Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats

BARRY E. LEVIN AND AMBROSE A. DUNN-MEYNELL

Neurology Service (127C), Veterans Affairs Medical Center, East Orange 07018; and Department of Neurosciences, New Jersey Medical School, Newark, New Jersey 07103

Levin, Barry E., and Ambrose A. Dunn-Meynell. Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R231–R237, 2000.—Half of Sprague-Dawley rats develop and defend diet-induced obesity (DIO) or diet resistance (DR) when fed a high-energy (HE) diet. Here, adult male rats were made DIO or DR after 10 wk on HE diet. Then half of each group was food restricted for 8 wk on chow to maintain their body weights at 90% of their respective baselines. Rate and magnitude of weight loss were comparable, but maintenance energy intake and the degree of sympathetic activity (24-h urine norepinephrine) inhibition were 17 and 29% lower, respectively, in restricted DR than DIO rats. Restricted DIO rats reduced adipose depot weights, plasma leptin, and insulin levels by 35%. Restricted DR rats reduced none of these. When fed ad libitum, both DR and DIO rats returned to the body weights of their respective chow-fed phenotype controls within 2 wk. This was associated with increased adipose mass and leptin and insulin levels only in DIO rats. Thus DR rats appear to alter primarily their lean body mass, whereas DIO rats primarily alter their adipose mass during chronic caloric restriction and refeeding.

diet-induced obesity; leptin; insulin; carcass fat; sympathetic activity; norepinephrine

THE PREVALENCE OF OBESITY continues to rise, whereas the success rate for the long-term treatment of obesity has remained dismally low at 10–15% (12, 36). There are a number of metabolic factors that tend to drive some postobese individuals back to their previously high body weight. In humans and rodents, caloric restriction can be associated with a reduction in both resting metabolic rate (16, 17) and sympathetic nervous system activity (1, 15, 37). When rats are weight restricted after having been made obese by intake of a variety of palatable and/or high-fat foods, and were designated as chow-fed controls. During the 10th wk on HE diet (week 12), 24-h urine NE collections were repeated (NE2) and caloric intake was assessed. At this time...
There was a clear distinction in body weights among the groups (Fig. 1). All DIO and DR rats were then switched to chow for 2 wk. Beginning week 14, 12 DIO and 12 DR rats were restricted to 50% of their baseline caloric intake on week 12. These rats were designated as “DIO-Restrict” and “DR-Restrict,” respectively. All of the food was given to restricted rats at the beginning of the dark period throughout the entire restriction period. By week 17, body weights in each group had fallen to 86–87% of their baseline on week 14, and food intake was reallocated to maintain a stable body weight at ~90% of their baseline for an additional 5 wk. Half of each group (n = 6/group) was killed at this time, and the remainder of restricted rats was allowed ad libitum access to chow for an additional 6 wk. All remaining rats were killed at week 28 (n = 6/group). Urine for norepinephrine (NE1–5) was collected at the time points shown, and tail blood was drawn for leptin and insulin assays as designated. Points are means ± SE body weight (g).

### RESULTS

Body weight, plasma leptin, and insulin levels and fat pad weights. By 3 wk on HE diet (week 5), body weights in DIO rats were significantly higher than chow-fed and DR rats and remained so for the remainder of the study (Fig. 1). On the other hand, DR rats weighed significantly less than chow-fed rats throughout the weight-gain period. Despite being more than 20% heavier, energy intake in DIO rats (100 ± 11 kcal/day) after 10 wk on HE diet (week 12) was not significantly different from that in DR (91 ± 10 kcal/day) or chow-fed rats (89 ± 10 kcal/day). During the 2-wk period after all rats were switched to chow ad libitum (weeks 13 and 14), body weights reached a plateau in both DIO and DR rats. When restricted on chow to 50% of their week 12 baseline caloric intake of HE diet, both DIO- and DR-Restrict rats rapidly dropped their body weights. By week 17, DIO-Restrict body weights had reached the level of chow-fed controls at 87% of their week 14 baseline weights. DR-Restrict weights decreased to 86% of their week 14 baseline by week 17. Caloric intake was readjusted in both restricted groups (on the basis of group mean body weights) to maintain their mean body weights at 90% of that baseline. This amounted to 94 kcal/day in DIO-Restrict and 78 kcal/day in DR-Restrict rats (P = 0.001). Thus DR-Restrict rats were eating significantly less than all other groups, although intake in DIO-Restrict rats was not significantly different from the other groups [Chow: 100 ± 5 kcal/day; DR: 102 ± 8 kcal/day; DIO: 110 ± 6 kcal/day; F(4,25) = 6.59; P = 0.001]. On week 18, 4 wk after caloric restriction began, leptin levels were significantly lower in both DR and DR-Restrict rats than all other groups and were 61 and 39% of chow-fed controls, respectively [Fig. 2; F(4,25) = 6.83; P = 0.001]. However, there was no significant difference in leptin levels between DR and DR-Restrict rats. Leptin levels in DIO rats were 50% higher than chow-fed controls, whereas DIO-Restrict rats had levels comparable to controls. There was a significant correlation between body weights and plasma leptin levels for both DR and DIO rats, but none was apparent for chow-fed, DR-Restrict, or DIO-Restrict rats (Table 1). Insulin levels in both DR and DR-Restrict rats were
After 8 wk of restricted intake (week 22), six rats in each group were killed. At this time, DIO rats had significantly heavier epidydimal, retroperitoneal, perirenal, mesenteric, and total fat pad weights than all other groups [Fig. 3; total pad weights $F(2,23) = 8.32; P = 0.001$]. DR and DR-Restrict rats had lighter pad weights than both DIO and DIO-Restrict rats at this time, although they were not significantly lower than chow-fed controls. Neither DR nor DR-Restrict rats differed significantly from each other in fat pad weights. During week 23, DIO- and DR-Restrict rats were allowed ad libitum access to chow. Within 2 wk, they increased their body weights to the level of their respective, ad libitum-fed DIO and DR controls and their body weights remained comparable to these groups for the remaining 4 wk of the study (week 28). At this time, both groups of DIO rats were 5% heavier and DR rats were 9% lighter than chow-fed controls $[F(2,23) = 8.32; P = 0.001]$. Despite this relatively small difference in body weight, total fat pad weights were 30% heavier in DIO rats from both groups than those from both groups of DR rats $[F(2,23) = 7.29; P = 0.0004]$. Despite the heavier body and fat pad weights of DIO rats, neither plasma leptin nor insulin levels differed among the five groups. There was, however, a positive correlation between final body weights and plasma leptin levels ($r = 0.59, P = 0.001$) and for body weight vs. insulin levels ($r = 0.43; P = 0.05$) across all five experimental groups.

Table 1. Pearson’s correlations between plasma leptin or insulin levels and body weight at week 18 (restricted intake) and week 28 (final values)

<table>
<thead>
<tr>
<th>Group</th>
<th>Restricted Intake</th>
<th>Final Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leptin r P</td>
<td>Insulin r P</td>
</tr>
<tr>
<td>Chow</td>
<td>0.10 NS 0.24 NS</td>
<td>0.68 0.05 0.41 NS</td>
</tr>
<tr>
<td>DR</td>
<td>0.65 0.05 0.85 0.05</td>
<td>0.69 0.05 0.97 0.01</td>
</tr>
<tr>
<td>DR-R</td>
<td>0.07 NS 0.47 NS</td>
<td>0.21 NS 0.75 0.05</td>
</tr>
<tr>
<td>DIO</td>
<td>0.82 0.01 0.15 NS</td>
<td>0.88 0.02 0.69 0.05</td>
</tr>
<tr>
<td>DIO-R</td>
<td>0.28 NS 0.29 NS</td>
<td>0.91 0.01 0.11 NS</td>
</tr>
</tbody>
</table>

DR, diet resistant; DR-R, diet resistant, restricted intake; DIO, diet-induced obese; DIO-R, diet-induced obese, restricted intake; NS, not significant, r, Pearson’s correlation.
leptin levels for chow-fed, DR, DIO, and DIO-Restrict rats and for insulin in DR, DR-Restrict, and DIO rats (Table 1). Again, there was a positive correlation between body weights and leptin \((r = 0.45, P = 0.009)\) and insulin levels \((r = 0.40; P = 0.05)\) across all experimental groups.

Twenty-four-hour urine NE levels. Figure 4 and Table 2 show 24-h urine NE levels. These collections were made as an indirect measure of sympathetic nervous system activity during the various stages of weight change. Initial NE (NE1) levels were assessed while all rats were fed chow and were 86% higher in DIO-prone than DR and chow-fed controls \([F(2,56) = 3.037; P = 0.05]\). NE2 levels were collected during the 10th week on HE diet for DIO and DR rats. At this point, there were no significant differences among the groups. After 3 wk on 50% caloric restriction (NE3), NE levels fell to 85% of controls in DR-Restrict and to 49% of Chow-fed controls in DIO-Restrict rats \([F(4,24) = 3.69; P = 0.01]\). However, after stabilizing body weights at 90% of their respective baselines for an additional 4 wk, NE levels (NE4) rose in DIO-Restrict rats to the level of Chow-fed controls while DR-Restrict NE levels rose to 125% of controls. After a total of 10 wk back on chow, urine NE levels rose in DIO rats to 152% of Chow-fed controls \([F(4,24) = 4.71; P = 0.006]\). During the final week (week 28), NE levels (NE5) in DIO-Restrict rats reached the level of DIO rats while levels in DR-Restrict rats fell to the level of DR and controls rats \([F(4,24) = 3.71; P = 0.01]\).

**DISCUSSION**

These studies were carried out to examine the way in which DIO rats defend their body weights against chronic food restriction on a low-fat diet as a model of diet therapy in humans. DR rats were examined for comparison to look for differences in the way in which they might defend their body weights under similar circumstances. This was based on the fact that DIO and DR rats are known to differ markedly in their weight gain patterns and metabolic responses to HE diet. Differences in weight gain cannot always be totally explained by differences in energy intake as shown in both previous (22) and current studies. Here, DIO rats had comparable intake to DR rats at the end of their period on HE diet, despite more than a 20% difference in body weight. DIO rats appear to increase their metabolic efficiency fairly soon after exposure to HE diet (22). Conversely, DR rats are selected for their low metabolic efficiency. This probably explains their reduced weight gain on HE diet compared with the Chow-fed controls, which were chosen from the intermediate group of weight gainers on HE diet. These animals represent the low and high end of DIO and DR weight gainers, respectively (20, 21). Differences in body weight gain between DIO and DR rats are primarily due to differences in carcass adiposity, because they have been shown to have similar lean body mass (20, 25, 26). This was supported here by the differences in total adipose depot weights and plasma leptin and insulin levels between DIO and DR rats. The similarity in lean body mass may explain why intakes do not differ between DIO and DR rats.

Not only do weight gain patterns differ between DIO and DR rats but so do weight loss patterns during prolonged caloric restriction. Both DIO and DR rats lost weight at equivalent rates and magnitude when comparably restricted to 50% of baseline intake. However, only DIO-Restrict rats reduced their fat pad weights, plasma leptin, and insulin levels during caloric restriction. Although we did not assess total carcass adiposity, the fat pads weights assessed here do represent a large.

**Table 2. Sequential 24-h urine NE levels**

<table>
<thead>
<tr>
<th>Group</th>
<th>NE1</th>
<th>NE2</th>
<th>NE3</th>
<th>NE4</th>
<th>NE5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>1.09 ± 0.10*</td>
<td>1.93 ± 0.22*</td>
<td>1.90 ± 0.14*</td>
<td>2.94 ± 0.23*</td>
<td>3.32 ± 0.35*</td>
</tr>
<tr>
<td>DR</td>
<td>1.09 ± 0.09*</td>
<td>1.80 ± 0.13*</td>
<td>1.91 ± 0.16*</td>
<td>2.82 ± 0.27*</td>
<td>3.62 ± 0.32*</td>
</tr>
<tr>
<td>DR-R</td>
<td>1.64 ± 0.13†</td>
<td>3.69 ± 0.29†</td>
<td>3.63 ± 0.32†</td>
<td>3.63 ± 0.32†</td>
<td>3.63 ± 0.32†</td>
</tr>
<tr>
<td>DIO</td>
<td>2.03 ± 0.05†</td>
<td>2.08 ± 0.14*</td>
<td>1.94 ± 0.18*</td>
<td>4.47 ± 0.25†</td>
<td>4.92 ± 0.39†</td>
</tr>
<tr>
<td>DIO-R</td>
<td>1.17 ± 0.16†</td>
<td>2.80 ± 0.21*</td>
<td>4.40 ± 0.35†</td>
<td>4.40 ± 0.35†</td>
<td>4.40 ± 0.35†</td>
</tr>
</tbody>
</table>

Data for a given collection period (means ± SE, µg/24 h) are from \(n = 12–24\) per group for NE1–4 and \(n = 6\) per group for NE5. NE1 baseline; NE2, after 10 wk on high-energy (HE) diet; NE3, after 3 wk on restricted chow intake for DIO- and DR-Restrict rats associated with a 15% weight reduction; NE4, after 7 wk of restricted chow intake for DIO- and DIO-Restrict rats kept at 10% of control baseline; NE5, after 3 wk back on ad libitum chow for all rats. Dissimilar superscripts differ from each other by \(P = 0.05\) by post hoc analysis after significant intergroup differences were found by ANOVA.
The apparent sympathetic response to chronic caloric restriction also differed between DIO and DR rats. Here, as in prior studies (18, 20), chow-fed DIO-prone rats had elevated 24-h urine NE levels before the development of DIO on HE diet. This elevation “normalized” with the development of DIO on HE diet and then returned to the prior elevated state after 10 wk back on chow. The DIO-Restrict rats showed a marked and early reduction in sympathetic activity associated with caloric restriction (37). Although this decrease became attenuated with prolonged caloric restriction, it remained relatively reduced compared with ad libitum-fed DIO rats. A similar reduction in sympathetic activity has been described during chronic weight loss in postobese humans (1). But the magnitude and pattern of change in urine NE levels were completely different in DR rats during diet switching and caloric restriction. Urine NE levels in ad libitum-fed DR rats on both chow and HE diet were comparable to chow-fed controls. Importantly, DR rats had only a small decrement in NE levels during the early stages of caloric restriction. After this, NE levels actually increased significantly above chow-fed control levels during prolonged caloric restriction. Thus DIO rats had a much more vigorous inhibition of sympathetic activity during caloric restriction than DR rats. Because maintenance intake requirements in DR rats were still lower than in all other groups during restriction, this suggests a dissonance between sympathetic activity and energy expenditure in restricted DR rats. Interestingly, and perhaps paradoxically, such a dissonance has been described in Pima Indians who have a high propensity to become obese (30). Finally, there was a gradual and progressive increase in urine NE levels in control groups over the course of the experiment, which is unexplained but did not affect the relative relationship of levels among the groups.

These differences in the regulation of sympathetic activity raise the important issue of the mechanism underlying the reduction in sympathetic activity and energy expenditure during caloric restriction. If these were a consequence of decreased lean body mass, then DR-Restrict rats should have had the lowest levels of urine NE. If a decrease in adiposity and leptin levels was responsible, where leptin normally activates the sympathetic nervous system (8, 28), this might explain the lower 24-h urine NE levels in DIO-Restrict rats versus the relatively meager reduction of NE levels in DR-Restrict rats. Although this is consistent with the idea that sympathetic activity and fat mass are related, it seems likely that some other factor common to both regulates the complex interaction between the two. However, this observation does suggest that the greater the loss of carcass fat, the greater the degree of conservation of energy in the form of increased metabolic efficiency.

Finally, and most importantly, both groups of restricted rats returned to the baseline of their respective phenotype controls within 2 wk of their ad libitum access to chow. This return to the higher body weight of the unrestricted DIO rats is similar to the almost inevitable regain of lost weight in most reduced obese humans after dieting is stopped (12). Although the expected correlation between body weight and plasma leptin levels was present in DIO, DIO-Restrict, and DR rats at the end of the study, it was not seen in recovered DR-Restrict rats. This may have been an artifact of the relatively high variance in final plasma leptin levels. For example, there were no significant intergroup differences in final leptin levels, whereas there were significant intergroup differences in total adipose pad weights. This appeared to be due to the large variance in leptin levels, because the relative magnitude of mean leptin levels for each group was almost identical to those for total fat depot weights. This variance aside, it is more likely that the lack of correlation between leptin levels and body weight in DR-Restrict rats reflected the fact that DR animals preferentially spared body fat during restriction. We previously showed (24) that DR rats could be made hyperphagic and obese on a highly palatable diet but that they would not defend this higher body weight when switched to a low-fat, low-palatability chow diet. Those DR rats spontaneously reduced their food intake and body weight to control levels within 2 wk of the switch to chow. But their indexes of carcass adiposity did not fully return to control levels even after 6 wk back on chow. On the other hand, fat mass and body weight declined in parallel in DIO rats restricted to the same intake as the previously obese DR rats (24). Taken together with the present findings, it appears that DIO rats alter their body weight and fat mass in parallel when driven off their metabolically stable defended body weight. This suggests that the DIO rats primarily defend their lean body mass. On the other hand, DR rats appear to alter body weight (and probably lean body mass) first and then fat mass only secondarily. Thus DR rats appear to defend their fat mass more rigorously than their lean body mass. Although it is far from clear what underlies these differences in body weight and carcass composition during weight gain, loss, and regain, further exploration of this question may provide important clues to the effective treatment of obesity.

Perspectives

The major dilemma in the long-term treatment of obesity is overcoming the remarkable resistance
mounted by the body when attempts are made to drive it away from its pathologically elevated body weight and carcass fat mass. When energy supplies are severely limited, this mechanism should confer enhanced survival on the individual. But, the excess availability of food stores in the developed world pushes such individuals inexorably toward ever higher body weights. The DIO rat acts as a reasonable surrogate for the study of this type of obesity. Obviously, human obesity is not homogeneous and the DIO model does not stand for all types of human obesity. For example, DIO rats have elevated sympathetic activity both before (DIO prone) and after they become obese. But obesity-prone Pima Indians have reduced sympathoadrenal function (35). However, there are important commonalities between obese humans and DIO rats, such as their low capacity to oxidize fatty acids (5, 33, 38). Also, reduced energy expenditure and/or sympathetic activity are seen in some weight-reduced obese humans (1, 15–17) and DIO rats (7, 11). Such factors would predispose postobese subjects to regain lost weight.

With the use of urine NE levels as an indirect measure of sympathetic activity, together with the other parameters examined, it is clear that DR rats respond very differently to chronic caloric restriction than do DIO rats. They had little or no reduction in fat mass or sympathetic activity when intake was restricted. This suggests that their weight loss resulted from a reduction in lean body mass. Despite these fairly profound differences in energy homeostasis, both DIO and DR rats lost and regained weight at comparable rates when restricted and refed. But they regained only to the level of their respective chow-fed phenotype controls. This return to these differing, phenotype-dependent body weights is also seen in overfed DR (24) and DIO rats (unpublished observation). But here again, only DIO rats do this by reducing carcass fat. Thus, in both over- and underfeeding situations, DIO rats appear to alter their fat mass, whereas DR rats appear to alter lean body mass. Although the reduction in plasma leptin levels seen in DIO-Restrict rats may be an important signal for spontaneous regain when food is readily available, it is clearly not the only signal for body weight regulation. DR rats regulate their body weight without apparent regard for plasma leptin levels or carcass adiposity. Here they regained lost body weight without ever showing significant reductions in plasma leptin levels during caloric restriction. But when DR rats were made obese and hyperphagic on a palatable diet and were then switched to chow, they spontaneously returned to, and perfectly maintained, the body weight of chow-fed DR controls long before their elevated leptin levels and carcass fat stores returned to that baseline level (24). Even obese Zucker rats, with their defect in leptin signaling (6), make the appropriate metabolic adjustments to caloric restriction (11). Such facts strongly suggest that there are other, as yet unidentified regulatory systems that are used by some animals.

Finally, even the intrinsic resistance of DR rats to obesity can be overcome by prolonged intake of an HE diet that does not cause hyperphagia (21). It may well be that there are similar obesity-resistant individuals within the population of obese humans. These may be the small proportion of obese individuals who have the most robust and successful long-term responses to a variety of obesity therapies. However, for the vast majority of individuals who are intrinsically obesity prone, it is likely that any successful therapy for their obesity will have to lower their defended body weight (27) and/or ameliorate the metabolic defenses provoked by weight reduction.

We thank Karen Brown for technical assistance. This work was funded by National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-30066 and the Medical Research Service of the Department of Veterans Affairs.

Address for reprint requests and other correspondence: B. E. Levin, Neurology Service (127C), VA Medical Center, 385 Tremont Ave., E. Orange, NJ 07018-1095.

Received 2 June 1999; accepted in final form 30 August 1999.

REFERENCES


