F-DIO obesity-prone rat is insulin resistant before obesity onset

Barry E. Levin,1,2 Christophe Magnan,3 Stephanie Migrenne,3 Streamson C. Chua, Jr.,4 and Ambrose A. Dunn-Meynell1,2

1Neurology Service, Veterans Administration Medical Center, East Orange, New Jersey; 2Department of Neurology and Neurosciences, New Jersey Medical School, Newark, New Jersey; 3Université Paris 7, Centre National de la Recherche Scientifique Unité Mixte de Recherche, Paris, France; 4Division of Molecular Genetics, Department of Pediatrics, Columbia University, New York, New York

Submitted 28 March 2005; accepted in final form 3 May 2005

Levin, Barry E., Christophe Magnan, Stephanie Migrenne, Streamson C. Chua, Jr., and Ambrose A. Dunn-Meynell. F-DIO obesity-prone rat is insulin resistant before obesity onset. Am J Physiol Regul Integr Comp Physiol 289: R704–R711, 2005.—We previously created a novel F-DIO rat strain derived by crossing rats selectively bred for the diet-induced obesity (DIO) phenotype with obesity-resistant Fischer F344 rats. The offspring retained the DIO phenotype through 3 backcrosses with F344 rats but also had exaggerated insulin responses to oral glucose before they became obese on a 31% fat high-energy (HE) diet. Here, we demonstrate that chow-fed rats from the subsequent randomly bred progeny required 57% higher glucose infusions to maintain euglycemia during a hyperinsulinemic clamp in association with 45% less insulin-induced hepatic glucose output inhibition and 80% lower insulin-induced glucose uptake than F344 rats. The DIO phenotype and exaggerated insulin response to oral glucose in the nonobese, chow-fed state persisted in the F6 generation. Also, compared with F344 rats, chow-fed F-DIO rats had 68% higher arcuate nucleus proopiomelanocortin mRNA expression which, unlike the increase in F344 rats, was decreased by 26% on HE diet. Further, F-DIO lateral hypothalamic orexin expression was 18% lower than in F344 rats and was increased rather than decreased by HE diet intake. Finally, both maternal obesity and 30% caloric restriction during the third week of gestation produced F-DIO offspring which were heavier and had higher leptin and insulin levels than lean F-DIO dam offspring. Third-gestational week dexamethasone also produced offspring with higher leptin and insulin levels but with lower body weight. Thus F-DIO rats represent a novel and potentially useful model for the study of DIO, insulin resistance, and perinatal factors that influence the development and persistence of obesity.

Rodent models have been used for many years to investigate the metabolic, hormonal and neural factors involved in the control of energy homeostasis. In the last few decades, such studies have become increasingly important as the incidence of obesity and type 2 diabetes mellitus has increased in both the developed and underdeveloped world (31, 49). Many insights have been gained from inbred strains, transgenic and knockout mice. However, a large percentage of human obesity and type 2 diabetes follows a polygenic mode of inheritance (3, 41). The rat model of diet-induced obesity (DIO) is a useful surrogate for the study of such polygenic traits. Both outbred rats and rats that were bred from these outbred strains to selectively express the DIO phenotype develop elements of the metabolic syndrome when fed diets with increased fat and caloric density. Such rats become obese and develop insulin resistance, hypertension, and hyperlipidemia (9, 22, 25, 40, 47). This model has also been useful for the study of maternal factors, which determine the development of obesity and insulin resistance in offspring. For example, when selectively bred DIO dams are made obese during gestation and lactation, their offspring become more obese, insulin resistant, and develop abnormalities of brain monoamine metabolism compared with offspring of lean DIO dams or dams that are diet-resistant (19, 26).

In an initial attempt to characterize the genotypic of the DIO rat, we crossed selectively bred DIO rats with the obesity-resistant inbred Fischer F344 strain (24). After this first crossing, offspring were bred back against the F344 strain three times with selection for the DIO trait in male progeny. This N3 generation was slightly heavier but not more obese than the F344 parent strain when fed a low-fat chow diet without weaning. We originally named these rats “F.DIO” to reflect their Fischer and DIO origins but have now renamed them “F-DIO,” so as not to connote a congenic strain. When fed a 31% fat, high-energy (HE) diet for 3 wk, these F-DIO rats became more obese than the F344 parent strain. Unexpectedly, the F-DIO rats also had a markedly exaggerated insulin response to an oral glucose tolerance test before they became obese. Exaggerated insulin release was further amplified and accompanied by an exaggerated glucose response with development of obesity after 3 wk on HE diet. These data suggested that the F-DIO rat, besides retaining the DIO phenotype through several cycles of backcrosses, had also developed an inherent insulin resistance in the nonobese state, which was not present in either of the DIO or F344 parent strains (22). Since that original report (24), we have successively bred six generations of the N3 rats (F1–F6) without selection for weight gain phenotype.

The current studies were carried out to further characterize this unique model by 1) establishing the stability of the DIO and insulin resistance phenotypes in the F-DIO rat; 2) confirming that preobese F-DIO rats are indeed insulin resistant by using the hyperinsulinemic euglycemic clamp technique; 3) assessing the expression of arcuate nucleus neuropeptide Y (NPY) and proopiomelanocortin (POMC) and lateral hypothalamic orexin mRNA for possible clues to the pathogenesis of the DIO and insulin resistance traits; 4) determining whether induction of maternal obesity would increase the development of obesity in F-DIO offspring as it did in the offspring of
selectively bred DIO dams (26, 48); and 5) exploring the possibility that maternal caloric deprivation (10, 14, 15, 35) and dexamethasone treatment (32, 33, 46), both of which produce abnormal glucose metabolism in normal rats, would exacerbate the obesity and abnormal glucose metabolism of the F-DIO rat and produce a true type 2 diabetes mellitus model.

We found that the DIO phenotype was indeed preserved after six generations of random matings, that the nonobese F-DIO rat had both hepatic and generalized insulin resistance, that both arcuate nucleus (ARC) POMC and lateral hypothalamic orexin mRNA were regulated much differently in the F-DIO than in either parent strain and that a variety of perinatal environmental manipulations produced marked effects on the development of obesity and abnormal glucose metabolism in the F-DIO rat.

METHODS

Animals and diets. Animal usage was reviewed and approved by the institutional animal care and use committee of the E. Orange Veterans Affairs Medical Center and complied with the guidelines of the American Physiological Society (1). All studies were carried out using F-DIO from our in-house colony and Fischer F344 rats from Charles River Labs (Kingston, NY). The F-DIO rats were originally bred by crossing our in-house colony of selectively bred DIO male rats (22) with female Fischer F344 rats. The offspring of these matings were designated as the N1 generation. The males from this generation were fed a defined high-energy (HE) (Research Diets #C11024F, New Brunswick, NJ) which 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 (27) for 3 wk. The N1 male rats that gained the most weight during this period were mated with Fischer F344 females, and their progeny were designated as N2. These rats were treated in the same way as their parents to produce the N3 generation. We have previously reported on the phenotype of this N3 generation (24). The male and female progeny of the N3 generation were then successively bred over 6 generations with each other without selection for weight gain phenotype. As with the original DIO breeding scheme, all crosses were made between males and females that were not first-degree relatives. These crosses were designated as F-DIO/F1, F-DIO/F2 . . . F-DIO/F6. Male rats from the F-DIO/F3 and F-DIO/F6 generations were used for the current studies.

Unless otherwise specified, rats were fed Purina rat chow (#5001), drank water ad libitum from weaning, and were housed on a 12:12-h light-dark schedule with lights on at 1800. Purina rat chow contains 3.30 kcal/g with 23.4% as protein, 4.5% as fat and 72.1% as carbohydrate which is primarily in the form of complex polysaccharide (27). For the majority of experiments, rats were weighed weekly. At the end of each study, rats were decapitated in the fed state between 0800 and 1100 h, and the brains were removed and frozen quickly on powdered dry ice. Terminally, trunk blood was taken for glucose and/or hormone determinations. In some studies, epididymal, retroperitoneal, perirenal, and mesenteric fat pads were removed and weighed as visceral depots and inguinal pads as subcutaneous depots.

Experiment 1. Glucose metabolism and insulin sensitivity

These studies were carried out in 7 chow-fed 360 –380 g (mean 378 ± 13 g) males of the F-DIO/F3 generation and 5 chow-fed 310 –360 g (mean 323 ± 9 g; P = 0.001) Fischer F344 males. Glucose turnover rate (GTR) was assessed in basal and euglycemic-hyperglycemic clamp conditions in the same rat by previously described methods (8). Rats were deprived of food for 4 h (from 0900 to 1300) and anesthetized with pentobarbital sodium (50 mg/kg ip). Catheters were inserted in the right jugular vein for blood sampling and infusions (insulin, labeled and unlabeled glucose) were carried out using butterfly needles inserted in the saphenous veins. To determine GTR in the basal state, a priming dose of [3-3H] glucose (4 μCi) was injected through the saphenous vein at −50 min, followed by a continuous infusion at a rate of 0.2 μCi/min throughout the study. Blood samples were drawn at −15, −10, and 0 min during the basal period and at time 70, 80, and 90 min during the evaluation of the steady state euglycemic clamp to determine the glucose disposal rate.

Euglycemic-hyperinsulinemic clamps were used to determine glucose kinetics. Before insulin infusion was begun, blood samples were drawn for determinations of basal blood glucose levels. Fasting glucose levels were 85 ± 6 mg/dl in F344 and 108 ± 7 mg/dl in F-DIO rats (P = 0.01). A priming dose of insulin (20 mU; Actrapid, Novo, Copenhagen, Denmark) dissolved in isotonic saline was then injected through the saphenous vein followed by a continuous infusion of insulin (0.4 U·kg⁻¹·h⁻¹) at a constant rate of 20 μl/min to maintain blood glucose levels at 90 mg/dl. Under these conditions, we have shown that insulin levels during the clamp procedure are maintained at −4 ng/ml (8). During the clamps, blood was sampled from the jugular catheters every 5 min to determine glucose levels and to adjust the rate of unlabeled glucose infusion to maintain euglycemia. The euglycemic conditions were attained within 30–40 min and then maintained for 2 h thereafter. Steady-state specific glucose radioactivity and plasma glucose and insulin concentrations were determined during the last 20 min of the clamp. In the basal steady state, the rate of glucose appearance (Ra), which reflects hepatic glucose production, is equal to the glucose disposal rate (Rd), which reflects glucose utilization. Rd is calculated according to the formula Rd = Ra = [3-3H] glucose infusion rate [disintegrations per minute (dpm/ min)] divided by blood glucose specific activity (dpm/mg). During the glucose clamp (50–70 min after the onset of insulin infusion), Rd = Ra + Ra'(i.e., amount of glucose expressed in milligrams per minute per kilogram necessary to maintain euglycemia) (8).

Blood glucose was determined by automated glucose oxidase method (Beckman) and insulin determinations by radioimmunnoassay (Linco). For the assay of [3-3H] glucose radioactivity, blood samples were deproteinized with Ba(OH)₂ and ZnSO₄, and the supernatant was evaporated to dryness at 50°C to remove tritiated water. The dry residue was dissolved in 0.5 ml water to which 10 ml scintillation solution was added (Aqualuma plus, Lumac, The Netherlands), and radioactivity was determined using a liquid scintillation counter.

Experiment 2. Effects of genotype and diet on ARC NPY and POMC and lateral hypothalamic orexin mRNA expression. Male Fischer F344 and F-DIO/F3 300–325 g rats were used for this study. They were weighed and then fed either chow (n = 6 per genotype) or HE diet (n = 6 per genotype) for 4 wk and then killed by rapid decapitation. The brains were quickly removed, frozen on dry ice, and kept at −80°C until being cut in 15-μm sections through the midportion of the ARC in a cryostat within 1 mo. Sections were processed for in situ hybridization by minor modifications of previously described methods (18, 28). Briefly, cRNA was synthesized, and radio-labeled probes for prepro-NPY (511-bp) (13), POMC (923-bp) (17), and prepro-orexin (287-bp) were made (42). The probes were hydrolyzed in 0.05 M Na₂CO₃ plus 0.033 M NaHCO₃ for 15–30 min and then subjected to our standard method for in situ hybridization (16, 21). After treatment with RNAase A (Calbiochem, San Diego, CA), sections were washed, dehydrated, dried and exposed to SB-5 X-ray film (Kodak, Rochester, NY). The resulting autoradiograms were read by an experimentally blinded observer using computer-assisted densitometry (Drexel University, Philadelphia, PA). The areas of NPY and POMC hybridization within the ARC, and orexin hybridization in the lateral hypothalamus at the same level, were measured from the autoradiograms. The three largest areas of hybridization in each brain were averaged for comparisons between groups.

Experiment 3. Effect of perinatal manipulations on offspring body weight gain, glucose, insulin and leptin levels. Because F-DIO rats have abnormal glucose metabolism even before they develop obesity, these studies were carried out with the hypothesis that manipulations reported to produce obesity and/or abnormal glucose metabolism in otherwise normal rats might produce a true picture of type 2 diabetes.

Nonobese F-DIO rats are insulin resistant.

R705

AJR-Regul Integ Comp Physiol • VOL 289 • SEPTEMBER 2005 • www.ajpregu.org

Downloaded from http://ajpregu.physiology.org/ by 10.220.33.6 on November 8, 2017.
mellitus with eventual islet cell fatigue and death in F-DIO offspring. Toward this end, matings were carried out for each study with 6 female and 3 male F-DIO/F3 rats. At birth, all litters were adjusted to 10 pups/dam. Four sets of maternal conditions were used: 1) Chow-fed (lean) dams were mated with males and carried through gestation and weaning. They were injected with dexamethasone vehicle (see Experiment 4 below) during the third week of gestation; 2) obese dams were produced by feeding them on a HE diet for 1 mo before mating. They were kept on the HE diet through gestation and until their pups were weaned at 3 wk; 3) maternal caloric deprivation was produced by mating chow-fed dams and then restricting their intake during the third week of gestation to 70% of their intake during the preceding 2 wk of gestation. At birth, dams were allowed unrestricted access to chow; and 4) pregnant females were treated daily during the third week of pregnancy with dexamethasone (100 μg·kg⁻¹·day⁻¹) dissolved in 4% ethanol-0.9% saline, 200 μg/ml sc (32). Male offspring from all four groups were weaned onto chow at 3 wk of age and carried out to 65 days of age. Tail blood samples for glucose were taken at weekly intervals after an overnight fast between 0900 and 1100 and terminally glucose, leptin, and insulin were assayed from trunk blood in overnight fasted animals.

Experiment 4. Persistence of the F-DIO phenotype. To ensure that the F-DIO phenotype was conserved after repeated cycles of random breeding without selection for the DIO phenotype, F-DIO/F6 (the F-DIO phenotype was conserved after repeated cycles of random trunk blood in overnight fasted animals. They were injected with dexamethasone vehicle (see Experiment 4 below) during the third week of gestation; 2) obese dams were produced by feeding them on a HE diet for 1 mo before mating. They were kept on the HE diet through gestation and until their pups were weaned at 3 wk; 3) maternal caloric deprivation was produced by mating chow-fed dams and then restricting their intake during the third week of gestation to 70% of their intake during the preceding 2 wk of gestation. At birth, dams were allowed unrestricted access to chow; and 4) pregnant females were treated daily during the third week of pregnancy with dexamethasone (100 μg·kg⁻¹·day⁻¹) dissolved in 4% ethanol-0.9% saline, 200 μg/ml sc (32). Male offspring from all four groups were weaned onto chow at 3 wk of age and carried out to 65 days of age. Tail blood samples for glucose were taken at weekly intervals after an overnight fast between 0900 and 1100 and terminally glucose, leptin, and insulin were assayed from trunk blood in overnight fasted animals.

Experiment 4. Persistence of the F-DIO phenotype. To ensure that the F-DIO phenotype was conserved after repeated cycles of random breeding without selection for the DIO phenotype, F-DIO/F6 (n = 10) and F344 rats (n = 10) were fed chow from weaning and were studied beginning at 6 wk of age. At that time, they were fasted overnight and, from 0800 to 1100, a baseline tail blood sample was taken for glucose and insulin levels. They were then gavaged with glucose (0.5 g/kg body wt), and repeated tail blood samples were taken at 30, 60, 90, and 120 min. They were then placed on HE diet for 6 wk. They were weighed weekly and, after 6 wk on HE diet, the glucose tolerance test was repeated. Terminally their epididymal, perirenal, retroperitoneal, mesenteric, and inguinal adipose depots were removed bilaterally and weighed.

Statistics. Parameters were compared among various genotypes, diet and perinatal manipulation groups by one- or two-way ANOVA with post hoc Bonferroni comparisons. For the glucose tolerance test and glucose infusion rates during the hyperinsulinemic clamps, areas under the curve (AUC) for glucose and/or insulin were calculated using GraphPad Prizm software. Glucose and insulin curves were also compared using repeated-measures ANOVA.

RESULTS

Experiment 1. Glucose metabolism and insulin sensitivity. Hyperinsulinemic euglycemic clamps were carried out in chow-fed F344 and F-DIO male rats from the F-DIO/F3 generation of breeding (Fig. 1A). Once steady state conditions were established during the clamp, the rate of glucose infusion required to maintain euglycemia, as calculated by AUC, was 57% lower for F-DIO (451 ± 32 mg/kg) than F344 rats (1,062 ± 65 mg/kg; P = 0.01). Although basal hepatic glucose output was comparable in F-DIO and F344 rats (Fig. 1B), output during the clamp reduced glucose output by 77% in F344 rats but by only 35% in F-DIO rats (F344 vs. F-DIO, P = 0.001). As with basal hepatic glucose output, total basal glucose uptake was comparable between F-DIO and F344 rats. But, while insulin increased glucose uptake by 92% in F344 rats, it was increased by only 19% in F-DIO rats (F344 vs. F-DIO, P = 0.001). Thus nonobese F-DIO rats showed insulin resistance at the level of both insulin-induced inhibition of hepatic glucose production and stimulation of total body glucose uptake compared with F344 rats.

Experiment 2. The effects of genotype and diet on ARC NPY and POMC and lateral hypothalamic orexin mRNA expression are shown in Table 1. There were no significant
differences in ARC NPY mRNA expression between F-DIO/F3 and F344 rats fed either chow or HE diet for 4 wk. However, intake of HE diet did significantly lower NPY expression in both F344 and F-DIO rats compared with their chow-fed conditions (chow vs. HE diet, \( P = 0.05 \)). On the other hand, POMC expression was 68% higher in chow-fed F-DIO than F344 rats. Furthermore, 4 wk on the HE diet was associated with a 49% increase in ARC POMC expression in F344 rats, but POMC expression was reduced by 26% in F-DIO rats on the HE diet compared with those on chow. Finally, orexin expression was 18% lower in chow-fed F-DIO than F344 rats, and HE diet exposure was associated with a 23% increase in expression in F-DIO but did not alter F344 expression. Thus, on chow, F-DIO rats had increased expression of POMC and decreased expression of orexin mRNA expression compared with F344 rats while only F-DIO rats exhibited a reduction in POMC and increase in orexin mRNA expression following 4 wk on the HE diet.

Experiment 3. The effect of perinatal manipulations on offspring body weight gain, glucose, insulin, and leptin levels is shown in Table 2 and Fig. 2. Offspring of F-DIO/F3 dams made obese during gestation and weaning by intake of a HE diet were 15% heavier at both 30 and 65 days of life than those of lean F-DIO dams. In fact, most of their increased weight gain had already occurred by 30 days of life, as there was no greater relative increase in body weight gain from 30 to 65 days compared with F344 rats. Terminally, they had 115% higher plasma leptin levels in association with 163% higher fasting insulin levels but no higher glucose levels than the offspring of lean F-DIO dams.

Similar to the offspring of obese dams, offspring of F-DIO dams, which were calorically restricted during the third trimester to 70% of their basal intake, were 20% heavier than offspring of lean dams at 30 days of age and were still 12% heavier and had 88% higher leptin levels at 65 days. As with maternal obesity, most of the increase in body weight gain had already occurred by 30 days of life, as there was no increase in the rate of weight gain between 30 and 65 days of life. Unlike the offspring of obese dams, offspring of calorically restricted dams had 52% higher fasting glucose levels at 30 days and 12% higher levels at 65 days than offspring of lean dams. These higher glucose levels at day 65 were associated with 152% higher insulin levels.

As opposed to maternal obesity and third-trimester caloric restriction, offspring of dams given third-trimester dexamethasone were 14% lighter at 30 days and 10% lighter at 65 days than offspring of lean dams. However, unlike maternal obesity and maternal deprivation, the offspring of dexamethasone-treated dams had a slightly higher (6%) rate of weight gain than offspring of lean dams from 30 to 65 days. Despite their reduced body weights, offspring of dexamethasone-treated dams had 45% higher leptin levels at 65 days than offspring of lean dams. Their fasting glucose levels were 62% higher at 30 days and 11% higher in association with 161% higher fasting insulin levels at 65 days.

Thus maternal obesity and third-trimester caloric restriction and dexamethasone treatment produced offspring that were hyperleptinemic and hyperinsulinemic compared with offspring of lean F-DIO dams. Although offspring of obese and maternally deprived dams were heavier, offspring of dexamethasone-treated dams had lower body weights but higher leptin and insulin levels, suggesting that they were obese but also possibly stunted in growth.

Experiment 4. Persistence of the F-DIO phenotype. To ensure that the DIO- and insulin-resistant traits first described in the N3 F-DIO rats (24) were not lost by further breeding without selection for body weight gain phenotype, male F-DIO/F6 rats were assessed for their body weight and fat pad weights and responses to an oral glucose load. As in the original N3 generation, chow-fed F-DIO/F6 males were

Table 2. Effect of perinatal manipulations on body weight gain and blood glucose levels in offspring of F-DIO/F3 offspring

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>Maternal Deprivation</th>
<th>Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 30 body wt. g</td>
<td>66±2(a)</td>
<td>76±2(b)</td>
<td>79±3(b)</td>
<td>57±1(b)</td>
</tr>
<tr>
<td>Day 65 body wt. g</td>
<td>250±4(a)</td>
<td>287±6(b)</td>
<td>280±6(a)</td>
<td>226±6(b)</td>
</tr>
<tr>
<td>Body wt gain. g</td>
<td>184±4(a)</td>
<td>211±6(b)</td>
<td>200±6(b)</td>
<td>169±5(b)</td>
</tr>
<tr>
<td>Body wt gain, % 30 days</td>
<td>282±6(a)</td>
<td>274±7(b)</td>
<td>255±7(a)</td>
<td>299±4(b)</td>
</tr>
<tr>
<td>Day 30 glucose, mg/dl</td>
<td>66±3(a)</td>
<td>54±3(b)</td>
<td>100±2(b)</td>
<td>107±2(b)</td>
</tr>
<tr>
<td>Day 65 glucose, mg/dl</td>
<td>90±3(a)</td>
<td>24±3(b)</td>
<td>112±2.3(b)</td>
<td>111±3(b)</td>
</tr>
<tr>
<td>Day 65 insulin, mg/ml</td>
<td>1.35±0.11(a)</td>
<td>3.55±0.30b(b)</td>
<td>3.40±0.29(b)</td>
<td>3.52±0.28(b)</td>
</tr>
<tr>
<td>Day 65 leptin, ng/ml</td>
<td>7.09±0.69(a)</td>
<td>15.3±0.2(b)</td>
<td>13.4±0.2(b)</td>
<td>10.3±0.2(b)</td>
</tr>
</tbody>
</table>

F-DIO/F3 dams were mated with males of the same generation. Lean dams were fed chow through gestation and weaning. Obese dams were fed HE diet for 4 wk before impregnation. Maternal deprivation was produced by restricting 3rd trimester dams to 70% of their caloric intake during the first 2 trimesters (weeks). Other dams were treated with dexamethasone daily during the last week of gestation. Male pups (n = 10 per group) were weaned onto chow and fed chow through 65 days of life. Body weights and glucose levels were assayed from tail blood at 30 and 65 days of life, and body wt gain was calculated from day 30 to 60. Body wt gain was also calculated as %body wt at 30 days of age. Data are presented as means ± SE. Data with different symbols differ from each other by \( P = 0.05 \) or less by post hoc t-test after 1-way ANOVA showed significant intergroup differences.
heavier (27%) and gained 19% more body weight over 6 wk on a HE diet than F344 males of the same age (Table 3). This increased body weight gain was associated with a 50–60% greater weight of total, visceral, and subcutaneous adipose depots. However, as a percent of body weight, total fat pad weights were only 23% higher in F-DIO than F344 rats. Also similar to the original N3 F-DIO rats, chow-fed F-DIO/F6 rats had 18% higher fasting glucose levels but no difference in the glucose AUC during the oral glucose tolerance test (Table 3, Fig. 3A). Importantly, chow-fed F-DIO/F6 rats also maintained their exaggerated insulin response to the glucose load compared with F344 rats. Fasting levels were 55% higher, and the insulin AUC during the glucose tolerance test was 37% higher than chow-fed F344 rats. After 6 wk on the HE diet, fasting glucose levels were comparable, but the glucose AUC was 39% higher than F344 rats. Fasting insulin levels were 27% higher, and the AUC during the glucose tolerance test was 35% higher than F344 rats. Thus, with only minor differences from the original N3 generation, male F-DIO/F6 rats still maintained apparent insulin resistance before onset of obesity and sustained the clear development of DIO associated with worsening of apparent insulin resistance after 6 wk on HE diet.

**DISCUSSION**

We originally bred the F-DIO strain as a test of the hypothesis that the DIO phenotype in selectively bred DIO rats was inherited as a polygenic trait, much as it is in many humans (3, 41). Unfortunately, in that original study (24), we were unable to identify any of the genes responsible for either the DIO or insulin resistance traits. This difficulty can be attributed to the fact that our physiological endpoints (DIO and insulin sensitivity) can have multiple causes, as well as being mutually interactive. Obesity produces insulin resistance in rodents (45), and defective insulin signaling can produce obesity (4). Although somewhat heavier than their obesity-resistant F344 parent strain, the F-DIO rats in that study were not significantly more obese than the F344 rats when fed chow from weaning (24). Nevertheless, they did become more obese than F344 rats when fed a HE diet. These results suggested that the insulin resistance of F-DIO rats was not due to underlying obesity and that insulin resistance alone did not cause obesity until dietary fat and caloric density were increased. The current study strongly reinforces our contention that we have successfully transmitted the DIO genes, as the DIO trait persisted through both the original three backcrosses and 6 subsequent breeding cycles performed without selection for high weight gainers. One unexpected consequence of the original breeding scheme was that the F-DIO rats had increased insulin secretion during an oral glucose tolerance test, even before they became obese (24). This suggested, but did not prove, that they were insulin resistant in the preobese state. Results from the use of the euglycemic hyperinsulinemic clamp technique in the current study confirm that chow-fed F-DIO rats have insulin resistance at the level of hepatic glucose production and total body glucose uptake, despite a lack of differences in these parameters in the basal state.

In addition to their insulin resistance in the nonobese state, F-DIO rats had very different expression of hypothalamic neuropeptides than the DIO and F344 parent strains. Outbred DIO rats have increased ARC NPY expression before they become obese (18). These elevated levels fall to levels seen in diet-resistant (DR) rats after 4 wk on HE diet in parallel with a 70% increase of leptin levels above those in DR rats (20). Also, NPY levels in outbred DIO rats were reduced by 17%, but only after developing marked hyperleptinemia during 14 wk on HE diet (16). Since leptin reduces ARC NPY expression (43), these results suggested that DIO rats might be leptin resistant. In fact, both outbred (20) and selectively bred DIO rats (23) do have central leptin resistance. Although the elevated ARC NPY

**Table 3. Effect of genotype and diet on body weight gain and insulin sensitivity**

<table>
<thead>
<tr>
<th></th>
<th>F344</th>
<th>F-DIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt on chow</td>
<td>179±3</td>
<td>227±2*</td>
</tr>
<tr>
<td>6-wk body wt gain on HE diet, g</td>
<td>105±4</td>
<td>125±2*</td>
</tr>
<tr>
<td>Fat Pads</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidydymal, g</td>
<td>9.2±0.7</td>
<td>13.4±0.7*</td>
</tr>
<tr>
<td>Perirenal, g</td>
<td>1.96±0.13</td>
<td>3.06±0.16*</td>
</tr>
<tr>
<td>Retroperitoneal, g</td>
<td>5.19±0.32</td>
<td>6.66±0.36*</td>
</tr>
<tr>
<td>Mesentric, g</td>
<td>5.07±0.46</td>
<td>10.1±0.4*</td>
</tr>
<tr>
<td>Inguinal, g</td>
<td>6.26±0.47</td>
<td>9.84±0.59*</td>
</tr>
<tr>
<td>Visceral pads, g</td>
<td>20.2±0.9</td>
<td>32.4±1.6*</td>
</tr>
<tr>
<td>Total fat pads, g</td>
<td>27.7±1.8</td>
<td>43.1±1.6*</td>
</tr>
<tr>
<td>Fasting glucose chow, mg/dl</td>
<td>93±3</td>
<td>110±3*</td>
</tr>
<tr>
<td>Glucose AUC chow, mg/dl -1•120 min -1</td>
<td>1227±200</td>
<td>997±180</td>
</tr>
<tr>
<td>Fasting insulin chow, ng/ml</td>
<td>0.85±0.09</td>
<td>1.32±0.11*</td>
</tr>
<tr>
<td>Insulin AUC chow, ng/ml •120 min -1</td>
<td>24±2</td>
<td>33±3*</td>
</tr>
<tr>
<td>Fasting glucose 6-wk HE diet, mg/dl</td>
<td>108±5</td>
<td>110±2</td>
</tr>
<tr>
<td>Glucose AUC HE diet, mg/dl -1•120 min -1</td>
<td>1905±180</td>
<td>2655±250*</td>
</tr>
<tr>
<td>Fasting insulin 6-wk HE diet, ng/ml</td>
<td>1.91±0.19</td>
<td>2.43±0.20*</td>
</tr>
<tr>
<td>Insulin AUC 6-wk HE diet, ng/ml •120 min -1</td>
<td>70.8±0.2</td>
<td>95.4±8.1*</td>
</tr>
</tbody>
</table>

Male 6-wk old F-DIO/F6 and F344 rats (n = 10 per group) underwent an oral glucose tolerance test while on chow and then were placed on HE diet for 6 wk and retested. Data are presented as means ± SE; *P = 0.05 or less compared with F344. Inguinal, subcutaneous fat pad visceral fat pads are epidydynamal, perirenal, retroperitoneal, and mesentric; total pads, visceral plus subcutaneous pads. AUC, area under the curve for insulin and glucose following an oral glucose load.
expression of preobese outbred DIO rats was not carried forward in either the selectively bred DIO (16, 40) or the F-DIO rats studied here, it is possible that F-DIO rats may also have central leptin resistance since F344 and F-DIO rats comparably reduced their ARC NPY expression by ~15% after 4 wk on HE diet, while F-DIO leptin levels were 90% higher in F-DIO than F344 rats (24).

The finding of elevated ARC POMC expression in chow-fed F-DIO vs. F344 rats and a selective reduction in F-DIO POMC levels after 4 wk on the HE diet suggests that F-DIO rats might retain normal sensitivity to leptin-induced regulation of POMC expression (44). F-DIO rats had 45% higher leptin levels on chow and those levels increased by 90% more than F344 rats after 3 wk on the HE diet (24). The supposition that F-DIO POMC neurons could retain their leptin sensitivity, while NPY neurons do not is supported by the recent finding that leptin may differentially affect signaling in NPY vs. POMC neurons (52). Finally, since leptin reduces orexin expression (30), the lower levels of lateral hypothalamic orexin mRNA expression in chow-fed F-DIO suggests that these neurons might also retain normal leptin sensitivity. However, the selective increase in orexin expression after HE diet in F-DIO vs. F344 rats suggests that there are other factors that regulate orexin. For example, high-fat diet alone can increase orexin expression (51).

Because POMC is the precursor for α-MSH, which improves peripheral insulin sensitivity (12, 34), increased expression in chow-fed F-DIO rats should lower their insulin levels and increase their insulin sensitivity. In fact, we found just the opposite. On the other hand, the further deterioration of insulin sensitivity after 4 wk on HE diet in F-DIO rats can be attributed to the deleterious effects of high-fat diets on leptin (11, 23), insulin (6), and melanocortin signaling (5). However, measurement of mRNA expression does not necessarily reflect the amount of active peptide released at the nerve terminal. In the case of POMC, mRNA expression does not even predict which of its many peptide products will be produced and released. In addition to α-MSH, POMC also is a prohormone for β-endorphin, a hormone which increases intake of palatable diets (7). Central administration of β-endorphin also produces a strain- and site-dependent elevation of blood glucose and possibly insulin levels (2, 37). Thus, if elevated ARC POMC expression were associated with increased β-endorphin production and release, this might predispose nonobese F-DIO rats to develop abnormal glucose metabolism and to eat more and gain more weight when exposed to the high corn oil and sucrose content of the HE diet.

The selectively bred DIO rat shows a strong interaction between maternal obesity and genetic background in the development of obesity in offspring. Offspring of obese DIO dams become more obese and insulin resistant than those of lean DIO dams, while maternal obesity in diet-resistant rats has no impact on adiposity or insulin sensitivity in their offspring (26). Such manipulations also have selective effects on the development of monoamine systems in the forebrains of offspring of DIO rats (19). Because F-DIO rats were bred from DIO rats and because they were demonstrably insulin resistant before the development of obesity, we reasoned that exposing them to perinatal manipulations that increase obesity and insulin resistance in offspring of normal rats might provoke a true type 2 diabetes mellitus phenotype in F-DIO offspring. The expectation was that offspring would become both hyperinsulinemic and hyperglycemic and that this would lead to eventual exhaustion of β-cells with resultant hypoinsulinemia. This did not turn out to be the case for the F-DIO offspring of dams made obese during pregnancy and lactation; here, the offspring developed fasting hyperinsulinemia without hyperglycemia.

Somewhat paradoxically, maternal undernutrition predisposes offspring to develop both obesity and insulin resistance. Generally, undernutrition throughout pregnancy or during the first two trimesters is associated with obesity in human (10, 38, 39) and rat offspring (14, 15, 35). However, we demonstrate
here that 70% third-trimester caloric restriction increased the body weight gain, plasma leptin, and insulin and glucose levels of chow-fed offspring of F-DIO dams. Similarly, third-trimester dexamethasone treatment induced offspring hyperleptinemia, hyperglycemia, and hyperinsulinemia. Thus, even though they gained less weight than those of lean dams, their elevated leptin levels suggest that they were much more obese but possibly growth retarded. Similar results have been demonstrated in other rat strains (33, 46). Therefore, the third trimester of pregnancy in F-DIO rats appears to be a particularly sensitive one for interventions that can produce changes in both energy homeostasis and neural development (29, 36, 50).

Despite the fact that both maternal caloric restriction and dexamethasone produced hyperinsulinemia and hyperglycemia compared with offspring of lean F-DIO dams, neither resulted in β-cell exhaustion and hypoinsulinemia as we had originally expected. Although it is possible that this might have occurred over a longer period of time, we actually did carry the offspring of maternally deprived dams out to 6 mo of life and found no reduction in plasma insulin levels or deterioration of fasting glucose levels (data not shown).

In summary, the F-DIO rat represents an obesity-prone strain that is also quite insulin resistant before exposure to a high-fat diet and the development of obesity. The persistence of the DIO phenotype through three backcrosses against obesity-resistant F344 rats and 6 subsequent cycles of breeding without selection for weight gain strongly supports our contention that the DIO trait is indeed polygenic in nature. Unlike the parent DIO or F344 strains, the lean and obese F-DIO rats also exhibit alterations in their expression of ARC POMC and lateral hypothalamic orexin expression. These alterations might provide some clue as to the pathogenesis of their insulin resistance. These differences in hypothalamic peptide expression are less likely to be responsible for the DIO phenotype, as they are not seen in the DIO parent strain. In conclusion, we suggest that the F-DIO rat provides an excellent model for the study of factors that predispose rats to develop DIO and insulin resistance in the nonobese state.

ACKNOWLEDGMENTS

We thank Antionette Moralishvili and Charlie Salter for technical assistance.

GRANTS

This work was supported by the Research Service of the Department of Veterans Affairs and National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-30066.

REFERENCES


