Dynamics of insulin sensitivity, β-cell function, and β-cell mass during the development of diabetes in fa/fa rats

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Topp BG, Atkinson LL, Finegood DT. The dynamics of insulin sensitivity, β-cell function, and β-cell mass during the development of diabetes in fa/fa rats. Am J Physiol Endocrinol Metab 293: E1730–E1735, 2007.—Both male Zucker Fatty (mZF) and lower-fat-fed female Zucker diabetic fatty (LF-fZDF) rats are obese but remain normoglycemic. Male ZDF (mZDF) and high-fat-fed female ZDF rats (HF-fZDF) are also obese but develop diabetes between 7 and 10 wk of age. Although these models have been well studied, the mechanisms governing the adaptations to obesity in the normoglycemic animals, and the failure of adaptation in the animals that develop diabetes, remain unclear. Here we use quantitative morphometry and our recently developed coupled β-cell function, mass, and replication rate (21). Slow expansion of β-cell mass during the development of diabetes in these animals is because of a lack of integrative longitudinal data. Traditional methodologies for estimating insulin sensitivity and pancreatic insulin secretory capacity (the hyperinsulinemic clamp and the perfused pancreas) are expensive, time consuming, require special experimental equipment, and would necessitate a different cohort of animals for each metabolic index and point in time. Although mathematical models such as the Minimal model and the homeostasis model assessment (HOMA) have vastly improved pancreas studies have shown β-cell function to be similar in mZDF and lean Zucker rats (16, 21). Studies of β-cell function in other animal models have also produced equivocal results. Chronic glucose infusion and partial pancreatectomy studies have clearly shown β-cell function to be plastic and responsive to acute changes (15, 27). However, other studies have shown chronic hypersecretion to be unsustainable, termed β-cell exhaustion (11). Overall, the relative contributions of βm and βc and function to the hyperinsulinemia of obesity remain unresolved.

In the prediabetic state, there is little to separate the mZDF from the mZF rat. Both are obese and insulin resistant. They display similar β-cell function, mass, and replication rates (21). However, by 12 wk of age, mZDF rats display reduced levels of both βm and β-cell function (21). The known effects of hyperglycemia and dyslipidemia on muscle glucose uptake would suggest that adult mZDF rats are more insulin resistant than mZF rats (18, 28). However, a direct comparison of insulin sensitivity levels between these animals has yet to be reported. It has been widely suggested that the transition from normoglycemia and prediabetes to overt hyperglycemia in mZDF rats occurs via lipotoxicity (14). Indeed, prediabetic mZDF rats have been shown to display high levels of plasma free fatty acid (FFA) and high susceptibility to β-cell lipotoxicity (14). Together these studies suggest that abnormal lipid buffering causes exacerbation of insulin resistance, decreased insulin secretion, and increased β-cell apoptosis. However, the longitudinal data required to support this evidence has yet to be reported.

Relatively little is known about fZDF rats. On a low-fat diet, they have been shown to display lower plasma FFA levels than mZDF rats, suggesting improved lipid partitioning and reduced susceptibility to lipotoxicity (14). Recently, the high-fat-fed fZDF (HF-fZDF) rat has been proposed as an animal model of diet-induced type 2 diabetes (6). The mechanisms responsible for the initiation and exacerbation of hyperglycemia in these animals remain largely unexplored.

Much of the controversy surrounding the relative contributions of insulin resistance, β-cell function, and βm to the pathogenesis of diabetes in these animals is because of a lack of integrative longitudinal data. Traditional methodologies for estimating insulin sensitivity and pancreatic insulin secretory capacity (the hyperinsulinemic clamp and the perfused pancreas) are expensive, time consuming, require special experimental equipment, and would necessitate a different cohort of animals for each metabolic index and point in time. Although mathematical models such as the Minimal model and the homeostasis model assessment (HOMA) have vastly improved
the practicality of measuring insulin sensitivity and pancreatic insulin secretory capacity in humans, they have not been adapted to the most commonly used laboratory animals (rats and mice; see Refs. 1 and 17). Recently, we developed a mathematical model of coupled $\beta_m$, insulin, and glucose (BIG) dynamics (25). Similar to the HOMA method, this model was designed to estimate insulin sensitivity and $\beta$-cell function from fasting glucose and insulin data. In addition, this model is capable of estimating net neogenesis (neogenesis − death) from estimates of $\beta_m$ and $\beta$-cell replication rates. With the use of human parameter values, BIG model estimates of insulin sensitivity and $\beta$-cell function showed strong correlation to the HOMA and Minimal models in a large human data set spanning the range of type 2 diabetes (26). Adjusting to rodent-specific parameter values, BIG model estimates of insulin sensitivity, $\beta$-cell function, and net neogenesis showed strong agreement with previously published in vivo measures of these indexes during chronic glucose infusion in Sprague-Dawley rats (27).

Here we use histological methods and the BIG model to estimate the full dynamics of insulin sensitivity, $\beta$-cell function, $\beta_m$, $\beta$-cell replication rates, and net neogenesis in two animal models of obesity [mZF and lower-fat-fed fZDF (LF-fZDF) rat] and two animal models of type 2 diabetes (mZDF and HF-fZDF).

**MATERIALS AND METHODS**

**Animals.** Thirty-two mZF, 32 mZDF, and 54 fZDF rats were obtained from Genetic Models (Indianapolis, IN). Animals were housed individually with free access to food and water. Male rats were fed Purina 3008 (16.5% fat by calorie) while female rats were randomly assigned either low-fat (Purina 5001, 12% fat by calorie) or high-fat (GMI 13004, 47.9% fat by calorie) chow. Weekly blood samples (~0.1 ml) were taken from the saphenous vein of nonfasted rats. Eight animals per group were killed biweekly. Before death (6 h), rats were given an intraperitoneal injection of 5-bromo-2’-deoxyuridine (BrdU; 100 mg/kg; Sigma-Aldrich, Oakville, ON, Canada). Glucose, insulin, $\beta_m$, and BrdU data from the mZF rats were previously reported (7). All procedures were performed in accordance with the standards set forth by the Canadian Council on Animal Care and were approved by the Animal Care Committee at Simon Fraser University.

**Measurement of glucose and insulin.** Plasma samples were centrifuged and stored at −20°C until assayed for glucose (Glucose Trinder; Sigma Diagnostics, St. Louis, MO) and insulin (rat insulin ELISA; Crystal Chem, Downers Grove, IL).

**Pancreatectomy.** Pancreatectomies were performed as previously described (1). Tissue samples were cut in two and then placed in a fixative [mixture of 75 ml water, 25 ml formaldehyde (37%), and 5 ml glacial acetic acid] for 48 h at room temperature. They were washed three times in PBS (pH 7.4) and then left in PBS at 4°C overnight. Samples were washed three times in 70% alcohol, placed in cassettes, and embedded in paraffin. Five serial sections (4 μm) from each block (2 sets/animal) were cut using an Olympus microtome (CUT 4060; Carsen Group, Markham, ON, Canada) and were mounted on poly-l-lysine-coated slides.

**Immunohistochemistry.** Two serial sections from each block were stained and analyzed. The first slide was stained with anti-insulin antibody (1:1,000; Dako Diagnostics, Mississauga, ON, Canada) at 37°C for 30 min, biotinylated anti-guinea pig antibody (1:500; Vector Laboratories, Burlington, ON, Canada) for 1 h; and avidin/biotin horseradish peroxidase complex (ABC-HP, 1:1,000; Vectorstain Elite ABC Kit; Vector Laboratories) for 1 h at room temperature. Samples were then developed in 3,3’-diaminobenzidine tetrahydrochloride (DAB) solution (Sigma-Aldrich) for 10 min. Sections were washed several times with PBS between incubations. After DAB development, sections were washed in running tap water, counterstained with hematoxylin (Harris, Sigma-Aldrich), and coverslipped with Permount mounting media (Fisher Scientific, Nepean, ON, Canada).

The sections double stained for BrdU and insulin were dewaxed and hydrated as described above and then incubated with anti-BrdU monoclonal antibody with nuclease (Amer sham Pharmacia Biotech, Baie d’Ur fe, Quebec, Canada) for 30 min at 37°C. Sections were washed with PBS and incubated serially with biotinylated goat antimouse antibody (Vector) for 1 h and ABC-HP (1:1,000; Vector) for 1 h at room temperature. Slides were washed in PBS between incubations. Sections were developed in DAB solution for 2–3 min. Slides were washed with PBS and incubated with guinea pig anti-insulin antibody (DAKO Diagnostics) for 30 min at 37°C. Next, they were serially incubated with biotinylated anti-guinea pig antibody (1:500; Vector) for 1 h and avidin/biotin alkaline phosphatase complex (Alkaline Phosphatase Standard Vectastain ABC Kit; Vector Laboratories) for 1 h at room temperature. PBS washes were carried out between incubations. Sections were developed in Dako Fuchsia Substrate-Chromogen System for 10 min (Dako Diagnostics). Slides were then washed in water, counterstained with hematoxylin, and coverslipped with Permount.

**Quantitative morphometry.** $\beta_m$ was determined via quantitative analysis of the insulin antibody-stained slides. An image analysis system consisting of an Olympus light microscope (model BX40; Carsen Group) attached to a Sony color video camera (model DXC-950; Sony) and MetaVue image analysis software (Universal Imaging, Downingtown, PA) was used to estimate the $\beta$-cell and non-$\beta$-cell area on each slide. A point counting system was used to estimate the ratio of adipose to nonadipose tissue on each slide. The ratio of $\beta$-cell to non-$\beta$-cell tissue was determined for each animal and then multiplied by the pancreatic mass to generate an estimate of total $\beta_m$. Ten slides from each group were used to determine the relationship between $\beta$-cell number and $\beta$-cell area. These correlations were then used to estimate the total number of pancreatic $\beta$-cells on each slide.

**BIG model analysis.** The equations for the BIG model (25) are as follows:

$$\frac{dG}{dt} = R_G - (E_G + S_G)G$$

(1)

$$\frac{dI}{dt} = \beta_m \frac{G^2}{(\alpha^2 + G^2)} - kI$$

(2)

$$\frac{d\beta_m}{dt} = (\text{replication} - \text{death} + \text{neogenesis})\beta_m$$

(3)

where $G$, $I$, $\beta_m$, and $t$ represent plasma glucose, insulin, $\beta_m$, and time, respectively. $R_G$, $E_G$, and $S_G$ represent the maximal rate of hepatic glucose output, glucose effectiveness at zero insulin, and insulin sensitivity, respectively. $\beta_m$, $\alpha$, and $k$ represent the $\beta$-cell secretory capacity (maximal rate of secretion per unit $\beta_m$), the glucose level that induces half-maximal insulin secretion rates, and the insulin clearance rate, respectively.

**Equation 1** was used to estimate insulin sensitivity for each animal at each point in time. Briefly, $dG/dt$ was assumed to be zero (i.e., the minute-to-minute changes were negligible relative to the 6-wk time frame of the study), and constant values were assigned to $R_G$ and $E_G$ based on the literature. The measured glucose and insulin levels could then be placed into equation 1 and used to estimate $S_G$. 

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was then calculated with the following formula:

$$\beta_m = k(\alpha^2 + G^2)/(\beta_m G^2)$$  \hspace{1cm} (5)

The standard error of \( \beta_m \) was estimated via the Taylor expansion method for propagating the standard errors from measured G, I, and \( \beta_m \) data at each point in time. Because the rats were not fasted, the calculated \( \beta_m \) reflects a pseudosteady state that is not specific to basal insulin secretion; some component is stimulated by glucose.

Equation 3 was used to estimate net neogenesis (neogenesis – death). Because \( \beta_m \) dynamics occur on a much slower time scale (days to weeks) relative to glucose and insulin dynamics (minutes), \( d\beta_m/dt \) could not be assumed to be zero. Thus \( d\beta_m/dt \) was approximated from the \( \beta_m \) measured data (\( \Delta\beta_m/\Delta t \)) and assigned to the time point in between the measured points (i.e., at 7, 9, and 11 wk of age). Propagation of error was used to estimate the uncertainty surrounding these calculated values. The percentage of \( \beta \)-cells replicating per day was assumed to be four times the percent of Brdu-positive cells (since Brdu is injected 6 h before termination). \( \beta_m \) and replication rates at 7, 9, and 11 wk of age were estimated by averaging the data measured at 6, 8, 10, and 12 wk of age, and propagation of error was used to estimate the standard errors on these calculated values. Net neogenesis was then calculated with the following formula:

$$\frac{(\Delta\beta_m/\Delta t)}{\beta_m} - \text{replication} = \text{neogenesis} - \text{death} = \text{net neogenesis} \hspace{1cm} (6)$$

The standard error about net neogenesis was estimated via propagating the standard errors of \( \Delta\beta_m/\Delta t \), \( \beta_m \), and replication rate data. The model structure used to determine insulin sensitivity in the Big model is the same as that used in the minimal model, which has been validated against glucose clamp data in many species. The time scale of the Big model is in days to weeks, whereas the time scale of the minimal model is minutes to hours; however, in the pseudosteady state, the Big model is similar to HOMA and QUICKIE. Although the Big model has not been compared directly with insulin sensitivity measurements in this study, published data from our laboratory are consistent with the data presented here (27).

Statistical analyses. Data are presented as means ± SE unless otherwise noted. Comparisons between points in time and treatment groups were performed via a repeated-measured two-way ANOVA with post hoc Tukey analysis. All statistical calculations were performed using SAS Version 7.0 (The SAS Institute, Cary, NC).

**RESULTS**

HF-fZDF consumed fewer calories than LF-fZDF rats (93.4 ± 1.1 vs. 97.4 ± 0.6 calories/day, \( P < 0.05 \)) despite being fed a high-calorie chow diet (4.9 vs. 3.9 calories/g) ad libitum (Fig. 1). Body weight differed only slightly between groups of female rats. At the beginning of the study, mZDF rats consumed fewer calories than mZF rats while displaying similar water intake and body weight. The development of diabetes in mZDF rats was associated with increased food and water intake as well as reduced weight gain. mZDF rats ingested more calories and put on more body weight than fZDF rats. In addition, despite similar levels of hyperglycemia at 12 wk of age (Fig. 2), HF-fZDF rats had less severe hyperphagia and glucosuria compared with mZDF rats.

Glucose and insulin dynamics for all four groups of animals are displayed in Fig. 2. LF-fZDF rats developed moderate hyperglycemia and hyperinsulinemia over the course of this study. HF-fZDF rats developed overt hyperglycemia between 8 and 11 wk of age while insulin levels display a biphasic pattern, increasing for the first 3 wk and then decreasing thereafter. Overt hyperglycemia (>20 mM) preceded the decrease in insulin levels. mZF rats remained normoglycemic throughout while displaying a three- to fourfold increase in plasma insulin levels. mZDF rats displayed 3 wk of rising glucose levels accompanied by biphasic insulin dynamics. Similar to HF-fZDF rats, overt hyperglycemia preceded the decrease in insulin levels in mZDF rats. Male rats had a higher insulin level relative to female rats at the beginning of the study. Also, the decrease in insulin levels was less pronounced and was initiated at a higher glucose level in HF-fZDF rats relative to mZDF rats.

Figure 3 shows the \( \beta_m \) and replication rate data for all four groups of Zucker rats. Both groups of fZDF rats displayed a two- to threefold increase in \( \beta_m \). This early rapid expansion was supported by high rates of \( \beta \)-cell replication (~8%/day) that fell to a moderately elevated level of ~4%/day by the end of the study. mZF rats displayed a three- to fourfold increase in pancreatic \( \beta_m \) between 6 and 10 wk of age that was due, at least in part, to elevated rates of \( \beta \)-cell replication (~8%/day) that subsided to moderately elevated levels (~4%/day) by the end of the study. During the first 2 wk of study, mZDF rats demonstrated a slow rate of \( \beta_m \) expansion relative to mZF rats (6.9 ± 0.5 vs. 2.0 ± 1.4%/day, \( P < 0.05 \)). This was followed
by an ~50% reduction in \( \beta_m \) between 8 and 12 wk of age \( (P < 0.05) \). These \( \beta_m \) dynamics are associated with reduced rates of \( \beta \)-cell replication throughout the study. mZDF rats displayed a higher \( \beta_m \) at the beginning of the study compared with fZDF rats, but this difference disappeared by 8 wk of age and was reversed by 12 wk of age. Replication rates were lower in mZDF rats throughout. Also, it should be noted that, despite similar end-study glucose levels, HF-fZDF rats did not display any significant reduction in \( \beta_m \). This discrepancy may be due to the fact that mZDF rats develop overt hyperglycemia 2 wk earlier than HF-fZDF rats.

\[ \beta_m \] model estimates of insulin sensitivity, \( \beta_{sc} \), and net neogenesis are presented in Fig. 4. LF-fZDF rats displayed an ~75% reduction in \( S_t \), whereas \( \beta_{sc} \) (an index of the maximal rate of glucose-induced insulin secretion/unit \( \beta_m \)) remained constant. There were no significant changes in net neogenesis in LF-fZDF rats. HF-fZDF rats quickly became more insulin resistant than LF-fZDF rats. \( \beta_{sc} \) was constant in HF-fZDF rats before the development of hyperglycemia but decreased thereafter. Net neogenesis was similar in both groups of female rats. mZF rats displayed an ~70% decrease in \( S_t \) between 6 and 8 wk of age while \( \beta_{sc} \) remained constant and net neogenesis decreased. mZDF rats were more insulin resistant than mZF rats during the initiation (week 7) and exacerbation (weeks 9–11) of hyperglycemia. \( \beta_{sc} \) remained constant during the initiation of hyperglycemia but decreased thereafter. Net neogenesis did not differ between mZDF and mZF. Male rats were more insulin resistant than female rats. Before the development of hyperglycemia, \( \beta_{sc} \) did not differ between mZDF and IzDF rats; however, male rats displayed more marked reductions in \( \beta_{sc} \) following the development of overt hyperglycemia.

Overall, these data suggest that, in obese control animals (mZF and LF-IzDF), \( \beta_m \) adaptation offset decreasing insulin resistance such that their disposition index (Fig. 5) remained relatively constant. However, excessive insulin resistance and insufficient \( \beta_m \) adaptation led to an initial decrease in the disposition index and the initiation of hyperglycemia in both
obese diabetic animals (mZDF and HF-fZDF). This hyperglycemia was followed by progressive insulin secretory defects that further reduced the disposition index and exacerbated hyperglycemia (Fig. 5).

**DISCUSSION**

Adaptation to obesity and the pathogenesis of type 2 diabetes are complex processes characterized by time-dependent changes in several key metabolic pathways. Here we used the tools of quantitative morphometry and mathematical modeling to estimate the dynamics of insulin sensitivity, \( \beta \)-cell function, and \( \beta_m \) during normal and pathological adaptation to obesity. In both obese control groups (mZF and LF-fZDF), we found adaptation to insulin resistance occurs via increased \( \beta_m \), whereas \( \beta \)-cell function remained relatively constant. During the initiation of hyperglycemia, mZDF and HF-fZDF rats displayed excessive insulin resistance and insufficient \( \beta_m \) adaptation. Progressive reductions in \( \beta \)-cell function occurred after overt hyperglycemia (plasma glucose levels >20 mM) was established. Together this suggests that excessive insulin resistance and abnormal \( \beta_m \) dynamics are important contributors to the pathogenesis of diabetes. Decreases in insulin secretion occurred secondary to hyperglycemia, although we cannot exclude a role for defects in secretion contributing to the development of hyperglycemia.

**Sufficient adaptation to obesity.** mZF rats were more insulin resistant than LF-fZDF rats throughout the study. Between 6 and 12 wk of age, insulin sensitivity dropped significantly in both obese control animals. This was accompanied by increased \( \beta_m \) and constant \( \beta \)-cell function. Although this suggests that \( \beta \)-cell function does not respond to the development of insulin resistance, it should be noted that 1) these animals were moderately obese and insulin resistant at the beginning of the study and 2) estimates of \( \beta \)-cell function reported here are \( \sim \)20-fold higher than \( \beta \)-G cell model estimates of \( \beta \)-cell function previously reported in adult Sprague-Dawley rats (27). This suggests that \( \beta \)-cell function may have adapted maximally before 6 wk of age.

Our finding of increased \( \beta_m \) in obese control animals is consistent with other animal and human studies, including other studies in mZF rats (2, 3, 16, 21). However, the literature on \( \beta \)-cell function in obesity is less clear. Glucose infusion and partial pancreatectomy studies have clearly demonstrated acute adaptation of \( \beta \)-cell function in response to increased insulin demand (15, 27). However, the concept of \( \beta \)-cell exhaustion would suggest that \( \beta \)-cell hypersecretion may not be sustainable in the long run (11, 13). Studies of function in obese Zucker rats have also generated mixed results. Some studies have reported a “left shift” in glucose-induced insulin secretion in islets from humans and Zucker rats (12, 30). Other studies have found \( \beta \)-cell function not to differ between obese and lean Zucker rats (16, 21). Finally, it should be noted that, because of the in vivo nature of our index of \( \beta \)-cell function (\( \beta_{sw} \)), differences between Sprague-Dawley and Zucker rat values for \( \beta_m \) may reflect differences in insulin clearance and/or non-glucose secretagogues rather than adaptation of glucose-induced insulin secretion per se. However, it is difficult to imagine that the 20-fold difference in \( \beta_{sw} \) for Sprague-Dawley and obese Zucker rats can be fully accounted for by differences in insulin clearance rates. Overall, these data support the concept of a feedback loop between \( \beta_m \) and insulin sensitivity and suggest that \( \beta \)-cell function adapted maximally before 6 wk of age.

**Insulin resistance during the development of hyperglycemia.** At the beginning of the study, insulin sensitivity did not differ between diabetes-prone and sex-matched obese controls. During the initiation of hyperglycemia, both groups of obese diabetic animals developed greater insulin resistance than their respective obese controls. Several hyperinsulinemic clamp studies have shown both the mZDF and mZF to be insulin resistant; however, this is the first study to compare the dynamics of insulin sensitivity in these animals. These observations suggest that additional insulin resistance may contribute to the development of hyperglycemia rather than only being a result of it. However, we can rule out the possibility that earlier changes in insulin secretory capacity could contribute to the eventual onset of hyperglycemia. Previous studies have also found that fatty acid levels increase in mZDF rats 2 wk before the development of hyperglycemia (14). Although we did not report plasma lipid dynamics here, these data are consistent with the notion that lipotoxicity plays a role in the excessive insulin resistance observed during the initiation of hyperglycemia (29).

**\( \beta \)-cell function during the development of hyperglycemia.** Although other studies have shown \( \beta \)-cell function to be normal or elevated in prediabetic mZDF rats (5, 21, 24), this is the first study to show \( \beta \)-cell function to remain constant during the initiation of hyperglycemia. It should be noted that, although several studies have suggested that mZDF rats display a primary defect in \( \beta \)-cell function, most of these studies have used lean islets as a control (9, 10, 20). Thus it is unclear if these previously reported defects are common to the whole Zucker family or if they occur only in ZDF rats. It is interesting to note that, despite previous reports of increased FFA in prediabetic mZDF rats and increased susceptibility of mZDF islets to lipotoxicity, \( \beta \)-cell function did not decrease in either diabetes-prone strains until glucose levels reached \( \sim \)20 mmol (14, 23). These findings are consistent with the argument that hyperglycemia is a required element of lipotoxicity (22).

**\( \beta_m \) dynamics during the development of hyperglycemia.** Despite the added insulin resistance incurred by the mZDF and HF-fZDF rats, \( \beta_m \) adaptation remained similar to, or slower than, rates observed in obese nondiabetic animals. Also, \( \beta_m \) increased in the mZDF rats but not the HF-fZDF rat and did so at a much higher glucose level than was associated with the initiation of insulin secretory defects. Our observation of reduced \( \beta_m \) in overtly diabetic mZDF rats is consistent with other studies (21). However, this is the first study to show that abnormal mass dynamics contribute to the initiation of hyperglycemia in these animals. Interestingly, Pick et al. (21) found \( \beta \)-cell replication rates to be similar in mZDF and mZF rats, suggesting that mZDF rats display elevated levels of \( \beta \)-cell apoptosis. Also, previous findings of increased FFA in prediabetic mZDF rats and increased susceptibility of mZDF islets to lipoapoptosis (23) would suggest that high rates of \( \beta \)-cell death play a primary role in the development of hyperglycemia. Here we calculated net \( \beta \)-cell death (neogenesis − death) and found it to be similar in all four animal models, although only \( \beta \)-cell replication rates were reduced in mZDF rats. However, because we calculated net cell death, it is possible that increased rates of \( \beta \)-cell death in the mZDF rats are “hidden” by increased rates of neogenesis.
Lipid partitioning during the development of hyperglycemia.

It is interesting to note that food intake and body weight were similar or reduced in diabetic animals during the initiation of hyperglycemia. This suggests that the additional insulin resistance observed in these animals was not likely a result of additional adiposity. One possible explanation for this is a defect in lipid partitioning that results in increased triglyceride accumulation in nonadipose tissue (8).

In summary, this study has used a combined mathematical and histological approach to generate a complete analysis of the normal and pathological adaptations to obesity in Zucker rats. As a result, we were able to show that both excessive insulin resistance and insufficient $\beta$ cell adaptation contribute to the initiation of hyperglycemia.

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