Defense of differing body weight set points in diet-induced obese and resistant rats

BARRY E. LEVIN1 AND RICHARD E. KEESEY2

1Neurology Service, Veterans Affairs Medical Center, East Orange 07018, and Department of Neurosciences, New Jersey Medical School, Newark, New Jersey 07103; and 2Psychology Department, University of Wisconsin, Madison, Wisconsin 53706

Levin, Barry E., and Richard E. Keesey. Defense of differing body weight set points in diet-induced obese and resistant rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R412–R419, 1998.—Among outbred Sprague-Dawley rats, approximately one-half develop diet-induced obesity (DIO) and one-half are diet resistant (DR) on a diet relatively high in fat and energy content (HE diet). Here we examined the defense of body weight in these two phenotypes. After HE diet for 13 wk, followed by chow for 6 wk, DR rats gained weight comparably but their plasma leptin levels fell to 54% of chow-fed controls. When a palatable liquid diet (Ensure) was added for 13 wk, other DR rats became obese. But when switched to chow, their weights fell by 60%, and body and retroperitoneal (RP) fat pad weights and plasma leptin and insulin levels all declined for 2 wk and then stabilized at control levels after 6 wk. In contrast, comparably obese DIO rats decreased their intake by only 20%, and their weights plateaued when they were switched to chow after 13 wk on HE diet. When a subgroup of these DIO rats was restricted to 60% of their intake, their weights fell to chow-fed control levels over 2 wk. But their leptin and insulin levels both fell disproportionately to 30% of controls. When no longer restricted, their intake and feed efficiency rose immediately, and their body and RP pad weights and leptin and insulin levels rose to those of unrestricted DIO rats within 2 wk. Thus diet and genetic background interact to establish high (DIO) or low (DR) body weight set points, which are then defended against subsequent changes in diet composition and/or energy availability. If leptin affects energy homeostasis, it does so differentially in DIO vs. DR rats since comparably low and high levels were associated with differing patterns of weight change between the two phenotypes.

METHODS

Animals and experimental protocol. The experiment began with 80 male Sprague-Dawley rats (Charles River), which were kept at 23–24°C on a 12:12-h light-dark cycle. They were brought into the facility at 225–250 g and kept on Purina lab chow (no. 5001) and water ad libitum for 1 wk. Food intake and body weights were then measured for 1 wk on chow before all rats were switched to a HE diet ad libitum. This diet is composed of 8% corn oil, 44% sweetened condensed milk, and 48% Purina rat chow (Research Diets) and 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 (18). Purina rat chow (no. 5001) contains 3.30 kcal/g, with 23% as protein, 12% as fat, and 65% as carbohydrate, which is primarily in the form of complex polysaccharide (18). After 2 wk on the HE diet (week 3), the 30 rats with the highest body weight gain were designated as DIO, and the 33 with the lowest weight gain were designated
as DR. The remaining 17 intermediate weight gainers were switched back to chow and were designated as chow-fed controls. The entire group of 33 DR rats was further subdivided at this time. Sixteen were given ad libitum access to Ensure (Ross Products) in addition to the HE diet (DR-Ensure rats) to produce obese DR rats. Ensure is a liquid diet that contains 1.06 kcal/ml with 14% of the metabolizable energy content as protein, 22% as fat, and 64% as carbohydrate. The remaining 17 DR rats were kept on HE diet (DR-HE diet rats). All rats were then kept on their respective diets until the body weights of the DR-Ensure rats reached those of DIO rats (week 14). This portion of the study is hereafter referred to as the weight gain phase. At the end of this phase (week 14), five or six rats from each of the four groups (chow, DR-HE diet, DR-Ensure, DIO) were killed by decapitation, without fasting, between 0900 and 1000. Their trunk blood was taken for plasma glucose, insulin, and leptin analysis by radioimmunoassays (Linco) using antibodies to authentic rat insulin and leptin, respectively. Samples of trunk blood were collected into heparinized tubes, and the plasma was removed for assay. Glucose was assayed by automated glucose oxidase method (Beckman), and both insulin and leptin were analyzed by radioimmunoassays (Linco) using antibodies to authentic rat insulin and leptin, respectively. Assays of glucose, insulin, and leptin were started on the chow diet, all rats were placed on HE diet. A control group of eight rats killed after 1 wk on chow had body weights of 336 ± 5 g, 1-wk Intakes of 612 ± 8 kcal, RP depots weighing 2.73 ± 0.2 g, plasma glucose levels of 145 ± 3 mg/dl, plasma leptin levels of 3.79 ± 0.47 ng/ml, and plasma insulin levels of 1.52 ± 0.21 ng/ml. These values served as the baseline for comparing subsequent changes in the remaining rats. After the first 2 wk on the HE diet, rats in the upper tertile of weight gain (101 ± 3 g) were designated as DIO and rats in the lower tertile of weight gain (80 ± 2 g; P = 0.001) as DR. The intermediate tertile weight gainers (95 ± 2 g) were placed back on the chow diet at the end of this 2-wk period and subsequently served as chow-fed controls. During the ensuing 12 wk, these chow-fed control rats gained weight progressively (Figs. 1 and 3) while maintaining a relatively stable intake of 623–735 kcal·rat−1·wk−1 (Figs. 2 and 3). During the same period, DR-HE diet rats had similar weight gain, whereas DR-Ensure (Ensure + HE diet) and DIO rats on HE diet both gained 40% more weight than chow-fed controls [Table 1; F(3,76) = 29.94; P = 0.001]. These intergroup differences were significant at each time point from weeks 4 to 14. Similarly, there were significant intergroup differences in energy intake during the weight gain phase [F(3,76) = 33.65; P = 0.001]. Although intake was increased by 7–11% in DR-HE diet rats over the first 3 wk on the HE diet, overall intake was equivalent to chow-fed controls for the entire 13-wk weight gain phase (Figs. 2 and 3; Table 1). DR-Ensure rats, on the other hand, increased their caloric intake to 146% of controls over the first 2 wk of exposure to Ensure and then reached a fairly stable plateau at 110–127% of controls for the remainder of the weight gain phase. Overall, they ingested 14% more energy than chow-fed controls during this period. Approximately 75% of their caloric intake was from the Ensure and the rest was from HE diet. DIO rats increased their intake to 119–125% of controls after 2 wk on HE diet. Then intake remained above control levels through week 11 and was 7% higher than chow-fed controls but 6% lower than DR-Ensure rats during the entire weight gain phase.

At 14 wk, when DIO rats on HE diet and DR-Ensure rats had comparable body weights, they each weighed 14% more [F(3,17) = 5.23; P = 0.01], and their RP pads each weighed 180% more [F(3,17) = 8.83; P = 0.001] than both the chow-fed control and DR-HE diet rats (Table 2, Fig. 3). Even though DR-HE diet rats had body weights comparable to chow-fed controls, their RP pad weights were 17% heavier and their plasma leptin...
levels were 170% higher. In keeping with their comparable body and RP pad weights, DR-Ensure and DIO rats on HE diet had similar plasma leptin levels, and these levels were each 260% greater than chow-fed controls [Table 2; Fig. 3; \( F(3,17) = 5.89, P = 0.007 \)]. Similarly, plasma insulin levels were 70 and 142% greater in DR-Ensure and DIO rats on HE diet, respectively, than chow-fed controls [Table 2; Fig. 3; \( F(3,17) = 5.13; P = 0.011 \)]. Finally, there were no intergroup differences in plasma glucose at the end of the weight gain phase (Table 2).

Weight loss phase (weeks 14–17). At the end of week 14, body weights of DIO rats on HE diet and DR-Ensure rats were equivalent, and all rats were switched to chow ad libitum (Figs. 1 and 3). Over the next 3-wk period, chow-fed controls gained 5% in body weight. In the other groups, the switch from HE diet and/or Ensure to chow was associated with significant intergroup changes in body weight [Table 2; Fig. 3; \( F(4,55) = 12.03, P = 0.001 \)] and energy intake [\( F(4,55) = 25.78; P = 0.001 \)].
DIO-Ad lib rats (Table 2). However, neither RP pad weights nor plasma leptin levels accurately reflected these differences in body weight after weight loss. Even though their body weights were comparable to the DR-HE diet and chow-fed control rats, DR-Ensure rats still had 70 and 48% heavier RP depot weights and 54 and 41% higher plasma leptin levels, respectively (Table 2; Fig. 3). Thus, despite their spontaneous loss of body weight on chow, DR-Ensure rats still had RP pad weights and plasma leptin levels comparable to the DIO-Ad lib rats, which had lost no weight on chow. On the other hand, weight loss brought about by energy restriction in DIO-Restrict rats was associated with a 25% reduction in RP pad weights compared with DIO-Ad lib rats. This restriction-induced weight loss was associated with a fall in plasma leptin levels to 53% of chow-fed controls and 33% of DIO-Ad lib rats [F(4,26) = 6.53; P = 0.001]. Also, plasma insulin levels in DIO-Restrict rats fell significantly below those in all of the other groups [F(4,26) = 7.86; P = 0.001]. Again, there were no significant differences in plasma glucose levels among the groups.

Weight regain phase (weeks 17–20). Once the body weights of DIO-Restrict rats reached those of DR-HE diet, DR-Ensure, and chow-fed control rats at week 17, the DIO-Restrict rats were given ad libitum access to chow. There was an immediate rebound in both their body weights (Figs. 1 and 3) and energy intakes (Figs. 2 and 3). They increased their energy intake by 49% and their body weights rose by 9% over the first week on unrestricted intake. This increase in body weight was not completely attributable to increased energy intake. When feed efficiency was calculated as the amount of “metabolic body mass” [body weight (kg^{0.75})] gained per kilocalorie of energy ingested, feed efficiency in DIO-Restrict rats was up to threefold greater than any other group during this period [DIO-Restrict: 14.2 ± 0.1; Chow-fed: 5.0 ± 0.0; DR-HE diet: 3.6 ± 0.1; DR-Ensure: 0.3 ± 0.0; DIO-Ad lib: 5.2 ± 0.1 kg^{0.75}/kcal × 10^{-5}/wk; F(4,24) = 18.51; P = 0.001]. For the remaining 2 wk of

![Figure 3](http://ajpregu.physiology.org/figures/AJ/ajrhe/ajrhe1911f3.png)
Data are means ± SE, n = no. of rats. Rats were fed chow or high in fat and energy (HE) diet alone (diet resistant [DR] HE diet, diet-induced obesity [DIO]) or HE diet ± Ensure (DR-Ensure) for 13 wk (phase 1). All rats were then switched to chow. DR-Ensure rats voluntarily restricted their energy intake by 60% and intake of the DIO-Restrict rats was paired to DR-Ensure intake until their body weights matched those of chow-fed controls (phase 2). Then DIO-Restrict rats were given ad libitum access to chow until their body weights matched those of the DIO-Ad lib group (phase 3). In a given data set, values with different superscripts in each data subset are significantly different at P < 0.05 by post hoc test where significant intergroup differences were found by ANOVA.

Table 2. Body and RP fat pad weights and plasma glucose, insulin, and leptin levels during weight gain, loss, and regain

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>RP Wt, g</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
<th>Leptin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chow</td>
<td>556 ± 12a</td>
<td>7.8 ± 1.7a</td>
<td>149 ± 3a</td>
<td>1.78 ± 0.17a</td>
<td>5.4 ± 1.2a</td>
</tr>
<tr>
<td>DR-HE diet</td>
<td>548 ± 20a</td>
<td>12.9 ± 1.9a</td>
<td>150 ± 6a</td>
<td>2.33 ± 0.12a</td>
<td>14.6 ± 1.4b</td>
</tr>
<tr>
<td>DR-Ensure</td>
<td>636 ± 25b</td>
<td>21.7 ± 3.6b</td>
<td>155 ± 4b</td>
<td>4.31 ± 0.41b</td>
<td>19.3 ± 5.0c</td>
</tr>
<tr>
<td>Loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chow</td>
<td>575 ± 12a</td>
<td>11.5 ± 1.6a</td>
<td>143 ± 3a</td>
<td>2.47 ± 0.13a</td>
<td>10.2 ± 2.4a</td>
</tr>
<tr>
<td>DR-HE diet</td>
<td>559 ± 13a</td>
<td>13.2 ± 1.8a</td>
<td>140 ± 6a</td>
<td>2.28 ± 0.25a</td>
<td>11.1 ± 1.9a</td>
</tr>
<tr>
<td>DR-Ensure</td>
<td>582 ± 17a</td>
<td>19.6 ± 1.3b</td>
<td>141 ± 5a</td>
<td>2.35 ± 0.23a</td>
<td>15.7 ± 2.1b</td>
</tr>
<tr>
<td>DIO-Ad lib</td>
<td>637 ± 19b</td>
<td>18.7 ± 1.4b</td>
<td>147 ± 3a</td>
<td>3.76 ± 0.32a</td>
<td>16.0 ± 1.4b</td>
</tr>
<tr>
<td>DIO-Restrict</td>
<td>580 ± 16b</td>
<td>13.9 ± 1.4b</td>
<td>145 ± 2a</td>
<td>1.17 ± 0.12a</td>
<td>5.4 ± 0.7c</td>
</tr>
<tr>
<td>Regain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chow</td>
<td>601 ± 12a</td>
<td>13.8 ± 1.8a</td>
<td>159 ± 5a</td>
<td>2.25 ± 0.19a</td>
<td>14.2 ± 1.9ab</td>
</tr>
<tr>
<td>DR-HE diet</td>
<td>582 ± 14a</td>
<td>9.6 ± 1.2b</td>
<td>155 ± 3a</td>
<td>1.98 ± 0.20a</td>
<td>7.7 ± 1.3c</td>
</tr>
<tr>
<td>DR-Ensure</td>
<td>579 ± 13b</td>
<td>17.1 ± 1.9a</td>
<td>149 ± 5a</td>
<td>1.74 ± 0.19a</td>
<td>10.9 ± 1.6b</td>
</tr>
<tr>
<td>DIO-Ad lib</td>
<td>665 ± 20b</td>
<td>18.9 ± 1.9b</td>
<td>156 ± 6a</td>
<td>3.85 ± 0.34a</td>
<td>17.0 ± 1.5a</td>
</tr>
<tr>
<td>DIO-Restrict</td>
<td>655 ± 19b</td>
<td>19.0 ± 1.7b</td>
<td>153 ± 3a</td>
<td>3.14 ± 0.34a</td>
<td>14.2 ± 2.9b</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rats were fed chow or HE diet alone (DR-HE diet, DIO) or HE diet ± Ensure (DR-Ensure) for 12 wk (phase 1; n = 5 or 6 rats/group). All rats were then switched to chow. DR-Ensure rats voluntarily restricted their energy intake by 60%, and intake of the DIO-Restrict rats was paired to DR-Ensure intake. Then rats (n = 5 or 6 per group) were killed when their body weights matched those of chow-fed controls (phase 2). Then DIO-Restrict rats were given ad libitum access to chow until their body weights matched the DIO-Ad lib group, and the remaining rats were killed (phase 3; n = 5 or 6 per group), RP, retroperitoneal fat pad. In a given data set, values with different superscripts in each data subset are significantly different at P < 0.05 by post hoc test where significant intergroup differences were found by ANOVA.
Terminal insulin levels also showed significant intergroup differences [Table 2; F(4,24) = 5.65; P = 0.003] with highest levels in DIO-Ad lib and DIO-Restrict rats. Again, there were no differences in plasma glucose levels.

Finally, analysis across all groups for the entire 20-wk experimental period revealed significant correlations among various parameters taken at the end of each phase. The highest correlation was seen between RP pad weights and plasma leptin levels (r = 0.84; P = 0.001). There were smaller correlations between RP pad and body weights (r = 0.71; P = 0.001), leptin and insulin levels (r = 0.65; P = 0.001), and between RP pad weights and plasma insulin levels (r = 0.55; P = 0.001).

**DISCUSSION**

Expression of the DIO and DR phenotypes represents the interface of environmental factors with genetic background. We have shown that the propensity of outbred Sprague-Dawley rats to become DIO or DR is expressed only when they are exposed to a diet relatively high in fat, energy, and sucrose content (17, 18, 19, 20). However, once the metabolic and physiological manifestations of these weight gain phenotypes are fully expressed, they appear to become relatively fixed (13, 18, 20). A strong genetic component underlying this interaction is suggested by the ease with which the two phenotypes can be selectively inbred to produce DIO and DR substrains, which then breed true to these weight gain phenotypes (16).

The current study addresses the defense of DIO and DR body weight phenotypes subsequent to their having been established on exposure to an HE diet. Here we have examined the response to overfeeding DR and underfeeding DIO rats. We specifically have not examined underfeeding normal weight DR rats or overfeeding already obese DIO rats. Nevertheless, the present study supports the contention that differential propensities for adjusting the body weight set point are unmasked when dietary composition and genetic background are allowed to interact. As found here and in previous studies (17), DR rats eventually do develop excess carcass fat when chronically fed a diet that contains increased fat content. Here, this increased adiposity was indicated by elevated RP pad weights (28) and was associated with a parallel increase in plasma leptin levels in the DR-HE diet rats. In addition, DR rats were made frankly obese by exposure to a highly palatable liquid diet that contained a moderate level of fat. However, although these obese DR rats were comparable to DIO rats in body and RP pad weights, plasma insulin, leptin, and glucose levels, switching their diet to chow caused them to spontaneously lower their energy intake sufficiently for all of these parameters to fall to the level of chow-fed controls. Even the DR rats fed HE diet spontaneously reduced their intake and body weight when switched to chow. Surprisingly, this was associated with a disproportionate reduction in RP pad weights and plasma leptin levels that were far in excess of the loss of body weight. Previous studies suggests that this fall in leptin was probably not due simply to a change in dietary composition (30). Although lowered leptin levels might be expected to stimulate increased energy intake and weight gain (1, 23), DR rats showed no such response. This suggests that DR rats are inherently resistant to diet-induced elevations in the set point for regulated body weight.

On the other hand, the DIO rat appears to be intrinsically sensitive to the influences diet can exert on the body weight set point. When rats are fed HE diet for 3 mo or more, their increased body weight and carcass fat persist for up to 3–4 mo after they are switched to chow (13, 18). Here we showed that forcibly lowering their body weights by restricting energy intake caused them to lose body weight and carcass fat at a rate comparable to obese DR rats. This ease of losing weight has been noted previously in obese rats (9, 22). Similar to DR HE-diet rats switched to chow, the restricted DIO rats showed an inordinate reduction in plasma leptin. Insulin levels were also comparably lowered. This disproportionate reduction in leptin levels relative to the loss of adipose mass has been well documented during weight loss in both rodents (8) and humans (30). As seen in studies where DIO was produced with other diets (9, 22), our weight-reduced DIO rats spontaneously and rapidly regained their lost body weight and fat. Increased energy intake was definitely a component of this regain. But the three- to fourfold increase in calculated feed efficiency in the now free-feeding DIO-Restrict rats over this period suggests that they had also become metabolically more efficient. This is in keeping with the reduced energy expenditure that accompanies both forced weight loss (4) and spontaneous regain (5). Here, reduced leptin and/or insulin levels might be stimulants for this regain. Since leptin levels fall during starvation (1), the primary role of leptin might be as a signal to the brain to increase intake and reduce expenditure in the face of lowered body energy stores (23). This might be more important in the regulation of energy than are the raised levels seen in obesity where the excess levels do not appear to be sensed appropriately by the brain (3, 24, 26). Regardless of its primary function, these studies make it clear that any role leptin (and/or insulin) might play in the regulation of energy balance are highly dependent on the intrinsic set point for regulated body weight of the animal.

In summary, this study presents new findings to show that, once rats of the DIO phenotype have expressed their propensity for obesity on the appropriate diet, they must be forcibly restricted to lower their body weights to control levels, even on a chow diet. Furthermore, they will then rapidly regain their lost weight when allowed free access to this chow diet. DR rats, on the other hand, can be made obese on palatable high-energy diets but readily return to a state of lowered carcass adiposity on a low-fat chow diet. This study thus represents a naturalistic example of defense of regulated body weight in a rodent model of DIO where environmental factors interact with genetic background to redefine the set point for the body’s energy...
homeostasis. What, if any, role leptin might play in this body weight regulation remains in question. Despite comparably high leptin levels at the end of the weight gain phase, only obese DR-Ensure rats spontaneously lowered their energy intake and lost weight back to control levels. On the other hand, during the weight loss and regain phases, both DR-HE diet and DIO-Restraint rats had comparably low levels of plasma leptin but only DIO rats overate and increased their metabolic efficiency and body and RP pad weights when given ad libitum access to chow. Despite their lack of weight gain on chow, DR-HE diet rats will gain excessive weight and carcass fat if reexposed to HE diet for a second time (13). Thus their lower leptin levels might predispose them to gain excess weight only if the fat content of their diet is increased. Thus any hypothesis regarding a causal link between plasma leptin levels and the regulation of body weight and energy homeostasis must take into account these differential responses of DIO vs. DR rats in the face of virtually identical high and low plasma levels. This might be related to differences in blood-brain barrier transport of leptin or sensitivity of central leptin receptors and/or the efficacy of efferent pathways responding to leptin as potential regulators of energy intake and expenditure (2, 3, 24, 26, 29).

Perspectives

The DIO model represents the crossroads of nature and nurture. Diet composition and genetic background interact to unmask underlying weight gain phenotypes, which are only manifest if rats are exposed to a HE diet. Once established, these higher set points for regulated body weight appear to be irrevocable (13, 18). The effects of either overfeeding DR rats to the point of obesity or restricting the intake of DIO rats so as to lower their weight to the level of DR rats are quickly reversed when ad libitum feeding of a low-fat chow diet is restored. The latter is reminiscent of obese humans subjected to low-calorie diet therapy (27). It lends further support to the idea that treatment failures are due to an elevated body weight set point. The location of the site at which this hypothetical set point is mediated remains controversial. Given previous work in the DIO model (7, 15, 19, 21, 31) and the striking changes in set point brought about by hypothalamic lesions (10), the brain seems a logical choice as the site for such a set point. A role for leptin is supported by the fact that it appears to cross the blood-brain barrier by carrier-mediated transport (2, 24) and there interacts with receptors through which it may reduce energy intake and body weight (23, 25). But the current studies suggest that underlying weight gain phenotype might be a critical factor in determining the response to leptin. In most obese humans, it is questionable whether high levels of plasma leptin are effective in regulating body weight since central transport of leptin appears to be markedly reduced in the face of high plasma levels (3, 24). Also, genetically obese rats have a relative central leptin insensitivity (26). On the other hand, leptin may be more important as a centrally directed signal of low body adipose and/or energy stores (1). It is also possible that plasma leptin levels may, under certain circumstances, simply reflect alterations in energy and/or metabolite flux (11, 12). It seems self-evident that these issues should be resolved before exogenous leptin is used to treat obese individuals, who clearly already have an excess of leptin in their circulation (3, 24).

The authors thank Elizabeth Govek and Karen Brown for expert technical assistance.

This work was funded by the National Institute of Diabetes and Digestive and Kidney Diseases and the Research Service of the Department of Veterans Affairs.

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

Received 22 July 1997; accepted in final form 1 October 1997.

REFERENCES


