Ontogeny of Diet-Induced Obesity in Selectively Bred Sprague-Dawley Rats

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ABSTRACT

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 ~5wk of age. Here we assessed the development of metabolic and neural functions for insights into the origins of their greater weight gain. From weeks 5-10, 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, 7wk old DIO rats had a 240% increase in plasma leptin levels after only 3d. 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 suggest that selectively bred DIO rats might have reduced leptin sensitivity similar to that seen in the outbred DIO parent strain.
INTRODUCTION

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 kcal % 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 to 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 week old selectively bred DIO rats weigh more and consume more calories than DR rats even on chow diet (26). Exposure to HE diet for two weeks exacerbates the weight gain and
energy intake differences and selectively bred DIO rats become more energy efficient, 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 3mo 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.

METHODS

Animals and Diets. Animal usage was in compliance with the animal care committee of the E. Orange VA Medical Center and the Guidelines of the American Physiologic Society Animal (1). 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 (P) 2. 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 sacrificed 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 post-weaning period, animals were singly housed for food intake measurements (powdered Purina 5001) and animals were sacrificed at $P_{28}$, $P_{35}$, $P_{42}$, $P_{49}$, $P_{56}$, $P_{63}$ and $P_{70}$.

During the pre-weaning period, a test of independent ingestion was performed to determine if DIO-prone rats display hyperphagia prior to chow or HE diet. This test has been used to identify adult-like ingestive behavior in pre-weaning 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 4h. Just prior to 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 20min, pups were removed, dried and weighed again. The difference in body weight was used to calculate food intake.

Terminally, all animals were weighed and sacrificed 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 post-weaning 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 $P_{42}$, a separate group of selectively bred DIO (n=11) and DR (n=19) rats was singly housed for one week and given powdered chow. On $P_{49}$, DIO and DR rats were randomly selected to remain on powdered chow or were switched to powdered HE diet for 14 d.
The HE diet is composed of 8% corn oil, 44% sweetened condensed milk and 48% Purina 5001 (Research Diets, Inc., 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. Since no direct measure of adiposity was made in this original study, a second set of selectively bred P42 DIO and DR rats were fed either chow or HE diet for 14d (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 X 6.25.

In situ hybridization. Serial 15 µm sections were taken through the rostro-caudal 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; 3559-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 hr (NPY, POMC, AgRP) or for ~3 weeks (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 rostro-caudal extent of the hypothalamus. Since 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 analysis of variance (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 weight 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

Though body weights of DIO and DR rats were similar prior to weaning, DIO rats became significantly heavier than DR rats beginning at $P_{35}$, or two weeks post-weaning (Table 1). They remained ~15% heavier for the remainder of the study period. Generally speaking, the increase in body weight of DIO compared to 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 $P_{35}$, they already had 8% more total carcass protein mass by 14d, and 43% more by $P_{28}$. The magnitude of this difference decreased with age such that DIO rats generally had 14-16% more carcass protein than DR rats from $d_{42}-70$. At no time did the relative amount (%) of carcass protein differ between DIO and DR rats. DIO rats were fatter than DR rats when carcass fat was considered as a percent 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 $P_{7}$ 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 post-weaning period and the sum of these weights was used to calculate a fat pad index (Table 2). During the post-weaning period, DIO rats had greater overall adiposity as a function of pad to carcass weights ($F[1,114]=23.9; P<0.001$), though 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 $P_{10}$ 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 $P56$ (+277%) and $P63$ (+207%) (Table 1). Plasma insulin levels were similar between the genotypes throughout the study period (Table 2).

*Energy intake and feed efficiency*

On average, DIO rats consumed about 14% more calories per week than DR rats during the post-weaning 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 (P49-55) and 8-9 (P56-62), respectively ($P<0.005$). While DIO rats were slightly more feed efficient (+7%) than DR rats during the post-weaning 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 pre-weaning 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 hours. Since independent ingestion behavior has more in common with adult ingestive behavior than with suckling (13), this method can be used to identify early hyperphagia of adult-like eating behaviors (15). Selectively bred DIO pups did not ingest more than selectively bred DR pups at any age studied (Figure 1). Interestingly, selectively bred DR pups were hyperphagic relative to selectively bred DIO pups on $P14$ (genotype x age interaction; $F[4,159]=2.9; P=0.02$; post-hoc at $P14; P=0.03$).
Hypothalamic expression of NPY, POMC, AgRP, OB-R mRNA

Given the early post-weaning 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 different between DIO and DR rats at any age (Figure 2). Similarly, expression of ARC OB-R mRNA expression did not differ between DIO and DR rats at any age (Figure 2D).

HE Diet Study

After being weaned onto chow, a subset of DIO and DR rats was given either chow or HE diet at 7wk of age and fed ad libitum for 14d. After just 1wk 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; Figure 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 (Figure 3B). On HE diet, DIO rats rapidly increased their energy intake compared to all other groups. By the end of the first week, average daily energy intake in DIO rats fed HE diet was 111±3 kcal as compared to 82±3 kcal, 83±4 kcal and 76±2 kcal for chow-fed DIO, and DR rats fed either HE diet or chow, respectively (F[3,27]=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 since they ate the same weight of HE diet (24.8±0.7g) as did DIO rats fed chow for that period (24.8±0.9g). 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±0.9g) than chow-fed DR rats (23.0±0.6g) over the first week. Thus, DIO rats on HE diet had a higher average daily energy intake than all other groups from day 2 and on (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 (Figure 3C). By the end of 14d on HE diet or chow (Table 3), DIO rats, regardless of diet, weighed more than DR rats (F[3,28]=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 to the other groups when assessed by either total weight of all 4 depots (F[3,28]=7.16; P=0.001) or as the weight of these depots expressed as a percent of body weight (F[3,28]=5.22; P=0.050).

After just 3d on HE diet (before the emergence of any differences in body weight), plasma leptin increased by 230% (P=0.046) in DIO rats versus chow-fed DIO rats, as well as both groups of DR rats (F[3,24]=5.9; P=0.004; Figure 4A). Thereafter, plasma leptin levels continued to rise and were 270% of those in chow-fed DIO rats after 14d 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 14d. Plasma insulin levels tended to increase by 3d 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 14d period. However, due to large variability in the data, there were no significant differences between groups at any time (Figure 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 post-weaning 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. Despite their hyperphagia, they became only ~5% fatter and generally had only slightly elevated plasma leptin levels when compared to DR rats. Thus, chow-fed DIO rats in the current study were larger but only slightly fatter than DR rats over the first 10wk 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 10wk 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 since the outbred DIO rats, from which the selectively bred DIO rats were derived, actually have reduced growth hormone secretion prior to (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 post-weaning period. Unfortunately, the assessment of ARC NPY, AgRP, POMC or Ob-R
mRNA expression failed to provide any clue 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 downregulate their caloric intake when the caloric density of the diet was increased. Unlike the selectively bred DR rats which 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 d. 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
S5B/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 (2). 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 expression, 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 (2;32) and to elevate POMC mRNA expression (2). 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 post-synaptic 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 which 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 3d 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 insulin between the groups in the present study make it unlikely that increased insulin drove the early increase in leptin. Glucocorticoids can increase (7) and sympathetic activity can decrease (38) leptin levels in rodents. However, selectively bred DIO adults have reduced basal corticosterone levels (29) and elevated 24h urine norepinephrine levels suggesting a generally elevated level of basal sympathetic activity (26). The most parsimonious explanation is that selectively bred DIO rats have a reduced ability to detect their early increases in plasma leptin and thus are unable to inhibit its release from adipose stores.

The lack of difference between the genotypes in the pre-weaning independent ingestion test suggests that, at least at an early age, DIO rats do not eat larger meals. However, it should be noted that this test would only detect differences in the ability of the animal to control individual meal size, not meal number. Also, it is possible that performing this test in the morning hours (when pups are less active) precluded us from finding real differences between the groups.
However, using a similar protocol, Kowalski et al. (16) found that fa/fu pups had larger single meals compared to wild-type littermates, suggesting that this method is valid for uncovering deficits in the control of single meals during the pre-weaning period. Clearly, post-weaning DIO rats eat more calories when given HE diet. Whether these extra calories are due to increased meal size and/or number and the developmental period during which this first occurs is presently unknown.

In summary, we have identified the early post-weaning period as the time when selectively bred DIO rats begin to increase their body weight and caloric intake on chow diet as compared to selectively bred DR rat. Unlike early generations of selectively bred DIO rats (26), weight gain in the current generation of DIO rats, produced by more than 20 breeding cycles, was due to a roughly proportional increase in both carcass protein and fat mass. It took exposure to the increased caloric density and fat content of the HE diet to increase plasma leptin and adipose stores in the current selectively bred DIO rats. Their propensity to become obese was not predated by differences in the mRNA expression of ARC NPY, POMC or AgRP suggesting that alterations in these neuropeptides might not play a causal role in DIO in this model. Nor do differences in the neuropeptide mRNA expression explain the onset of spontaneous hyperphagia of DIO rats on a low fat diet during the early post-weaning period. However, the early rise in leptin levels and failure to respond by decreasing energy intake on a HE diet suggest that reduced leptin sensitivity might underlie their propensity to develop DIO on a diet of increased caloric density and fat content.
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REFERENCES


FIGURES

**Figure 1:** Intake (% of initial body weight) of half-and-half milk cream in a 20-min test of ingestion independent of the dam. Selectively-bred DIO (n=8-27) and DR (n=7-26) rat pups were deprived of the dam (but not siblings) for 4 hrs prior to testing. Data are mean ± SEM. \(^a\) P=0.05 or less when P14 DIO rats were compared to P10 DIO rats. \(^b\) P = 0.05 or less when P14 DR rats were compared to P14 DIO rats.

**Figure 2:** (A) Neuropeptide Y (NPY), (B) agouti related peptide (AgRP), (C) proopiomelanocortin (POMC), and (D) leptin receptor (OB-R [all forms]) mRNA expression by in situ hybridization was assessed in serial sections through the arcuate nucleus (ARC) of the hypothalamus from selectively-bred DIO and DR rats fed chow from weaning. Data are mean ± SEM. NPY: (DIO: n=7-12; DR: n=9-11); AgRP (DIO: n=8-12; DR: n=9-11); POMC: (DIO: n=5-12; DR: n=7-11); OB-R: (DIO: n=4-11; DR: n=2-10).

**Figure 3:** (A) Body weight gain (% of initial body weight), (B) average daily energy intake (kcal) and (C) feed efficiency (body weight in kg\(^{0.75}\) divided by the number of calories ingested) in selectively-bred, 7 wk old DIO (Chow: n=5; HE: n=6) and DR (Chow: n=10; HE: n=10) rats given either chow or HE diet for 14 days. Data are mean ± SEM. \(^a\) P=0.05 or less within DIO groups at same time-point. \(^b\) P = 0.05 or less between DIO rats fed HE and both DR groups at same time-point. \(^c\) P = 0.05 or less within DR groups at same time-point.

**Figure 4:** (A) Plasma leptin (ng/ml) and (B) plasma insulin (ng/ml) in selectively-bred, 7 wk old DIO (Chow: n=5; HE: n=6) and DR (Chow: n=10; HE: n=10) rats given either chow or HE diet for 14 days. Data are mean ± SEM. \(^a\) P=0.05 or less within DIO groups at same time-point. \(^d\) P = 0.05 or less between DIO rats fed HE and all other groups.
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<td>5.4±0.1 b</td>
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<td>6.1±0.1 b</td>
<td>6.5±0.2 b</td>
<td>6.5±0.2 b</td>
<td>7.0±0.2 b</td>
<td>8.4±0.3 b</td>
<td>8.2±0.5 b</td>
<td>8.0±0.3 b</td>
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<tr>
<td>Protein (%)DIO</td>
<td>0.1±0.01 b</td>
<td>0.7±0.03 b</td>
<td>0.9±0.1 b</td>
<td>1.3±0.02 b</td>
<td>2.0±0.1 b</td>
<td>3.6±0.2 b</td>
<td>6.5±0.3 b</td>
<td>6.5±0.2 b</td>
<td>7.0±0.2 b</td>
<td>12.9±0.5 b</td>
<td>22.3±1.2 b</td>
<td>26.2±1.2 b</td>
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<td>Water (%) DIO</td>
<td>14.9±0.3 b</td>
<td>16.0±0.2 b</td>
<td>18.0±0.2 b</td>
<td>18.6±0.4 b</td>
<td>19.1±0.1 b</td>
<td>20.1±0.2 b</td>
<td>20.4±0.3 b</td>
<td>21.1±0.4 b</td>
<td>20.4±0.3 b</td>
<td>21.7±0.2 b</td>
<td>21.7±0.2 b</td>
<td>21.7±0.2 b</td>
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<tr>
<td>Protein (%) DIO</td>
<td>0.7±0.03 b</td>
<td>1.9±0.05 b</td>
<td>2.5±0.1 b</td>
<td>4.1±0.1 b</td>
<td>6.1±0.2 b</td>
<td>11.0±0.8 b</td>
<td>19.2±0.3 b</td>
<td>29.9±1.1 b</td>
<td>38.0±1.6 b</td>
<td>55.9±1.2 b</td>
<td>61.2±2.2 b</td>
<td>70.7±1.3 b</td>
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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. Groups of selectively bred DIO and DR rats were observed from 2-70 days of age. Rats were weaned onto chow on day 21 and groups were sacrificed on the observation days shown. Data are mean ± SEM with the number of rats in parentheses. a = Significantly different ($P\leq0.05$) from DR group overall by ANOVA. b = Significantly different ($P\leq0.05$) from DR group at corresponding age by post hoc t test after two way ANOVA showed significant intergroup differences.
Table 2: Post-weaning fat pad index (FatFP), plasma insulin, energy intake (EI) and feed efficiency (Feed Eff) in selectively bred DR and DIO rats on chow. Groups of selectively bred rats DIO and DR rats were observed weekly from 28-70 days of age. Rats were weaned onto chow on day 21 on chow and groups were sacrificed on the observation days. Data are mean ± SEM with the number of rats in parentheses. FatFP = % body fat (calculated as described in the Methods). a = Significantly different (P≤0.05) from DR group overall by ANOVA. b = Significantly different (P≤0.05) from DR group at corresponding age by post hoc t test after ANOVA showed significant intergroup differences.
Table 3. Body and fat pad weights in DIO and DR rats chow or HE diet. At P42, selectively bred DIO and DR rats were continued on chow or fed HE diet for 14d. Terminally, their epididymal, retroperitoneal, perirenal and mesenteric fat pads were removed and weighed. Data are mean ± SEM. Data with differing superscripts differ from each other by P=0.05 or less by post hoc t test after significant intergroup differences were found by ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>DR Chow</th>
<th>DR HE diet</th>
<th>DIO Chow</th>
<th>DIO HE Diet</th>
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<tr>
<td><strong>Final Body weight (g)</strong></td>
<td>241±8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>239±7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>249±6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>280±8&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Fat pad weight (g)</strong></td>
<td>3.61±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.19±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.27±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.64±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>% Body weight</strong></td>
<td>1.50±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.75±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.71±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.72±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>
FIGURE 1
Figure 2
FIGURE 4