 4- and 24-h food intake. After an additional 3 days, rats were fasted for 24 h and injected with murine leptin (Calbiochem) in aCSF, and their food intake was assessed. An initial group of 8 rats was assessed in this manner for dose responses to leptin at 3, 6, and 10 μg spaced at 3-day intervals. Only the 10-μg dose produced significant and reliable inhibition of food intake in at least half of the animals under these conditions (3- and 6-μg data not shown). Because this was the minimal effective dose to produce significant reductions in intake in more than half the animals, we considered this a threshold dose and therefore appropriate to test our hypothesis. Thus a second group of 15 rats was then submitted to the same procedure using the 10-μg dose. At the end of testing for food intake, rats were weighed and then placed on HE diet for 3 wk with weekly weighing. Terminally, brains were removed and examined histologically for cannula placements and any damage. A total of 11 rats completed this entire procedure.

ARC NPY mRNA in situ hybridization. Brains were processed for in situ hybridization by minor modifications of previously described methods (19). Briefly, the 511-bp probe (derived from the original probe of Higuchi et al. (8)) for NPY was subcloned into a pBluescript SK+ vector at an EcoRI site. Radiolabeled cRNA was synthesized in vitro from BamHI linearized plasmids. Sense and antisense probes were transcribed with T3 and T7 promoters, respectively, using [α32P]UTP (1,000 Ci/mmol; New England Nuclear, Beverly, MA). The probes were hydrolyzed in 0.5 M NaHCO3 for 30 min. Frozen sections of brain were freeze-thawed onto gel-coated slides and fixed in 4% paraformaldehyde. They were treated with acetic anhydride for 10 min and dehydrated through six steps of graded ethanol solutions. Prehybridization was carried out at 50°C for 30 min, and then sections were hybridized with labeled sense and antisense probes at 50°C overnight. After treatment with RNase A (Calbiochem), sections were washed, dehydrated, dried, and opposed to SB-5 X-ray film (Kodak, Rochester, NY) for 3 days. The resulting autoradiograms were read by an experimentally “blinded” observer using computer-assisted densitometry (Drexel University, Philadelphia, PA). Area and optical density measures were made of that portion of the ARC showing maximal NPY mRNA expression. Readings from the sections with the three largest areas were averaged for comparison among groups.

Plasma leptin and insulin levels. Leptin and insulin concentrations from plasma were determined by radioimmunoassays (Linco, St. Charles, MO) using antibodies to authentic rat insulin and leptin, respectively.

Chemicals and reagents. Unless otherwise specified, all chemicals and reagents were purchased from Sigma (St. Louis, MO).

Statistics. Intergroup comparisons were made by one-way ANOVA for body weight gain, plasma leptin, and ARC NPY mRNA expression. In the leptin infusion studies, the inhibition of intake produced by intracerebroventricular leptin was compared with that of animal's subsequent body weight gain on HE diet over 3 wk by Pearson's correlation coefficient test. Also, DR and DIO rats were identified retrospectively by the weight gain after 3 wk on HE diet (23). The percent inhibition of intake by leptin vs. aCSF was then calculated for each animal at 4 and 24 h. These results were compared by ANOVA for repeated measures. After a significant intergroup difference was found, actual intake in the same rats between aCSF and leptin was compared by one-way ANOVA at 4 and 24 h. When significant differences were found (P < 0.05), post hoc Scheffé’s multiple comparison tests were carried out.

RESULTS

Experiment 1: establishment of the defended body weight. In this experiment, rats were subjected to a series of dietary manipulations to ascertain when DIO rats would assume and maintain their higher body weights after exposure to HE diet. After only 1 wk on HE diet (Fig. 1; Table 1), the body weights of the six highest weight gainers (DIO rats) were not significantly different from those of the six lowest weight gainers (DR rats). However, when body weight gain was considered, the DIO rats gained significantly more weight (63 ± 6 g) than the DR rats [36 ± 5 g; F(1,10) = 4.62; P = 0.025] after 1 wk on HE diet. They were then switched to low-fat chow diet for 3 wk. After 3 wk on chow, their body weights were comparable, but their total fat pad weights were 51% higher, their plasma insulin levels were 102% higher, and their leptin levels were 96% higher than DR rats switched to chow. At this point, the areas of ARC NPY mRNA by in situ hybridization were comparable in DIO (0.525 ± 0.079 mm2) and DR rats (0.462 ± 0.070 mm2). Thus 1 wk of exposure to HE diet was sufficient to establish a higher body weight gain and to set the rats on a trajectory of elevated carcass adiposity, plasma leptin, and insulin
levels. Importantly, with leptin levels at 196% of DR controls, DIO ARC NPY mRNA expression was normalized to DR levels.

After 2 wk on HE diet (Fig. 2), the 12 highest weight-gaining DIO rats were 12% heavier (448 ± 7 g) than the 6 lowest weight-gaining DR rats (402 ± 3 g; P = 0.01). When both DIO and DR rats were switched to chow and a subset of six DIO rats was restricted to 50% of its baseline intake on HE diet, their body weights fell to the level of DR rats within 3 days. When allowed ad libitum intake again, these rats regained the body weight of the unrestricted DIO rats within 1 wk. After 4 wk on HE diet, the highest 12 weight-gaining DIO rats were 10% heavier (572 ± 11 g) than the 6 lowest weight-gaining DR rats (520 ± 14 g; P = 0.01) (Fig. 3). This amounted to a 67% greater gain in body weight by DIO vs. DR rats over these 4 wk. When all rats were switched to chow after 4 wk on HE diet and six DIO rats were restricted to 50% of their baseline caloric intake on HE diet, their body weights of the restricted rats fell to those of DR rats within 1 wk. As with rats studied after 2 wk on HE diet, those in the 4-wk group regained the body weight of unrestricted DIO rats within an additional 1 wk of ad libitum access to chow.

Experiment 2: relationship between ARC NPY mRNA and plasma leptin. Original data reported here are for rats fed HE diet for 1, 2, and 4 wk (all data) and for ARC NPY mRNA expression in the brains of rats fed HE diet for 12 wk and then switched to chow for an additional 10 wk (Table 2; Fig. 4) (20) as well as for DIO rats from that study which were weight matched to the body weights of DR rats for 8 wk (Table 3) (20).

Tables 2 and 3 and Fig. 4 summarize the body weight, plasma leptin and insulin, and additional ARC NPY data points from previously published studies (18–20, 24). When fed chow from weaning, DIO-prone rats weighed the same and had the same fat pad mass and leptin levels as DR-prone rats. Despite this, their ARC NPY levels were 38% higher than DR-prone rats (19). After 1 wk on HE diet, DIO became 3% heavier. Although the total weight of four adipose depots did not differ, these DIO rats had 37% higher plasma leptin levels than DR rats (Table 2; Fig. 4). Despite this, their ARC NPY expression was still 34% higher than DR levels. After 2 wk on HE diet, DIO rats were 6% heavier and had 69% heavier fat pads and 37% higher leptin levels. These changes were insufficient to lower ARC NPY expression, which remained at 34% higher than DR rats.

ARC NPY expression in DIO rats remained higher than in DR rats until DIO rats had been exposed to HE diet for 4 wk (Table 2; Fig. 4). At that time, DIO rats were 10% heavier and had 110% heavier fat pads and 68% higher leptin levels than DR rats. However, after 12 wk on HE diet, DIO rats maintained a 16% higher body weight and had 173% higher adipose depot weights and 79% higher leptin levels (24). At this point, their ARC NPY expression was actually 17% lower than DR rats (18). Finally, when comparable rats were made obese after 12 wk on HE diet and then switched to chow for 10 wk, they maintained a 15% heavier body weight while their fat pad mass fell to 153% and their leptin fell to 145% of DR levels (20). In association with this reduction in plasma leptin levels,

Table 2. Summary of body and fat pad weights, plasma leptin levels, and ARC NPY mRNA expression

<table>
<thead>
<tr>
<th>Weeks (Ref. No.)</th>
<th>Final Body Weight, g</th>
<th>Total Fat Pad Weight, g</th>
<th>Plasma Leptin, ng/ml</th>
<th>NPY Area, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DR</td>
<td>DIO</td>
<td>DR</td>
<td>DIO</td>
</tr>
<tr>
<td>0(19)</td>
<td>436 ± 11</td>
<td>433 ± 9</td>
<td>6.9 ± 0.5</td>
<td>6.7 ± 0.7</td>
</tr>
<tr>
<td>1</td>
<td>420 ± 3</td>
<td>432 ± 3*</td>
<td>7.3 ± 0.4</td>
<td>8.4 ± 1.7</td>
</tr>
<tr>
<td>2</td>
<td>472 ± 8</td>
<td>499 ± 8*</td>
<td>10.3 ± 1.0</td>
<td>17.4 ± 1.9*</td>
</tr>
<tr>
<td>4</td>
<td>520 ± 14</td>
<td>573 ± 11*</td>
<td>12.8 ± 2.2</td>
<td>26.9 ± 2.9*</td>
</tr>
<tr>
<td>12(18, 24)</td>
<td>548 ± 20</td>
<td>636 ± 25*</td>
<td>15.8 ± 2.0</td>
<td>43.2 ± 4.2*</td>
</tr>
<tr>
<td>14(18, 24)</td>
<td>543 ± 16</td>
<td>630 ± 22*</td>
<td>14.3 ± 2.3</td>
<td>46.3 ± 4.4*</td>
</tr>
<tr>
<td>16(18, 24)</td>
<td>542 ± 31</td>
<td>628 ± 18*</td>
<td>16.2 ± 2.8</td>
<td>45.7 ± 4.1*</td>
</tr>
<tr>
<td>22(20)</td>
<td>545 ± 5</td>
<td>625 ± 6*</td>
<td>19.0 ± 2.9</td>
<td>48.3 ± 5.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Groups of 6–8 DIO and DR rats were fed HE diet for 1, 2, 4, or 12 wk; body and fat pad weights, plasma leptin, and arcuate nucleus (ARC); neuropeptide Y (NPY) data for the 0- and 2-wk group are from Ref. 19 and 12, 14, and 16-wk groups are from Refs. 18 and 24. Chow-fed rats at 0 wk were defined as DR prone vs. DIO prone by their low and high urinary norepinephrine levels, respectively (19). The 22-wk rats were divided into 12 DIO and 12 DR rats based on their respective weight gains after 12 wk on HE diet. At that point, all rats were switched to chow for an additional 10 wk (body and fat pad weights and plasma leptin levels are from Ref. 20). *P ≤ 0.05 when DIO rats were compared with DR rats by t-test.

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Table 1. Terminal body and total fat pad weights, plasma insulin, and leptin levels for rats fed HE diet for 1 wk and then switched to chow for 3 wk

<table>
<thead>
<tr>
<th>Phen</th>
<th>BWf, g</th>
<th>Epi, g</th>
<th>RP, g</th>
<th>PR, g</th>
<th>Mes, g</th>
<th>Fat Pad Total, g</th>
<th>Insulin, ng/ml</th>
<th>Leptin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR</td>
<td>501 ± 15</td>
<td>6.6 ± 0.7</td>
<td>5.5 ± 0.9</td>
<td>1.2 ± 0.1</td>
<td>4.5 ± 0.5</td>
<td>17.8 ± 0.1</td>
<td>1.18 ± 0.36</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td>DIO</td>
<td>525 ± 10</td>
<td>8.9 ± 1.2</td>
<td>8.5 ± 1.5</td>
<td>2.2 ± 0.3</td>
<td>7.4 ± 1.2</td>
<td>26.9 ± 0.4*</td>
<td>2.38 ± 0.11*</td>
<td>10.4 ± 1.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. A total of 18 rats was placed on high-energy (HE) diet for 1 wk. The 6 highest and 6 lowest weight gainers were designated as diet-induced obesity (DIO) and diet-resistant (DR) rats, respectively. These 12 rats were then switched to chow for 3 wk, and their final body weight (BWf) and epididymal (Epi), retroperitoneal (RP), perirenal (PR), and mesenteric (Mes) fat pad weights were assessed along with trunk blood insulin and leptin levels. *P ≤ 0.05 for a given parameter in DIO compared with DR rats by t-test. Phen, phenotype.
Experiment 3: relationship between leptin-induced anorexia and the development of DIO. Intraventricular administration of 10 μg of leptin was less effective in reducing 4-h chow intake in rats with a propensity to develop DIO on HE. Thus there was a significant inverse correlation between leptin-induced inhibition of chow intake at 4 h and the subsequent body weight gain of that animal over 3 wk on HE diet (Fig. 5B; r = 0.71; P = 0.014; 95% confidence intervals: slope: −2.10 to −0.24; y-intercept: 20.3 to 75.6). There was no such correlation between inhibition of 24-h intake and subsequent weight gain. When the 11 rats that completed the entire protocol were retrospectively divided into DR (n = 4; <15% weight gain) and DIO (n = 7; >20% weight gain) groups according to their weight gain over 3 wk on HE diet, DR-prone rats showed a significantly greater leptin-induced inhibition of intake at both 4 and 24 h when compared as a percentage of their intake after aCSF at the same time intervals (F[1,9] = 5.74; P = 0.03). When absolute intakes were compared, only the DR-prone rats showed significant inhibition of 4-h intake (35.5 ± 6.2% compared with aCSF (Fig. 5A). Leptin also tended to have a greater absolute inhibitory effect on 24-h intake in DR (43.2 ± 7.7%) than DIO rats (26.6 ± 7.8%; P = 0.1).

DISCUSSION

DIO-prone rats overexpress ARC NPY mRNA, yet they are neither hyperphagic nor obese when fed only low-fat diet (17). However, once the fat and caloric density of the diet is increased, they overeat and begin to accrue excess weight and carcass fat (20, 23, 24). Obesity-prone rats rapidly increase their ratio of carbohydrate to fat oxidation when fed a high-fat diet (4). Central NPY injections in nonobese rats produce a similar metabolic shift (25). Such a reduced oxidation of dietary fat favors adipose deposition and suggests a causal link between the overexpression of ARC NPY mRNA and the rapid increase in the defended body weight of obesity-prone rats placed on HE diet. After only 1 wk on HE diet, DIO rats increased their rate of

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**Table 3. Relationship of plasma leptin to ARC NPY mRNA expression during restriction of food intake in DIO rats**

<table>
<thead>
<tr>
<th>Time Restricted (Ref. No.)</th>
<th>Plasma Leptin, ng/ml</th>
<th>%DR Control</th>
<th>NPY Area, mm²</th>
<th>%DR Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 day (19)</td>
<td>3.2 ± 0.3</td>
<td>60*</td>
<td>0.607 ± 0.050</td>
<td>131*</td>
</tr>
<tr>
<td>2 wk (18, 24)</td>
<td>5.4 ± 0.7</td>
<td>49*</td>
<td>0.838 ± 0.027</td>
<td>134*</td>
</tr>
<tr>
<td>8 wk (20)</td>
<td>13.1 ± 1.2</td>
<td>99</td>
<td>0.337 ± 0.038</td>
<td>92</td>
</tr>
</tbody>
</table>

Values are means ± SE. These data are derived from 3 previously published studies. One group of 5 rats was defined as DIO prone by their elevated 24-h urine norepinephrine levels and then were restricted to 50% of their baseline intake of chow for 5 days (19). An additional group of 6 rats was made obese by intake of HE diet for 12 wk. They were switched to chow for 2 wk and then were restricted to 50% of their baseline intake of chow for 5 days (19). An additional group of 6 rats was made obese by intake of HE diet for 12 wk. They were switched to chow for 2 wk and then were restricted to 50% of their baseline intake of chow for 8 wk (20). *P < 0.05 when values for plasma leptin and ARC NPY areas are by in situ hybridization in these diet-restricted DIO rats were compared with DR controls fed chow ad libitum. All data except the NPY area for the 8-wk restricted DIO rats have been previously published (18–20, 24).
weight gain and then continued to deposit adipose tissue, even when switched back to a low-fat diet for 3 wk. After only 2 wk on HE diet, DIO rats defended their higher body weight when restricted to the weight of DR rats. Despite rising plasma leptin levels, DIO ARC NPY expression remained elevated until 4 wk on HE diet when their leptin levels were 68% higher than DR rats. In addition, DIO rats fed HE diet for 1 wk and then chow for 3 wk had leptin levels that were 93% higher than DR rats, and their ARC NPY expression was also at DR levels. However, even after NPY expression “normalized” after 4 wk of HE diet, DIO rats continued to gain more weight and adipose tissue than DR rats on HE diet. This suggests that elevated ARC NPY expression primes DIO-prone rats for an early increase in their weight and adiposity on HE diet, but, once the weight gain pattern is well established, this overexpression is not required for further weight gain or defense of a higher body weight.

Leptin normally inhibits ARC NPY expression (26, 28). The current studies, taken in the context of our prior studies (18–20, 24), suggest that DIO-prone rats have a reduced sensitivity to this inhibitory effect. NPY expression is elevated in DIO-prone rats that have normal leptin levels (18, 19, 24) and did not fall to DR levels until plasma leptin levels were almost 70% higher than DR rats. When leptin levels reached 180% of DR levels after 12 wk on HE diet, ARC NPY expression was actually decreased by 25% (18, 24). At that point, when DIO rats were switched to a low-fat diet, their body weights plateaued while their fat pad weights and plasma leptin levels fell slowly over 10 wk (20, 24). Once DIO plasma leptin fell to <50% above DR levels, ARC NPY mRNA again rose above DR NPY expression. In addition to this apparent reduction of sensitivity to the suppressive effect of leptin on NPY expression, obesity-prone rats appear to be less sensitive to the anorectic effects of centrally administered leptin. There was a significant inverse correlation between leptin’s effect on decreasing food intake and subsequent weight gain after 3 wk on HE diet in chow-fed rats. This means that the less sensitive to leptin’s anorectic effect a rat was, the more weight it gained when fed a diet relatively high in fat and caloric content. Also, although the overall number of rats per group was small, when retrospectively identified as DIO prone or DR prone by their weight gain on HE diet, DIO-prone rats had a significantly reduced anorectic effect of centrally administered leptin. This suggests that obesity-prone rats have a reduced sensitivity to central leptin signaling pathways.

During short-term energy restriction, plasma leptin levels fall and ARC NPY mRNA expression rises (19, 26, 28). A causal relationship is suggested by the fact that leptin replacement reduces NPY expression to fed levels (28). DIO-prone rats did not increase their ARC NPY expression when restricted for up to 5 days, probably because their basal expression was already maximized (19). However, once NPY expression was normalized after 12 wk on HE diet, DIO rats showed the expected increase in ARC NPY expression associated with reduced leptin levels after 2 wk of restricted intake (18, 24). The lower leptin levels are postulated to be responsible for the elevated ARC NPY expression which, in turn, is thought to be responsible for the rapid regain of weight when they were allowed free access to food (26, 28). However, this mechanism cannot be responsible for the rapid regain of body weight that occurred in DIO rats held on restricted intake for 8 wk and then given free access to food (20). In those animals, both plasma leptin levels and ARC NPY expression returned to the level of unrestrained rats. To
our knowledge this is the first demonstration of the eventual normalization of both plasma leptin levels and ARC NPY expression after prolonged (comparable to ~7–8 human years) lowering of body weight by food restriction. Obviously, there are many other factors besides ARC NPY and leptin that contribute to the defense of body weight. For example, NPY-deficient mice have relatively normal regulation of their body weight (7), and Zucker rats, which have deficient leptin signaling (5) and elevated ARC NPY expression (14), defend their elevated body weight normally when food restricted (12).

In summary, DIO rats appear to have an intrinsic elevation in their threshold for leptin signaling that antedates the development of obesity. Their ARC NPY expression remains elevated during the development of obesity until plasma leptin levels exceed DR levels by >50%. ARC NPY expression rises again as DIO rats lose carcass fat and leptin levels fall below the 50% threshold on low-fat diet. In addition, DIO-prone rats show a reduced sensitivity to the anorectic effects of centrally administered leptin. This suggests a causal link between reduced leptin sensitivity, ARC NPY overexpression, and the early weight gain and defense of an obese body weight in DIO-prone rats when dietary fat and caloric content is increased. However, it does not fully explain their defense of a higher body weight.

Perspectives

Both obese humans (16) and rats (20, 24) defend their elevated body weight and adiposity against chronic dietary restriction. Our studies suggest that a defect in central leptin signaling might help determine the level of defended body weight and the predisposition of DIO rats to become obese when dietary energy and fat content are increased. Reduced leptin signaling of one sort or the other is seen in some obese humans and rodents. Obese humans (27) and some obese mice (6) appear to have reduced blood-brain barrier transport of leptin. Some obesity-prone mice show reduced leptin production during the development of DIO (31), while ob/ob mice produce no leptin at all (26). A variety of genetically obese mice and rats have defects in their leptin receptor signaling pathway (5, 13, 26). ARC NPY overexpression is a common feature in many of these rodents that have reduced leptin receptor signaling (5, 13, 14, 26). Although no specific defect in receptor-mediated signaling has been identified to date, the DIO rat appears to fall into this latter category. A raised threshold for leptin in DIO rats would promote overexpression of ARC NPY expression and lead to increased intake and fat storage when dietary fat and calories were increased (3, 15, 30). Although we use the DIO rat as a model for the adverse effects of human obesity, reduced leptin sensitivity would actually be an advantage for survival in the wild when food was in short and intermittent supply. It would have the same effect in humans with restricted access to food. A raised threshold for leptin signaling should also raise the defended level of carcass adiposity (and body weight) required to generate sufficient leptin for detection and regulation of homeostatic processes at a physiological level. In fact, this is exactly the way in which the DIO rat responds.

Obviously, leptin and NPY are not the whole story. Chronically food-restricted DIO animals quickly regained lost weight with unrestricted feeding in the presence of normal ARC NPY and plasma leptin levels. Similarly, animals with abnormalities in leptin signaling pathways regulate their body weights at higher levels (12). In such cases, other adipose-derived factors or insulin might act as a second, but less effective, line of defense. Leptin, insulin, glucose, and other metabolic signals are sensed by a specialized class of metabolic sensing neurons in the brain (22). In addition to defective leptin signaling, DIO rats also have several defects in central glucosensing mediated by these metabolic sensing neurons (22). Thus DIO rats, like the genetically obese Zucker rat (1, 9, 10, 29, 33), may have a genetically programmed elevation in their threshold for many nutrient and hormonal feedback signals. When sufficient nutrient is readily available, such rats would increase their adipose stores until these signals were raised above that elevated detection threshold. This predicts that such animals (and humans) will regulate and defend their energy homeostasis at such a raised level and could explain the great difficulty encountered in reducing their body weight by dieting.

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