Ontogeny of diet-induced obesity in selectively bred Sprague-Dawley rats

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Ricci, Matthew R., and Barry E. Levin. Ontogeny of diet-induced obesity in selectively bred Sprague-Dawley rats. Am J Physiol Regul Integr Comp Physiol 285: R610–R618, 2003—Outbred Sprague-Dawley rats selectively bred for their propensity to develop diet-induced obesity (DIO) become heavier on low-fat diet than those bred to be diet resistant (DR) beginning at ~5 wk of age. Here we assessed the development of metabolic and neural functions for insights into the origins of their greater weight gain. From week 5 to week 10, chow-fed DIO rats gained 15% more body weight and ate ~14% more calories but had only slightly greater adiposity and plasma leptin than DR rats. From day 3 through week 10, DIO and DR rats had similar mRNA expression of arcuate nucleus neuropeptide Y, proopiomelanocortin, agouti-related peptide, and all splice variants of the leptin receptor (OB-R). When fed a high-energy (HE; 31% fat) diet, 7-wk-old DIO rats had a 240% increase in plasma leptin levels after only 3 days. Despite this early leptin rise, they maintained a persistent hyperphagia and became more obese than chow-fed DIO rats and DR rats fed chow or HE diet. Their failure to reduce caloric intake, despite high levels of leptin, suggests that selectively bred DIO rats might have reduced leptin sensitivity similar to that seen in the outbred DIO parent strain.

leptin receptor; neuropeptide Y; proopiomelanocortin; agouti-related peptide; arcuate nucleus

RODENT MODELS OF OBESITY are valuable tools for studying the underlying factors that contribute to the initiation and maintenance of the obese state in humans. The model of diet-induced obesity (DIO) in rodents is particularly suited to this task as DIO rats share a number of traits with human obesity. These include polygenic inheritance (28, 36), insulin resistance (28, 34), hyperleptinemia (9, 31), lowered growth hormone secretion (4, 19), proclivity to preferentially oxidize carbohydrate over fat (8, 17), and the ability to decrease metabolic rate when calorically restricted (10, 20), leading to weight regain after restriction (21, 23). Moreover, the recent and rapid increase in obesity in developed countries points to the important interaction between genes that predispose to obesity and an environment that facilitates expression of the obese phenotype, another trait shared with DIO rodent models (6, 30).

In outbred Sprague-Dawley rats fed a 31% fat [high energy (HE)] diet, about one-half develop DIO, while the rest are resistant to obesity and gain no more weight than chow-fed controls (diet resistant, DR) (27). Adult outbred DIO-prone rats overexpress neuropeptide Y (NPY) in the arcuate nucleus (ARC) of the hypothalamus relative to DR-prone rats (22). Once the DIO genotype is achieved after exposure to HE diet, ARC NPY becomes “normalized” or even lower in DIO compared with DR rats (23, 27). This suggests that elevated NPY predisposes DIO rats to gain weight when the energy density of the diet is increased.

While this outbred model is useful for studying adult-onset obesity, it is less useful for examining the early developmental aspects of DIO (30). To address this issue, we selectively bred rats to express either the DIO or DR traits with 100% penetrance (28, 29). Unlike outbred rats, 10-wk-old selectively bred DIO rats weigh more and consume more calories than DR rats even on chow diet (26). Exposure to HE diet for 2 wk exacerbates the weight gain and energy intake differences, and selectively bred DIO rats become more energy efficient and have higher adiposity and plasma insulin levels than selectively bred DR rats (26). Relative to selectively bred DR rats, adult selectively bred DIO rats also underexpress ARC NPY when they become obese (23). This reduced ARC NPY expression is also seen when outbred DIO rats become fully obese after 3 mo on HE diet (22).

Because the data on the early development of these selectively bred DIO and DR substrains are sparse, the present studies were performed to characterize the early development of these rats with regard to weight gain, body composition, and hypothalamic neuropeptide expression. One goal was to determine the developmental period at which body weight and energy intake first differed between the genotypes and to determine if any alterations in NPY, proopiomelanocortin (POMC), agouti-related peptide (AgRP), and leptin receptor (OB-R) mRNA expression might predate these changes. A second aim of this study was to examine the early body weight, energy intake, and plasma leptin response to HE diet in these selectively bred substrains.

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METHODS

Animals and Diets

Animal usage was in compliance with the animal care committee of the E. Orange Veterans Affairs Medical Center and the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society (2). Male selectively bred DIO and DR rats raised in our own vivarium were used (26). To reduce potential effects of litter size on body weight gain, litters were culled to n = 10 on postnatal day 2 (P2). Pups were weaned onto Purina 5001 rodent chow and water ad libitum on P21. All rats were housed at 23−24°C on a 12:12-h light-dark cycle (lights off at 1800).

Ontogeny Study

Animals from both selectively bred genotypes were killed from P2 to P70. Pups were removed from litters at P2, P7, P10, P14, and P21 for various terminal measurements. At each period, pups from three unrelated litters were used. The pups remaining in the litters were not used for experimental purposes. During the postweaning period, animals were singly housed for food intake measurements (powdered Purina 5001), and animals were killed at P28, P35, P42, P49, P56, P63, and P70.

During the preweaning period, a test of independent ingestion was performed to determine if DIO-prone rats display hyperphagia before chow or HE diet. This test has been used to identify adultlike ingestive behavior in preweaning Zucker fa/fa rat pups (15). Pups (3–4 from the same litter) were removed from the dam at ~0800 and placed in an incubator at 37°C for 4 h. Just before the test, pups were voided by gently brushing the anogenital region with a soft cotton-tipped applicator and weighed to the nearest 0.01 g. Pups were then placed in a beaker containing a Kimwipe soaked with warm, commercially available half-and-half milk cream. After 20 min, pups were removed, dried, and weighed again. The difference in body weight was used to calculate food intake.

Terminally, all animals were weighed and killed between 0800 and 1100 by decapitation. Trunk blood was collected for measurement of plasma leptin and insulin. Brains were rapidly dissected and immediately frozen on powdered dry ice and stored at −80°C for later sectioning. During the postweaning period, the perirenal, retroperitoneal, mesenteric, and epididymal fat pads were dissected and weighed to the nearest 0.01 g. The gastrointestinal tract contents were removed, and all fat pads were returned to the carcass. All carcasses were frozen at −80°C for later compositional analysis.

HE Diet/Chow Study

On P42, a separate group of selectively bred DIO (n = 11) and DR (n = 19) rats was singly housed for 1 wk and given powdered chow. On P49, DIO and DR rats were randomly selected to remain on powdered chow or were switched to powdered HE diet for 14 days. The HE diet is composed of 8% corn oil, 44% sweetened condensed milk, and 48% Purina 5001 (Research Diets, C11024) and has an energy density of 4.47 kcal/g with 21% of the metabolizable energy as protein, 31% as fat, and 48% as carbohydrate, 50% of which is sucrose. Food intake and body weight were measured after 1, 2, 3, 7, 10, and 14 days on diet. Tail blood samples (0.5 ml) were taken after 0, 3, 7, 10, and 14 days on diet. Because no direct measure of adiposity was made in this original study, a second set of selectively bred P42 DIO and DR rats was fed either chow or HE diet for 14 days (n = 8/group). Terminally, their epididymal, retroperitoneal, perirenal, and mesenteric fat pad and body weights were obtained.

Plasma Leptin and Insulin Levels

Blood was collected in heparin-coated tubes, and the plasma was assayed by RIA for leptin and insulin (Linco).

Carcass Composition

Analyses were performed as previously described (5). Samples of the ground carcass were sequentially analyzed for water, fat, and ash content. Independent samples were taken for nitrogen analysis. Water content was determined by desiccation. The desiccated sample was analyzed for fat content by extraction in a Soxhlet apparatus, with ethyl ether as the solvent. The defatted samples were combusted in a muffle furnace at 810°C to assess residual ash content. Nitrogen was determined by Kjeldahl analysis on a separate sample. Body protein was calculated from nitrogen wherein protein = nitrogen × 6.25.

In Situ Hybridization

Serial 15-μm sections were taken through the rostralcaudal extent of the arcuate (ARC) hypothalamic nuclei. Sections were processed for in situ hybridization by minor modifications of previously described methods (23). Frozen sections of brain were freeze thawed onto gel-coated slides and fixed in 4% paraformaldehyde. The slides were treated with acetic anhydride for 10 min and dehydrated through six steps of graded ethanol solutions and frozen at −80°C until use. In situ hybridization was carried out as previously described (24). Briefly, cRNA was synthesized and radiolabeled from probes for NPY (511 bp), POMC (923 bp), AgRP (348 bp), and a probe that recognizes all splice variants of the leptin receptor (OB-R; 3,559 bp). The probes were hydrolyzed in 0.5 M NaHCO3 for 15−30 min. The probes were then subjected to our standard method for in situ hybridization (23). On completion of hybridization, slides were opposed to SB-5 X-ray film (Kodak, Rochester, NY) for 24−48 h (NPY, POMC, AgRP) or for ~3 wk (OB-R). The resulting autoradiograms were read by an experimentally “blinded” observer using computer-assisted densitometry (Drexel University, Philadelphia, PA). Areal and optical density measures were made through the entire rostralcaudal extent of the hypothalamus. Because the product of optical density and area did not alter the results, the data are presented as area alone.

Statistics

Parameters were compared between DIO and DR groups over time by one-way ANOVA. Where significant intergroup differences were found at a given time point, data were compared with post hoc Bonferroni comparisons. Data for the study assessing responses to chow vs. HE diet were assessed using two-way ANOVA with repeated measures. In addition to direct carcass composition, we calculated fat pad index by dividing the sum of four fat pads (retroperitoneal, perirenal, mesenteric, and epididymal) by body weight. There is very good agreement between the sum of these four fat pads and the total grams of fat as determined by carcass analysis (correlation coefficient r = 0.98). Feed efficiency was estimated by dividing the presumed gain in metabolic mass (body wt in kg0.75) by the number of calories ingested over the same period of observation.
RESULTS

Ontogeny Study

Body weight, adiposity, plasma leptin, and insulin. Although body weights of DIO and DR rats were similar before weaning, DIO rats became significantly heavier than DR rats beginning at P35, or 2 wk postweaning (Table 1). They remained ~15% heavier for the remainder of the study period. Generally speaking, the increase in body weight of DIO compared with DR rats was due to a proportional increase in both carcass protein and fat content. Over the entire period of assessment, DIO rats had greater carcass protein content than DR rats (Table 1; F(1,74) = 98.13; P = 0.001). While DIO rats did not have heavier body weights than DR rats until P35, they already had 8% more total carcass mass by age of DIO rats generally had 14–16% more carcass protein than DR rats from day 42 to day 70. At no time did the relative amount (%) of carcass protein differ significantly between DIO and DR rats. DIO rats were fatter than DR rats when carcass fat was considered as a percentage of total carcass weight (6.3 vs. 6.0%; F(1,174) = 8.8; P = 0.003). Even so, post hoc analysis revealed significance only on P7, and they never had more total carcass fat mass than DR rats. In addition, four fat pad weights (retroperitoneal, perirenal, mesenteric, and epididymal) were recorded in the postweaning period, and the sum of these weights was used to calculate a fat pad index (Table 2). During the postweaning period, DIO rats had greater overall adiposity as a function of age of total carcass weight (F(1,114) = 23.9; P < 0.001), although there were no significant differences at any selected time point by post hoc analysis for this measure of adiposity.

Plasma leptin levels were consistently detectable from P10 and on. In general agreement with the measures of adiposity, DIO rats had slightly increased plasma leptin levels overall (1.69 ± 0.12 vs. 1.22 ± 0.07 ng/ml; F(1,164) = 19.6; P < 0.001). However, post hoc testing showed higher levels in DIO rats only at P56

Table 1. Body weight, carcass composition (fat, protein, and water) on a relative (%) and absolute (g) basis, and plasma leptin in selectively bred DR and DIO rats on chow

<table>
<thead>
<tr>
<th>Age, days</th>
<th>2</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
<th>56</th>
<th>63</th>
<th>70</th>
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<tbody>
<tr>
<td>Body wt, g</td>
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</tr>
<tr>
<td>DIO</td>
<td>6.7 ± 0.2</td>
<td>15.1 ± 0.4</td>
<td>19.0 ± 0.5</td>
<td>26.5 ± 0.7</td>
<td>39.3 ± 1.2</td>
<td>76.9 ± 2.4</td>
<td>125.2 ± 2†</td>
<td>215 ± 5†</td>
<td>226 ± 8†</td>
<td>313 ± 9†</td>
<td>350 ± 14†</td>
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<tr>
<td>DR</td>
<td>7.0 ± 0.1</td>
<td>13.5 ± 0.3</td>
<td>18.5 ± 0.4</td>
<td>26.9 ± 0.5</td>
<td>38.3 ± 0.9</td>
<td>58.0 ± 2.7</td>
<td>99.5 ± 4.0</td>
<td>152.4 ± 10</td>
<td>197 ± 4</td>
<td>245 ± 8</td>
<td>291 ± 5</td>
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<tr>
<td>Fat, g</td>
<td></td>
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<tr>
<td>DIO</td>
<td>0.2 ± 0.1</td>
<td>0.16 ± 0.1</td>
<td>0.15 ± 0.1</td>
<td>0.20 ± 0.1</td>
<td>0.36 ± 0.2</td>
<td>6.5 ± 0.0</td>
<td>9.6 ± 0.4</td>
<td>12.9 ± 0.5</td>
<td>22.3 ± 12</td>
<td>24.8 ± 21</td>
<td>26.2 ± 1.2</td>
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<tr>
<td>DR</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>3.0 ± 0.3</td>
<td>8.0 ± 0.3</td>
<td>15.7 ± 12</td>
<td>19.1 ± 9</td>
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<tr>
<td>Protein, g</td>
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<tr>
<td>DIO</td>
<td>0.17 ± 0.05</td>
<td>0.5 ± 0.02</td>
<td>0.7 ± 0.02</td>
<td>1.3 ± 0.01</td>
<td>1.9 ± 0.01</td>
<td>2.0 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>3.0 ± 0.3</td>
<td>8.0 ± 0.3</td>
<td>15.7 ± 12</td>
<td>19.1 ± 9</td>
</tr>
<tr>
<td>DR</td>
<td>0.16 ± 0.05</td>
<td>0.5 ± 0.02</td>
<td>0.7 ± 0.02</td>
<td>1.3 ± 0.01</td>
<td>1.9 ± 0.01</td>
<td>2.0 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>3.0 ± 0.3</td>
<td>8.0 ± 0.3</td>
<td>15.7 ± 12</td>
<td>19.1 ± 9</td>
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<tr>
<td>Fat pad index</td>
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</tr>
<tr>
<td>DIO</td>
<td>0.1 ± 0.02</td>
<td>0.2 ± 0.01</td>
<td>0.3 ± 0.01</td>
<td>0.5 ± 0.02</td>
<td>0.7 ± 0.02</td>
<td>1.2 ± 0.01</td>
<td>1.8 ± 0.02</td>
<td>2.0 ± 0.03</td>
<td>3.0 ± 0.05</td>
<td>4.0 ± 0.07</td>
<td>5.0 ± 0.09</td>
</tr>
<tr>
<td>DR</td>
<td>0.1 ± 0.02</td>
<td>0.2 ± 0.01</td>
<td>0.3 ± 0.01</td>
<td>0.5 ± 0.02</td>
<td>0.7 ± 0.02</td>
<td>1.2 ± 0.01</td>
<td>1.8 ± 0.02</td>
<td>2.0 ± 0.03</td>
<td>3.0 ± 0.05</td>
<td>4.0 ± 0.07</td>
<td>5.0 ± 0.09</td>
</tr>
</tbody>
</table>

Data are means ± SE; nos. in parentheses are no. of rats. Groups of selectively bred diet-induced obesity (DIO) and diet-resistant (DR) rats were observed from 2 to 60 days of age. Rats were weaned onto chow on day 21, and groups were killed on the observation days shown.

†Significantly different (P < 0.05) from DR group overall by ANOVA. ††Significantly different (P < 0.05) from DR group at corresponding age by post hoc t-test after 2-way ANOVA showed significant intergroup differences. ND, not determined.
rats during the postweaning period (F during the postweaning period (fi were slightly more feed ef

Energy intake and feed efficiency. On average, DIO rats consumed ~14% more calories per week than DR rats during the postweaning period ($F_{1,114} = 72.1; P < 0.001$; Table 2). However, post hoc testing showed that DIO rats consumed 18 and 13% more calories than DR rats only during weeks 7–8 (P49–P55) and weeks 8–9 (P56–P62), respectively ($P < 0.005$). While DIO rats were slightly more feed efficient (+7%) than DR rats during the postweaning period ($F_{1,114} = 3.96; P = 0.05$), there were no significant differences between the genotypes at any given week (Table 2).

To determine whether the hyperphagia that selectively bred DIO rats display in response to a high-energy diet as adults (26) was present during the preweaning period, we employed a model of ingestion independent of the dam (13). The intake of half-and-half was measured over 20 min in thermoneutral pups who were deprived of the dam (but not siblings) for 4 h. Because independent ingestion behavior has more in common with adult ingestive behavior than with suckling (15), this method can be used to identify early hyperphagia of adultlike eating behaviors (15). Selectively bred DIO pups did not ingest more than selectively bred DR pups at any age studied (Fig. 1). Interestingly, selectively bred DR pups were hyperphagic relative to selectively bred DIO pups on P14 (genotype × age interaction; $F_{4,159} = 2.9; P = 0.02$; post hoc at P14; $P = 0.03$).

Hypothalamic expression of NPY, POMC, AgRP, and OB-R mRNA. Given the early postweaning increases in body weight and energy intake in DIO rats, we examined whether alterations in gene expression of key hypothalamic neuropeptides or receptors might predate, and therefore possibly play a causal role in, these phenotypic differences. By hybridization, ARC NPY, POMC, or AgRP mRNA expression was no

![Fig. 1. Intake (% of initial body wt) of half-and-half milk cream in a 20-min test of ingestion independent of the dam. Selectively bred diet-induced obesity (DIO; n = 8–27) and diet-resistant (DR; n = 7–26) rat pups were deprived of the dam (but not siblings) for 4 h before testing. Data are means ± SE. *$P < 0.05$ when postnatal day 14 (P14) DIO rats were compared with P10 DIO rats. †$P < 0.05$ when P14 DR rats were compared with P14 DIO rats.](http://ajpregu.physiology.org/)

Table 2. Postweaning fat pad index, plasma insulin, energy intake, and feed efficiency in selectively bred DR and DIO rats on chow

<table>
<thead>
<tr>
<th>Age, days</th>
<th>28</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>56</th>
<th>63</th>
<th>70</th>
</tr>
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<tbody>
<tr>
<td>FatFP, %</td>
<td></td>
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<tr>
<td>DIO*</td>
<td>1.1 ± 0.05 (12)</td>
<td>1.1 ± 0.03 (11)</td>
<td>1.2 ± 0.05 (10)</td>
<td>1.5 ± 0.06 (12)</td>
<td>2.1 ± 0.1 (5)</td>
<td>2.3 ± 0.2 (7)</td>
<td>2.7 ± 0.2 (8)</td>
</tr>
<tr>
<td>DR</td>
<td>0.8 ± 0.03 (10)</td>
<td>1.2 ± 0.5 (12)</td>
<td>1.1 ± 0.04 (10)</td>
<td>1.3 ± 0.4 (10)</td>
<td>1.9 ± 0.1 (7)</td>
<td>2.0 ± 0.09 (9)</td>
<td>2.3 ± 0.2 (5)</td>
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<tr>
<td>Insulin, ng/ml</td>
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<tr>
<td>DIO</td>
<td>0.1 ± 0.02 (12)</td>
<td>0.3 ± 0.04 (11)</td>
<td>0.5 ± 0.2 (9)</td>
<td>1.7 ± 0.3 (12)</td>
<td>2.0 ± 0.3 (5)</td>
<td>1.4 ± 0.2 (7)</td>
<td>1.2 ± 0.2 (8)</td>
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<tr>
<td>DR</td>
<td>0.2 ± 0.04 (9)</td>
<td>0.2 ± 0.07 (10)</td>
<td>0.4 ± 0.07 (12)</td>
<td>1.9 ± 0.3 (10)</td>
<td>1.2 ± 0.2 (7)</td>
<td>1.3 ± 0.3 (9)</td>
<td>1.7 ± 0.1 (5)</td>
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<tr>
<td>EI, kcal/wk</td>
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</tr>
<tr>
<td>DIO*</td>
<td>222 ± 8 (12)</td>
<td>350 ± 8 (11)</td>
<td>451 ± 10 (10)</td>
<td>527 ± 12 (12)</td>
<td>627 ± 16† (5)</td>
<td>663 ± 32† (7)</td>
<td>625 ± 6 (8)</td>
</tr>
<tr>
<td>DR</td>
<td>167 ± 13 (10)</td>
<td>299 ± 9 (10)</td>
<td>415 ± 10 (12)</td>
<td>474 ± 11 (10)</td>
<td>513 ± 9 (5)</td>
<td>575 ± 21 (7)</td>
<td>587 ± 18 (5)</td>
</tr>
<tr>
<td>Feed Eff</td>
<td></td>
<td></td>
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<tr>
<td>DIO*</td>
<td>3.7 ± 0.07 (10)</td>
<td>3.0 ± 0.06 (10)</td>
<td>2.8 ± 0.2 (12)</td>
<td>2.2 ± 0.09 (10)</td>
<td>1.8 ± 0.06 (5)</td>
<td>1.6 ± 0.03 (7)</td>
<td>1.4 ± 0.06 (8)</td>
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<tr>
<td>DR</td>
<td>3.4 ± 0.2 (10)</td>
<td>2.9 ± 0.05 (10)</td>
<td>2.5 ± 0.09 (12)</td>
<td>2.0 ± 0.05 (10)</td>
<td>1.7 ± 0.05 (5)</td>
<td>1.5 ± 0.04 (7)</td>
<td>1.5 ± 0.09 (5)</td>
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</table>

Data are means ± SE; nos. in parentheses are no. of rats. Groups of selectively bred DIO and DR rats were observed weekly from 28 to 70 days of age. Rats were weaned onto chow on day 21, and groups were killed on the observation days. Fat pad index (FatFP) = % body fat (calculated as described in METHODS). EI, energy intake; Feed Eff, feed efficiency. *Significantly different ($P < 0.05$) from DR group overall by ANOVA. †Significantly different ($P < 0.05$) from DR group at corresponding age by post hoc t-test after ANOVA showed significant intergroup differences.

(+277%) and P63 (+207%) (Table 1). Plasma insulin levels were similar between the genotypes throughout the study period (Table 2).

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different between DIO and DR rats at any age (Fig. 2). Similarly, expression of ARC OB-R mRNA expression did not differ between DIO and DR rats at any age (Fig. 2D).

**HE Diet Study**

After being weaned onto chow, a subset of DIO and DR rats was given either chow or HE diet at 7 wk of age and fed ad libitum for 14 days. After just 1 wk on diet, DIO rats fed HE diet increased their body weight by 23%, while DR rats fed HE diet and chow-fed DIO and DR rats gained 17, 19, and 19%, respectively ($F_{3,27} = 10.8; P < 0.001$; Fig. 3A). The response to HE diet was genotype specific as DR rats fed either chow or HE diet showed no difference in percent body weight gain. The relatively large increase in body weight in DIO rats fed...
HE diet was due, in part, to increased caloric intake (Fig. 3B). On HE diet, DIO rats rapidly increased their energy intake compared with all other groups. By the end of the first week, average daily energy intake in DIO rats fed HE diet was 111 ± 3 kcal compared with 82 ± 3, 83 ± 4, and 76 ± 2 kcal for chow-fed DIO rats and DR rats fed either HE diet or chow, respectively (F3,27 = 22.0; P < 0.001). The increased caloric intake of DIO rats on HE diet was entirely due to the increased caloric density of the HE diet because they ate the same weight of HE diet (24.8 ± 0.7 g) as did DIO rats fed chow for that period (24.8 ± 0.9 g). Importantly, while DR rats did increase their caloric intake when first exposed to HE diet, they reduced their caloric intake to the level of chow-fed DR rats by the end of the first week. Unlike DIO rats on HE diet, DR rats fed HE diet compensated for the increased caloric density of the HE diet and ate significantly less HE diet by weight (18.6 ± 0.9 g) than chow-fed DR rats (23.0 ± 0.6 g) over the first week. Thus DIO rats on HE diet had a higher average daily energy intake than all other groups from day 2 onward (P < 0.005 for all) and never compensated for the increased caloric density of the HE diet. This increase in caloric intake appeared to be primarily responsible for the increased weight gain in DIO rats on HE diet as their feed efficiency did not differ from any of the other groups at any time (Fig. 3C). By the end of 14 days on HE diet or chow (Table 3), DIO rats, regardless of diet, weighed more than DR rats (F3,28 = 6.34; P = 0.002). However, this greater overall weight was due to the fact that DIO rats on HE diet weighed more than all other groups. Similarly, only the DIO rats on HE diet had elevated adipose mass compared with the other groups when assessed by either total weight of all four depots (F3,28 = 7.16; P = 0.001) or as the weight of these depots expressed as a percentage of body weight (F3,28 = 5.22; P = 0.050).

After just 3 days on HE diet (before the emergence of any differences in body weight), plasma leptin increased by 230% (P = 0.046) in DIO rats vs. chow-fed DIO rats, as well as both groups of DR rats (F3,24 = 5.9; P = 0.004; Fig. 4A). Thereafter, plasma leptin levels continued to rise and were 270% of those in chow-fed DIO rats after 14 days on HE diet. By comparison, intake of HE diet had no significant effect on plasma leptin levels in DR rats at any time during the 14 days. Plasma insulin levels tended to increase by 3 days in both DIO (70%) and DR (61%) rats on HE diet, but these levels did not differ statistically from their chow-fed controls. On average, plasma insulin levels were higher in DIO rats fed HE diet over the course of the 14-day period. However, due to large variability in the data, there were no significant differences between groups at any time (Fig. 4B).

DISCUSSION

Selectively bred DIO and DR Sprague-Dawley rats clearly have divergent metabolic responses to diets of both low and moderate energy density and fat content. Chow-fed selectively bred DIO rats begin to overeat and gain more weight than selectively bred DR rats during the early postweaning period. This increase in body weight is preceded by an increase in carcass protein content, and their weight gain was composed of a largely proportional increase in both protein and fat content. Both DIO and DR rats rapidly increased their energy intake when first exposed to HE diet, they reduced their caloric intake to the level of chow-fed DR rats by the end of the first week. Unlike DIO rats on HE diet, DR rats fed HE diet compensated for the increased caloric density of the HE diet and ate significantly less HE diet by weight (18.6 ± 0.9 g) than chow-fed DR rats (23.0 ± 0.6 g) over the first week. Thus DIO rats on HE diet had a higher average daily energy intake than all other groups from day 2 onward (P < 0.005 for all) and never compensated for the increased caloric density of the HE diet. This increase in caloric intake appeared to be primarily responsible for the increased weight gain in DIO rats on HE diet as their feed efficiency did not differ from any of the other groups at any time (Fig. 3C). By the end of 14 days on HE diet or chow (Table 3), DIO rats, regardless of diet, weighed more than DR rats (F3,28 = 6.34; P = 0.002). However, this greater overall weight was due to the fact that DIO rats on HE diet weighed more than all other groups. Similarly, only the DIO rats on HE diet had elevated adipose mass compared with the other groups when assessed by either total weight of all four depots (F3,28 = 7.16; P = 0.001) or as the weight of these depots expressed as a percentage of body weight (F3,28 = 5.22; P = 0.050).

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Table 3. Body and fat pad weights in DIO and DR rats on chow or HE diet

<table>
<thead>
<tr>
<th></th>
<th>DR Chow</th>
<th>DR HE Diet</th>
<th>DIO Chow</th>
<th>DIO HE Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>241 ± 8*</td>
<td>239 ± 7*</td>
<td>249 ± 6*</td>
<td>280 ± 8†</td>
</tr>
<tr>
<td>Fat pad weight, g</td>
<td>3.61 ± 0.41*</td>
<td>4.19 ± 0.21*</td>
<td>4.27 ± 0.23*</td>
<td>7.64 ± 0.43†</td>
</tr>
<tr>
<td>%Body weight</td>
<td>1.50 ± 0.13*</td>
<td>1.75 ± 0.25*</td>
<td>1.07 ± 0.17*</td>
<td>2.72 ± 0.31†</td>
</tr>
</tbody>
</table>

Data are means ± SE. At postnatal day 42, selectively bred DIO and DR rats were continued on chow or fed high-energy (HE) diet for 14 days. Terminally, their epididymal, retroperitoneal, perirenal, and mesenteric fat pads were removed and weighed. Data with differing superscripts differ from each other at P ≤ 0.05 by post hoc t-test after significant intergroup differences were found by ANOVA.
content. Despite their hyperphagia, they became only ~5% fatter and generally had only slightly elevated plasma leptin levels compared with DR rats. Thus chow-fed DIO rats in the current study were larger but only slightly fatter than DR rats over the first 10 wk of life. This differs from the original description of these substrains in which chow-fed selectively bred DIO rats had 44% more carcass fat than selectively bred DR rats at 10 wk of age (26). However, those rats were the fifth generation of these substrains, and the current rats represent over 20 generations of selective breeding. Obviously, continued breeding has changed the way in which these animals regulate their carcass composition. The increase in body weight and lean body mass in the current DIO rats is somewhat puzzling because the outbred DIO rats, from which the selectively bred DIO rats were derived, actually have reduced growth hormone secretion before (18) and after (19) the expression of DIO on HE diet. Regardless of the underlying mechanism, hyperphagia is probably the primary reason for the increased weight gain of these animals. Their failure to become obese on chow in the face of hyperphagia is probably related to the combination of the low fat content of chow (4.5%) and their rapid growth during the early postweaning period. Unfortunately, the assessment of ARC NPY, AgRP, POMC, or OB-R mRNA expression failed to provide any clues as to why these rats become spontaneously hyperphagic on chow.

However, while the current selectively bred DIO rats no longer become obese as a result of their hyperphagia on chow, their propensity to become obese on HE diet has clearly been retained over the successive breeding cycles. An interesting feature of the development of DIO on HE diet was the failure of DIO rats to down-regulate their caloric intake when the caloric density of the diet was increased. Unlike the selectively bred DR rats that quickly reduced the weight of food consumed to compensate for the increased caloric density of the HE diet, DIO rats failed to compensate for their increased caloric intake over 14 days. This occurred despite an early and marked increase in plasma leptin levels that should have led to reduced intake (33). Therefore, the current selectively bred DIO rats require increased caloric density and fat content of their diet to become obese. This obesity develops largely because of hyperphagia, which is unabated by an early increase of plasma leptin levels.

This finding suggested that selectively bred DIO rats might have reduced leptin sensitivity similar to that seen in outbred DIO rats (25). Therefore, it was unexpected that chow-fed selectively bred DIO rats did not exhibit the same increase in arcuate NPY expression seen in chow-fed outbred DIO rats (22, 24), nor did the selectively bred DIO rats have altered leptin receptor mRNA expression using a probe that recognizes all splice variants of the leptin receptor (OB-R). Even so, the fact that selectively bred DIO rats on HE diet remained hyperphagic and had an early elevation of plasma leptin levels, before adipose stores become significantly elevated (31), suggests that they have a reduced ability to sense and regulate leptin. A similar early increase in plasma leptin levels has been reported in unselected (by weight gain genotype) outbred Sprague-Dawley rats (40), both obesity-prone Osborne-Mendel and obesity-resistant SS/Pl rats (31), and obesity-prone mice (37) fed a high-fat diet. Such increased leptin levels might reasonably be expected to inhibit intake of the HE diet (1). Even without a significant increase in leptin levels, DR rats quickly compensated for the increased caloric density of the HE diet and reduced their total energy intake. However, DIO rats never made this compensation and continued to overeat and become obese on HE diet. Thus, despite normal expression of hypothalamic OB-R mRNA, selectively bred DIO rats appear to have a defect in their ability to monitor and regulate leptin levels, i.e., reduced leptin sensitivity comparable to that seen in the outbred DIO parent strain (25). It is quite possible that, despite their comparable OB-R expression to DR rats, selectively bred DIO rats might have reduced leptin sensitivity based on decreased mRNA and/or receptor protein expression of the long (signaling) form (OB-RB) of the leptin receptor.

Leptin acts through the OB-Rb in the ARC (39) to reduce the expression of the anabolic peptides NPY and AgRP (1, 32) and to elevate POMC mRNA expression (1). Increased POMC expression should lead to increased synthesis of α-melanocyte-stimulating hormone, the presumptive agonist for the catabolic melanocortin-3 and -4 receptors (11). Thus we had predicted that selectively bred DIO rats would have elevated ARC NPY expression, as well as possibly elevated AgRP and reduced POMC expression, preceding the onset of their hyperphagia. In fact, there were no differences in the expression of any of these neuropeptides between selectively bred DIO and DR rats throughout their early postnatal or early adult life. Despite a lack of difference in neuropeptide mRNA expression, there might still be differences in actual neuropeptide synthesis or release or in postsynaptic receptor function. There might also be a more generalized imbalance between anabolic NPY/AgRP and catabolic melanocortin systems that were not detected by in situ hybridization. Alternatively, there might well be differences in the expression of other neuropeptides or transmitters that were not assessed here.

The factors responsible for the rapid and sustained increase in plasma leptin in selectively bred DIO rats on HE diet or in other rodents on high-fat diet (12, 14, 34, 37) are presently unknown. It is possible that selectively bred DIO rats have reduced renal clearance of leptin. While long-term elevations in leptin are likely due to increased fat mass, hormonally driven short-term changes in plasma leptin levels can occur in the face of unaltered adipose tissue stores. After 3 days on diet, when leptin levels had increased by 240% in DIO rats on HE diet, there were no differences in body weight [and presumably adipose tissue mass (31)]. While insulin acutely increases leptin release from rat adipose tissue in vitro (3) and plasma leptin levels in vivo in rodents (35), the lack of differences in plasma
DISCLOSURES

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