Development of insulin resistance and hyperphagia in Zucker fatty rats

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Durham, Holiday A., and Gary E. Truett. Development of insulin resistance and hyperphagia in Zucker fatty rats. Am J Physiol Regul Integr Comp Physiol 290: R652–R658, 2006. First published October 13, 2005; doi:10.1152/ajpregu.00428.2004.—The onset of hyperphagia in the Zucker fatty (fa/fa) rat occurs on a single day in postnatal development and could be driven by an increase in insulin sensitivity. To test this hypothesis, we performed insulin tolerance tests at several points in development. In rapidly growing juvenile rats, fatty rats are as insulin sensitive as lean rats at 4 wk of age but become increasingly insulin resistant as they became obese. During the suckling to weaning transition, fatty rats are insulin resistant at 2 wk of age, when they are exclusively suckling; they are also insulin resistant at 3 wk of age, when they are suckling and consuming solid food, but not hyperphagic. By 4 wk of age, when fatty rats are hyperphagic, they are as insulin sensitive as their lean littermates. These data indicate that fatty rats experience two phases of insulin resistance, punctuated by a brief period of insulin sensitivity that follows the onset of hyperphagia. To determine whether the increase in insulin sensitivity could be driving the onset of hyperphagia, insulin tolerance tests were performed from 21 to 27 days of age. Obese and lean rats became increasingly insulin resistant from 21 to 23 days of age and then became as insulin sensitive as lean rats by 25 days of age. These data show that increased insulin resistance precedes the onset of hyperphagia and increased insulin sensitivity follows the onset of hyperphagia. This pattern suggests that developmental perturbations in insulin signaling are likely to be involved in the onset of hyperphagia.

obesity; leptin receptor; weaning; food intake; ontogeny

ZUCKER FATTY (FA/FA) RATS inherit a leptin receptor mutation that causes early onset obesity (12). Fatty rats accumulate a significant amount of excess adipose mass during the first 3 wk of life by increased energy efficiency (4, 7, 8); however, they grow at the same rate as their lean littermates for the first 22 days of age (17), and they are not visibly obese. During this period, fatty rats are typically referred to as preobese to acknowledge that they are phenotypically different from their lean littermates (6), but not overtly obese.

The grow rate of fatty rats increases dramatically after 22 days of age (17). This large increase in growth rate on a single day in postnatal development corresponds with the onset of robust hyperphagia in fatty rats and is accompanied by the emergence of hyperinsulinemia. Fatty rats become visibly distinguishable from their lean littermates at this age by the expansion of their bellies with food, and within a few days they also become visibly obese as their adipose mass expands more rapidly. This increase in growth is easily detectable when fatty rats remain with their dams through the 4th wk of life but is obscured when fatty rats are separated from their dams at 3 wk of age, which is a common husbandry practice. The fact that the onset of hyperphagia occurs at such a precise age without the introduction of environmental changes suggests that a developmentally regulated mechanism determines the timing of the onset of hyperphagia.

The weaning transition is associated with many physiological changes, including changes in insulin sensitivity in adipose tissue in normal rats. Suckling rats consume a high-fat, low-carbohydrate diet and are somewhat insulin resistant (1–3). Furthermore, the expression of peroxisome proliferator receptor (PPAR)-γ, a transcription factor that controls the expression of insulin-sensitizing pathways, is very low during the first 2 wk of life and increases to adult levels during the weaning transition (13). An increase in insulin sensitivity during this period could contribute to the onset of hyperphagia by stimulating pathways for glucose disposal for lipid synthesis.

An analogous situation occurs when adult fatty rats are treated with PPARγ receptor agonists. Fatty rats become increasingly insulin resistant as they become obese (18), and their hyperphagia also lessens as they become obese and insulin resistant (19). However, treatment of insulin-resistant fatty adult rats with thiazolidinediones increases insulin sensitivity by stimulating glucose utilization for lipogenesis and stimulates hyperphagia (21). These observations suggest that an increase in insulin sensitivity could also play a role in the emergence of hyperphagia in fatty rats during the weaning transition. To determine the relationship between insulin resistance and the emergence of hyperphagia in fatty rats, we measured insulin tolerance under several developmental conditions.

MATERIALS AND METHODS

Animals. Animals were produced in the principal investigator’s colony of partially inbred (F15–F17) Zucker fa rats. Animals are housed in plastic breeder boxes with ad libitum access to Teklad 8640 Rodent Diet and tap water. The light cycle was maintained at 12 h of dark, followed by 12 h of light. The room temperature was maintained at 68–74°F and 55–60% humidity. All animal studies were reviewed and approved by the University of Tennessee Institutional Animal Care and Use Committee.

The population was maintained with brother by sister matings to minimize extraneous genetic variation and with selection for large litter size to minimize inbreeding depression. Sires were removed when the dams appeared to be pregnant. The day of birth was considered 0 days of age. Neonates were genotyped for fa during the first wk of life, as described in detail below. Litters were reduced to eight pups during the first 10 days of life by decapitating excess heterozygotes. Litters with no fatty offspring were discarded. Pups were weighed daily from 14 days of age to the end of each experiment to acclimate them to handling and to generate growth curves. Pups remained with their dams until 28 days of age. Growth data were analyzed under mixed linear models using Proc Mixed of SAS version

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9.1. Genotype and sex were treated as fixed-effects factors. Age and litter were treated as random-effects factors.

Genotype detection. Pups were marked by toe-clipping, and a single toe was saved to prepare DNA by the hotSHOT method (16, 17). Briefly, toes were collected in 200-μl thermal cycler tubes. Seventy-five microliters of 25 mM NaOH-0.2 mM EDTA (pH 12) were added to each tube. Samples were heated to 95°C for 30 min and then cooled to room temperature. Seventy-five microliters of 40 mM Tris-HCl (pH 8) were added to each tube to adjust the pH to 8. The DNA was then ready for PCR amplification.

The fa mutation creates a restriction site for Msp I that can be used to detect the fa genotype (12). However, Msp I occasionally fails to cut DNA to completion, which can cause genotype misclassification. To overcome this problem, we engineered an alternative restriction site by a strategy that has been described previously (20). PCR primers were designed to amplify 101 base pairs (bp) of DNA flanking the fa mutation. The reverse primer, RLepr, was selected to anneal adjacent to the site of the mutation, and a single base substitution (C→G) was made in the second base from the 3′-end of the primer to create a Pvu II restriction site in the wild-type allele and no restriction site in the mutant allele. The primer sequences were forward Lepr primer, 5′-CGATGGAAATCATACAGA-3′ and RLepr, 5′-GAATTCTTCTAATATTTACG-3′; the base substitution in RLepr is underlined. Primers were mixed at 500 nM each in 50 mM KCl, 10 mM Tris-HCl (pH 9 at 25°C), 0.1% Triton X-100, 1.5 mM MgCl2, 0.2 mM each dATP, dCTP, dGTP, and dTTP, 0.25 units Taq DNA polymerase and 2 μl of hotSHOT DNA in a 22-μl volume. PCR amplification was carried out in a Robocycler thermal controller (Stratagene, La Jolla, CA) at 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 45°C for 45 s, and 72°C for 30 s. The wild-type genotype produced an 80-bp band, and the mutant genotype produced a 101-bp band; both bands were visible in heterozygotes. The reverse primer, RLepr, was selected to anneal adjacent to the site of the mutation, and a single base substitution (C→G) was made in the second base from the 3′-end of the primer to create a Pvu II restriction site in the wild-type allele and no restriction site in the mutant allele. The primer sequences were forward Lepr primer, 5′-CGATGGAAATCATACAGA-3′ and RLepr, 5′-GAATTCTTCTAATATTTACG-3′; the base substitution in RLepr is underlined. Primers were mixed at 500 nM each in 50 mM KCl, 10 mM Tris-HCl (pH 9 at 25°C), 0.1% Triton X-100, 1.5 mM MgCl2, 0.2 mM each dATP, dCTP, dGTP, and dTTP, 0.25 units Taq DNA polymerase and 2 μl of hotSHOT DNA in a 22-μl volume. PCR amplification was carried out in a Robocycler thermal controller (Stratagene, La Jolla, CA) at 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 45°C for 45 s, and 72°C for 30 s. PCR product (20 μl) was digested with 1 unit Pvu II (New England Biolabs, Beverly, MA) in a 30-μl volume for 1 h at 37°C. Pvu II-treated PCR product (30 μl) was loaded on a 3% 3:1 high-resolution blend agarose (Amresco) gel and electrophoresed for 30 min at 5 volts/cm. Gels were stained with ethidium bromide and exposed to ultraviolet light. The wild-type genotype produced an 80-bp band, and the mutant genotype produced a 101-bp band; both bands were visible in heterozygotes.

Insulin tolerance tests. Insulin tolerance tests were performed after a 2-h fast. Experiments were performed between 10:00 AM and 5:00 PM, which begins 3 h into the light phase and ends 2 h before the dark phase. Animals were always housed with their cagemates, except that the dams were removed from their littermates in experiments 2 and 3. Fasting took place under the same conditions the rats were housed under, except for the absence of food and the dam. The room temperature was maintained at 68–74°F and 55–60% humidity. Animals were briefly removed from their cage for the tail bleeds and insulin injection, and then returned. The fast was kept brief to limit stress on young rats. A 2-h was sufficient to eliminate differences in stomach contents and plasma insulin between fatty rats and their lean littermates during the 3rd and 4th wk of life (unpublished data). However, as fatty pups become older they develop fasting hyperinsulinemia. The impact of a brief fast is greater on young rats because of their lower glycogen stores.

After a 2-h fast, the tip of the tail was nicked with a scalpel blade, a drop of blood was expressed on a blood glucose test strip, and glucose values were measured with a glucose meter (Prestige Smart System; Valleylab). After fasting glucose was measured, rats were injected subcutaneously at the back of the neck with 0.5 units/kg insulin. Pilot experiments determined that subcutaneous subscapular injection of insulin was less stressful to rats than intraperitoneal injections and gave more consistent results with lower variation. A pilot insulin tolerance test was performed on five lean rats at 20 days of age to confirm the effectiveness of the insulin dose. In this test, blood glucose was sampled at times 0, 15, 30, 45, 60, 90, and 120 min after insulin injection. If necessary, the tail wound was reopened with a scalpel at these time points. This test determined that blood glucose levels reach a nadir 30 min after injection of 0.5 units/kg insulin (Humulin R; Eli Lilly Pharmaceuticals), then began to rise back toward the baseline (Fig. 1). The peak at 30 min is a good measure of the response of blood glucose to insulin, whereas the return to baseline is a response to the counterregulatory release of epinephrine.

Because the return to baseline is not a measure of insulin sensitivity, but of hepatic glycogenolysis, it provides no additional information about insulin sensitivity. In addition, we found that young rats were more stressed by the repeated sampling associated with a 120-min insulin tolerance test, and that even longer sampling intervals would be required to detect a complete return to baseline. Because the return to baseline is not particularly informative, we chose to use a simplified insulin tolerance test to assess the development of insulin resistance in fatty rats. In the simplified insulin tolerance test, blood glucose is measured at 0 and 30 min after insulin injection. The 30-min glucose value is used as an indicator of insulin resistance. A similar approach has been used clinically to evaluate insulin resistance in humans (9). After the second glucose reading, rats were returned to their home cage with ad libitum access to food and water. This simplified insulin tolerance test reduces the stress to the animals. It also limits the number of incisions that must be made in the tail and enables the same subjects to be tested repeatedly across development.

Statistical analysis and interpretation of insulin tolerance test data. The insulin tolerance test gives two measures of blood glucose (0 and 30 min after insulin injection). We analyzed the effects of genotype on these variables separately under a mixed linear model that accounts for genotype, sex, and age as fixed-effects factors and litter as a random-effects factor. When insulin tolerance tests were also performed on the same subjects at more than one age (experiments 1 and 2), the within-subject covariance among ages was accounted for as a random-effects factor with an unstructured covariance structure. We never observed an effect of fa genotype on the 0-min glucose measurements, indicating that the genotypes shared the same baseline glucose. Insulin sensitivity was interpreted based on the differences in glucose 30 min after insulin injection; when one group had a higher mean glucose at 30 min after insulin, they were considered to be the more insulin-resistant group. We also considered analyzing these data by subtracting the 0-min glucose value from the 30-min value and analyzing the change in glucose over 30 min. Because this method essentially combines the variance in two measures into one, and only one of those measures is actually influenced by insulin (30 min is influenced by insulin and 0 min is not), there is a loss in statistical power with this approach that could lead to an error in statistical inference; therefore, this method was deemed inappropriate.
Experiments. An initial experiment was performed to establish the course of development of insulin resistance in fatty rats under our experimental conditions. Sex-matched fatty and wild-type littermates from five litters were separated from their dams at 24 days of age and housed in pairs. There were initially six rats in each of the four sex-matched by genotype groups. One female fatty rat was removed from the study because of health problems that were unrelated to the experiment, leaving 23 animals from five litters. Insulin tolerance tests were performed at 28, 35, 42, 49, and 56 days of age on the same set of rats. Rats were moved to clean cages with no food but ad libitum access to water. After 2 h, insulin tolerance tests were performed as described above.

The transition from suckling to weaning includes substantial changes in diet composition and food intake in fatty animals and their lean littermates. At 14 days of age, pups obtain all their nutrients from the dam. At 21 days of age, pups are suckling and consuming solid food, and fatty pups are not yet hyperphagic, hyperinsulinemic, or visibly obese. By 28 days of age, pups obtain most of their nutrients from solid food, and fatty animals are hyperphagic, hyperinsulinemic, and visibly obese. To test the hypothesis that changes in insulin sensitivity occur across this interval, insulin tolerance tests were performed at 14, 21, and 28 days of age on the same set of rats. Rats were housed with their dams throughout this period, except during fasting and insulin tolerance tests, to minimize investigator-induced changes in experimental conditions. Five litters were used in this experiment, and data were collected from all 8 members of each litter at each age, for a total of 40 animals. These litters included 6 female wild types, 11 female heterozygotes, 9 female fatty rats, 4 male wild types, 5 male heterozygotes, and 5 male fatty rats.

The results of the previous experiment suggested that changes in insulin sensitivity could be driving the onset of hyperphagia. To test this hypothesis, rats were housed with their dams throughout this period, except during fasting and insulin tolerance tests. Insulin tolerance tests were performed at 21, 22, 23, 24, 25, 26, and 27 days of age, using different litters for each age group. It was necessary to use different sets of rats for each age because it takes a couple of days to recover from insulin tolerance tests. This experiment included 241 rats in 26 litters. Because of the large number of subjects required for this experiment, litter size was not reduced for this experiment, and every member of the litters was tested. The distribution of litters by age and subjects by genotype, sex, and age are presented in Table 1.

RESULTS

A 120-min pilot insulin tolerance test displays the changes in blood glucose in response to insulin injection (Fig. 1). Blood glucose drops sharply from 0 to 30 min. This represents the effect of insulin on the translocation of glucose transporters from the cytosol to the plasma membrane of adipose tissue and muscle, and the resulting uptake of blood glucose. After 30 min, blood glucose rises gradually toward the baseline but does not fully return to the baseline, even by 120 min. This represents the effect of epinephrine on hepatic glycogenolysis in response to hypoglycemia. The level of blood glucose at 30 min after insulin injection can be used as a measure of insulin resistance, whereas the level of blood glucose from 30 to 120 min reflects the counterregulatory response to hypoglycemia. Because we are specifically interested in insulin resistance, we chose to use blood glucose 30 min after insulin injection as an indicator of insulin resistance in subsequent experiments. Blood glucose at 0 min was also measured to verify that the genotype groups started from the same baseline. This simplified insulin tolerance test reduced the amount of stress on the subjects and allowed animals to be tested at several points in development without excessive damage to their tails from the incisions used to sample tail blood.

Table 1. Distribution of litters by age and subjects by age, sex, and genotype in experiment 3

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<th>Day of Age</th>
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A total of 241 rats from 26 litters were used in experiment 3. Insulin tolerance tests were performed on every member of a litter on the same day. Each litter was used one time for an insulin tolerance test.
similar in wild type and fatty pups. By 42 days of age, blood glucose values after insulin injection are higher in fatty animals, and by 56 days of age, fatty rats do not lower their blood glucose in response to this dose of insulin. These data demonstrate that fatty pups are as insulin sensitive as their wild-type littermates at 4 wk of age and become increasingly insulin resistant in parallel with the increase in body weight during this period of rapid growth. These results are consistent with expectations.

From the 2nd wk of life to the 4th wk of life, rat pups change from reliance on their dam for all their nutrients to feeding independently on solid food. To establish the response to insulin across this transition, we performed insulin tolerance tests daily from 21 to 27 days of age. At 21 days of age, all genotypes have similar levels of blood glucose 30 min after insulin injection are higher in fatty rats compared with their lean littermates (Fig. 7). Although this pattern is maintained at 22 and 23 days of age, 30 min blood glucose shifts upward in both lean and fatty rats, suggesting that both groups become slightly more insulin resistant at these ages. After 23 days of age, insulin resistance decreases. By 25 days of age, fatty rats and their lean littermates have similar levels of blood glucose 30 min after insulin injection as detected by an increased in the weight of the stomach contents and by elevated insulin levels in fatty rats (17). Blood glucose levels 30 min after insulin are notably lower in experiment 3 than in experiments 1 and 2. We believe this is attributable to the use of a different bottle of insulin in this experiment. At 21 days of age, blood glucose levels 30 min after insulin are higher in fatty rats compared with their lean littermates (Fig. 7). Although this pattern is maintained at 22 and 23 days of age, 30 min blood glucose shifts upward in both lean and fatty rats, suggesting that both groups become slightly more insulin resistant at these ages. After 23 days of age, insulin resistance decreases. By 25 days of age, fatty rats and their lean littermates have similar levels of blood glucose 30 min after insulin injection as detected by an increased in the weight of the stomach contents and by elevated insulin levels in fatty rats (17).

In experiment 3, daily gain of fatty rats exceeds that of lean rats at 23 days of age, precisely the same age as in many previous experiments (Fig. 6). We previously showed that this increase in daily gain is associated with increased food intake as detected by an increased in the weight of the stomach contents and by elevated insulin levels in fatty rats (17). Blood glucose levels 30 min after insulin are notably lower in experiment 3 than in experiments 1 and 2. We believe this is attributable to the use of a different bottle of insulin in this experiment. At 21 days of age, blood glucose levels 30 min after insulin are higher in fatty rats compared with their lean littermates (Fig. 7). Although this pattern is maintained at 22 and 23 days of age, 30 min blood glucose shifts upward in both lean and fatty rats, suggesting that both groups become slightly more insulin resistant at these ages. After 23 days of age, insulin resistance decreases. By 25 days of age, fatty rats and their lean littermates have similar levels of blood glucose 30 min after insulin injection as detected by an increased in the weight of the stomach contents and by elevated insulin levels in fatty rats (17).
Fig. 5. Insulin tolerance during the suckling to weaning transition. Values represent sex-averaged means ± SE. There were no significant effects on 0-min glucose of fa genotype \( [F(2,90) = 0.93, P = 0.40] \) or fa genotype by age \( [F(4,90) = 1.65, P = 0.13] \); however, there was a large effect of age \( [F(2,90) = 33.59, P < 0.0001] \). This age effect reflects the fact that the mean 0-min glucose value was lower at 28 days of age \( [99.6 ± 1.6] \) than at 14 days of age \( [114.2 ± 1.5, n(90) = 7.8, P < 0.0001] \) or 21 days of age \( [111.5 ± 1.5, n(90) = 6.4, P < 0.0001] \). There were significant effects of fa genotype \( [F(2,90) = 29.92, P < 0.0001] \), age \( [F(4,90) = 20.44, P < 0.0001] \), and fa genotype by age \( [F(2,90) = 9.11, P < 0.0001] \) on 30-min glucose. Thirty-minute glucose value was higher in fatty rats compared with lean rats at 14 days of age \( [F(2,90) = 31.27, P < 0.0001] \) and 21 days of age \( [F(2,90) = 20.51, P < 0.0001] \), but not at 28 days of age \( [F(2,90) = 0.08, P = 0.92] \).

Fig. 6. Daily gain during the 4th wk of life. Values are sex-averaged means ± SE. There were significant effects of fa genotype \( [F(2,241) = 9.96, P < 0.0001] \), sex \( [F(1,241) = 7.82, P < 0.0056] \), age \( [F(6,689) = 206, P < 0.0001] \), and fa genotype by age \( [F(6,241) = 8.69, P < 0.0001] \) on daily gain. Fatty rats gain less weight than lean rats at 21 days of age. At 22 days of age, daily gains of fatty and lean rats are similar. At 23–26 days of age, daily gains of fatty rats are significantly higher than lean rats \( [P = 0.05] \). These data replicate a pattern we have described previously (17), i.e., daily gain increases in fatty rats after 22 days of age. This corresponds with the onset of hyperphagia. Asterisks indicate means differ significantly between fatty and lean rats at that day.

Fig. 7. Insulin tolerance during the 4th wk of life. Insulin tolerance tests were performed on fatty rats and their lean littermates from 241 subjects from 26 litters at 21–27 days of age. Because recovery requires >1 day, each litter was used on only 1 day. The distribution of litters by age and subjects by age, sex, and genotype is given in Table 1. There were no significant effects of fa genotype, sex, age, or their interaction on 0-min blood glucose in this experiment. Therefore, all subjects can be assumed to share a mean 0-min glucose value of 102.5 mg/dl. For clarity, only glucose values 30 min after insulin injection are shown. Genotype \( [F(1,194) = 23.11, P < 0.0001, \text{age} \ [F(6,195) = 4.26, P = 0.0005], \text{and genotype by age} \ [F(6,194) = 12.86, P < 0.0001] \) had significant effects on 30-min glucose. At 21 days of age, fatty rats had greater 30-min glucose values than lean rats \( [F(1,194) = 13.40, P = 0.0003] \). At 22 days of age, fatty rats had greater 30 min glucose values than lean rats \( [F(1,194) = 40.21, P < 0.0001] \). At 23 days of age, fatty rats had greater 30 min glucose values than lean rats \( [F(1,194) = 14.20, P = 0.0002] \). At 24 days of age, fatty rats had greater 30 min glucose values than lean rats \( [F(1,194) = 5.19, P = 0.0238] \). At 25 days of age, fatty rats had greater 30 min values than lean rats \( [F(1,194) = 1.40, P = 0.2229] \). At 26 days of age, fatty rats had 30 min glucose values similar to lean rats \( [F(1,194) = 0.40, P = 0.5285] \). At 27 days of age, fatty rats had 30 min glucose values similar to lean rats \( [F(1,194) = 10.27, P = 0.0016] \). Asterisks indicate mean glucose is significantly different between fatty and wild type rats on that day after insulin injection.

**DISCUSSION**

There are many ways to evaluate insulin resistance, depending on the objectives of the study (9). The insulin tolerance test is one of the oldest and most direct methods. When injected at the appropriate dose, insulin stimulates insulin signaling pathways that cause the translocation of GLUT4 glucose transporter from the intracellular vesicles to the plasma membrane in adipose tissue and muscle (14). Glucose uptake causes blood glucose to fall, resulting in hypoglycemia. Hypoglycemia triggers a counterregulatory release of epinephrine, which stimulates hepatic glycogenolysis to return glucose to baseline levels. Clinically, the level of blood glucose at 60 min after an insulin injection has been used to screen for insulin resistance (9); however, this method is less desirable than other methods because of the potential health consequences of hypoglycemia and because counterregulatory hormone release can complicate the interpretation of the results.

We evaluated a 120-min insulin tolerance test for evaluation of insulin resistance in rats. Our pilot data indicated that the nadir of blood glucose in rats occurred at 30 min after a subcutaneous insulin injection. From 30 to 120 min, blood glucose rose steadily toward the baseline. Because we are interested in insulin effects, and not the effects of counterregu-
latory hormones, we chose to use the 30-min glucose value as a measure of insulin resistance. This method has the advantage of being a simple and direct measure of insulin resistance. It is particularly suited to this study because it reduced the stress on the experimental subjects compared with a 120-min insulin tolerance test and because it allowed the same subjects to be used at different points in development, without damaging their tails with multiple incisions. This method is also suitable for use with large numbers of subjects, where more complex methods are logistically challenging.

The glucose tolerance test is another popular method of measuring insulin resistance in animal models. In the glucose tolerance test, glucose is given by injection or by gavage and blood glucose is measured at 15- to 30-min intervals over 120 min (9). In the glucose tolerance test, the initial rise in glucose reflects absorption, and the subsequent return to baseline reflects the influence of endogenous insulin on the clearance of blood glucose. The interpretation of a glucose tolerance test is more complicated because it reflects the subject’s ability to detect elevated glucose, to release insulin, to stimulate insulin signaling, and to take up glucose from the blood. In this case, a longer time period and more time samples are important for interpreting insulin resistance.

Using this approach, we found that the Zucker fatty rat (fa/fa) experiences insulin resistance during two phases of development. The early phase of insulin resistance occurs during the suckling period before the onset of hyperphagia, hyperinsulinemia, and overt obesity. Hyperphagia emerges in fatty rats at 23 days of age and is accompanied by elevated plasma insulin and rapid development of overt obesity (17). After the onset of hyperphagia, fatty rats rapidly become as insulin sensitive as their lean littermates. This insulin-sensitive phase is fairly brief; as they become more obese over the next few weeks, fatty rats gradually become insulin resistant again.

The early phase of insulin resistance probably reflects a lack of leptin signaling uncomplicated by hyperinsulinemia and obesity. Although fatty rats have high leptin levels, their leptin signaling is low because of a defect in the leptin-binding domain of the leptin receptors (12). In this respect, they are similar to mice that are leptin deficient because they lack adipose tissue. These mice produce very little leptin and are also insulin resistant. Treatment with exogenous leptin improves insulin resistance in this model, and in humans with lipodystrophies (11, 15). Because leptin signaling cannot be restored in fatty mutants after the onset of hyperphagia, the development of insulin sensitivity in this period must be mediated by mechanisms that are independent of leptin signaling.

The transition from the early phase of insulin resistance to the insulin-sensitive phase is more complex than a simple increase in responsiveness to insulin. Instead, the response of blood glucose to insulin injection resembles a roller coaster ride, i.e., there is a gradual increase in insulin resistance before onset of hyperphagia, a peak at the onset of hyperphagia, and a drop in insulin resistance after the onset of hyperphagia. This pattern has two implications. First, it suggests that increased insulin sensitivity does not drive the onset of hyperphagia as we initially proposed. Instead, increased insulin sensitivity seems to follow the onset of hyperphagia. Second, the fact that an increase in insulin resistance precedes the onset of hyperphagia and peaks at the onset of hyperphagia suggests that the mechanism that activates hyperphagia is related to increased insulin resistance.

An increase in lipogenesis in adipose tissue is one factor that probably contributes to the development of insulin sensitivity after the onset of hyperphagia. The increase in insulin secretion that accompanies a higher level of food intake contributes to the increase in lipogenesis at this age. However, the onset of hyperphagia and hyperinsulinemia emerges with striking amplitude at 23 days of age. We have shown elsewhere that the weight of the stomach contents increases in fatty rats by ~80% at 23 days of age, and insulin levels increase by 140% at this age (17). In contrast, the transition from an insulin-resistant state to an insulin-sensitive state requires a period of adaptation of a couple of days. This is likely to involve changes in other signaling molecules that improve the ability to respond to the increased insulin levels, such as PPARγ.

PPARγ is a transcription factor that improves insulin sensitivity by stimulating the expression of genes for glucose disposal and lipogenesis in adipose tissue. PPARγ mRNA levels are low at 15 days of age and reach a peak at 23 days of age in wild-type rats (13). This corresponds with the ages when rats in our studies become increasingly insulin resistant, which suggests that the changes in insulin resistance and PPARγ mRNA expression could be linked. Furthermore, when insulin-resistant adult fatty rats or humans with diabetes are treated with thiazolidinediones, a class of PPAR agonists, their insulin sensitivity improves and their appetite is also stimulated (10, 21). These observations suggest that developmental changes in PPARγ expression could be an important part of the changes in insulin sensitivity in response to hyperphagia, or perhaps a part of the mechanism that triggers the onset of hyperphagia.

Although we have shown that the onset of hyperphagia occurs at 23 days of age in fatty rats raised under standard husbandry conditions, it should be noted that others have demonstrated that fatty rats will consume greater amounts of food earlier than this age when individual food intakes are measured under other experimental conditions. When rats are separated from their dams for 4 h and housed with their siblings, then transferred to individual test chambers with access to half and half (milk and cream), fatty animals consumed more half and half at 12, 15, and 18 days of age (5). This experiment suggests that the pathways that stimulate hyperphagia can influence food intake earlier than 23 days of age. The fact that fatty animals are not hyperphagic under standard husbandry conditions suggests that the onset of hyperphagia is also dependent on other factors. These factors could be related to diet composition, the thermal environment, maternal interactions, or developmentally programmed changes in metabolism. On this note, the increase in PPARγ expression during the weaning transition does not differ between rats weaned on high-carbohydrate vs. high-fat diets but is independent of diet composition. Thus PPARγ expression is regulated by developmental factors rather than diet during the weaning transition (13).

These data show a dynamic relationship between insulin resistance and the fatty phenotype that is closely related to hyperphagia. Not only are there long-term changes in insulin resistance from the age when fatty animals at not obese or hyperinsulinemic to the age when they are massively obese, but there are also short-term changes in insulin resistance around the onset of hyperphagia that suggest that some devel-
opmental perturbation of insulin signaling is related to the onset of hyperphagia. The increase in expression of PPARγ at this age, and the fact that PPARγ agonists not only improve insulin signaling but also stimulate lipogenesis and hyperphagia in adult fatty rats (21), suggest that this transcription factor could be a critical component of the activation of hyperphagia.

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REFERENCES